RESEARCH PAPER

Oestradiol influences on dopamine release from the nucleus accumbens shell: sex differences and the role of selective oestradiol receptor subtypes

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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 oestradiol, which enhances dopamine release in the dorsal striatum. In this study, we tested the acute effect of oestradiol on dopamine release in the nucleus accumbens (NAc) shell after cocaine administration and investigated which oestradiol receptors (ERs) contribute to sex differences in the response to cocaine.

EXPERIMENTAL APPROACH

The ability of oestradiol benzoate (EB) to acutely modulate the effect of cocaine on phasic dopamine release in the NAc shell was measured by fast-scan cyclic voltammetry in anaesthetized male and female rats. The roles of ER subtypes, ERα and ERβ, was determined with selective agonists.

KEY RESULTS

EB acutely enhanced the effect of cocaine on stimulated dopamine release from the NAc shell in females but not in male rats only at levels of stimulation expected to optimally saturate dopamine transporters. Enhanced dopamine 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 dopamine reuptake in males but not in females.

CONCLUSIONS AND IMPLICATIONS

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

LINKED ARTICLES

This article is part of a themed section on The Importance of Sex Differences in Pharmacology Research. To view the other articles in this section visit http://onlinelibrary.wiley.com/doi/10.1111/bph.v176.21/issuetoc

Abbreviations

DPN, diarylpropionitrile; EB, oestradiol benzoate; ER, oestradiol receptor; NAc, nucleus accumbens; OVX, ovariectomy/ ovariectomized; p, pulse; PPT, propyl pyrazole triol; Tau, τ; VTA, ventral tegmental area

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Introduction

Sex differences in striatal circuitry affect both the initial response to psychomotor stimulants as well as the propensity to develop addiction-like behaviours 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-induced 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 behavioural 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). Oestradiol replacement in OVX females results in enhanced responses to psychomotor stimulants, underscoring the importance of oestradiol 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, oestradiol treatment did not enhance cocaine self-administration or stimulant-induced behaviour in males (Becker et al., 2001; Jackson et al., 2006), indicating a sex difference in the activational effects of oestradiol and organizational sex differences in the neural systems mediating drug taking.

Oestradiol enhancement of the behavioural responses to psychomotor stimulants has been linked to changes in striatal dopamine release. Within the dorsal striatum, oestradiol acutely enhances dopamine release in females but not in males (Cummings et al., 2014; Shams et al., 2016). A substantial body of work has demonstrated that oestradiol acts directly on GABAergic striatal tissue to disinhibit dopaminergic terminals and promote dopamine release within the striatum (Yoest et al., 2018). However, while the effects of oestradiol in dorsal striatum have been well established, studies on the rapid effects of oestradiol on dopamine release within the nucleus accumbens (NAc) have produced mixed results. Some studies have shown that oestradiol is able to act directly on NAc circuitry to rapidly enhance stimulated dopamine release (Thompson and Moss, 1994), others have demonstrated an effect of the oestrous cycle but no effect of direct oestradiol application (Calipari et al., 2017) and still others have failed to show an acute effect of oestradiol 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 oestradiol or by measurement of oestradiol across the oestrous cycle, have measured dopamine using fast-scan cyclic voltammetry, while studies that failed to show an effect used microdialysis. Fast-scan cyclic voltammetry measures phasic dopamine release on the scale of seconds within discrete subregions of the NAc, while experiments using microdialysis measure slower changes in tonic release within the NAc as whole. Oestradiol may have opposite effects within the NAc core and shell, and decreased regional specificity in experiments using microdialysis is likely to contribute to the lack of effects in these studies (Peterson et al., 2015).

Additionally, for studies using fast-scan cyclic voltammetry, differences in oestradiol dose may account for discrepancies in findings. The range at which oestradiol 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 oestradiol would enhance phasic dopamine release specifically within the NAc shell.

Here, we sought to characterize the receptor mechanism by which oestradiol could modulate dopamine release within the NAc shell. There are multiple oestradiol receptor (ER) subtypes; the most 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 dopaminergic 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 ER subtypes may mediate oestradiol's ability to modulate stimulated dopamine release after systemic cocaine treatment.

Methods

Animals

All animal care and experimental 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. Animal studies are reported in compliance with the ARRIVE guidelines (Kilkenny et al., 2010; McGrath and Lilley, 2015). Male ($n = 28$) and female ($n = 29$) Sprague Dawley rats (Charles River Breeding Laboratory, Portage, MI, USA, RRID: RGD_734476) 50–55 days of age were maintained on 14:10 light:dark cycle (lights on at 09:00 h) 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, USA). Animals were gonadectomized as described previously (Cummings et al., 2014). Absence of the oestrous cycle in ovariectomized females was verified by daily vaginal lavage, and animals were left otherwise undisturbed in their home cage for 2 weeks following gonadectomy.

Treatments

Oestradiol benzoate (EB) was suspended in peanut oil at a concentration of 5 μg 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 oestradiol ester that is commonly used in hormone priming experiments due to its increased stability when compared with free oestradiol. In previous work from the Becker laboratory, we have found that plasma oestradiol was significantly increased at 30 min and 1 h following s.c. administration of EB (Hu *et al.*, 2004). Additionally, we have repeatedly shown an effect of EB on stimulated dopamine in microdialysate within 30 min, indicating that this time is sufficient to induce an oestrogenic effect on the dopamine 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, USA, Cat No. 1426, CAS #263717-53-9) and the ERβ selective agonist diarylpropionitrile (DPN, Tocris, Cat. No. 1494, CAS #1428-67-7) were suspended in gelatin at a concentration of 1 mg·mL^{-1}. Agonists were stored at 4°C until the day of use, at which time syringes were prepared and brought to room temperature. All hormones and agonists were used within 2 weeks of preparation.

Fast-scan cyclic voltammetry surgery

On the day of surgery, animals were anaesthetized with urethane (1.5 g·kg^{-1}, i.p. in 0.9% sterile saline; Sigma-Aldrich) and prepared in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). A glass-encased cylindrical carbon fibre electrode was lowered into the dorsal portion of the NAc shell (AP, +1.7; ML, ±0.8; and DV, −6), and a silver/silver chloride reference electrode was placed in the contralateral cortex (AP, −2.3; ML ±2.7). Dopamine release was recorded by oxidation and reduction of dopamine in response to the application of a triangular waveform (oxidative scan, 0.4–1.3 V; reductive scan, 1.3–0.4 V; 400 V·s^{-1}) applied to the carbon fibre electrode. Prior to taking recordings of stimulated release, this waveform was repeatedly cycled at a rate of 60 Hz for 30 min, followed by another 10 min 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 dopamine release using 60 Hz 60 pulse (p) biphasic stimulations. Once release was optimized, three recordings were taken during application of a 20 Hz 24p stimulation to the VTA in order to verify stability of the dopamine 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 an s.c. intrascapular injection of either EB (5 μg per 0.1 mL peanut oil) or vehicle (peanut oil; 0.1 mL). The ERα selective agonist PPT (1 mg·kg^{-1}) or the ERβ selective agonist DPN (1 mg·kg^{-1}) was used to determine the role of ER subtypes. For all groups, baseline recordings were taken 30 min following treatment. The experimental timeline is outlined in Figure 1A.

Fast-scan cyclic voltammetry recordings

For recordings of stimulated release, 10 recordings were taken for each stimulation parameter. Stimulations of 20 Hz 24p, 20 Hz 12p, 10 Hz 24p and 10 Hz 12p were applied in order to determine whether the effect of oestradiol on dopamine signalling after cocaine administration is dependent on the amount of dopamine release. The highest stimulation parameter, 20 Hz 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 colour plots (Figure 1C,D) are presented in Figure 1. Following baseline recordings, animals received an i.p. injection of cocaine HCl (10 mg·kg^{-1}, NIDA). After cocaine administration, a second set of recordings was taken using the same stimulation parameters. All recordings were taken by an experimenter blinded to the animal treatment groups.

Data processing and analysis

Analysis of stimulated dopamine 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 dopamine concentration was extracted as a measure of dopamine release, and Tau (τ) was calculated as a measure of dopamine reuptake. τ is an exponential decay constant that has been recommended for quantifying dopamine reuptake after stimulated release (Yorgason *et al.*, 2011). τ is inversely proportional to the decay rate so that an increase in τ corresponds to a decrease in reuptake. Therefore, treatment with cocaine, which blocks the dopamine transporters (DAT) and prevents reuptake, leads to an increase in τ. This method of quantifying dopamine release and reuptake is recommended when stimulation does not induce substantial synaptic overflow and therefore does not fulfil the assumptions required for Michaelis–Menten modelling techniques. At lower levels of stimulation (10 Hz 24p and 10 Hz 12p), the dopamine signal was not sufficient to reliably extract release and reuptake values for all animals; such animals (10 Hz 24p: $n = 2$; 10 Hz 12p: $n = 6$) were excluded from further analysis for these parameters. At the 10 Hz 12p level of stimulation, this resulted in less than five animals in one group (DPN females). As such, these 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 dopamine release measured both before and after cocaine administration (Table 1). This variability may be due to individual differences in dopamine signalling 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 % change from baseline was used to analyse group differences in the effect of cocaine on NAc dopamine release. Average dopamine 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
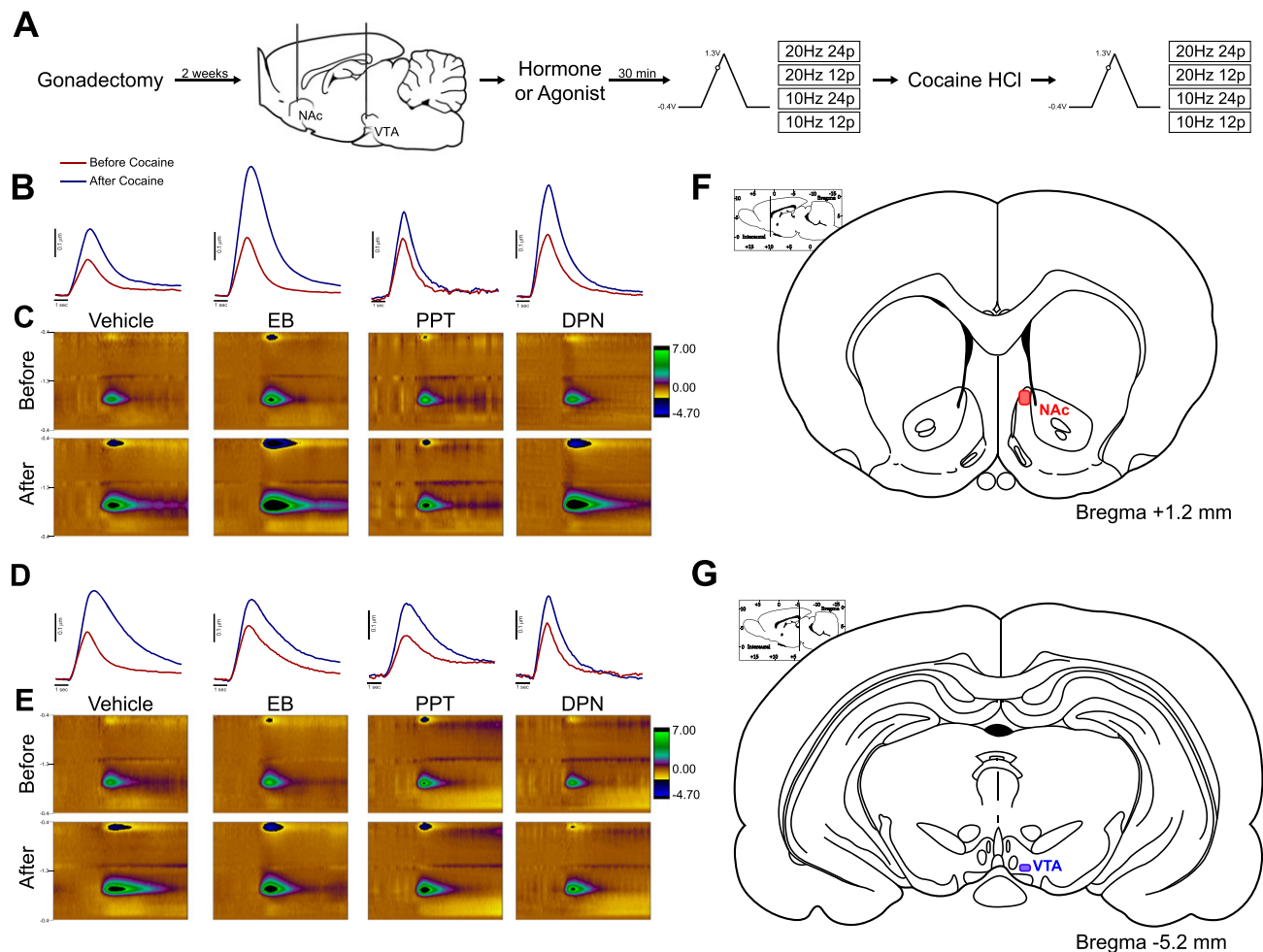
Figure 1

Measurement of stimulated dopamine release by fast-scan cyclic voltammetry. Experimental timeline for voltammetry collection (A). Animals are gonadectomized at least 2 weeks prior to being anaesthetized and implanted with a glass encased carbon fibre 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 and after administration of 10 mg·kg^{-1} cocaine HCl. Representative false colour plots for females (D) and males (E) before (upper rows) and after (lower rows) administration of cocaine HCl. Colouring in false color plots range from -4.7nA (blue) to +7.0nA (green), with yellow indicating baseline current output. Coronal diagrams displaying the placement of recording electrodes in the NAc shell (F) and VTA (G). NAc, nucleus accumbens; VTA, ventral tegmental area; EB, oestradiol benzoate; PPT, propyl pyrazole triol; DPN, diarylpropionitrile.

electrodes used or individual differences in baseline dopamine 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 ($\alpha = 0.05$; $1\text{-}\beta = 0.9$) using an estimated effect size of 1 indicated a minimum necessary sample size of six animals per treatment group. Male and female animals were assigned to receive 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 anaesthetized voltammetry experiments due to missed probe sites, lack of recording stability

or premature mortality, animals were tested in pseudorandom order until the minimally required group size was reached for each condition.

Data and statistical analysis

The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology (Curtis *et al.*, 2018). Group comparisons were performed using GraphPad Prism v7.0a (GraphPad, San Diego, CA, USA; RRID:SCR_002798). Data were analysed using two-way ANOVA with Holm–Sidak *post hoc* tests to compare animals treated with hormones or agonists with controls within sex, as well as to determine if there were sex differences in the effect of each treatment. The accepted value

Table 1

Average stimulated dopamine release during baseline collections and after administration of cocaine at different stimulation rates

	20 Hz 24p			20 Hz 12p			10 Hz 24p			10 Hz 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	$n = 4$

Values shown are mean ± SD after conversion to dopamine concentration (shown as μM).

for statistical significance was set to $P < 0.05$, and *post hoc* tests were only run when initial F values reached this threshold.

Materials

Sigma-Aldrich (St. Louis, MO) supplied EB (Cat No. E8515, CAS #50-50-0). PPT (Cat No. 1426, CAS #263717-53-9) and the ERβ selective agonist DPN (Cat. No. 1494, CAS #1428-67-7) were supplied by Tocris, Minneapolis, MN,

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*., 2017a,b,c).

Results

Effect of oestradiol on stimulated dopamine release after cocaine administration

The effect of oestradiol on stimulated dopamine release after cocaine administration was assessed at four levels of stimulation. Stimulation of VTA with 20 Hz 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 dopamine 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 dopamine 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 oestradiol, where EB-treated females showed a significantly greater effect of cocaine on dopamine concentration, compared with EB-treated males (Figure 2A). The cocaine-induced increase in dopamine overflow was also greater in EB-treated females, compared with vehicle-treated females. Together, these results demonstrate that treatment with oestradiol enhanced the effect of cocaine on stimulated dopamine release in females, but not in males, when stimulation parameters promoted maximal DAT occupancy.

At the 20 Hz 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 towards significance where EB-treated females showed slightly enhanced dopamine release compared with vehicle ($P < 0.10$).

After 10 Hz 24p stimulation (Figure 2C), there was a significant main effect of treatment on dopamine release ($F(3, 47) = 5.85$) but no effect of sex and no significant
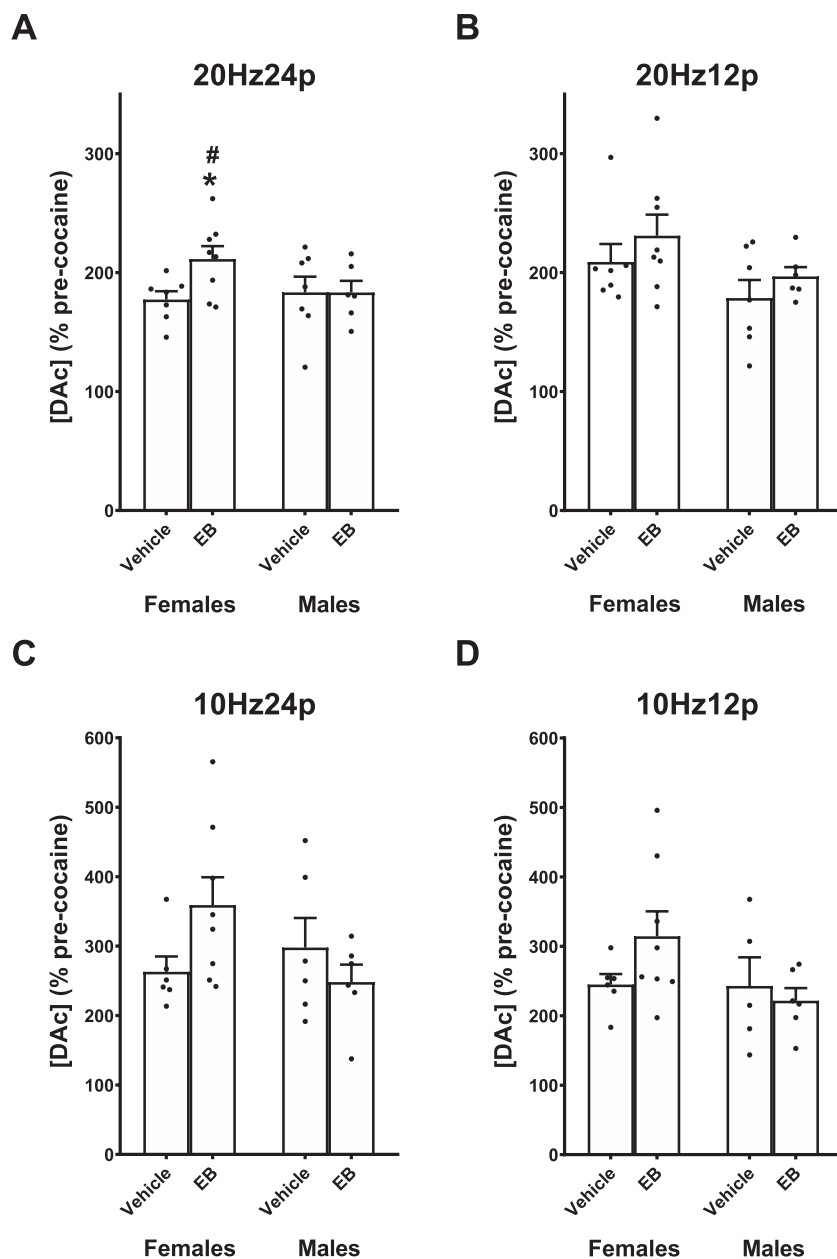
Figure 2
Oestradiol enhances the effect of cocaine on NAc dopamine release in females but not in males. Oestradiol-treated females show significantly greater dopamine release after cocaine administration during (A) 20 Hz 24p stimulation of the VTA but not (B) 20 Hz 12p, (C) 10 Hz 24p or (D) 10 Hz 12p stimulation. Bars show mean (+SEM) % change in [DAc] after i.p. administration of 10 mg·kg^{-1} cocaine HCl. Individual subject values are also shown. 20 Hz 24p: $n = 6$ for EB-treated males; $n = 7$ for vehicle-treated females and males; and $n = 8$ for EB-treated females. 20 Hz 12p: $n = 6$ for EB-treated males; $n = 7$ for vehicle-treated females and males; and $n = 8$ for EB-treated females. 10 Hz 24p: $n = 6$ for vehicle-treated females, vehicle-treated males and EB-treated males; $n = 8$ for EB-treated females. 10 Hz 12p: $n = 5$ for vehicle-treated males; $n = 6$ for vehicle-treated females and EB-treated males; and $n = 8$ for EB-treated females. *$P < 0.05$, significantly different, within sex, from vehicle effect. $^{\#}P < 0.05$, significantly different between sexes.

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 dopamine release after 10 Hz 12p stimulation of the VTA nor a significant interaction between the two (Figure 2D).

Oestradiol enhancement of cocaine's effect on dopamine release is mediated by ERβ
Treatment with the ERβ selective agonist DPN increased the effect of cocaine on stimulated dopamine release in females at the highest stimulation parameter (Figure 3). There was also a sex difference in animals treated with
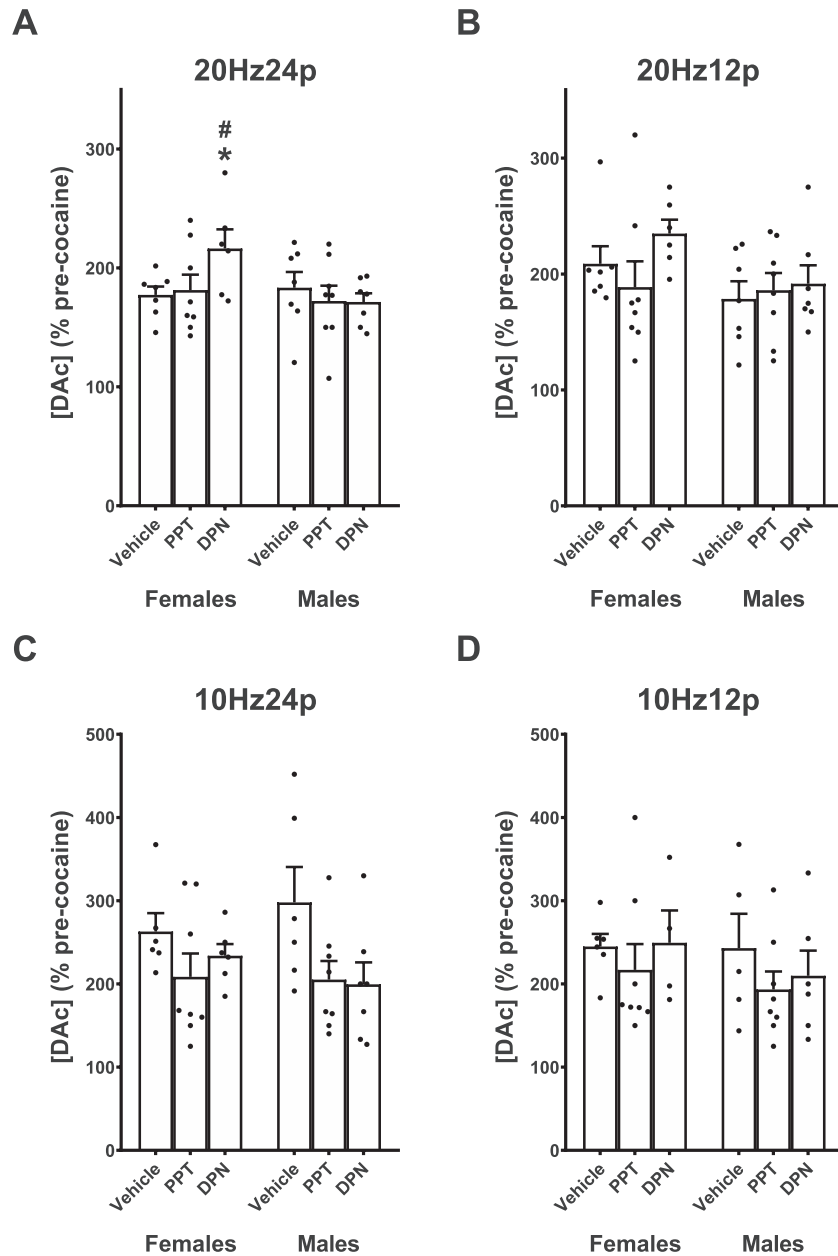
Figure 3

The effect of oestradiol on dopamine release after cocaine is mediated by ERβ. DPN-treated females show significantly greater dopamine release after cocaine administration during (A) 20 Hz 24p stimulation of the VTA but not (B) 20 Hz 12p, (C) 10 Hz 24p or (D) 10 Hz 12p stimulation. Bars show mean (+SEM) % change in [DAc] after i.p. administration of 10 mg·kg^{-1} cocaine HCl. Individual subject values are also shown. 20 Hz 24p: $n = 6$ for DPN-treated females; $n = 7$ for vehicle-treated females, vehicle-treated males and DPN-treated males; and $n = 8$ for PPT-treated females and PPT-treated males. 20 Hz 12p: $n = 6$ for DPN-treated females; $n = 7$ for vehicle-treated females, vehicle-treated males and DPN-treated males; and $n = 8$ for PPT-treated females and PPT-treated males. 10 Hz 24p: $n = 6$ for vehicle-treated females, DPN-treated females and vehicle-treated males; $n = 7$ for DPN-treated males; and $n = 8$ for PPT-treated females and PPT-treated males. 10 Hz 12p: $n = 4$ for DPN-treated females; $n = 5$ for vehicle-treated males; $n = 6$ for vehicle-treated females and DPN-treated males; and $n = 8$ for PPT-treated females and PPT-treated males. *$P < 0.05$, significantly different, within sex, from vehicle. #$P < 0.05$, significantly different between sexes.

DPN, where DPN-treated females showed greater enhancement of dopamine release following cocaine compared with 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 towards a significant enhancement of dopamine release after DPN in females after 20 Hz 12p stimulation of the VTA.

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

Effect of oestradiol on dopamine reuptake after cocaine administration

After the 20 Hz 24p stimulation, there was a significant interaction between sex and treatment on dopamine reuptake as indicated by an effect on τ ($F(3, 49) = 3.34$;

Figure 4). There was no pre-existing sex difference in the effect of cocaine on dopamine reuptake. However, there was a sex difference in the effect of oestradiol, where males showed reduced sensitivity to the effect of cocaine on dopamine reuptake compared with within-subject vehicle,

A

20Hz24p

B

20Hz12p

C

10Hz24p
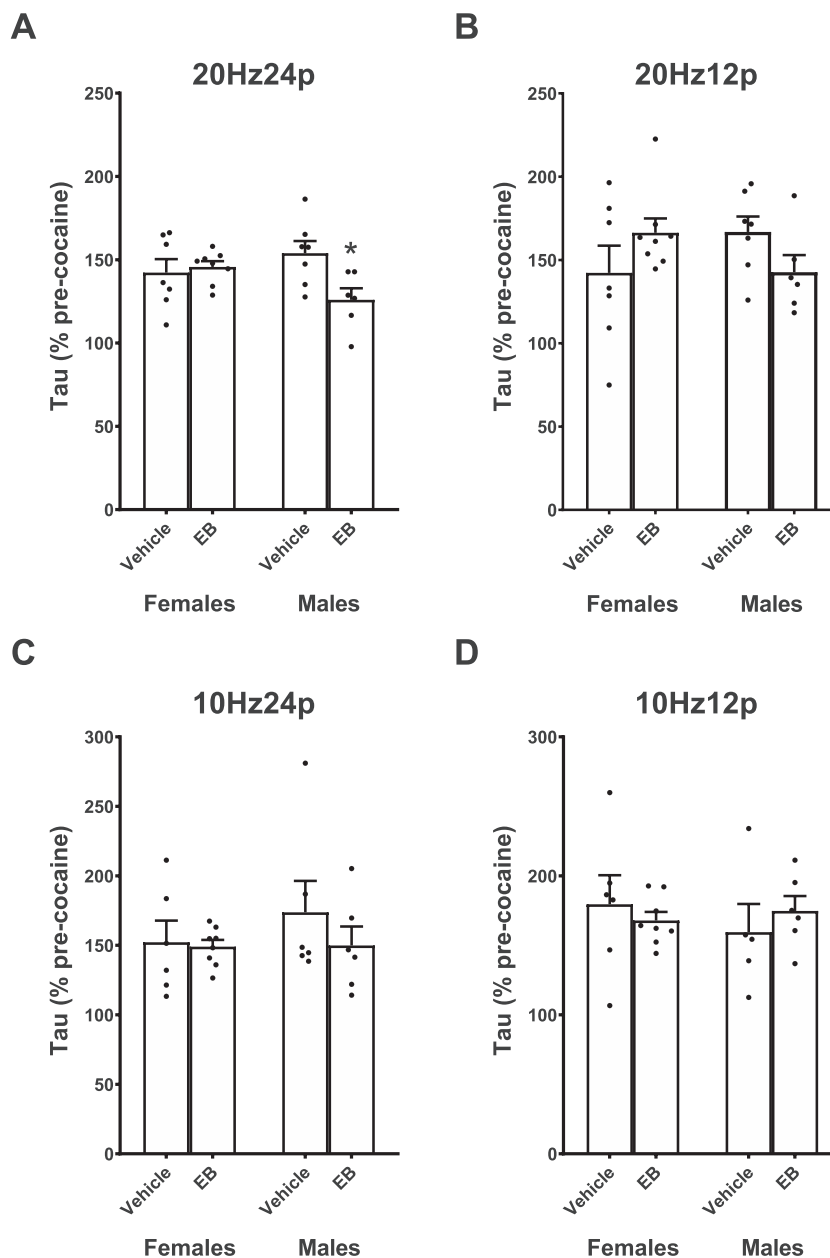
D

10Hz12p

Figure 4

Oestradiol attenuates the effect of cocaine on NAc dopamine reuptake in males but not in females. EB-treated males show significantly less of an effect of cocaine on dopamine reuptake after (A) 20 Hz 24p stimulation of the VTA. Reuptake is represented by the exponential decay constant τ (Tau). τ is inversely proportional to the decay rate so that an increase in τ corresponds to a decrease in reuptake. There was no difference in the effect of cocaine on dopamine reuptake during (B) 20 Hz 12p, (C) 10 Hz 24p or (D) 10 Hz 12p stimulation of the VTA. Bars show mean (+SEM) % change in τ after i.p. administration of 10 mg·kg^{-1} cocaine HCl. Individual subject values are also shown. Group sizes – 20 Hz 24p: $n = 6$ for EB-treated males; $n = 7$ for vehicle-treated females and males; and $n = 8$ for EB-treated females. 20 Hz 12p: $n = 6$ for EB-treated males; $n = 7$ for vehicle-treated females and males; and $n = 8$ for EB-treated females. 10 Hz 24p: $n = 6$ for vehicle-treated females, vehicle-treated males and EB-treated males; $n = 8$ for EB-treated females. 10 Hz 12p: $n = 5$ for vehicle-treated males; $n = 6$ for vehicle-treated females and EB-treated males; and $n = 8$ for EB-treated females. *$P < 0.05$, significantly different, within sex, from vehicle

while females did not (Figure 4). Neither the ERα selective agonist PPT nor 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 (Figures 4B–D and 5).
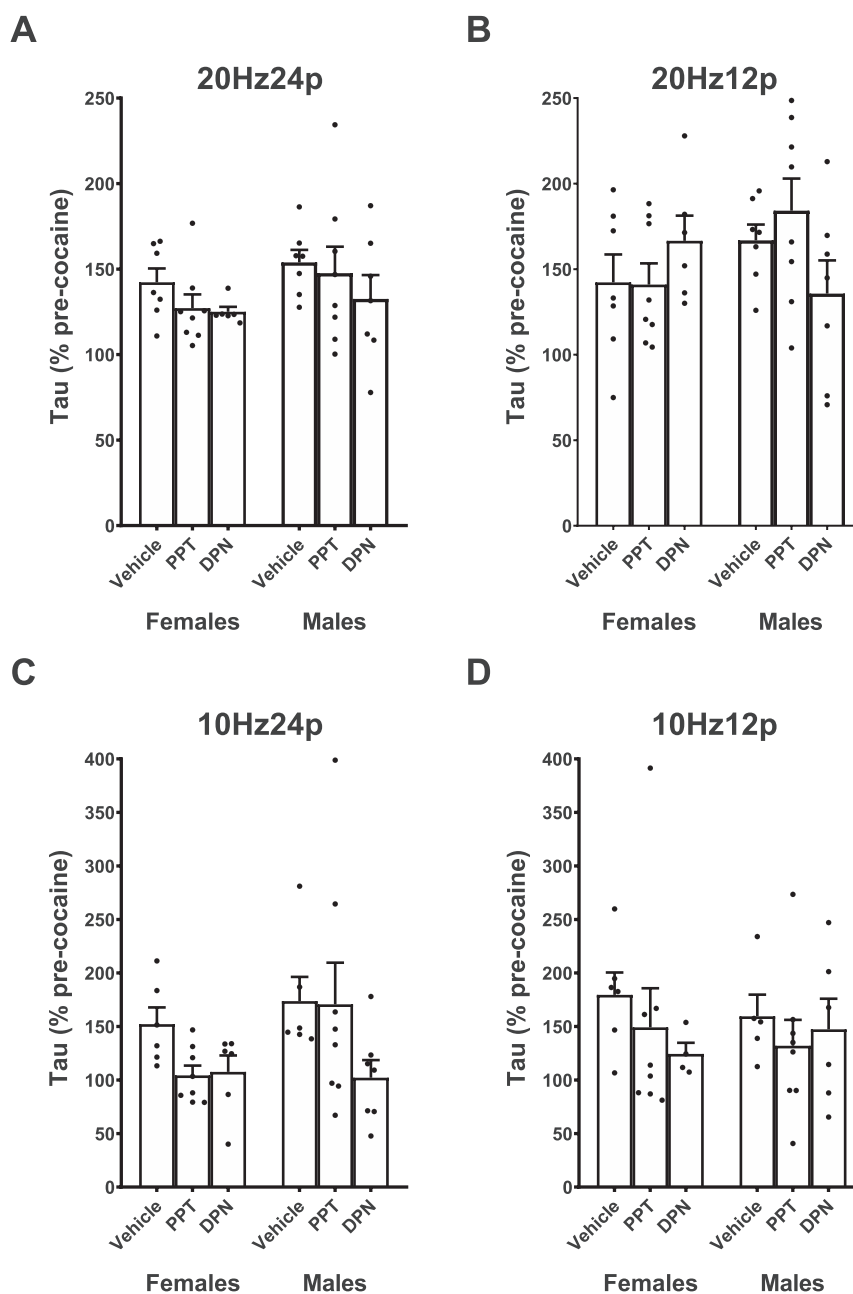

Figure 5
There was no effect of selective ER activation on dopamine reuptake in males or females. Reuptake is represented by the exponential decay constant τ, which is inversely proportional to the decay rate so that an increase in τ corresponds to a decrease in reuptake. There is no difference in the effect of cocaine on dopamine reuptake during (A) 20 Hz 24p, (B) 20 Hz 12p, (C) 10 Hz 24p or (D) 10 Hz 12p stimulation of the VTA. Values show mean % change in τ after i.p. administration of 10 mg·kg^{-1} cocaine HCl. Error bars represent SEM. Scatter dots represent individual subject values. Group sizes – 20 Hz 24p: $n = 6$ for DPN-treated females; $n = 7$ for vehicle-treated females, vehicle-treated males and DPN-treated males; and $n = 8$ for PPT-treated females and PPT-treated males. 20 Hz 12p: $n = 6$ for DPN-treated females; $n = 7$ for vehicle-treated females, vehicle-treated males and DPN-treated males; and $n = 8$ for PPT-treated females and PPT-treated males. 10 Hz 24p: $n = 6$ for vehicle-treated females, DPN-treated females and vehicle-treated males; $n = 7$ for DPN-treated males; and $n = 8$ for PPT-treated females and PPT-treated males. 10 Hz 12p: $n = 4$ for DPN-treated females; $n = 5$ for vehicle-treated males; $n = 6$ for vehicle-treated females and DPN-treated males; and $n = 8$ for PPT-treated females and PPT-treated males.

Discussion

Oestradiol increases dopamine release in response to cocaine in females by an ERβ-mediated mechanism

Our data showed that oestradiol treatment rapidly enhanced the effect of cocaine on stimulated dopamine release in the NAc shell of female rats by an ERβ-mediated mechanism. Oestradiol increased dopamine 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 oestradiol on ventral striatal dopamine release and provide a potential mechanism by which oestradiol renders females more susceptible to drug abuse.

The effect of oestradiol 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 oestradiol on dopamine release after cocaine administration was still apparent at lower levels of stimulation in females. These results suggest that the rapid effects of oestradiol in the NAc shell of females affected the stimulated increase in dopamine release, as the effect was only seen when DAT was fully occupied. VTA dopaminergic neurons show both tonic (0.5–8 Hz) and phasic (15–50 Hz) patterns of activity (Zhang and Sulzer, 2004). Phasic burst firing of dopaminergic neurons has been linked to the detection and prediction of salient environmental events, while tonic dopamine release 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). Oestradiol enhancement of cocaine's effects on phasic dopamine release only at this highest level of stimulation may indicate that oestradiol heightens the ability of cocaine to enhance dopamine 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 dopamine 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 oestradiol on physiological conditions of dopamine release drawn from these data are speculative, and future work using methods that selectively 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 oestradiol modulation of cocaine's enhancement of NAc dopamine release.

The reinforcing properties of cocaine and other psychomotor stimulants are strongly linked to their ability to enhance striatal dopamine transmission. Blockade of D_1 dopamine receptors in both the NAc and the 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 behaviours are likely due to sex differences in the underlying dopamine 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 oestradiol 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 dopaminergic neurons during oestrus 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 dopamine release. On the other hand, cocaine-induced increases in NAc dopamine release are still apparent in mice lacking DAT, so other mechanisms may regulate dopamine signalling within this brain area (Carboni et al., 2001). Importantly, DAT is not required for behavioural 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 dopamine release or cocaine reinforcement, it is still necessary for changes in dopamine reuptake after cocaine administration (Budygin et al., 2002). This underscores the importance of changes in dopamine release, and not reuptake, in drug taking behaviours. This is further supported by our findings that oestradiol, which enhances drug taking in females but not males, alters dopamine release without an effect on reuptake in female rats.

Oestradiol may also be able to rapidly modulate dopamine signalling by altering binding of D_2 dopamine receptors. Within the dorsal striatum, oestradiol rapidly decreases D_2 dopamine receptor binding by altering the proportion of high versus low affinity D_2 dopamine receptors (Lévesque and Di Paolo, 1988; Bazzett and Becker, 1994). Decreased D_2 dopamine receptor binding may lead to reduced autoreceptor-mediated suppression of dopamine release and subsequent increases in dopamine 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 D_2 dopamine 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 oestradiol on cocaine-induced dopamine release are mediated by ERβ

We tested whether selective activation of ER subtypes would also modulate the effects of cocaine on dopamine release in male and female rats. Activation of ERβ alone was sufficient to enhance the effect of cocaine on stimulated dopamine release. The ERβ selective agonist, DPN, but not the ERα selective agonist, PPT, duplicated the effects of oestradiol on cocaine-induced dopamine release in females, where selective activation of ERβ enhanced the effect of cocaine on dopamine release, compared with 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 oestradiol on expression of D_2 receptors and DAT within the NAc (Morissette et al., 2008). Activation of ERβ within the NAc also mediates oestradiol'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 oestradiol enhances dopamine 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 oestradiol enhances excitability of VTA dopaminergic 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 dopamine release after direct application of oestradiol to NAc (Thompson and Moss, 1994). The discrepancy in these results may indicate that oestradiol alters dopamine release *via* multiple mechanisms. Importantly, studies using intact cycling females have produced results indicating that oestradiol enhances excitability of VTA dopaminergic neurons, while experiments on the acute effect of exogenously applied oestradiol have not. It is possible that oestradiol acutely enhances dopamine release *via* direct actions on striatal circuitry, while the slower changes in levels of oestradiol and progesterone seen in intact cycling females lead to increased excitability of VTA dopaminergic neurons, perhaps *via* actions in hypothalamic nuclei that project to the striatum, including the medial preoptic area (McHenry *et al.*, 2017). The results reported here do not directly address where oestradiol acts within striatal circuitry, particularly as we nondiscriminately stimulated all neurons and fibres of passage within the VTA in order to induce NAc dopamine release.

However, the ability of selective activation of ERβ to enhance dopamine release after cocaine administration may provide further indication of the specific mechanism by which oestradiol regulates dopamine 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 oestradiol on cocaine-conditioned place preference, as well as oestradiol enhancement of cocaine-induced NAc cFos (Satta *et al.*, 2018). Further work, utilizing site-specific microinjections to either the NAc or the VTA, could clarify where oestradiol is acting to alter the dopamine response to cocaine. It is also possible that ERs within hypothalamic or amygdala circuitry indirectly modulate dopamine release, leading to increased dopamine signalling in response to cocaine.

Oestradiol modulates the effect of cocaine on dopamine reuptake after VTA stimulation in males

Consistent with previous research, we did not see an effect of oestradiol on dopamine release in males. However, males treated with oestradiol showed a decreased effect of cocaine on dopamine reuptake after electrical stimulation of the VTA. To our knowledge, this is the first evidence that oestradiol rapidly modulates NAc dopaminergic transmission in males. Neither the ERβ nor the ERα selective agonist alone reduced changes in dopamine reuptake after cocaine. There are multiple potential explanations for this inconsistency. The decreased dopamine reuptake after oestradiol

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. In particular, ERβ often regulates the effects of ERα, which could explain the differences after selective and non-selective ER activation seen here. Alternatively, oestradiol may act through another ER to attenuate the effect on dopamine reuptake seen after cocaine treatment. The G-protein oestradiol receptor-1 (GPER-1) is expressed in the NAc in female rats, and GPER-1 activation may be responsible for the attenuated changes in dopamine reuptake seen in EB-treated males in this study.

In conclusion, our present data add to the growing evidence for sex differences in the neurochemical and behavioural responses to psychomotor stimulants. We have provided further evidence of an effect of oestradiol on dopamine release within the NAc mediated by ERβ and establish that this effect was absent in males. In addition, the findings reported here are the first to determine the ER subtype responsible for the effects of oestradiol on NAc dopamine release after cocaine. Dopamine release within the NAc is integral to the rewarding and addictive properties of cocaine, and sex differences in the cocaine-induced dopamine release are likely to contribute to the greater vulnerability to addictive behaviours in females. As oestradiol is known to regulate the dopamine response to drugs of abuse in females, anti-oestradiol treatments have been proposed as a possible therapeutic approach to treatment of 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 have shown that ERβ mediated the effects of oestradiol on dopamine release within the NAc and thus may be a better target for development of pharmacotherapies for stimulant abuse in females. Further work characterizing how oestradiol mediates acute responses to drugs of abuse should be prioritized in understanding sex differences in addiction. In particular, determining whether oestradiol similarly modulates dopamine 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 and 5 and analysed the data for all the experiments; J.A.C. collected the data for

Figures 2 and 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.

Conflict of interest

The authors declare no conflicts of interest.

Declaration of transparency and scientific rigour

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research recommended by funding agencies, publishers and other organisations engaged with supporting research.

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