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Influence of Preoptic Estradiol on Behavioral and Neural Response to Cocaine in Female Sprague-Dawley Rats

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Abstract

Rationale—Systemic estradiol (E2) increases the behavioral and neural response to cocaine. Where in the brain E2 acts to modulate cocaine response is not entirely clear. Evidence supports a role in this modulation for several candidate regions, including the medial preoptic area (mPOA).

Objectives—This study examined whether manipulation of E2 in the mPOA modulates differing behavioral responses to cocaine and whether this is reflected in differing levels of c-Fos in the NAc following cocaine administration.

Methods—Female rats received ovariectomies and bilateral cannulations of the mPOA. They then received either artificial cerebrospinal fluid (aCSF) or E2 microinjections into the mPOA the day before receiving systemic injections of saline or cocaine (10 mg/kg). Conditioned-place preference (CPP) to cocaine and locomotor activation were then obtained.

Results—Animals receiving cocaine developed significant CPP, with those receiving E2 into the mPOA expressing greater CPP than those receiving microinjections of only aCSF ($p < 0.05$, $d = 0.80$). Cocaine also caused significant psychomotor activation, but this was not dependent on microinjection of E2 in the mPOA. Finally, animals that received cocaine had increased NAc core and shell c-Fos relative to animals that received saline, with animals receiving both E2 microinjections and systemic cocaine expressing the highest activation in the caudal NAc, compared to rats receiving aCSF microinjections and systemic cocaine ($p = 0.05$, $d = 0.70$).

Conclusions—These results indicate that E2 in the mPOA facilitates the behavioral response and neural activation that follows cocaine administration. Furthermore, they confirm the close relationship between the mPOA and cocaine response.

Keywords

cocaine; medial preoptic area; nucleus accumbens; estradiol; neuroendocrine; cpp

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Introduction

The medial preoptic area (mPOA) is a region at the rostral end of the hypothalamus most widely recognized for its vital role in the regulation of male sexual behavior (Hull and Dominguez 2015), female sexual behavior (Graham and Pfaus 2010), and parental behaviors (Lee and Brown 2007). Recent evidence indicates that the mPOA also has a more generalized role in motivated behaviors, such as the response to drugs of abuse, namely cocaine. Bilateral lesions of the mPOA significantly increased cocaine-induced c-Fos within the nucleus accumbens (NAc) without affecting baseline expression and enhanced cocaine-induced conditioned place preference (CPP) in female rats (Tobiansky et al. 2013). Lesions of the mPOA also potentiate the cocaine-induced increase of dopamine in the NAc, as evidenced by microdialysis experiments performed following lesions (Tobiansky et al. 2016).

Sex steroids are potent regulators of the cocaine response. In both human and rodent studies, estradiol (E2) has been shown to potentiate the psychoactive effects of cocaine (Carroll et al. 2004; Festa and Quinones-Jenab 2004). In humans and non-human primates, sex steroids are a significant determinant of the cocaine response (Evans and Foltin 2010). Rodent studies show a similar hormonal effect, where E2 increases measures of cocaine response, including self-administration (Larson et al. 2007). Conversely, in the Japanese quail testosterone potentiates the locomotor response to cocaine, whereas E2 does not (Gill et al. 2015). This regulation by sex steroid, especially E2, may occur via the mPOA.

The mPOA is one of several hypothalamic nuclei, including the arcuate nucleus and ventromedial hypothalamus, containing densest populations of estrogen receptor alpha (ER α)-expressing neurons (Chakraborty et al. 2003). ER α downregulation in the mPOA results in increased sexual and locomotor behaviors and decreased anxiety behaviors, indicating that this cell population is part of an inhibitory circuit of the reward system (Spiteri et al. 2012). This is concordant with recent results from our laboratory indicating that ER α -containing cells in the mPOA project to dopaminergic neurons in the ventral tegmental area (VTA), and that the microinjection of E2 into the mPOA increases the dopaminergic response to cocaine in the NAc Shell (Tobiansky et al. 2016). Furthermore, the mPOA innervates the VTA far more extensively than any other hypothalamic area outside of the lateral hypothalamus (Mahler and Aston-Jones 2012), further implicating it as a potential regulatory target.

The aim of the current study is to determine whether mPOA E2 is responsible for sensitizing the psychomotor and motivational aspects of cocaine administration and to determine whether these changes are reflected in differing c-Fos expression within the NAc. Given previous observations with systemic E2 (Festa and Quinones-Jenab 2004), we used a female rat model to determine whether E2 microinjected into the mPOA is sufficient to increase either CPP or locomotor activity in response to cocaine, and whether observed behavioral differences were reflected by differing c-Fos expression within the NAc.

Materials & Methods

Animals

Female Sprague-Dawley rats (2.5 months old, 215–240 g; Envigo, Indianapolis, IN) were single-housed in a temperature-controlled room on a 12:12 reverse light/dark cycle (lights off at 1000 hours) with food and water available ad libitum. The rats were received in two cohorts (animals born late Fall, 2015 and early Spring, 2016, respectively), and each cohort was divided into two sub-cohorts that were tested on successive weeks. All experimental procedures were approved by the Institutional Animal Care and Use Committee at The University of Texas at Austin and carried out in accordance with the NIH Guide for the Care and Use of Animals in Research (8th Edition). Rats were randomly assigned to one of four groups: animals that received an aCSF microinjection and systemic saline (aCSF Sal; n=19), an aCSF microinjection and 10 mg/kg systemic cocaine (aCSF Coc; n=22), an E2 microinjection and systemic saline (E2 Sal; n=20), or an E2 microinjection and 10 mg/kg systemic cocaine (E2 Coc; n=22). An additional cohort of rats was tested separately at a lower 5 mg/kg cocaine dose (aCSF Sal n=8; aCSF Coc n=14; E2 Sal n=8; E2 Coc n=15) and is assessed here as a second experiment.

Surgeries

Animals were sequentially ovariectomized and cannulated while under isoflurane anesthesia. Rats were anesthetized using 4% isoflurane until they did not register a toe pinch reflex and were maintained at 2–2.5% until the end of surgery. Ovariectomies (OVX) were accomplished via abdominal incision during which time they were given Gentamicin antibiotic (10 mg/kg; Aspen Veterinary Resources) and ketoprofen analgesic (10 mg/kg; Zoetis). Immediately after OVX, rats were bilaterally cannulated to the mPOA (+5.0 mm elevation at the incisor bar; relative to bregma, +2.3 mm A/P; relative to the midline $\pm$ 0.5 mm M/L; relative to the cranial surface –6.8 mm D/V) and cannulated using dual 26 ga stainless steel guide cannulae (Plastics One, Roanoke, VA) secured in place using dental acrylic. Animals were returned to their home cage and placed on heat pads until fully ambulatory after surgery. Animals were given three daily shots of ketoprofen (10 mg/kg) and were monitored postoperatively for at least three days until the animal returned to its presurgical weight. Surgical staples were removed from the abdomen after 1 week, and all animals were given at least 10 days to recover before behavioral testing.

Hormone Administration

To maintain normal ER α expression, animals were given systemic E2 injections (10 μ g E2 in sesame oil s.c.) 9 and 5 days prior to the first day of CPP training in a schedule identical to what we have previously reported (Tobiansky et al. 2016). After CPP testing, animals were again given E2 9 and 5 days prior to locomotor testing.

Conditioned Place preference Testing

Animals were given CPP testing in an automated two-chamber apparatus (San Diego Instruments Place Preference System, San Diego, CA) with ambient white noise (70 dB) using a biased CPP paradigm we have used previously (Tobiansky et al. 2013). Each

conditioning chamber measured 35 cm wide by 21 cm deep and could be differentiated by the animals on the basis of floor texture and lighting condition. Photocells around the apparatus enabled for the detection of locomotor activity. On an initial habituation day, animals were given 20 minutes to freely explore the entire apparatus. Any animals that initially preferred one channel by a greater than 2:1 ratio (that is, more than 800 of the 1200 total seconds) were excluded from CPP analysis; a total of 4 animals were excluded on this basis. The next day, a partition was used to bisect the CPP apparatus and all animals were given an injection of saline and left to explore the preferred chamber for 30 minutes. After the apparatus was cleaned, animals received an injection of saline or cocaine hydrochloride (10 mg/kg i.p.) on the non-preferred side, and were left to explore the non-preferred chamber for 30 minutes. This procedure was repeated for a second day of training. Approximately 3.5 hours after the completion of habituation testing and the first day of CPP training, animals were given hormone microinjections (described below). The day after the second training trials, animals received a CPP test where the partition was removed and the animals were given 20 minutes to explore the CPP apparatus. The change in preference was calculated by subtracting the time spent in the non-preferred side during habituation from the time spent in the same side during testing. An additional cohort of animals was tested in an identical procedure with either saline or a lower cocaine dose (5 mg/kg i.p.).

Hormone Microinjections

20 hours prior to each CPP training session, animals received bilateral microinjections of either aCSF (0.25 μ l, Harvard Apparatus, Cambridge, MA) or 2.5 μ g of water-soluble E2 (cyclodextrin-encapsulated β -estradiol, cat# E4389, Sigma-Aldrich St. Louis, Mo) dissolved in 0.25 μ l aCSF. Microinjections were carried out over the course of 2 minutes, using dual 33 ga microinjection needles projecting 2.0 mm below the level of the cannulae (thus, -8.8 mm relative to the surface of the cranium). The microinjection needles were kept in the head for 2 minutes after microinjections to allow for proper diffusion of hormone.

Locomotor Testing

Animals were tested in locomotor chambers identical to those used in CPP tests, but with non-differentiated chambers (that is, no differentiation in lighting or floor texture). Subjects were injected with either cocaine (10 mg/kg or 5 mg/kg) or saline, depending on their group designation, and immediately placed in the testing apparatus for 20 minutes. Locomotion was calculated by measuring consecutive beam breaks of a 16 $\times$ 4 photocell array on the apparatus and summing the results in 5-minute blocks.

Tissue Preparation

One hour after the final drug injection of cocaine or saline, animals were injected with 1.5 ml/kg Euthasol (Med-Pharmex, Inc.). Once deep anesthesia was achieved, as measured by an absence of toe pinch or corneal blink reflexes, the animal was perfused via transcardial perfusion using 100 ml ice cold 0.1 M phosphate buffer (PB; pH 7.35) followed by 350 ml ice cold 4% paraformaldehyde (dissolved in PB). Brains were removed after perfusion and post-fixed for 90 minutes in 4% paraformaldehyde before being transferred to a solution of 30% sucrose in PB at 4° C, and then switched to fresh 30% sucrose 24 hrs later. Once the brains were saturated, as indicated by sinking in the sucrose solution, they were coronally

sectioned at 35 μm on a freezing microtome and stored in cryoprotectant (30% ethylene glycol and 30% sucrose dissolved in PB with 200 $\mu\text{g/l}$ sodium azide) until use.

Placement Assessment

Sections were examined under a dissection microscope at 40x magnification for cannula tracts and injection placement. Placement of the tract, injection site, and any unusual brain features were noted, and a selection of 4–5 sections indicating surgical placement was set aside for methyl green staining to confirm this assessment. Surgical placements were judged to be either bilateral hits, unilateral hits, or bilateral misses on the basis of placement (Figure 1). The majority of placements were directly dorsal to the medial preoptic nucleus. Subjects with two missed placements were excluded from analysis. In the first experiment, five animals that had bilateral misses were excluded from analysis, leaving final group sizes of aCSF Sal (n=18), aCSF Coc (n=22), E2 Sal (n=16), and E2 Coc (n=18) for behavioral analysis. Likewise in the second experiment, five animals were excluded, leaving final group sizes of aCSF Sal (n=7), aCSF Coc (n=12), E2 Sal (n=7), and E2 Coc (n=12) for analysis.

Immunohistochemistry

Tissue was immunostained for c-fos expression using nickel-enhanced diaminobenzidine visualization. Briefly, free-floating sections containing NAc and corresponding to the approximate bregma coordinates +2.5 mm, +2.0 mm, +1.5 mm, and +1.0 mm (Paxinos and Watson 2009) were processed with extensive rinsing in PB or PBT (PB with 0.1% Triton X-100) between incubations. Sections were incubated in 1:8000 rabbit anti-c-Fos antibody (SC-52, Santa Cruz) overnight at room temperature, followed by 1:500 biotinylated goat anti-rabbit secondary (BA-1000, Vector Laboratories). Peroxidase complex was conjugated to the secondary antibody using 1:500 ABC reagent (PK-6100, Vector Laboratories), and then visualized with exposure to nickel-enhanced DAB (0.02% 3,3'-diaminobenzidine, 0.03% H_2O_2 in 0.1M PB, and 2% nickel sulfate). Stained tissues were mounted to slides and coverslipped with DPX mounting medium (VWR).

Cell Counting

Photomicrographs of the stained tissue were taken with a Zeiss AX10 microscope. Four pictures were taken for each brain region in each animal at the coronal coordinates specified above. These photomicrographs were cropped to $0.5 \times 0.5 \text{ mm}$ (0.25 mm^2) and processed using ImageJ (NIH) to convert the images into high-contrast binary images revealing cell nuclei but not tissue background. Processed images were counted using an automated counting procedure validated against human counts.

Statistical Analysis

All statistics were conducted in SPSS version 17.0. For CPP data, group comparisons were made via a mixed model ANOVA to discriminate between drug and hormone effects, and individual group comparisons were made using Tukey's HSD test with statistical significance set to $p < 0.05$. Locomotor activity was assessed using a RM-ANOVA to assess main effects of time, drug, and hormone and a mixed model ANOVA to perform groupwise comparisons at each time point. Cell counts were averaged across the relevant

photomicrographs for each animal (e.g. all four NAc core pictures for the total NAc core statistic). All error bars are presented $\pm$ SEM.

Results

CPP Behavior

Cocaine induced CPP and this was enhanced by E2 microinjection. CPP was calculated on the basis of preference shift between the habituation trial and the post-training trial. A mixed-model ANOVA revealed a significant main effect of drug ($F(2,69)=15.181$, $p<0.001$), no main effect of hormone, and a drug x hormone interaction ($F(2,69)=3.641$, $p=0.031$). Post-hoc analysis indicates that aCSF-Coc animals expressed more CPP than all animals receiving saline ($p<0.001$, $d=0.63$), but not aCSF-Sal alone, E2-Coc animals expressed more CPP than animals receiving E2-Sal ($p<0.001$, $d=1.79$), and E2-Coc animals expressed more CPP than aCSF-Coc ($p<0.05$, $d=0.80$) (Figure 2A). These effects were primarily seen in the first 10 minutes of the test, where the drug x hormone interaction was more pronounced ($F(1,70)=7.387$, $p=0.008$), aCSF-Coc was significantly higher than aCSF-Sal ($p<0.05$, $d=0.71$), and the difference between E2- and aCSF-Coc animals expressed greater significance ($p<0.01$, $d=0.89$) (Figure 2B).

In the second experiment, a lower 5-mg/kg dose of cocaine was used. A mixed-model ANOVA revealed a significant main effect of drug ($F(2,37)=6.913$, $p=0.013$) on CPP. Post-hoc analysis indicates that E2-Coc animals receiving 5 mg/kg cocaine expressed more CPP than all animals receiving E2-Sal ($p<0.05$, $d=1.49$) and animals receiving aCSF-Coc ($p<0.05$, $d=0.99$). Animals receiving aCSF and 5 mg/kg cocaine were not significantly different from saline animals in any comparisons (Figure 3). Unlike with the 10 mg/kg dose, group differences were not amplified in the first 10 minutes. While there was also a main effect of drug ($F(1,37)=8.206$, $p=0.007$) and E2-Coc expressed more CPP than E2-Sal ($p<0.01$, $d=2.15$), there was no significance with any other groupwise comparison. Thus, there is a dose-dependent effect of cocaine administration on the acquisition of CPP, and this is exacerbated by E2 pretreatment within the mPOA.

Locomotor Activity

Cocaine increased total locomotor activity, but was not affected by E2 microinjection. Locomotor data was computed from the number of beam breaks within a CPP apparatus for 20 minutes after drug injection (Figure 4). Across the entirety of the test, there was a significant treatment effect of drug ($F(2,64)=20.393$, $p<0.001$, $d=1.47$), with no significant effect of hormone or drug x hormone interaction. Within subjects, there was a significant effect of time on the basis of comparison between 5-minute time bins ($F(3,63)=168.465$, $p<0.001$) and a significant time x drug interaction ($F(3,63)=5.811$, $p=0.001$), with animals generally expressing less locomotor activity later in the test, and animals receiving cocaine expressing a less dramatic drop in activity than animals receiving saline. There was no significant effect of time x hormone and no three-way time x drug x hormone interaction. Post-hoc analysis indicates that aCSF-Coc expressed more locomotion than animals receiving E2 or aCSF and saline ($p<0.05$ and $p<0.01$, respectively) and E2-Coc animals expressed more locomotion than animals receiving aCSF or E2 and saline ($p<0.001$).

However, aCSF-Coc animals did not differ from E2-Coc, and aCSF-Sal did not differ from E2-Sal. When analyzed at individual time points, E2-Coc animals expressed significantly more ambulatory activity than E2-Sal or aCSF-Sal animals at all time points ($p < 0.05$), while aCSF-Coc expressed a significant difference from E2-Sal or aCSF-Sal only at the last two time points ($p < 0.01$).

The locomotor effects of cocaine were less pronounced in the 5 mg/kg experiment (Figure 5). Across the entirety of the test, there was a main effect of drug ($F(1,38)=12.919$, $p=0.001$, $d=1.22$). When analyzed across time points, aCSF-Coc animals expressed significantly more ambulatory activity than aCSF-Sal animals at the last two time points ($p < 0.05$) and E2-Coc animals expressed significantly more than E2-Sal only in the last time point ($p < 0.05$). Thus, there is a robust effect of cocaine on locomotor activity at 10 mg/kg, but this does not appear to be influenced by E2 within the mPOA of female rats.

c-Fos Expression

Cocaine increased c-Fos in the NAc shell and core, and E2 microinjection potentiated this increase in the caudal shell. Photomicrographs were quantified for c-Fos immunoreactivity and statistically evaluated for the entire NAc (shell + core), as well as for six discrete anatomical subregions of the NAc: the NAc core (NAcCo), further subdivided into the rostral and caudal NAcCo, and the NAc shell (NAcSh), further subdivided into the rostral and caudal NAcSh (Figure 6). There was a significant main effect of drug on total-NAc cFos ($F(7,68)=11.315$, $p < 0.001$, $d=1.98$), with no significant effect of hormone or drug x hormone interaction. There was a significant omnibus group effect for all examined regions and subregions ($p < 0.001$), with drug displaying a significant effect for all areas ($p < 0.001$), and hormone displaying significance only within the caudal NAcSh ($F(1,69)=4.719$, $p=0.033$, $d=0.39$). Post-hoc analysis indicates that both groups receiving cocaine expressed more cFos-ir than either group receiving saline in all subregions examined ($p < 0.01$). Interestingly, the only subregion displaying a significant difference between aCSF-Coc and E2-Coc animals was the caudal NAcSh, where E2-Coc animals expressed more cFos-ir than aCSF-Coc animals ($p < 0.05$, $d=0.70$); in all other examined regions, the two cocaine groups were not statistically differentiated.

At 5 mg/kg cocaine, the differences between E2 and aCSF on cocaine-induced c-Fos were more pronounced (Figure 7). There was a significant omnibus effect for all examined regions ($p < 0.05$). Drug presented a significant main effect for all areas ($p < 0.01$), but hormone and hormone x drug effects were only seen within the caudal shell ($F(1,35)=9.330$, $p=0.004$ and $F(1,35)=5.567$, $p=0.024$, respectively). Post-hoc analysis indicates that aCSF-Coc was not significantly different from aCSF-Sal within either the rostral or caudal shell, and E2-Coc expressed significantly greater c-Fos than E2-Sal only within the caudal shell ($p < 0.001$, $d=2.49$); moreover, E2-Coc also expressed greater c-Fos than aCSF-Coc ($p < 0.001$, $d=1.55$). Both aCSF-Coc and E2-Coc induced significant c-Fos within all core subregions compared to their respective saline controls ($p < 0.05$). Thus, any E2-driven difference in the primary response to cocaine appears to be centered within the caudal NAcSh.

Discussion

The mPOA is a site of interest in the estrogenic potentiation of cocaine response because it is one of several hypothalamic areas that densely expresses the estrogen receptor, ER α . This is of special interest because ER α , but not ER β , knockout mice exhibit an attenuated cocaine response similar to that of ovariectomized animals (Van Swearingen et al. 2013). The distribution of ER α includes the mPOA, bed nucleus of the stria terminalis, arcuate nucleus, and ventromedial hypothalamus, and this distribution is sexually dimorphic (Mori et al. 2008; Brock et al. 2015). As far back as 1986, it was observed that estrogen-concentrating neurons in the mPOA projected to the vicinity of the VTA (Fahrbach et al. 1986), and more recent anatomical studies by our laboratory show that many of these VTA-projecting neurons express ER α , and appose upon VTA-projecting neurons that express TH (Tobiansky et al. 2013). Thus, our observation that E2 microinjections influence the behavioral and cellular response to cocaine, including an ability to potentiate subthreshold levels of cocaine, dovetails with previous findings indicating an integral role for the mPOA in endocrine-mediated modulation of cocaine response.

Considerable evidence indicates that sex steroids regulate psychostimulant drug response (Festa and Quinones-Jenab 2004). Females generally exhibit greater motivational and behavioral sensitivity to psychostimulants than males, and at least some of this effect seems to be regulated by ovarian steroids. For example, female rats in estrus have a higher break point on a progressive ratio task to self-administer cocaine than males or non-estrus females (Roberts et al. 1989) and E2 replacement sensitizes the reinstatement of cocaine self-administration after extinction (Larson et al. 2005). Behaviorally, E2 replacement potentiates cocaine-induced stereotypical behaviors in ovariectomized (OVX) females, while progesterone decreases them, even when coadministered with E2 (Souza et al. 2014). It is not entirely clear from this research what brain regions and hormone receptors are engaged to drive these effects, but the results we report here implicate the mPOA as playing a major role in this regulation.

Concordant with previous work, our findings indicate that the mPOA is an important regulator of the reward response, and that the manipulation of the mPOA results in a potentiated response to cocaine in terms of both behavior and downstream effects within the brain's reward system. Here, we observed that E2 microinjections within the mPOA result in enhanced CPP in response to cocaine relative to animals receiving aCSF-vehicle microinjections. In a second set of experiments using a lower dose of cocaine, we saw that this estrogenic potential extended to inducing CPP at cocaine doses that were otherwise subthreshold in our model. These results are similar to those seen in previous studies, wherein bilateral lesions of the mPOA resulted in a similarly enhanced CPP (Tobiansky et al. 2013). Furthermore, we also observed a robust increase in NAc c-Fos immunoreactivity in response to cocaine that was enhanced in the caudal NAc shell by E2 microinjections to the mPOA. This, again, is similar to previous findings that demonstrated an increase in NAc cFos in response to mPOA lesions (Tobiansky et al. 2013). Interestingly, this previous study saw increased cocaine-induced c-Fos expression in lesion animals within both the core and shell, perhaps indicating a difference between overall mPOA function in the reward circuit, and estrogenic influence upon this function. Our data is also consistent with microdialysis

studies showing that lesions and E2 microinjections to the mPOA increase cocaine-induced dopamine release within the NAc (Tobiansky et al. 2016). The mPOA also contains estrogen-responsive GABAergic VTA projections that regulate social reward (McHenry et al. 2017). This neuronal population may overlap significantly with those regulating drug response.

While our findings provide insight into the causes of sex differences in psychostimulant response, they are unlikely to comprise the entirety of observed differences. Since estrogen replacement increases the locomotor response to cocaine (van Haaren and Meyer 1991; Van Swearingen et al. 2013), and we did not observe a locomotor effect, mPOA E2 appears to be insufficient to reinstate this estrogenic effect. Acute (30 min before drug administration) E2 treatment enhances both the locomotor response to cocaine and cocaine-induced dopamine release within the dorsolateral striatum, but not the NAc (Cummings et al. 2014). Furthermore, E2 is capable of directly affecting the striatum, where D2 receptors and transporters are regulated by ER β in the medial striatum (Morissette et al. 2008), and driving sex differences in spine density within the NAc core via mGluR5 (Wissman et al. 2012; Peterson et al. 2015). Our data may indicate dissociation between hedonic aspects of the estrogenic effect via the mPOA and effects exerted through changes to striatal function or through efferents from other brain areas. For instance, the ablation of β -endorphin-containing cells in the arcuate nucleus attenuates cocaine response (Nguyen et al. 2012).

Our results may also be relevant within the broader context of reward. Various natural rewards, most notably sex and maternal behavior, are incorporated into the reward system via the mPOA. Bilateral lesioning of the mPOA slows paced mating in females and blocks the ability of a phosphodiesterase inhibitor to regulate pacing (Meerts et al., 2012). Within the mPOA, systemic hormonal priming alters D1 and D2 receptor expression (Graham et al., 2015), mu opioid receptors (Eckersell et al., 1998) and pharmacological manipulation of these receptors variously influences appetitive behaviors (Graham and Pfaus, 2012; Garcia-Horsman et al., 2008). This putative circuit is also relevant for maternal care, where oxytocinergic neurons that project from the mPOA to the VTA regulate dopamine release within the NAc (Shahrokh et al., 2010).

Another notable point in our findings is that only the caudal portions of the NAc shell expressed significantly more c-Fos-ir in animals that received E2 and cocaine than animals that received aCSF and cocaine. Interestingly, this area has previously been identified as a hedonic “hotspot” for drugs of reward including opioids and cannabinoids (Zangen et al. 2006; Terashvili et al. 2008). The NAc core and shell are differentiable along a rostrocaudal gradient in terms of what pharmacological manipulations they respond to and what behaviors these elicit (Reynolds and Berridge 2003). Interestingly, the microinjections of amphetamine into the rostral shell induces greater locomotor activation than into the caudal shell (Heidbreder et al. 1999), though this does not necessarily imply a difference in hedonic value. Thus, while there is functional and pharmacological variability across NAc subregions, there is not yet sufficient data to conclude that our c-Fos results indicate this particular role for mPOA E2.

In summary, these data support previous observations regarding the role of E2 and the mPOA in the mPOA-VTA-NAc arm of the mesolimbic dopamine system. E2 generally inhibits estrogen-sensitive projection neurons in the mPOA (Sakuma 2015). This effect was not seen in intact males or androgenized females, and other studies have reported that systemic E2 facilitates self-administration in females but not males (Jackson et al. 2006), so this E2 effect may be sex-specific. Estradiol has a complex effect on inhibitory tone within the mPOA, maximally inhibiting GABAergic activity around 24 hours after administration (Wagner et al. 2001). Since the mPOA projections to the VTA are largely GABAergic cells that terminate upon TH-positive neurons (Tobiansky et al. 2013), our behavioral findings here illustrate how hormonal inputs through the mPOA can drive motivational outputs through the NAc. In this model, the hyperpolarization of inhibitory efferents from the mPOA via E2 results in the disinhibition of dopaminergic neurons in the VTA that project to the caudal NAc shell and drive the hedonic aspects of cocaine response (Figure 5). This provides a candidate mechanism to explain the underlying sex differences in drug response and may be useful for predicting clinical outcomes and guiding treatments on the basis of these differences.

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Fig. 1.

Surgical placement of bilateral cannulae within the mPOA. Coronal sections are based upon diagrams from (Paxinos and Watson 2009). The photomicrographs on the left illustrate typical bilateral cannula placements.

Fig. 2.

Shift in chamber preference after saline or cocaine exposure in estradiol- or vehicle-pretreated animals. (A) In a 20-minute CPP trial, E2-Coc animals expressed significantly greater-preference shift than both aCSF-Sal and E2-Sal animals, as well as aCSF-Coc animals (Tukey's HSD post hoc, letters denote significance against a=aCSF Sal; b=E2 Sal; c=aCSF Coc; d=E2 Coc). (B) Behavioral differences were most prominent in the earlier phase of the test, where aCSF-Coc animals expressed a greater preference shift than animals receiving saline, and E2-Coc animals expressed a significantly greater preference shift than all other groups.

Fig. 3.

Shift in chamber preference after saline or cocaine exposure in estradiol- or vehicle-pretreated animals. In a 20-minute CPP trial, E2-Coc animals expressed significantly greater-preference shift than both aCSF-Sal and E2-Sal animals, as well as aCSF-Coc animals (Tukey's HSD post hoc, letters denote significance against a=aCSF Sal; b=E2 Sal; c=aCSF Coc; d=E2 Coc).

Fig. 4.

Locomotor activity in an open field after acute cocaine or saline exposure. In a 20 minute locomotor task binned into 4 5-minute segments, both aCSF-Coc and E2-Coc animals expressed greater overall activity than aCSF-Sal or E2-Sal animals, as determined in a mixed model ANOVA. The cocaine groups did not differ from each other. A repeated measures ANOVA revealed a main effect of time, as well as a time x drug interaction, with animals receiving cocaine displaying less proportional reduction in locomotor activity than saline animals. Post hoc analysis revealed that E2-Coc animals expressed greater locomotion than saline groups at all time points, while aCSF-Coc differed significantly from saline groups only at the last two segments (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Fig. 5.

Locomotor activity in an open field after acute cocaine or saline exposure. In a 20 minute locomotor task binned into 4 5-minute segments, both aCSF-Coc and E2-Coc animals expressed greater overall activity than aCSF-Sal or E2-Sal animals, as determined in a mixed model ANOVA. The cocaine groups did not differ from each other. A repeated measures ANOVA revealed a main effect of time, as well as a time x drug interaction, with animals receiving cocaine displaying less proportional reduction in locomotor activity than saline animals. Post hoc analysis revealed that E2-Coc animals expressed greater locomotion than E2 saline at the last time point, while aCSF-Coc differed significantly from saline groups at the last two segments (* $p < 0.05$).

Fig. 6. c-Fos immunoreactivity in NAc subregions after acute cocaine or saline exposure. (A) 60-minutes after systemic administration of saline, animals receiving either aCSF or E2 and cocaine expressed significantly more c-Fos-ir in all NAc subregions than animals receiving saline ($p < 0.01$). There was no significant difference between aCSF-Coc and E2-Coc animals, except within the caudal NAc Shell, where E2-Coc animals expressed significantly more c-Fos-ir than aCSF-Coc animals ($p < 0.05$). (B) Demonstrative photomicrographs illustrate mean c-Fos-ir differences between groups within the caudal NAc core and shell. While there is no significant difference between aCSF-Coc and E2-Coc animals within the caudal core, aCSF-Coc animals are intermediate in c-Fos-ir between saline animals and E2 Coc animals in the caudal shell.

Fig. 7.

c-Fos immunoreactivity in NAc subregions after acute cocaine or saline exposure. (A) 60-minutes after systemic administration of saline, animals receiving either aCSF or E2 and cocaine expressed significantly more c-Fos-ir in all NAc subregions than animals receiving saline ($p < 0.01$). There was no significant difference between aCSF-Coc and E2-Coc animals, except within the caudal NAc Shell, where E2-Coc animals expressed significantly more c-Fos-ir than aCSF-Coc animals ($p < 0.05$). (B) Demonstrative photomicrographs illustrate mean c-Fos-ir differences between groups within the caudal NAc core and shell. While there is no significant difference between aCSF-Coc and E2-Coc animals within the caudal core, aCSF-Coc expression resembled that of saline animals in the caudal shell.

Fig. 8. Model of mPOA estrogen effects on mesolimbic dopamine circuitry. Increased estradiol within the mPOA activates estrogen receptors. This has a generally inhibitory effect on mPOA efferents. Since a majority of these outputs to the VTA are GABAergic cells that terminate upon TH-positive neurons, this reduces tonic inhibition upon dopaminergic projections from the VTA to the NAc shell. Thus, when cocaine blocks dopamine reuptake, there is an increase in available dopamine, relative to animals that did not receive estradiol. This results in increased measures of cocaine response such as CPP. Modified with permission from Tobiansky et al., (2016).