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Estradiol as a Mechanism for Sex Differences in the Development of an Addicted Phenotype following Extended Access Cocaine Self-Administration

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Women progress more rapidly after initial cocaine use to addiction as compared with men. Similarly, female rats appear to require less cocaine exposure before developing an addicted phenotype with evidence implicating estradiol as a potential mechanism. The goals of this study were to determine whether there are sex differences in the magnitude of the addicted phenotype under optimized conditions that induce its development in both males and females and to determine the role of estradiol in this effect. Following acquisition, intact male and intact and ovariectomized (OVX) female rats with and without estradiol replacement were given access to cocaine (1.5 mg/kg per infusion) under either extended access (ExA; discrete trial procedure, 4 trials/h, 24 h/day, 10 days) or short access (ShA) conditions (20 infusions maximum/day, 3 days). Motivation to obtain cocaine (0.5 mg/kg/infusion), as assessed under a progressive-ratio schedule, was then examined following a 2-week abstinence period. Results showed that following ExA self-administration, both males and females developed an addicted phenotype, with 9 of 11 males and 8 of 10 females showing a greater than 15% increase in levels of motivation to obtain cocaine as compared with ShA controls. In contrast, within the OVX groups, responding was enhanced from control levels after ExA self-administration in estradiol-replaced rats only. These results suggest that while females may have an enhanced vulnerability to developing an addicted phenotype, they may be similar to males once addiction has developed. These results also suggest that estradiol is critically involved in the development of an addicted phenotype in females.
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INTRODUCTION

Despite higher rates of cocaine use, abuse, and dependence among men, recent trends support the idea that women may be more vulnerable than men on certain aspects of cocaine addiction. One of the most striking examples is 'the telescoping effect', where following initial cocaine use, women meet criteria for abuse/dependence or enter treatment programs after fewer years of drug use as compared with men (Hass and Peters, 2000; McCance-Katz *et al*, 1999; Griffin *et al,* 1989; White *et al,* 2006). This is a robust effect that has been reported not only for cocaine but also for alcohol, heroin, and amphetamine (Anglin *et al,* 1987; Westermeyer and Boedicker, 2000; Brady and Randall, 1999; Hernandez-Avila *et al,* 2004).

Little information is available on the biological basis for this accelerated time-course to addiction in women. Part of the reason for this knowledge gap, is that 'addiction' paradigms have only recently been developed in laboratory animals. Currently, and for many decades, the majority of preclinical studies have examined cocaine self-administration behavior under short access (ShA) conditions (1–2 h/day), which results in relatively low and stable levels of intake. In contrast, the goal of newer addiction paradigms is to more fully capture features that are critical to human cocaine addiction, such as excessive drug use and an enhanced subsequent motivation to use the drug (Ahmed, 2012; Roberts *et al,* 2007). This latter characteristic, an enhanced level of motivation to obtain the drug as compared with baseline or ShA controls, has been used to define the development of an addicted phenotype (Roberts *et al,* 2007; Lynch and Taylor, 2004). The use of extended access (ExA) conditions (6–24 h/day) coupled with a protracted abstinence period (7 days or more) appear to be necessary for inducing this phenotype in animals. Results from the few studies that have used these newer addiction models provide support for the idea that females have an enhanced vulnerability to developing an addicted phenotype as compared with males. Specifically, female rats self-administer more cocaine under ExA conditions (Lynch and Taylor, 2005; Roth and Carroll, 2004), and importantly, following ExA self-administration, they show an enhanced motivation for cocaine under conditions that do not affect

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motivation for cocaine in male rats (ie, following 7 days of ExA and a 10-day abstinence period; Lynch and Taylor, 2004). This effect can be observed in males when conditions are optimized; that is, when they are given either longer periods of ExA to cocaine or when they are tested after longer periods of abstinence (Roberts *et al,* 2007). These findings suggest that, similar to the telescoping effect in women, females have an accelerated course to developing an addicted phenotype as compared with males. It is not yet known whether sex differences are maintained under optimized conditions that lead to the development of an addicted phenotype in both sexes.

While the mechanism for this enhanced vulnerability in females is not clear, findings in both humans and animals suggest that estradiol may have a critical role (Segarra *et al,* 2010). In women, results have revealed that the subjective effects of stimulants vary across the menstrual cycle (Terner and de Wit, 2006), with the greatest subjective effects observed when estrogen levels are high and relatively unopposed by progesterone (Justice and de Wit, 1999). Animal studies have directly manipulated estradiol with results showing that while removing estradiol either surgically (ie, ovariectomy, OVX) or pharmacologically (estrogen receptor blockade) reduces cocaine self-administration; estradiol replacement enhances it (Hu and Becker, 2008; Lynch *et al,* 2002). Although estradiol appears to affect both short and ExA cocaine self-administration (Larson *et al,* 2007; Lynch and Taylor, 2005), its role in mediating the enhanced subsequent motivation for cocaine, and hence the development of an addicted phenotype, is not yet known.

The goals of the present study were to determine whether there are sex differences in the magnitude of the addicted phenotype under conditions that induce its development in both males and females and to determine the role of estradiol in this effect. We hypothesized that by extending the access conditions (10 days of access) and abstinence length (2 weeks), both males and females would show an enhanced motivation for cocaine as compared with ShA controls, and that this effect would be greater in females with estradiol (ie, intact females and OVX females treated with estradiol) as compared with males and females without estradiol (ie, OVX females treated with vehicle).

MATERIALS AND METHODS

Subjects

Adult, sexually mature, intact male ($N=24$), intact female ($N=24$), and OVX female ($N=34$) Sprague-Dawley rats (Charles River) were used as subjects. Rats were age-matched and started the experiment at $\sim$3 months of age. Rats were individually housed in operant testing chambers (ENV-018M; Med-Associates, St Albans, VT) for the duration of the experiment. Rats were maintained on a 12-h light/dark cycle (lights on at 0700 hours) and had *ad libitum* access to food and water except as noted below. To ensure that cocaine self-administration was acquired rapidly, rats first pre-trained to lever press for sucrose pellets (45 mg) under a fixed ratio (FR)1 schedule using methods previously described (Lynch, 2008). Each rat was anesthetized and implanted with a catheter using methods previously described (Lynch *et al,* 2000). Rats were weighed three times per week and health was monitored daily. All protocols were approved by the University of Virginia Animal Care and Use Committee and were conducted in accordance with guidelines set by the NIH.

Estradiol Replacement and Vaginal Cytology

OVX rats were purchased from Charles River and arrived at the facility within 3 days of the OVX surgery. Rats were then randomly assigned to either an estradiol (OVX + E) or a vehicle group (OVX + Veh). Hormone treatments began within 5 days of the OVX surgery, with rats receiving subcutaneous injections of estradiol or an equal volume of sesame oil at 11:30 a.m. 5 days per week. The dose of estradiol selected (5 μg/day) has been shown to enhance acquisition cocaine self-administration (Hu *et al*, 2004) and to reinstate sexual receptivity (Pfaus and Pfaff, 1992). Successful ovariectomies were verified by daily vaginal swabbing using methods previously described (Lynch and Taylor, 2005). We also monitored the estrous cycle phase in intact females; however, there was not enough data to reliably analyze the effects of estrous cycle phase by access group. Future research is needed to address this question.

Self-Administration Procedures

Cocaine self-administration training. Rats were trained to self-administer cocaine (1.5 mg/kg per infusion) under a FR1 schedule with a maximum of 20 infusions available per day using methods previously described (Lynch *et al,* 2010). This relatively high dose of cocaine was selected to ensure rapid rates of acquisition (Lynch and Taylor, 2004). Acquisition was defined as the first two consecutive sessions in which all 20 infusions available were obtained. When necessary, moderate levels of food restriction were used to encourage acquisition (ie, 16 g for females and 20 g for males). These brief periods of food restriction (2–3 days) did not significantly impact any of the subsequent measures of cocaine self-administration. All groups acquired rapidly, and although the OVX + Veh groups required approximately two additional training sessions (mean ± SEM number of training sessions were 4.04 ± 0.43, 4.21 ± 0.70, 3.47 ± 0.36, and 6.18 ± 0.76 for males, females, OVX + E, OVX + Veh, respectively), the number of training sessions run did not differ between the ShA and ExA conditions.

ExA cocaine self-administration. Rats assigned to the ExA condition were given 24-h access to cocaine (1.5 mg/kg per infusion) under a discrete trials procedure using methods previously described (10-min trials, 4 trials/h; Lynch *et al,* 2010). These access–dose conditions were selected based on previous work in both males and females showing that such conditions produce high and dysregulated patterns of cocaine intake with limited toxicity (Lynch and Taylor, 2004; 2005; Lynch and Roberts, 2004; Lynch *et al,* 2007; 2010; Morgan *et al,* 2002; Roberts *et al,* 2002). Trials initiated every 15 min for a total of 10 days allowing for 4 infusions/h and 96 infusions/24-h period. Following the last discrete trial session, cocaine infusions were again available under a FR1 schedule with a maximum of

20 infusions available per session for two consecutive sessions. Every rat obtained all 20 infusions available on both days. These FR sessions served a control to minimize the predicted between-group differences in levels of intake before abstinence. A 14-day abstinence period began following the second FR1 session, wherein rats remained in their chambers with both levers retracted, and all lights except the house light (illuminated from 0700 to 1900 hours) were switched off. This length of abstinence was selected to maximize the likelihood that both males and females would display an enhanced subsequent motivation to obtain cocaine (Roberts *et al*, 2007).

ShA cocaine self-administration. Following acquisition, rats assigned to the ShA control group were given access to cocaine for an additional three sessions using the same conditions that were used for cocaine self-administration training. After the third session in which all 20 infusions were obtained, the cocaine-associated lever retracted and a 2-week abstinence period began using the same conditions as described above.

Progressive-ratio (PR) cocaine self-administration. A PR schedule was used to measure motivation for cocaine following abstinence from ExA *vs* ShA cocaine self-administration. With this schedule, the ratio requirement to obtain an infusion increases progressively and the final ratio completed is believed to be a sensitive measure of motivation for the drug. Additionally, because the PR schedule generates a linear dose–effect curve, changes in responding can be readily interpreted (Arnold and Roberts, 1997). ShA controls were selected for comparison with the ExA groups based on previous work showing that motivation for cocaine does not change from baseline following abstinence from ShA cocaine self-administration (Roberts *et al*, 2007), and based on results showing that while responding under a PR schedule varies between animals, levels of responding within animals are incredibly stable over time (Arnold and Roberts, 1997). The inclusion of ShA controls also allowed us to identify the potential between-group differences at baseline.

PR sessions began following the 14th day of abstinence using methods previously described (Doyle *et al*, 2012). Sessions were run for at least 3 days to establish a stable baseline (defined as 3 consecutive days with no increasing or decreasing trend in the number of infusions obtained). Although stability was typically reached within the first three sessions, some animals showed a trend for increase or a decrease over sessions and needed an additional one or two sessions to reach this criterion. Importantly, the number of sessions run was similar between groups. We selected a modest-to-high cocaine dose (0.5 mg/kg per infusion) to examine levels of motivation for cocaine based on previous work showing that while modest-to-high cocaine doses produce similar levels of responding between males and females following ShA self-administration (Lynch, 2006), they are sensitive enough to detect a sex difference, once an 'addicted phenotype' had developed (Lynch and Taylor, 2004, 2005).

Drugs

Cocaine hydrochloride was obtained from NIDA. The concentration (g/ml) was maintained throughout the experiment with mg/kg dose adjusted three times/week based on body weight (2 s/100 g body weight). β-Estradiol 3-benzoate was purchased from Sigma-Aldrich (St Louis, MO) and dissolved in sesame oil (administered as 5 μg/0.1 ml).

Data Analysis

Separate analyses were conducted to compare male *vs* female rats and the two OVX groups, as sex differences can be present independent of hormonal status. Intake under ExA conditions was compared between groups across each of the 10 days of access using repeated-measures ANOVA. The first three sessions were also compared with the last seven sessions based on our previous work showing that intake was maximal during the first 3 days for males and females, but then reduced to lower levels in males, but not females (Lynch and Taylor, 2004). Repeated-measures ANOVAs were used to examine the effect of sex/estradiol on motivation for cocaine following abstinence from ShA *vs* ExA self-administration with the number of infusions obtained during the three PR sessions as the dependent measure. On the basis of our previous findings in intact males and females, an addicted phenotype was defined on an individual basis as greater than 15% difference from ShA controls in the number of infusions obtained following ExA self-administration. ShA control values were obtained by calculating the average number of infusions obtained over the three PR sessions for each ShA animal and then calculating the average within each of the ShA control groups. Percent difference from ShA control was then calculated for each of the ExA animals and for each of the three PR sessions using the following formula:

$$\left(\frac{\text{Individual rat (X) on PR session (X) in group (X)} - \text{average of ShA group (X)}}{\text{Individual rat (X) on PR session (X) in group (X)}}\right)\times 100 = \ \%\ \text{difference}$$

Data were compared between groups and over time using repeated-measures ANOVA. The average percent difference from ShA control was calculated similarly using the average number of infusions obtained across the three PR sessions for each ExA animals and analyzed using ANOVA. The one-sample *t*-test was used to determine significant differences from ShA controls. The one-tailed *t*-test was used for all *a priori* predicted hypotheses and the Bonferroni correction was used to control for multiple comparisons. Statistical analyses were performed with IBM SPSS Statistics with alpha set at 0.05.

RESULTS

Effects of Sex and Estradiol on ExA Cocaine Self-Administration

Figure 1 shows daily cocaine intake during the 10-day ExA period under the discrete trials procedure. Under this schedule, sex and hormone effects were apparent. Males and females self-administered cocaine in a similar pattern, taking maximal levels of cocaine during the first 3 days

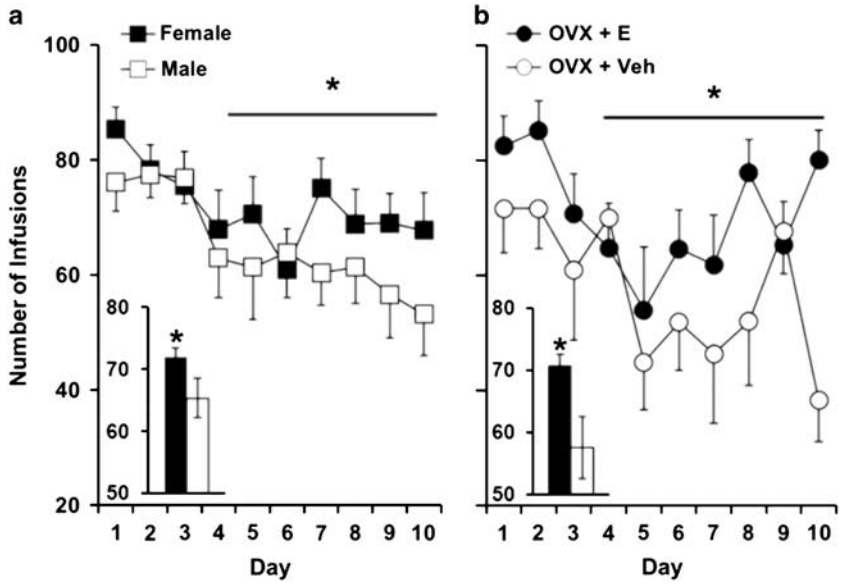

Figure 1 Effect of sex and estradiol on extended access (ExA) cocaine self-administration. Mean (±SEM) number of cocaine infusions for (a) intact female (filled squares; $n=12$) and male rats ($n=12$, open squares), and for (b) ovariectomized (OVX) + estradiol (E) (filled circle; $n=8$), and OVX + vehicle (Veh) (open circles; $n=8$) rats for each of the 10 days of ExA. The insets show the mean (±SEM) number of infusions averaged across the 10-day ExA period for (a) intact females (filled bar), males (open bar), and for (b) OVX + E (filled bar) and OVX + Veh (open bar). *Significant group difference ($p<0.05$).

and then showing progressive decrease in intake. Females, however, self-administered more cocaine than males, particularly following the initial 3 days. Results from a repeated-measures ANOVA revealed a significant overall effect of group ($F_{1,22}=7.386$, $p<0.05$) and day ($F_{9,198}=2.835$, $p<0.01$), and an analysis of intake in the first 3 days and the last 7 days revealed a significant group effect for the last 7 days only ($p>0.05$; $F_{1,22}=7.64$, $p<0.05$, respectively). Similarly, OVX + E and OVX + Veh groups self-administered high levels of cocaine during the initial three sessions and then decreased their intake in subsequent sessions. OVX + E rats self-administered higher levels of cocaine as compared with OVX + Veh rats, particularly following the initial 3 days of access. Results from a repeated-measures ANOVA revealed a significant overall effect of group ($F_{1,14}=12.254$, $p<0.05$) and day ($F_{9,126}=2.924$, $p<0.05$), and an analysis of intake in the first 3 days and the last 7 days revealed a significant group effect for the last 7 days only ($p>0.05$; $F_{1,14}=10.71$, $p<0.05$, respectively). Thus, intact and OVX + E females took more cocaine under ExA conditions as compared with males and OVX + Veh females.

Effects of Sex and Estradiol on Motivation for Cocaine following ExA Self-Administration

As predicted, following ShA self-administration and abstinence, all groups responded at similar levels under the PR schedule (Figure 2a and b left panels). Specifically, male and female rats obtained a similar number of infusions under the PR schedule (11.17 ± 0.99 and 11.81 ± 0.90, respectively), with results from a repeated-measures ANOVA revealing nonsignificant effects of sex and sex by day ($p>0.05$). Similarly, the number of infusions obtained were equivalent between OVX + E and OVX + Veh rats (11.89 ± 1.31 and 13.28 ± 0.99, respectively) and did not differ significantly by hormone group or by hormone group by day ($p>0.05$). Thus, under these moderate-dose conditions, motivation to obtain cocaine did not differ between males and females or between OVX + E and OVX + Veh rats following abstinence from ShA self-administration.

Following ExA self-administration and a 2-week abstinence period, males and females responded at similar rates to obtain cocaine (Figure 2a right panel), while OVX + E responded significantly higher than OVX + Veh rats (Figure 2b right panel). Specifically, males and females obtained a similar number of infusions under the PR schedule (14.82 ± 0.95 and 14.81 ± 0.62 infusions, respectively) with results from a repeated-measures ANOVA revealing nonsignificant effects of sex and sex by day ($p>0.05$). In contrast, OVX + E self-administered significantly more cocaine than OVX + Veh rats (17.44 ± 1.22 and 13.27 ± 0.67, respectively). A repeated-measures ANOVA revealed a significant overall effect of hormone group ($F_{1,14}=13.494$, $p<0.01$), but a nonsignificant interaction of hormone group by day ($p>0.05$). Thus, following abstinence from ExA self-administration, males and females were equally motivated to obtain cocaine, whereas a robust difference was observed between the two OVX groups where estradiol significantly increased motivation for cocaine.

The effects of ExA self-administration on subsequent motivation for cocaine were further assessed by comparing percent difference from ShA control conditions (Figure 3). Relative to controls, PR responding was increased in both males and females (Figure 3a), with results from ANOVA revealing nonsignificant effects of sex, day, and sex by day ($p>0.05$). Subsequent comparison within males and females revealed that responding was significantly enhanced from control levels for each of the three PR sessions (within males, session 1, $t=2.962$, df = 10, $p<0.05$; session 2,

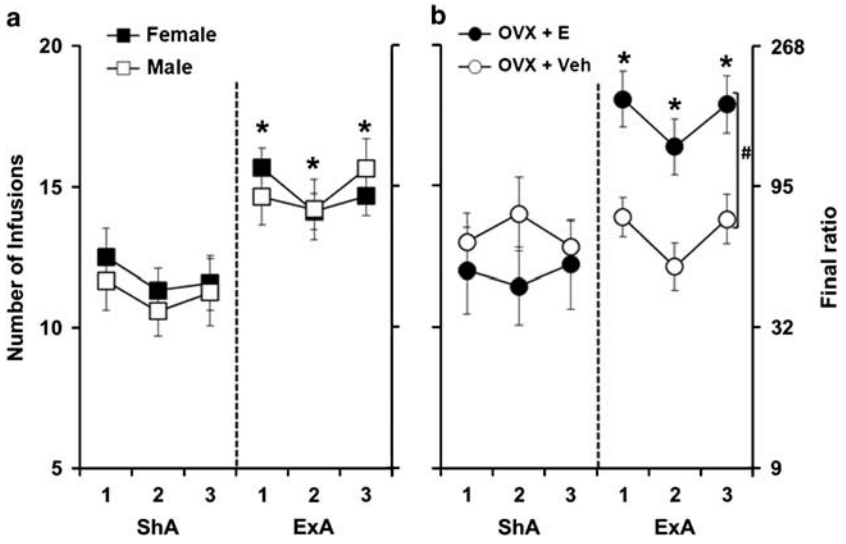

Figure 2 Effect of sex and estradiol on motivation for cocaine following short access (ShA) *vs* extended access (ExA) self-administration. (a) Mean (±SEM) number of cocaine infusions obtained during the three PR self-administration sessions for intact females (filled squares) and males (open squares) tested following ShA (left panel) or ExA (right panel) self-administration and a 2-week abstinence period ($n=12$, and 10 for females, respectively; $n=12$, and 11 for males, respectively). (b) Mean (±SEM) number of cocaine infusions obtained during the three PR self-administration sessions for ovariectomized (OVX) + estradiol (E) (filled circles) and OVX + vehicle (Veh) (open circles) rats tested following ShA (left panel) or ExA (right panel) self-administration and a 2-week abstinence period ($n=9$, and 8 for OVX + E, respectively; $n=9$, and 8 for OVX + Veh, respectively). *Significant difference from control; #Significant group difference ($p<0.05$).

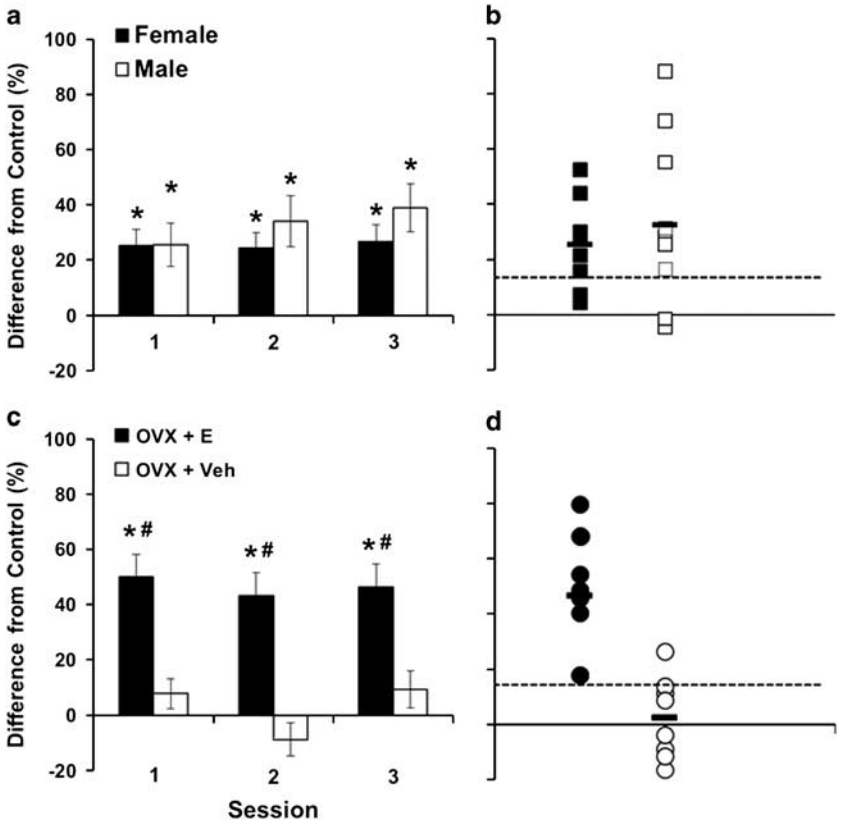

Figure 3 Effect of sex and estradiol on motivation for cocaine following extended access (ExA) self-administration. (a and c) Percent change in the number of infusions obtained following ExA self-administration relative to ShA control conditions. ((a) Female, filled bars; male, open bars; (c) Ovariectomized (OVX) + estradiol (E), filled bars; OVX + vehicle (Veh), open bars). *Significant difference from control; #Significant group difference ($p<0.05$). (b and d) Scatterplot of each individual rats data on percent change in the number of infusions obtained following ExA self-administration relative to ShA control conditions. ((b) Female, filled squares; male, open squares. (c) OVX + E, filled circles; OVX + Veh, open circles). Black solid bars represent the average values within each group, and dotted lines represent a 15% increase.

$t = 3.341$, df = 10, $p < 0.05$; session 3, $t = 4.090$, df = 10, $p < 0.05$; within females, session 1, $t = 4.478$, df = 8, $p < 0.05$; session 2, $t = 4.385$, df = 8, $p < 0.05$; session 3, $t = 4.60$, df = 8, $p < 0.05$, respectively). Motivation for cocaine averaged across the 3 days was increased by 15% or more in 9 out of 11 males and 7 out of 9 females (Figure 3b). In contrast, within the OVX groups, responding was enhanced from control levels in OVX + E rats, but not in OVX + Veh rats (Figure 3c), with results from the ANOVA revealing a significant overall effect of hormone group ($F_{1,14} = 26.424$, $p < 0.05$) but a nonsignificant interaction of hormone group by session ($F_{2,28} = 12.86$, $p > 0.05$). Subsequent comparison within the OVX + E group revealed that responding was significantly enhanced from control levels for each of the three PR sessions (session 1, $t = 6.110$, df = 7, $p < 0.05$; session 2, $t = 5.027$, df = 7, $p < 0.05$; session 3, $t = 5.513$, df = 7, $p < 0.05$). OVX + Veh rats did not differ significantly from controls on any session ($p > 0.05$). Motivation for cocaine averaged across the 3 days was increased by 15% or more in each of the eight OVX + E rats, but only one of the eight OVX + Veh rats (Figure 3d). Taken together, these findings demonstrate an increase in motivation for cocaine in male, female, and OVX + E rats, but not in OVX + Veh rats, following abstinence from ExA self-administration.

The vaginal swab data confirmed the hormonal status for each of the OVX groups. Metestrus/diestrus-like cells and proestrus-like cells were the predominant cell types observed in vaginal smears obtained from the OVX + Veh and the OVX + E group, respectively.

DISCUSSION

The goals of this study were to examine sex differences in the development of an addicted phenotype, and to determine the role of estradiol in this effect. We optimized the self-administration conditions to maximize the likelihood that all animals tested would develop an addicted phenotype, and under these conditions, both males and females did display this phenotype. However, in contrast to our hypothesis, no sex difference was observed for the magnitude of this effect and both males and females showed a similar change in levels of motivation for cocaine following ExA self-administration and abstinence. As predicted, estradiol was involved in this effect, and following ExA self-administration, OVX + E rats showed markedly increased levels of motivation to obtain cocaine. However, an unexpected finding from the OVX groups was that following ExA self-administration, not only did OVX + Veh rats show lower levels of motivation to obtain cocaine as compared with OVX + E rats, but they also did not show an increase in levels of motivation for cocaine as a result of ExA self-administration. These results demonstrate that while both males and females developed an addicted phenotype under these optimized conditions, within females, estradiol appeared to be critical for its development.

Under these conditions, both males and females developed an addicted phenotype, with 9 of the 11 males and 8 of the 10 females showing a greater than 15% increase in levels of motivation as a result of ExA self-administration. However, in contrast to our hypothesis, no sex difference was observed for the degree to which this phenotype was displayed with both males and females showing a similar increase in levels of motivation for cocaine. The lack of a sex difference is surprising given our previous work showing that females developed an addicted phenotype following less exposure to cocaine and/or a shorter abstinence period as compared with males (Lynch and Taylor 2004), and given our current and previous findings showing that females self-administer more cocaine than males during ExA self-administration (Lynch and Taylor 2004). However, these results do parallel findings in humans, which show that although women take less time than men to progress from initial use of a substance to abuse of that substance (ie, cocaine, alcohol, methamphetamines), once dependent, women and men report similar levels of drug use (McCance-Katz *et al*, 1999; Hass and Peters 2000). Taken together, these results suggest that while females have an enhanced vulnerability to developing an addicted phenotype, they may be similar to males once addiction has developed.

One of the most surprising findings from this study was that while ExA self-administration robustly increased subsequent motivation to obtain cocaine in OVX + E rats (46.5%), it was without effect in OVX + Veh rats (1.7%). Although we did predict that this group would show less of an increase in motivation as a result of ExA self-administration as compared with OVX + E rats, we did not expect to see no enhancement, particularly under these optimized conditions. These findings suggest that estradiol may be required for the development of an addicted phenotype in female rats. In support of this idea, within the OVX + E group, all animals tested showed a greater than 15% increase motivation for cocaine following ExA self-administration. In contrast, only one of the eight animals in the OVX + Veh group showed a greater than 15% increase in motivation relative to ShA controls. Although this finding appears to contradict the idea that estradiol is required for this phenotype to develop, it is still a possibility given that the data from this one animal may be an artifact of our between-subject design (ie, this subject could have been 'high responder' at baseline even without a history of ExA cocaine self-administration). Even so, these findings indicate that estradiol is critically involved in the development of an addicted phenotype in females, but whether it is required is not yet known. Future studies using within-subject designs, and perhaps even higher access conditions and/or longer abstinence periods, would be useful for addressing this possibility.

Notably, the OVX model we used has limitations. This model does not attempt to mimic normal cyclicity observed in intact females, and it does not account for the role of other ovarian hormones, such as progesterone, which appears to have a key role in modulating vulnerability to cocaine addiction in females (Anker and Carroll, 2010). Despite its limitations, however, this model provides a powerful tool for assessing estradiol's specific contribution to cocaine addiction vulnerability. Our results not only suggest that estradiol may be necessary for the development of an addicted phenotype in females but also suggest that different mechanisms/circuits may be utilized in its development in males *vs* females. One likely mechanisms is through dopamine signaling in the reward pathyway (ie, striatum), which is enhanced in females compared with

males in response to cocaine, and enhanced by estradiol in females, but not males (Lynch *et al* 2007; Becker 1999; Xiao and Becker, 1994; Jackson *et al*, 2006). While these findings indicate estrogen–dopamine interactions as a potential sexually dimorphic mechanism for cocaine addiction, further research is necessary to determine their role in the development of an addicted phenotype given that most of the evidence in this regard is for initial, not later, vulnerability.

Together with past studies, these results suggest that while females may have an enhanced vulnerability to developing addiction, once addiction has developed, the behavioral phenotype may be similar between the sexes. Importantly, these results suggest that within females, estradiol may be critically involved in the development of cocaine addiction, which may have important implications for women. In particular, further work is needed to understand changes in vulnerability to addiction in women taking hormone-based birth control and in women at hormone transition phases, such as puberty, pregnancy, and the postpartum period. Additionally, a particularly understudied area of research is on changes in vulnerability to cocaine addiction at menopause. While our findings predict that older post-menopausal women would be less vulnerable to cocaine addiction than men, this possibility has not yet been examined. Further research also needed to understand the neurobiological mechanisms underlying these behavioral and hormonal differences found here.

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DISCLOSURE

The authors declare no conflict of interest.

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