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Research

17β-Estradiol is necessary for extinction of cocaine seeking in female rats

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Human and preclinical models of addiction demonstrate that gonadal hormones modulate acquisition of drug seeking. Little is known, however, about the effects of these hormones on extinction of drug-seeking behavior. Here, we investigated how 17β-estradiol (E_2) affects expression and extinction of cocaine seeking in female rats. Using a conditioned place preference (CPP) paradigm, ovariectomized rats were maintained throughout conditioning with 2 d of E_2 treatment followed by 2 d of vehicle treatment, or were injected with E_2 daily. Hormone injections were paired or explicitly unpaired with place conditioning sessions. Expression of a cocaine CPP was of equal magnitude regardless of conditioning protocol, suggesting that E_2 levels during conditioning did not affect subsequent CPP expression. During extinction, daily E_2 administration initially enhanced expression of the cocaine CPP, but resulted in significantly faster extinction compared to controls. Whereas E_2-treated rats were extinguished within 8 d, vehicle-treated rats maintained CPP expression for more than a month, indicative of perseveration. To determine whether E_2 could rescue extinction in these rats, half were given daily E_2 treatment and half were given vehicle. E_2-treated rats showed rapid extinction, whereas vehicle-treated rats continued to perseverate. These data demonstrate for the first time that E_2 is necessary for extinction of cocaine seeking in female rats, and that it promotes rapid extinction when administered daily. Clinically, these findings suggest that monitoring and maintaining optimal E_2 levels during exposure therapy would facilitate therapeutic interventions for female cocaine addicts.

Gonadal hormones render women more susceptible than men to developing compulsive patterns of psychostimulant use. Natural fluctuations in levels of the primary ovarian hormones, estrogens and progesterone, account for this increased abuse liability. Higher levels of estrogens, and lower levels of progesterone, are associated with greater sensitivity to the euphorigenic properties of cocaine in women (Evans 2007). This increased sensitivity may contribute to why women, compared with men, start using cocaine regularly at a younger age (Chen and Kandel 2002), transition from use to abuse more quickly (McCance-Katz et al. 1999), experience greater craving in response to drug-associated cues (Robbins et al. 1999) and stress (Potenza et al. 2012; Waldrop et al. 2012), and exhibit more severe drug-seeking behavior upon relapse (Gallop et al. 2007). In contrast, more women than men remain abstinent after treatment (Weiss et al. 1997). Thus, women are both more susceptible to cocaine abuse and, paradoxically, more responsive to treatment.

A substantial literature supports that estrogens mediate the enhancement of drug seeking observed in females (Febo et al. 2002; Larson et al. 2007; Segarra et al. 2010; Kerstetter and Kippin 2011), but little is known regarding the role of estrogens during treatment. Preclinically, treatment is modeled through extinction learning, which results in the formation of a new inhibitory memory that suppresses drug seeking (Quirk and Mueller 2008; Torregrossa and Taylor 2012). Whether estrogens are necessary for extinction of drug seeking, however, remains unknown. Extinction is a form of new learning, and the potent estrogen 17β-estradiol (E_2) has been shown to enhance learning and memory across paradigms (Daniel 2006; Frick 2012). For example, E_2 enhances the consolidation of object recognition and spatial memory in ovariectomized female rats (Gresack and Frick 2006a) and,

importantly, higher levels of E_2 in intact female rats and humans are associated with enhanced consolidation and recall of fear extinction (Milad et al. 2009, 2010). Therefore, E_2 may be a key mediator of both acquisition and extinction of cocaine seeking in female rats. The effects of E_2 on extinction of cocaine seeking in females, however, have not yet been examined.

Here, we assessed the necessity of E_2 for extinction of cocaine seeking in female rats. Using a conditioned place preference (CPP) procedure, ovariectomized female rats were administered vehicle or E_2 during acquisition and extinction. We first determined the effects of E_2 on expression and extinction of cocaine seeking. We then investigated whether E_2 administration would rescue an extinction deficit in perseverating rats. Our results demonstrate that E_2 is necessary for extinction in female rats, and can facilitate extinction even after long-term perseveration of cocaine seeking.

Results

Paired E_2 during conditioning does not augment cocaine seeking

We first examined the effects of E_2 during conditioning on the magnitude of cocaine seeking on the first CPP test. To avoid buildup of E_2 levels across days, we used a water-soluble form of E_2 that is metabolized within 24 h (Pitha and Pitha 1985). E_2 injections (0.2 mg/kg, i.p.) were either paired (Paired) or explicitly unpaired (Unpaired) with conditioning trials (see Table 1, Experiment 1). After conditioning, rats were given 4 d with no E_2 treatment to eliminate carryover effects on the first day of CPP testing (extinction day 1). Both groups were injected with E_2 and tested 1 h later in the absence of cocaine for a cocaine-induced CPP. Both groups exhibited robust and virtually identical cocaine-induced CPPs (Fig. 1A). ANOVA revealed a significant effect of Chamber for both the Paired ($F_{(2,20)} = 12.64$, $P < 0.001$) and Unpaired groups ($F_{(2,22)} = 15.02$, $P < 0.001$), but

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Table 1. Timeline and treatment conditions

	Conditioning						Extinction	
	(Days 1–2)	(Days 3–4)	(Days 5–6)	(Days 7–8)	...	(Days 15–16)	Trial 1	Trial 2+
Experiment 1								
Paired	E_2 + Cond	Veh	E_2 + Cond	Veh	...	Veh	E_2	E_2
Unpaired	E_2	Veh + Cond	E_2	Veh + Cond	...	Veh + Cond	E_2	Veh
			(Days 1–8)				Trial 1	Trial 2+
Experiment 2								
E_2			Daily E_2 + Cond				E_2	E_2
Vehicle			Daily E_2 + Cond				Veh	Veh

(E_2) 17β-estradiol, (Veh) vehicle, (Cond) conditioning.

no Group × Chamber interaction ($F_{(2,42)} = 0.54$, $P = 0.59$). Post hoc tests confirmed that both groups spent significantly more time in the cocaine-paired chamber than either the saline-paired or center chambers (P's < 0.05). Moreover, E_2 treatment during conditioning had no effect on motor behavior, as both Paired and Unpaired groups displayed equivalent levels of photobeam breaks during the initial CPP trial ($t_{(21)} = 1.96$, $P > 0.05$) (Fig. 1B). Taken together, these data indicate that levels of E_2 at the time of conditioning do not affect subsequent cocaine seeking.

E_2 enhances expression and extinction of cocaine seeking

To determine the extent to which E_2 affects extinction of cocaine seeking, rats received daily injections of vehicle or E_2 before each extinction trial. Repeated E_2 treatment enhanced expression of the cocaine CPP initially, but promoted rapid extinction relative to vehicle-treated controls (Fig. 2). A mixed model three-way ANOVA (Group × Chamber × Extinction Trial) revealed a significant three-way interaction ($F_{(28,588)} = 1.53$, $P = 0.045$), indicating that E_2-treated rats showed a greater CPP magnitude initially but faster extinction than vehicle-treated rats. Post hoc tests confirmed that E_2-treated rats exhibited a significantly greater CPP than vehicle-treated rats on trials 4 and 6 (P's < 0.05) and a lack of CPP after trial 9 (P's > 0.05). Vehicle-treated rats, however, exhibited a marked deficit in extinction across trials (Fig. 2), with post hoc tests confirming expression of a CPP through trial 16 (P's < 0.05). Furthermore, expression of the CPP continued to persist through trial 25 (data not shown). Taken together, these data suggest that E_2 facilitates both expression and extinction of cocaine seeking in female rats and that the absence of E_2 prevents extinction.

Acute E_2 does not augment cocaine seeking

Although the magnitude of the initial CPP was not affected by paired or unpaired E_2 treatment during conditioning, an effect could have been masked because all rats received E_2 prior to the first extinction trial. To eliminate the possibility that acute E_2 affects expression of CPP, two groups of ovariectomized rats were injected daily with E_2 (0.2 mg/kg) during conditioning followed by 4 d with no treatment, and then one group received E_2 before the first CPP trial while the other group received vehicle (Table 1, Experiment 2). Both groups exhibited robust and virtually identical cocaine-induced CPPs (Fig. 3A), indicating that acute E_2 injection did not increase expression of a cocaine CPP. Repeated measures ANOVAs revealed a significant effect of Chamber for both groups (E_2, $F_{(2,20)} = 7.17$, $P = 0.005$; Vehicle, $F_{(2,22)} = 8.18$, $P = 0.003$), but no Group × Chamber interaction ($F_{(2,42)} = 0.12$, $P = 0.88$). Post hoc tests confirmed that both groups spent significantly more time in the cocaine-paired chamber than in either the saline-paired or center chambers (P's < 0.05). Moreover, E_2 treatment prior to the initial CPP trial had no effect on motor behavior, as both E_2- and vehicle-treated groups displayed equivalent

amounts of photobeam breaks during the initial CPP trial ($t_{(21)} = 1.11$, $P > 0.05$) (Fig. 3B). Collectively, these data indicate that the timing of E_2 administration during conditioning does not affect CPP magnitude, and that expression of drug seeking does not depend on the acute effects of E_2 at test.

E_2 prevents perseverative cocaine seeking

Although daily E_2 treatment during extinction enhanced extinction of drug seeking relative to vehicle treatment, these effects may be altered by the shift from cycling E_2 on and off during conditioning to daily E_2 treatment during extinction. To test whether the noncycling groups (Experiment 2) exhibit similar extinction patterns to those of the cycling groups (Experiment 1) and to confirm the extent to which daily E_2 enhances extinction of a CPP, rats treated with E_2 on day 1 of extinction continued during extinction with daily injections of E_2, whereas rats treated with vehicle on day 1 of extinction continued with daily vehicle injections. As in Experiment 1, we found that daily E_2 treatment augmented cocaine seeking before rapidly enhancing its extinction (Fig. 4). ANOVA revealed a significant three-way interaction (Group × Chamber × Extinction Trial, $F_{(28,588)} = 1.80$, $P = 0.008$) and post hoc tests confirmed that E_2-treated rats exhibited a consistent preference for the cocaine-paired chamber on extinction trials 2–6 that was significantly higher than that of vehicle-treated rats on trials 4 and 5 (P's < 0.05). Importantly, this CPP rapidly extinguished and was no longer evident by trial 7 ($Ps > 0.05$). Without E_2 treatment, however, the vehicle group exhibited a marked deficit in extinction learning across trials (Fig. 4). Post hoc tests confirmed this observed deficit, as these animals exhibited an inconsistent CPP
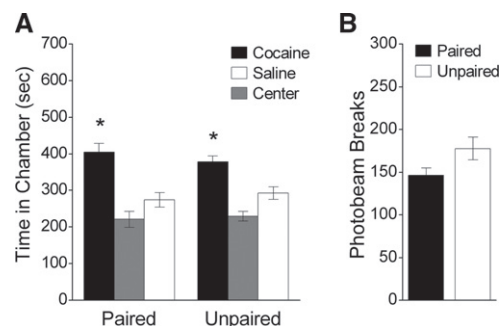

Figure 1. Paired and unpaired E_2 treatment during conditioning did not affect initial CPP expression. (A) E_2 administered 1 h before (Paired) or during the 2 d between (Unpaired) conditioning trials resulted in a similar preference for the cocaine-paired chamber compared with either the saline-paired or center chambers. (B) E_2 treatment had no effect on locomotor activity, as measured by photobeam breaks, when administered prior to a CPP trial regardless of E_2 treatment during conditioning. (*) $P < 0.05$ relative to the saline-paired and center chambers.
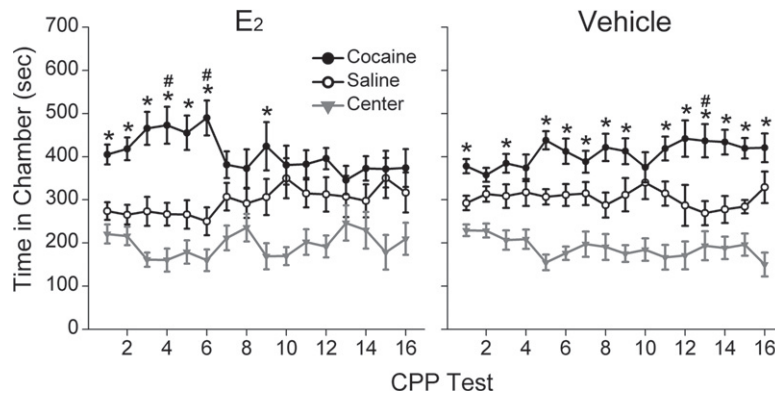
Figure 2. E_2-enhanced expression and extinction of cocaine seeking. Ovariectomized rats injected daily with E_2 (0.2 mg/kg, i.p.) during extinction initially expressed a larger cocaine-induced CPP than vehicle-treated rats, but this extinguished more rapidly than in vehicle-treated rats. Vehicle-treated rats initially expressed a weaker CPP and then exhibited a pronounced deficit in extinction learning that led to a larger CPP in later trials. (*) $P < 0.05$, Cocaine vs. Saline; (#*) $P < 0.05$, E_2 vs. Vehicle.

during early trials that grew more consistent as extinction training continued (P's < 0.05). Indeed, the CPP was significantly larger than the extinguished preference of their E_2-treated counterparts on trials 9 and 16 (P's < 0.05). Repeated testing revealed that the vehicle-treated rats failed to extinguish drug seeking across 36 daily trials, and the magnitude of the CPP increased across trials (Fig. 4). ANOVA revealed a significant two-way interaction (Chamber × Extinction Trial, $F_{(70,770)} = 1.43$, $P = 0.015$) and post hoc tests confirmed that vehicle-treated rats continued to express a significant cocaine-induced CPP on trials 17–36 (P's < 0.05). Together, these data indicate that E_2 is sufficient to prevent perseverative cocaine seeking in ovariectomized female rats, and that a lack of E_2 replacement prevents extinction learning in this paradigm altogether.

E_2 rescues a persistent deficit in extinction of cocaine seeking

Perseveration of drug seeking is a hallmark of addiction (American Psychiatric Association, 2000), and our data show that without E_2 replacement, ovariectomized female rats persistently seek out the cocaine-paired chamber in spite of 25 additional extinction trials. Whether E_2 would facilitate extinction in these perseverating rats, however, remained unknown. Thus, we asked whether E_2 treatment could rescue extinction learning in these rats. Perseverating rats were divided into two groups receiving either vehicle or E_2 1 h before further extinction training. We found that daily E_2 treatment rescued extinction learning within seven additional extinction trials (trials 37–43), even after 5 wk of perseverative drug seeking (Fig. 5). ANOVA revealed a significant Chamber × Trial interaction ($F_{(2,6)} = 9.93$, $P = 0.013$) between trial 36 (last trial without E_2) and the terminal trial 43 (last trial with E_2) for rats injected with E_2. Post hoc tests for E_2-treated rats confirmed that the cocaine CPP on day 36 was no longer significant by trial 43; moreover, the amount of time spent on the cocaine-paired side on trial 36 was significantly greater than that of trial 43 (P's < 0.05). In contrast, ANOVA revealed no difference in the expression of CPP of vehicle-treated rats between trials 36 and 43 ($F_{(2,6)} = 0.62$, $P = 0.57$) (Fig. 5). Indeed, there was a significant Chamber effect on trial 43 ($F_{(2,6)} = 7.36$, $P < 0.024$), and post hoc tests confirmed that vehicle-treated rats continued to spend significantly more time in the cocaine-paired chamber than either the saline-paired or center chambers (P's < 0.05). Taken together,

these results demonstrate that E_2 can rescue the capacity to extinguish drug seeking.

Discussion

We found that E_2 facilitates extinction of cocaine seeking in ovariectomized female rats independently of its well-characterized enhancement of cocaine's unconditioned hedonic effects (Becker and Hu 2008). The magnitude of the initial CPP was identical regardless of whether E_2 was explicitly paired or unpaired with cocaine (Experiment 1) or administered daily (Experiment 2) during conditioning. Moreover, acute E_2 treatment just prior to the first extinction trial had no effect on the magnitude of CPP expression. Therefore, the E_2-enhanced expression of cocaine seeking observed during extinction is not likely due to E_2-mediated enhancements of either cocaine reward or learning during conditioning. Rather, our results indicate that E_2 treatment during extinction training leads to both greater expression and faster extinction through a mnemonic mechanism. Moreover, without E_2 replacement, ovariectomized female rats fail to suppress cocaine-seeking behavior under extinction conditions. Remarkably, this extinction learning deficit can be rescued by E_2 treatment even after 5 wk of perseverative cocaine seeking. As such, daily E_2 treatment is sufficient to enhance the cognitive processes necessary for extinction learning to proceed. These data suggest that although E_2 promotes cocaine intake in female rats self-administering cocaine (Zhao and Becker 2010), subsequent treatment with this hormone also enhances the learning required to suppress cocaine seeking during extinction.

The finding that E_2 enhances extinction of cocaine seeking in females is consistent with previous studies showing that E_2 enhances performance on a wide range of cognitive tasks, including extinction learning in other paradigms (Milad et al. 2009; Phan et al. 2012; Zhao et al. 2012). E_2 replacement or elevated E_2 levels are associated with improved object and social recognition (Gresack and Frick 2006a; Phan et al. 2012), spatial reference memory in a water maze (Gresack and Frick 2006a), and extinction of fear in female rats and humans (Milad et al. 2009, 2010).
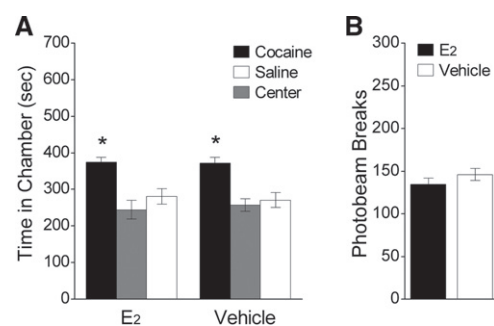

Figure 3. Acute E_2 did not affect expression of a cocaine-induced CPP. (A) E_2- and vehicle-treated rats both exhibited a similar preference for the cocaine-paired chamber compared with either the saline-paired or center chambers. (B) E_2 injection had no effect on locomotor activity, as measured by photobeam breaks, when administered prior to a CPP trial. (*) $P < 0.05$ relative to the saline-paired and center chambers.
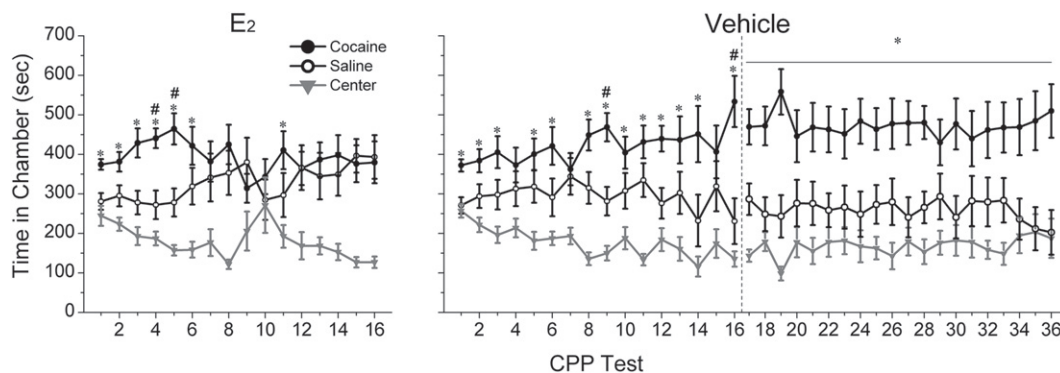
Figure 4. E_2 administered 1 h before daily extinction trials enhanced expression and extinction of a cocaine CPP in rats that were maintained on E_2 during conditioning. Ovariectomized rats injected daily with E_2 during extinction initially expressed a larger cocaine-induced CPP than vehicle-treated rats, but extinguished more rapidly than in vehicle-treated rats. Vehicle-treated rats initially expressed a weaker CPP and then exhibited a pronounced deficit in extinction learning that led to larger CPP in later trials. Vehicle-treated rats failed to exinguish drug-seeking behavior in 36 trials. (*) $P < 0.05$, Cocaine vs. Saline; ([#]*) $P < 0.05$, E_2 vs. Vehicle on the same day.

Similarly, low estrogen levels predict resistance to extinction of fear-potentiated startle in women diagnosed with post-traumatic stress syndrome (Glover et al. 2012), and recent evidence shows that low levels of E_2 in both rats and humans leads to resistance to extinction of conditioned fear (Graham and Milad 2013). Extinction depends on new learning that suppresses the expression of the original memory (Bouton 2004). Therefore, E_2 could enhance extinction of cocaine seeking by bolstering acquisition, retrieval, and/or consolidation of the new extinction memory or, perhaps, interfering with reconsolidation of the former drug associations (Torregrossa and Taylor 2012). The latter is unlikely because E_2 would have also interfered with reconsolidation of extinction and our design employed repeated and prolonged exposure to the context, which favors the formation of extinction memories (Mamiya et al. 2009). Moreover, there is substantial evidence that E_2 treatment enhances memory consolidation in other learning paradigms (Packard and Teather 1997; Gresack and Frick 2006a), and emerging evidence that cognitive enhancers administered during the consolidation window can facilitate extinction of cocaine (Botreau et al. 2006; Paolone et al. 2009) or amphetamine CPP (Schroeder and Packard 2003). Although the mnemonic and neural mechanisms underlying E_2-induced enhancement of extinction remain unknown, the present experiments are the first to demonstrate that E_2 facilitates extinction of cocaine seeking in female rats.

Our findings suggest that E_2 is required for females to extinguish cocaine seeking. Ovariectomized female rats failed to extinguish for over a month, but extinction in these rats could be rescued with E_2 replacement. The observed perseverative drug seeking was unexpected, and indicative of the necessity of E_2 for extinction. The fact that E_2 rescues these extinction deficits suggests that E_2 enhances synaptic plasticity associated with extinction learning. Estrogen receptors α and β are expressed in the medial prefrontal cortex, amygdala, and hippocampus (Shughrue et al. 1997; Ostlund et al. 2003), structures that mediate extinction learning and consolidation (Quirk and Mueller 2008). E_2 stimulates intracellular signaling cascades, growth factor induction, synaptogenesis, and protein synthesis (Spencer et al. 2008; Frick 2012). Importantly, there is a synergistic overlap between the effects of E_2 and synaptic plasticity associated with extinction learning. For example, E_2 increases activation of mitogen-activated protein kinase (MAPK) cascades during memory consolidation (Fernandez et al. 2008), and MAPK signaling is required for extinction (Hugues et al. 2004). Thus, E_2 may promote extinction through stimulation of MAPK cascades. Similarly, levels of brain-

derived neurotrophic factor are increased following both E_2 administration (Luine and Frankfurt 2012) and successful extinction (Peters et al. 2010). Collectively, these findings suggest that E_2 acts to strengthen synaptic plasticity associated with extinction learning. As such, the absence of E_2 could suppress synaptic plasticity, thereby impairing extinction.

Our finding that E_2 transiently enhanced expression of cocaine seeking is congruent with the vast majority of literature describing the enhanced response to cocaine exhibited by E_2-treated female rats. Female rats exhibit enhanced responding to psychostimulants in multiple paradigms in a manner both dependent and independent of ovarian sex hormones (Hu et al. 2004; Jackson et al. 2006). In ovariectomized rats, E_2 enhances the sensitization of cocaine-induced locomotor behavior and CPP (Segarra et al. 2010), self-administration of cocaine on both fixed and progressive ratio schedules of reinforcement (Lynch and Taylor 2005; Zhao and Becker 2010), and preference for cocaine over food reinforcement in a discrete trials procedure (Kerstetter and Kippin 2011), and lowers thresholds for electrical brain stimulation of the ventral tegmental area (Galankin et al. 2010). Thus, E_2-enhanced expression of CPP could be interpreted as further evidence in females of "enhanced abuse liability to cocaine," yet this interpretation is at odds with the finding that E_2 also enhanced extinction of drug seeking. In fact, because E_2 augmented CPP expression only after four extinction trials, the data point
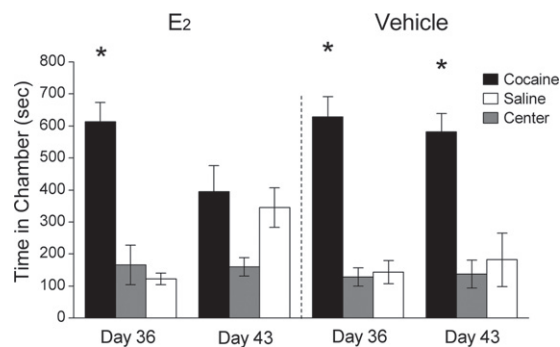

Figure 5. E_2 treatment rescues an extinction learning deficit in ovariectomized rats that perseverate on cocaine seeking. A cocaine-induced CPP that persisted for 5 wk was abolished by 7 d of E_2 administration with extinction training. Vehicle-treated rats, however, continued to express a cocaine CPP. (*) $P < 0.05$ relative to the saline-paired and center chambers.

toward an alternative explanation, specifically that a common neural and mnemonic mechanism mediates both enhanced expression and extinction. Because E_2 enhances memory in a variety of paradigms, E_2 may act to enhance working memory and/or retrieval of the original cocaine association, thereby augmenting the incentive salience of the cocaine-associated context and enhancing expression of the CPP. The same E_2-mediated cognitive enhancement, then, would also enhance extinction learning and lead to more rapid extinction of cocaine seeking. Neurobiologically, both enhanced expression and extinction would likely be influenced by E_2 modulation of mesocorticolimbic dopamine (Bazzett and Becker 1994; Wyvell and Berridge 2001; Jacobs and D'Esposito 2011) or norepinephrine signaling (Mueller et al. 2008; Mueller and Cahill 2010; Otis and Mueller 2011). Thus, it is conceivable that previous reports demonstrating enhanced expression of cocaine-induced CPP assign too much importance to estrogen's undisputed enhancement of cocaine reward with little attention paid to the cognitive enhancements mediated by E_2.

The present findings have clear implications for basic and clinical research, and for clinical treatment of women addicted to cocaine. Estrogens are key hormones that regulate the response to cocaine in both female rats and humans (Segarra et al. 2010). Changes in estrogen levels in cycling females may account for the apparent greater severity of cocaine addiction, or faster transition from use to abuse, relative to that of men (Becker et al. 2012). Nevertheless, more men than women initiate illicit drug use (55.1% vs. 44%; and for cocaine, 18.1% vs. 11.4%), which may account for the higher rate overall of drug abuse among men (3.6% vs. 2.0%; SAMHSA 2010). These findings suggest a differential path to abuse for men and women (for review, see Becker et al. 2012) or, alternatively, reflect the greater propensity for women to remain abstinent following treatment for drug abuse (Weiss et al. 1997). Our finding that E_2 treatment enhanced extinction of cocaine seeking even in ovariectomized rats that were perseverating for up to 5 wk suggests a potentially vital role for E_2 in the recovery from cocaine abuse. The importance of E_2 is underscored by our finding that female rats in the absence of E_2 failed to suppress cocaine seeking during extinction, and may explain why gonadally intact female rats in estrus (low E_2 levels) are more resistant to extinction of drug seeking than are male rats (Kerstetter et al. 2008; Anker et al. 2010). Therefore, low E_2 levels may increase the risk of relapse in some women, and this risk may be exacerbated by cocaine-induced menstrual cycle disorders (Mello and Mendelson 1997). Because E_2 levels may predict individual differences in response to treatment, our data suggest a potential benefit of routine assessment of hormonal status as part of treatment of cocaine addiction in women. Moreover, our findings suggest a need to maintain optimal levels of E_2 to enhance therapeutic outcome in cocaine-addicted women.

Materials and Methods

Subjects and surgery

Female Long-Evans rats (Harlan Laboratories, 175–200 g) were individually housed in clear plastic cages. Rats were maintained on a 14-h light/10-h dark cycle (lights on at 07.00 h), and had unlimited access to water and standard laboratory rat chow (Teklad, Harlan Laboratories). Rats were weighed and handled daily. All experimental protocols were approved by the Institutional Animal Care and Use Committee at the University of Wisconsin-Milwaukee in accordance with National Institutes of Health guidelines.

Rats were handled for a minimum of 3 d prior to surgery. They were then anesthetized with a mixture of ketamine/xylazine (90 mg/kg/10.5 mg/kg, i.p.), and underwent bilateral ovariecto-

my using a dorsal approach (Frick et al. 2004). Through a single incision along the spine, the ovary was isolated, the tip of the uterus was clamped and ligated, and the ovary was removed with a scalpel. The remaining tissue was returned to the abdomen, the procedure repeated on the other ovary, and the incision closed with sterile silk suture and wound clips. Following surgery, rats were given an antibiotic (penicillin G procaine, 75,000 units in 0.25 mL) and an analgesic (carprofen, 5.0 mg in 0.1 mL) subcutaneously, and were given 7 d to recover.

Drugs

Cocaine HCl (National Institute on Drug Abuse) was dissolved in sterile 0.9% saline (10 mg/mL), and administered at a dose of 10 mg/kg, i.p. To ensure that E_2 levels would not build up over time from repeated infusions, we used a water-soluble form of E_2 dissolved in 2-hydroxypropyl-β-cyclodextrin (HBC) that is metabolized within 24 h (Pitha and Pitha 1985). The HBC vehicle and HBC-encapsulated E_2 (Sigma Aldrich) were dissolved in sterile 0.9% saline (0.2 mg/mL) and injected i.p. 1 h before testing at a dose of 0.2 mg/kg. This dose produces levels of E_2 in the physiological range (Gresack and Frick 2006b). Post-training i.p. injection of this dose of HBC-encapsulated E_2 enhances spatial memory in the water maze in ovariectomized rats (Packard and Teather 1997) and mice (Gresack and Frick 2006a) and object recognition in ovariectomized mice (Gresack and Frick 2006a).

Conditioning and testing

Conditioning and testing were conducted as described previously (Otis and Mueller 2011). Briefly, a three-chamber apparatus was used in which two larger conditioning chambers were separated by a smaller center chamber. Baseline preferences were determined by placing the rats into the center chamber with free access to the entire apparatus for 15 min. Rats spent less time in the center chamber before conditioning than in either of the larger conditioning chambers ($F_{(2,94)} = 62.8$, $P < 0.001$; post hoc P's < 0.001) and an equivalent amount of time was spent in the conditioning chambers ($P > 0.05$). Therefore, an unbiased procedure was used. Rats were randomly assigned to associate one large chamber with cocaine and the other with saline in a counterbalanced fashion over four conditioning trials. Rats were injected immediately before each 20-min conditioning session and confined to their respective chambers. For extinction training, rats were placed into the center chamber and allowed free access to the entire apparatus for 15 min. A CPP was determined when significantly more time was spent in the previously cocaine-paired chamber than in the saline-paired chamber.

Hormonal treatment and experimental design

Experiment 1

Rats were maintained on an experimenter-controlled, repeating 4-d treatment cycle consisting of 2 d of E_2 followed by 2 d of HBC vehicle (see Table 1). Conditioning trials were either paired (Paired, $n = 11$) or explicitly unpaired (Unpaired, $n = 12$) with E_2 over 16 d, resulting in four pairings with cocaine and four with saline. After conditioning, rats were given 4 d with no hormone injections before CPP testing. On the first CPP test trial, both groups were injected with E_2 and tested for a cocaine-induced CPP. For the remainder of the daily extinction trials, rats received either E_2 ($n = 11$) or HBC vehicle ($n = 12$).

Experiment 2

Rats were maintained on daily E_2 treatment throughout the conditioning phase (Table 1). Conditioning trials were conducted daily over 8 d, resulting in four pairings with cocaine and four with saline. After conditioning, rats were given 4 d with no hormone injections before CPP testing. Rats were injected with E_2 ($n = 11$) or HBC vehicle (Vehicle, $n = 12$) prior to each CPP test trial. These conditions were maintained for the remainder of extinction

testing through trial 36. To determine whether E_2 treatment could rescue extinction learning in perseverating rats, the rats treated with HBC vehicle during extinction were divided into two groups receiving either vehicle ($n = 4$) or E_2 ($n = 4$) 1 h before further extinction training. These rats received either daily E_2 treatment or vehicle for an additional seven extinction trials (trials 37–43).

Data analysis

Drug seeking during single or across multiple CPP trials was analyzed by comparing time spent in the previously cocaine-paired, saline-paired, and center chambers using multivariate mixed model and repeated measures ANOVA. When appropriate main and interaction effects were detected, Fisher's LSD post hoc tests were used to make pairwise comparisons. All data were analyzed using Statistica 64 (Statsoft, Inc.).

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