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Effect of short- vs. long-term estrogen on reinstatement of cocaine-seeking behavior in female rats

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Abstract

Estrogen effects on cocaine-induced reinstatement of lever responding were examined in sham-operated, vehicle-treated (SH+VEH), ovariectomized (OVX+VEH), and OVX female Wistar rats with estrogen replacement (OVX+EB). The effect of long- (64±1.56 days) and short-term (9 days) EB treatment on reinstatement of cocaine-seeking behavior was compared in Experiment 1 and 2, respectively, in order to compare the effect of EB when it was present during the development vs. expression of reinstatement of cocaine-seeking behavior. Rats were trained to self-administer 0.4 mg/kg/inf cocaine. After the acquisition criteria were met, rats continued to respond for cocaine for 2 h/day for a 14-day maintenance period. Cocaine was then replaced with saline and the 21-day extinction period commenced. Subsequently, rats were tested for reinstatement of lever responding on the previously drug-paired lever after alternating daily injections of saline or cocaine. In both experiments, there were no differences between groups in self-administration behavior during training, maintenance, or extinction. In Experiment 1, SH+VEH and chronically treated OVX+EB rats had greater cocaine-induced reinstatement than OVX+VEH rats. In Experiment 2, short-term treated OVX+EB rats also showed enhanced cocaine-induced reinstatement compared to OVX+VEH rats. The results indicate that EB-mediated enhancement of cocaine-induced reinstatement is dependent on EB presence during the expression of reinstatement but not during the formation of stimulus–reward associations during the development of cocaine-reinforced behavior.

Keywords: Cocaine; Estrogen; Female; Self-administration; Reinstatement

1. Introduction

Clinical studies indicate that women may be more vulnerable to drug abuse than men. For example, it has been reported that women initiate cocaine use at a younger age (Griffin et al., 1989), and they have more complications related to their cocaine use than men (Griffin et al., 1989; Kosten et al., 1993; Weiss et al., 1997; Brady and Randall, 1999). Women also have higher levels of cue-induced cocaine craving (Robbins et al., 1999; Elman et al., 2001), and they are more likely to relapse after an abstinence period than males (Griffin et al., 1989). The idea that females are more prone to drug abuse is supported by preclinical self-administration studies in which sex differences have been reported across various phases of addiction (Carroll et al., 2004; Roth et al., 2004). Of particular interest is the finding that females that had been previously self-administering fentanyl (Klein et al., 1997) or cocaine (Lynch and Carroll, 2000) were more likely than males to reinstate their drug-seeking behavior after exposure to stress or cocaine primes, respectively. Similarly, behavioral sensitization, which has been associated with the reinstatement of drug seeking after an abstinence period (De Vries et al., 1998, 2002), is more pronounced in females compared to males (Becker, 1999). Together, these findings suggest that females may be more vulnerable to relapse and more sensitive to the stimuli that induce it (e.g., cues, drug intake, and stress). Relapse

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prevention is a major goal of drug abuse research, but currently there are no treatments that successfully prevent relapse to cocaine abuse. Thus, it is important to identify factors that mediate sex differences in drug-seeking and drug-taking behavior, particularly during a drug abstinence period.

Estrogen is one factor thought to mediate sex differences in response to cocaine administration. For example, removal of circulating estrogen by ovariectomy (OVX) or blockade of estrogen receptors by tamoxifen attenuated acquisition of cocaine self-administration compared to sham-operated (SH) controls. Conversely, administration of estradiol benzoate (EB) to OVX rats enhanced acquisition of cocaine self-administration (Lynch et al., 2001; Hu et al., 2004). A role for estrogen has also been established for locomotor and ambulatory activity, rearing, and stereotypy after acute or chronic cocaine administration (Peris et al., 1991; Quiñones-Jenab et al., 2000; Perrotti et al., 2001; Becker et al., 2001; Sell et al., 2000, 2002; Chin et al., 2002), cocaine-induced conditioned place preferences (Russo et al., 2003), and cocaine-induced behavioral sensitization (Glick and Hinds, 1984; Haney et al., 1994; Sircar and Kim, 1999; Chin et al., 2002; Febo et al., 2003; Hu and Becker, 2003).

The ability of estrogen to modulate the behavioral responses to cocaine in female rats is also supported by evidence suggesting that cocaine-induced behavioral responses vary across the phases of the estrous cycle. For example, higher levels of behavioral activation after cocaine, as well as self-administration of cocaine, occur during proestrus (high estrogen) or estrus (the phase immediately following proestrus when estrogen levels are declining from their peak) (Becker et al., 1982; Quiñones-Jenab et al., 1999; Sell et al., 2000, 2002). Similarly, breakpoints (the highest ratio completed in an increasing succession of fixed-ratios–responses/drug delivery) under a progressive-ratio (PR) schedule of reinforcement are highest in female rats in the estrus phase (Roberts et al., 1989). Furthermore, in a study in which rats increased their cocaine dose by responding on one lever and decreased the dose by responding on another lever, dose dysregulation and elevated levels of cocaine intake (mg/kg) occurred during the estrus phase (Lynch et al., 2000).

While it has been shown that estrogen mediates sex differences in cocaine self-administration during acquisition and regulation/dysregulation phases of drug addiction (Lynch et al., 2000, 2001), the effect of estrogen during another critical phase of cocaine abuse, reinstatement, has not been examined, yet this is the phase of drug abuse that is the most challenging to treat. Therefore, in the present study, the role of estrogen on cocaine-induced reinstatement of cocaine-seeking behavior was examined by comparing SH or OVX female rats with or without EB replacement. A drug priming-induced reinstatement procedure modified from De Vries et al. (1998) was used, as it employs a long-term withdrawal period (i.e., 21 days), and it has been shown to produce robust reinstatement of lever responding for cocaine. In Experiment 1, EB treatment of OVX rats was long-term (present during training, maintenance, extinction, and reinstatement, 60–70 days). Based on previous findings of sex differences (F>M) during reinstatement, it was hypothesized that SH+VEH and OVX+EB groups would show greater cocaine-induced reinstatement of lever responding than the OVX+VEH group.

While studies examining EB effects on psychostimulant-induced behaviors and dopamine functioning have often employed long-term replacement methodology (Di Paolo et al., 1981, 1982; Hruska, 1986; Lynch et al., 2001; Sell et al., 2002), estrogen effects have also been reported under short-term treatment durations (Becker, 1990a, 1999; Xiao and Becker, 1998; Becker and Rudick, 1999; Thompson, 1999), and differential effects of short- and long-term estrogen treatment have been reported (Becker, 1990b, 1999). Additionally, there is evidence that chronic estrogen treatment can modulate acquisition and retention of certain memory tasks (e.g., Luine et al., 1998; Gibbs, 2000; Korol and Manning, 2001; Foster et al., 2003), that may have influenced the magnitude of reinstatement of lever responding in Experiment 1. In order to determine whether elevated reinstatement of cocaine-seeking behavior is related to the presence of EB during the development of cocaine-seeking behaviors, during the expression of cocaine-induced reinstatement, or both, Experiment 2 examined reinstatement of lever responding when EB was given only during reinstatement and 3 days before (9 days of treatment). It was hypothesized that short-term EB treatment of OVX rats would also enhance reinstatement of lever responding after cocaine compared to VEH-treated controls. It was also expected that, if EB enhanced associative learning as well as the motivation for cocaine seeking during reinstatement, reinstatement of lever responding in OVX+EB rats in Experiment 1 would be greater than for OVX+EB rats in Experiment 2.

2. Methods

2.1. Animals

Forty-four sexually mature (>90 days) female Wistar (Harlan Sprague–Dawley) rats weighing between 240 and 350 g were used as subjects. Upon arrival at the laboratory, rats were pair-housed in plastic bins and they had ad libitum access to Purina Laboratory Chow (Purina Mills, Minneapolis, MN) and water. They were allowed to acclimate to the laboratory for a minimum of 3 days prior to surgery. After acclimation, each rat was implanted with a chronic indwelling catheter into the right jugular vein following procedures previously described (Carroll and Boe, 1982) and they received either bilateral OVX or a SH surgery. Following surgery, rats were placed in individual operant chambers and allowed a recovery period of 10 days. Rats remained in the operant chambers for the duration of the experiment with free access to water. Rations of 16 g ground

rat chow were given daily at 1100 hours in order to maintain rats at 85% of their free-feeding body weights (weights were measured weekly). Rooms were kept at constant temperature (24 °C) and humidity and a 12/12-h light/dark cycle was used with lights on at 0600 hours. The University of Minnesota Institutional Care and Use Committee approved the experimental protocol under protocol number 0112A13581. Laboratory facilities were accredited by the American Association of the Accreditation of Laboratory Animal Care (AAALAC) and experiments were carried out in accordance with the Principles of Laboratory Animal Care (National Research Council, 2003).

2.2. Apparatus

Rats were housed and tested in individual operant chambers that were each enclosed in ventilated sound-attenuating wooden boxes. Chambers were octagonal in shape with fitted insertions for a drinking spout, a food receptacle, and two response levers (MedAssociates, St. Albans, VT). Three colored stimulus lights (4.76 W) were placed 5 cm above each lever, and a house light (4.76 W) was positioned near the top of the chamber. An infusion pump (RHSYOCKC, Fluid Metering, Oyster Bay, NY) was attached to a 500-ml aspirator bottle containing the cocaine solution. Both the pump and the reservoir were mounted on the outside of the wooden enclosure. The infusion pump was fitted with Tygon tubing (1.52 mm o.d., 0.51 mm i.d., Fisher Scientific, Springfield, NJ) which was connected to a swivel (050-0022, Alice King Chatham, Hawthorne, CA) that was positioned at the top (center) of every test chamber. The swivel was attached to a tether (C313CS, Plastic Products, Roanoke, VA) that extended into the chamber and connected to the rat via a metal cannula (C3236, Plastics One, Roanoke, VA). The metal cannula was embedded in the center of a plastic infusion harness (Instech Laboratories, Plymouth Meeting, PA), and it attached to the end of the rat's indwelling catheter. Programming, data collection, and data storage were conducted with IBM-compatible computers with Med-PC interfaces (Med Associates, St. Albans, VT).

2.3. Drugs

Cocaine HCl was provided by the National Institute on Drug Abuse (Research Triangle Institute, Research Triangle Park, NC), and it was dissolved in a sterile 0.9% saline solution. Heparin (5 USP units/ml saline) was added to cocaine and saline solutions in order to improve catheter patency. Cocaine infusions (0.4 mg/kg) were delivered at a rate of 0.025 ml/s, and the infusion duration was 1 s/100 g of body weight. Infusion durations ranged from 2.4 to 3.5 s and volumes ranged from 0.06 to 0.08 ml. The cocaine solution was refrigerated until it was added to the 500-ml reservoirs covered with aluminum foil outside each test chamber. The 17 β-estradiol (EB) and peanut oil vehicle (VEH) were purchased from Sigma-Aldrich (St. Louis, MO). EB was dissolved in peanut oil (0.0006 g EB/ml oil), and both EB and VEH were injected subcutaneously (s.c.) on the dorsal side of the rat. The dose of EB used (0.05 mg/kg/day) has previously been shown to result in plasma estrogen levels comparable to estrogen peaks in intact rats in the proestrus phase of the estrous cycle (Cheng and Johnson, 1974).

2.4. Surgery

All rats underwent a catheterization procedure and at the same time they were given either a bilateral or SH OVX. Prior to the surgical procedure, they were anesthetized with a combination of ketamine (90 mg/kg) and nembutal (10 mg/kg), and atropine (0.15 cc) was given to facilitate respiration. For cannulation, a modified silicone catheter containing two small silicone anchors was fed into the right jugular vein and secured with 3 silk sutures (Genzyme, Fall River, MA). The unfastened end of the catheter was led subcutaneously to and passed through a puncture hole 1 cm caudal to the scapulae, where it was then connected to a metal cannula embedded in the center of an external covance infusion harness (Instech Laboratories, Plymouth Meeting, PA). For the OVX surgery, a bilateral incision was made and ovaries were localized, externalized, and removed. Muscle wall that had been blunt cut and the outer incisions were sutured with chromic gut. The procedure was the same for SH surgeries, except the ovaries were not removed. Immediately following surgery and for the following 2 days, rats received heparinized saline (0.3 ml) and gentamicin (2.0 mg/kg, i.v.), followed by 7 days of injections of 0.3 ml heparinized saline alone. The tether/swivel attachment was connected to the rat's harness the day after surgery and the infusion pump tubing was attached on the morning of the first training session. The equipment remained installed throughout the experiment and was visually checked daily for defects. Catheter patency was assessed approximately every 7 days by an injection of sodium pentobarbital (25 mg/ml, i.v.). If an immediate loss of the righting reflex was observed, it was assumed the catheter was patent. If a loss in the righting reflex did not manifest following the injection, the rat was implanted with a catheter in the left jugular vein, and it was returned to the experiment after 3 days of recovery.

2.5. Procedure

Fig. 1 summarizes the experimental procedures, that proceeded in 5 phases: cocaine self-administration training/acquisition, maintenance, drug extinction, pre-reinstatement, and reinstatement.

2.5.1. Cocaine self-administration training

All rats were trained to self-administer cocaine (0.4 mg/kg/inf) under a fixed-ratio 1 (FR 1) schedule with 2-h

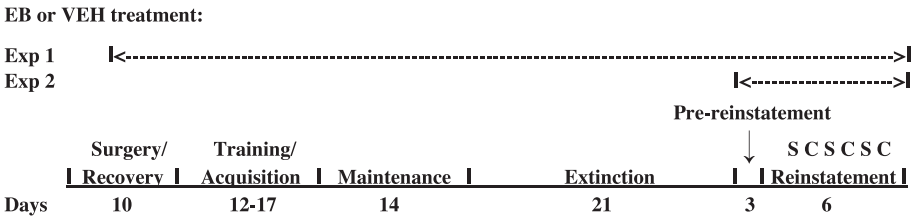

Fig. 1. Summary of the experimental procedure (adapted from De Vries et al. (1998)). Phases of the experiments are shown with the phase duration presented underneath. S C S C S C: priming injection order during reinstatement, S=saline priming injection and C=cocaine priming injection (10 mg/kg, i.p.). The dashed lines represent the duration of treatment with VEH or EB in Exp. 1 (~64 days) and Exp. 2 (9 days).

access beginning at 0900 hours. The house light was extinguished 30 min prior to the daily session and illuminated at 0900 hours to signal session onset. Responses on the active lever resulted in illumination of the stimulus lights above the lever and an immediate infusion of cocaine, followed by a 20-s timeout period where the stimulus lights remained on, but lever responding had no other consequence. Responses on the inactive lever also illuminated the stimulus lights above the lever for 20 s; however, inactive lever presses did not result in a cocaine infusion. Light-reinforcement on the inactive lever served as a control for the active lever (i.e., conditions on both levers were identical except for the delivery of the drug), thus differences in responding on the levers were due solely to the presence of the drug. Responses on the active and inactive lever, as well as infusions, were recorded throughout the session.

During training, rats were administered 2–3 cocaine priming infusions at the start of the session. If rats exhibited minimal responding (i.e., 0–3 lever presses per session) for more than a week, a small amount of food (~0.5 g) was placed on the levers to facilitate lever pressing behavior. Rats that earned ≥30 infusions/day and exhibited an active : inactive lever response ratio of at least 2 : 1 subsequently had the priming infusions and/or food on levers discontinued. If the rats continued to respond at ≥30 infusions a day the following day, they were advanced to the maintenance phase of the experiment (procedure modified from DeVries et al. (1998)). Cocaine priming injections were only given during training in order to promote operant lever responding, as without these primes, the majority of the rats did not self-initiate lever pressing behavior. Cocaine priming at the session onset may have initially acted as an "occasion setter", predicting contingent cocaine availability; however, only rats that continued to respond without those primes were allowed to progress into the maintenance phase.

2.5.2. Maintenance

Rats were allowed to lever press under a FR 1 schedule for cocaine (0.4 mg/kg/inf) in 2-h sessions (0900–1100) for 14 days. Stimulus conditions were the same as in training; however, no cocaine priming infusions were given during this phase. During maintenance, lever responses and infusions continued to be monitored and recorded.

2.5.3. Extinction

The extinction phase of the experiment commenced after the 14-day maintenance period and lasted for 21 days. During this time, cocaine solutions were replaced with saline. All other stimulus conditions remained identical to those in the maintenance phase.

2.5.4. Pre-reinstatement

In order to eliminate the influence of conditioned stimuli during the reinstatement phase, stimulus lights and infusion pumps were turned off after drug extinction (3 days prior to reinstatement testing), and they remained off throughout the rest of the experiment. Additionally, the house light was turned off, thus eliminating confounds from the potential "occasion setter" effects of this stimulus. However, the rat's equipment (harness and tether/swivel attachment) remained on during pre-reinstatement as well as during reinstatement testing.

2.5.5. Reinstatement

Reinstatement testing for cocaine-seeking behavior consisted of 6 alternating days of saline (S) or cocaine (C, 10 mg/kg, i.p.) priming injections (i.e., SCSCSC). Injections were experimenter-delivered immediately prior to session onset (0900 hours). After receiving injections, rats were immediately returned to their individual operant chambers, and responding on the previously active and inactive levers was recorded for the duration of the 2-h session. Cocaine was given based on previous studies indicating that this dose and route of administration produced robust cocaine-induced reinstatement of lever responding (De Vries et al., 1998; Schenk and Partridge, 1999). Additionally, this procedure had the advantage that catheter patency was not needed after the extinction phase.

2.6. Hormone treatment

2.6.1. Experiment 1. Effects of long-term EB treatment on reinstatement

Rats were randomly assigned to one of three groups: 1) SH+VEH (n=9), 2) OVX+VEH (n=9), or 3) OVX+EB (n=10). They received daily injections of 0.05 mg/kg EB or an equal volume of VEH s.c. at 0830 hours each day, starting a week before training and continuing until the termination of the experiment (mean treatment duration=64 (±1.56) days).

To verify that the rats' hormonal status was consistent with their treatment group, they received a daily vaginal swab at 1430 hours. Swab samples were placed on slides, stained with methylene blue, cover slipped, and examined under light microscopy. Upon viewing the slides, it was noted that OVX+EB animals had cytology that displayed a predominance of cornified epithelial cells (similar to estrus in intact females) and OVX+VEH slides were characterized by a predominance of leukocytes (similar to diestrus in intact females), similar to what has been previously shown (Montes and Luque, 1988). Cycle phase was not controlled for in SH+VEH rats; however, all SH+VEH animals had normal 3–5 day estrous cycles verified by cytologic examination of vaginal mucosa samples.

2.6.2. Experiment 2. Short-term EB effects on reinstatement of lever responding

This experiment was identical to Experiment 1, with 2 exceptions. One of these was the duration of EB/VEH treatment. In Experiment 2, rats were randomly assigned to one of two treatment groups: 1) OVX+VEH ($n=8$) or 2) OVX+EB ($n=8$). They received daily injections of 0.05 mg/kg EB or an equal volume of VEH (s.c.) at 0830 hours starting 3 days prior to reinstatement and continuing on until the termination of the experiment, a total of 9 days. The second difference was the schedule of vaginal swabbing. In contrast to Experiment 1, rats in Experiment 2 had vaginal smears taken for 5 days prior to training to confirm successful OVX. Smears were then discontinued and they were resumed during short-term VEH or EB treatment to confirm OVX+VEH or OVX+EB group status, respectively.

2.7. Data analysis

Statistical analyses were conducted with GB Stat (Dynamic Microsystems, Silver Springs, MD). Independent variables were treatment, day of maintenance or extinction, and priming injection type (saline or cocaine) during reinstatement. Day of maintenance and extinction were considered to be independent variables as the number of days in these phases was determined by the experimenters and because self-administration could potentially have changed as a function of day. For example, in extinction, one group could have extinguished more rapidly than the other group; thus, we considered the number of saline infusions administered (dependent variable) as being dependent on the day of extinction (independent variable). Dependent measures were infusions during maintenance and extinction and responses during reinstatement. The effect of treatment during maintenance and extinction was examined using a two-way repeated measures analysis of variance (ANOVA) (treatment and day). The effects of group and priming injection type during reinstatement were analyzed using a three-way repeated measures ANOVA (treatment, priming agent type, and day). All post-hoc tests were done using Fisher's LSD protected *t*-tests. The comparison of cocaine-induced reinstatement responding in OVX+EB groups from Experiment 1 and 2 was done using a two-tailed Student *t*-test. Comparisons of inactive and active lever responses during reinstatement were conducted with one- or two-way ANOVAs. Results were considered to be significant if $p<0.05$.

3. Results

3.1. Experiment 1. Effects of long-term EB treatment on reinstatement

There were no group differences in the number of days for rats to meet training criteria for cocaine self-administration. The mean (±SEM) days to reach the acquisition criteria was 14.22 (±3.75), 13.56 (±2.33), and 12.20 (±2.15) for SH+VEH, OVX+VEH, and OVX+EB rats, respectively. Fig. 2 illustrates the mean (±SEM) daily infusions self-administered across the 14-day maintenance period. Statistical analysis revealed no effect of treatment or day on the number of cocaine infusions self-administered during this phase. The mean (±SEM) number of infusions self-administered per day was 42.67 (±7.42), 38.98 (±4.49), and 42.96 (±5.09) for SH+VEH, OVX+VEH, and OVX+EB rats, respectively.

Fig. 3 illustrates the mean (±SEM) number of saline infusions across the 21-day extinction phase for the 3 groups. Statistical analysis revealed a significant effect of day [$F_{(20, 587)}=20.47$, $p<0.05$], but not a significant treatment effect. Rats in all groups initially had high responding that diminished over the extinction period. Saline self-administration was minimal by Day 10, and it remained low for the rest of the 21 days. There was a significant day × treatment interaction [$F_{(40, 587)}=1.71$, $p<0.05$], and post-hoc analysis using Fisher's LSD protected *t*-tests indicated that SH+VEH rats self-administered more saline infusions than OVX+VEH or OVX+EB rats on Days 1–3 (*$p<0.05$).

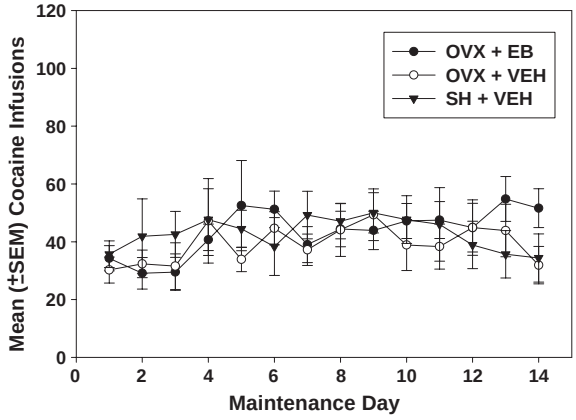

Fig. 2. Experiment 1. Cocaine self-administration behavior during 14-day maintenance period. The daily mean (±SEM) cocaine infusions self-administered are shown for SH+VEH (closed triangles), OVX+VEH (open circles), and OVX+EB (closed circles) rats.

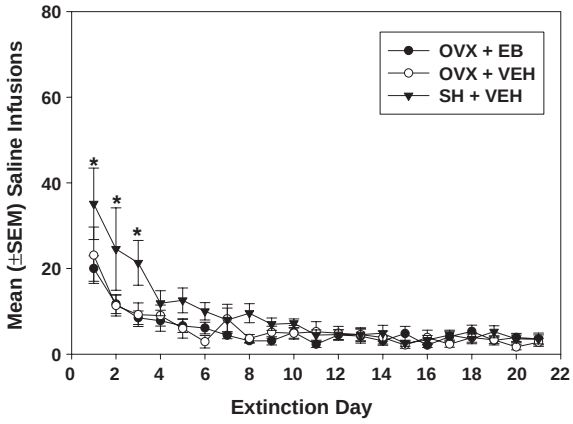

Fig. 3. Experiment 1. Saline self-administration behavior across the 21-day extinction period. The mean (±SEM) number of saline infusions earned each day are shown for SH+VEH (closed triangles), OVX+VEH (open circles), and OVX+EB (closed circles) rats. The asterisk depicts that SH+VEH rats had more saline infusions than OVX+VEH or OVX+EB rats on Day 3 ($p<0.05$).

Fig. 4 illustrates the mean (±SEM) number of responses on the active lever during the 2-h reinstatement tests after priming injections of saline (S) or cocaine (C, 10 mg/kg, i.p.) for the 6 days of reinstatement testing. Statistical analyses revealed a significant main effect of priming agent [$F_{(1,167)}=27.61$, $p<0.05$], with responding on the active lever being higher after cocaine compared to saline. There was also a significant treatment effect [$F_{(2,167)}=3.49$, $p<0.05$] and a priming agent × day interaction [$F_{(2,167)}=3.49, p<0.05$]. Post-hoc analyses revealed that all groups had higher responding on the previously active lever after cocaine than after saline (*$p<0.05$). The one exception to this was in OVX+VEH rats, where cocaine failed to induce significant

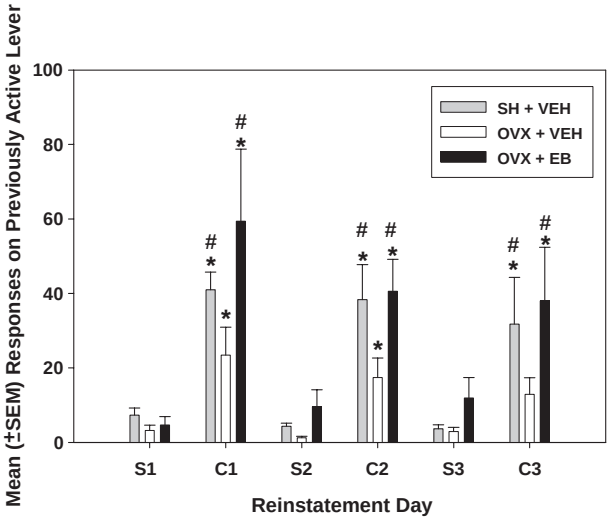

Fig. 4. Experiment 1. Reinstatement of lever responding induced by saline (S) or cocaine (C, 10 mg/kg, i.p.). The mean (±SEM) number of responses made on the previously active lever are shown for SH+VEH (gray bars), OVX+VEH (white bars), and OVX+EB (black bars) rats after saline (S1, S2, and S3) or cocaine (C1, C2, and C3) priming injections. *Significantly different from saline priming days ($p<0.05$). #Significantly different from OVX+VEH on cocaine priming days ($p<0.05$).

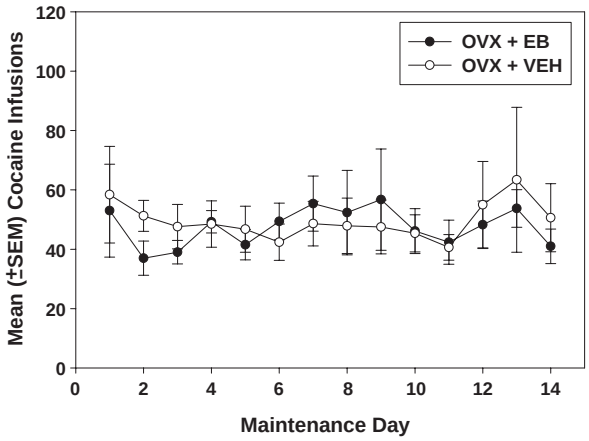

Fig. 5. Experiment 2. Cocaine self-administration behavior during the 14-day maintenance period. The mean (±SEM) number of cocaine infusions self-administered each day are shown for OVX+VEH (open circles) and OVX+EB (closed circles) rats.

reinstatement of lever responding on Day 3 of testing (see S3 and C3). After cocaine priming, SH+VEH and OVX+EB rats responded more on the previously active lever than OVX+VEH-treated rats for all days of testing (#$p<0.05$). There was no significant difference between SH+VEH and OVX+EB rats in responding on the previously active lever after cocaine priming injections. There were no group differences in inactive lever pressing after either saline or cocaine priming injections (data not shown). Inactive lever pressing was higher on cocaine days than on saline days [$F_{(1,55)}=14.64$, $p<0.05$]. However, lever pressing on the active lever was higher than inactive lever pressing on cocaine days [$F_{(1,55)}=5.69, p<0.05$], but not on saline days.

3.2. Experiment 2. Short-term EB effects on reinstatement of lever responding

As in Experiment 1, there were no differences in the number of days for the rats to meet training criteria. The

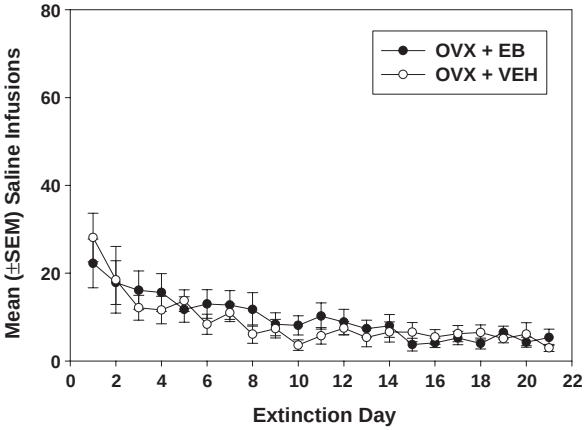

Fig. 6. Experiment 2. Saline self-administration behavior across the 21-day extinction period. The mean (±SEM) number of saline infusions earned each day are shown for OVX+VEH (open circles) and OVX+EB (closed circles) rats.

mean (±SEM) days to meet acquisition criteria was 17.0 (±4.1) and 15.9 (±4.1) for OVX+VEH and OVX+EB rats, respectively. Fig. 5 illustrates the daily mean (±SEM) number of cocaine infusions self-administered during maintenance. There were no significant differences between groups for the number of cocaine infusions self-administered during maintenance and no effect of day. OVX+VEH rats self-administered an average of 49.56 (±7.5) infusions of cocaine per day; whereas, OVX+EB rats averaged 47.30 (±4.9) infusions per day.

Fig. 6 shows the mean (±SEM) number of saline infusions self-administered over the 21-day extinction period. There was a significant effect of day [$F_{(20,335)}$= 9.97, $p<0.05$], but there were no significant group differences in the overall ANOVA or on any one of the 21 days. The pattern of responding across extinction and its magnitude were similar to that shown in Fig. 4. By Day 13, responding was minimal.

Fig. 7 depicts reinstatement of lever responding in OVX+VEH and OVX+EB rats after priming injections of either saline or cocaine. There was a significant main effect of priming agent type [$F_{(1,95)}$=31.10, $p<0.05$], with active lever pressing being greater on cocaine than saline days. There was also a significant treatment effect [$F_{(1,95)}$=5.65, $p<0.05$] and a treatment × priming agent interaction [$F_{(1,95)}$=5.29, $p<0.05$]. Post-hoc analyses indicated that reinstatement of lever responding was significantly higher in OVX+EB rats compared with OVX+VEH rats after cocaine priming on Days 1 and 3 of testing (C1 and C3; $^{\#}p<0.05$). There were no group differences in inactive lever pressing after priming injections of saline or cocaine and, although inactive lever pressing was higher on cocaine days than on saline days, this effect was not significant (data not shown).

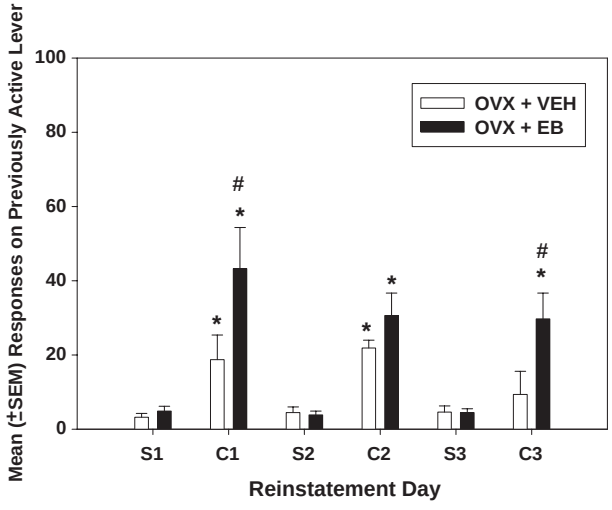

Fig. 7. Experiment 2. Reinstatement of lever responding induced by saline (S) or cocaine (C, 10 mg/kg, i.p.). The mean (±SEM) number of responses made on the previously active lever are depicted for OVX+VEH (white bars) and OVX+EB (black bars) rats after saline (S1, S2, and S3) or cocaine (C1, C2, and C3) priming injections. *Significantly different from saline priming days ($p<0.05$). $^{\#}$Significantly different from OVX+VEH on cocaine priming days ($p<0.05$).

In order to compare the magnitude of reinstatement of lever responding after cocaine in OVX rats receiving long (Exp.1) vs. short-term (Exp. 2) EB replacement, an additional analysis was conducted using a two-tailed Student *t*-test. The analysis revealed no significant difference in the magnitude of reinstatement of lever responding in OVX+EB rats, suggesting that the enhanced reinstatement of cocaine-seeking behavior in EB-treated animals was specific to EB effects during reinstatement and that EB was not significantly affecting the development of cocaine-reinforced behavior during the training or maintenance phases.

4. Discussion

We hypothesized that estrogen would enhance cocaine-induced reinstatement of lever responding in female rats. In Experiment 1, removal of endogenous estrogen by OVX resulted in an attenuation of cocaine-induced reinstatement of lever responding compared to SH+VEH rats. In addition, chronic, long-term EB replacement in OVX rats restored the magnitude of cocaine-induced reinstatement to the level of SH+VEH animals. There were no group differences in saline self-administration during extinction or in lever responding after priming injections of saline during reinstatement testing, which indicates that EB-mediated enhancement of cocaine-induced reinstatement was not due to estrogen-induced elevations of general activity. During reinstatement, inactive lever pressing was higher on cocaine days compared with saline days, indicating that cocaine increased general locomotor activity. However, active lever presses were significantly higher than inactive presses on cocaine, but not saline days, suggesting that rats were selectively reinstating their cocaine-seeking behavior, and increased responding on the active lever was not purely a result of psychomotor activation after a cocaine injection. Therefore, the ability of estrogen to enhance the resumption of a behavior (lever pressing) previously associated with cocaine self-administration indicates that estrogen is a strong factor mediating drug-seeking behavior in females. It also suggests that estrogen may underlie the previously observed sex differences in cocaine-induced reinstatement of cocaine-seeking behavior (Lynch and Carroll, 2000).

One explanation of the results from Experiment 1 is that the enhanced reinstatement in SH+VEH and OVX+EB rats was a consequence of stronger associative cue learning in these animals, as studies have shown that EB can enhanced learning and retention of certain memory tasks, such as the Morris Water Maze task (e.g., Foster et al., 2003; Luine et al., 1998; Gibbs, 2000). During training, when rats learned to associate lever pressing (stimulus) with cocaine intake (reward), there were no between-group differences in the number of days to acquire cocaine self-administration,

which suggests that EB did not affect the learning of stimulus–reward associations. However, it is possible that the procedure used during training may not have been sensitive enough to detect subtle differences during this phase. For example, using an autoshaping procedure that is sensitive to individual differences in acquisition, EB increased the amount and rate of acquisition of cocaine self-administration (Lynch et al., 2001), and EB effects during acquisition have also been found using other procedures (Hu et al., 2004). Although these EB effects on acquisition were thought to be due to an enhanced rewarding value of cocaine (Lynch et al., 2001; Hu et al., 2004), it is also possible that EB enhanced acquisition by strengthening associative learning in these animals. In Experiment 1, EB was not only present during training, when stimulus–reward associations were being learned, but it was also present during maintenance, when this association was being maintained, and during extinction, when a second association (i.e., lever pressing no longer resulted in cocaine reward) was being formed. Therefore, although there were no overt effects of EB during training, maintenance, or extinction, it is still possible that EB was influencing associative learning in some way that may have resulted in enhanced reinstatement upon reexposure to the cocaine stimulus.

In order to further assess the possibility that EB-mediated enhancement of cocaine-induced reinstatement was a product of EB effects on associative learning, rats were treated with EB after the training, maintenance, and extinction phases in Experiment 2. It was hypothesized that short-term treatment with EB would also enhance reinstatement of lever responding after cocaine priming injections. This hypothesis was supported, as short-term EB treatment in OVX rats significantly increased the cocaine-induced reinstatement of lever responding above that of OVX+VEH rats. Furthermore, the magnitude of cocaine-induced reinstatement in OVX+EB rats in Experiment 2 was similar to that seen in OVX+EB and SH+VEH rats in Experiment 1 and OVX+VEH rats had similar performance during reinstatement testing in both experiments. Although the short- vs. long-term EB treatments were tested in separate experiments using different rats, it is possible that there is some mix between acute and chronic effects of EB on cocaine self-administration or reinstatement. However, this appears unlikely, as self-administration during maintenance and the magnitude of reinstatement responding were similar for rats receiving short- or long-term EB.

The finding that EB treatment promoted reinstatement of cocaine-seeking behavior under short-term treatment conditions is consistent with other studies that have shown that EB can have rapid effects on both neurological function and behavioral output (Becker and Rudick, 1999; Thompson 1999; Xiao and Becker, 1998; Becker, 1990a). That both long- and short-term EB treatment enhanced cocaine-induced reinstatement to a similar extent also indicates that EB can mediate the motivation for cocaine seeking in the absence of any facilitatory effects on associative learning. Even so, as EB was present during reinstatement in both experiments, the possibility that estrogen enhanced the expression (recall) of learned stimulus–reward associations cannot be excluded. Therefore, it would have been interesting to compare the OVX+EB group in Experiment 2 with OVX+EB rats that had estrogen replacement during training, maintenance, and extinction but no supplements during reinstatement. Another limitation of the present study was that only one dose of cocaine (10 mg/kg) was used to induce reinstatement. Therefore, it is unknown how estrogen would have affected dose–response functions for cocaine-induced reinstatement (e.g., shift upward or to the left) or whether the short- or long-term EB treatment groups would differ on this measure. Such comparisons require either larger group designs or repeated measures in which initial dose exposure may influence subsequent performance. However, in future studies priming dose will be a variable of interest.

The enhanced cocaine-induced reinstatement of lever responding found in rats with exogenous or endogenous estrogen was not a result of differential cocaine self-administration histories, as all rats showed comparable performance during training, maintenance, and extinction in both Experiments 1 and 2. For maintenance, the lack of group differences in cocaine self-administration in Experiment 1 is consistent with other studies showing that there are no sex differences in self-administration behavior under short access (e.g., 2–3 h) conditions and/or conditions with low response requirements (e.g., FR1) (Lynch et al., 2000; Lynch and Carroll, 2000; Carroll et al., 2004, 2005; Roth et al., 2004). In Experiment 2, OVX+VEH and OVX+EB rats were both untreated during maintenance, so differences in cocaine self-administration were not expected. Therefore, the enhanced reinstatement of cocaine-seeking behavior in SH+VEH and OVX+EB rats in these experiments was not due to enhanced cocaine intake during maintenance but was specifically due to the presence of estrogen during reinstatement. It should be noted that even when maintenance and extinction responding differ across groups, it does not affect subsequent reinstatement levels (Comer et al., 1995).

It is also possible that the enhanced reinstatement in EB-treated females is related to increased impulsivity in these animals. For example, high impulsivity is associated with enhanced acquisition of cocaine self-administration (Perry et al., 2005). Also, extinction responding has been viewed by some as impulsive behavior (Bouton and Swartzentruber, 1991; Shaham et al., 2000) and as a factor separate from motivation and drug seeking (Deroche-Gamonet et al., 2004). In Experiment 1, there were no overall group differences in extinction responding (saline self-administration) after the discontinuation of cocaine availability. Although SH+VEH rats had higher initial responding compared to OVX+VEH and OVX+EB rats, this difference was eliminated by Day 4 and all animals had completely extinguished their responding prior to reinstatement testing. This is consistent with Lynch et al. (2000),

who failed to find significant sex differences in extinction responding using a within-session, same day extinction–reinstatement procedure. Together these findings suggest that circulating gonadal hormones play a limited role in extinction responding. In Experiment 2, both OVX+VEH and OVX+EB rats were untreated during extinction and differences in self-administration were not expected. This again indicates that effects found in reinstatement were not due to differences in performance in phases that preceded it. It also suggests that, under the present conditions, estrogen enhancement of cocaine-induced lever responding during reinstatement was not a product of increased impulsive responding for cocaine.

The most likely explanation for the enhanced cocaine-induced reinstatement of lever responding in SH+VEH and OVX+EB rats is that estrogen increased the motivation for cocaine seeking in these animals. This idea is consistent with the finding of Roberts et al. (1989), who demonstrated that female rats in estrus achieved higher breakpoints than during other phases of the estrous cycle, indicating that estrogen enhanced the motivation for cocaine as measured by a PR schedule of reinforcement. Estrogen has also been shown to influence mechanisms thought to mediate the motivation for drug seeking in drug abstinent animals. For example, Robinson and Berridge (2003) postulate that, upon repeated drug exposure, brain reward systems become sensitized to the incentive-motivational properties of these drugs, and sensitization has been shown to be associated with increased reinstatement of lever responding after cocaine (De Vries et al., 1998, 2002). Female rats not only reinstated their cocaine-seeking behavior more readily than males (Lynch and Carroll, 2000), but they also showed greater sensitization of the psychomotor activating effects of cocaine (Febo et al., 2003; Haney et al., 1994; Hu and Becker, 2003; Chin et al., 2002; Glick and Hinds, 1984; Becker et al., 2001), as well as having more pronounced sensitization of dopaminergic functioning in the striatum (Peris et al., 1991). Whereas estrogen has been shown to modulate many of these effects (Sircar and Kim, 1999; Hu and Becker, 2003; Quiñones-Jenab et al., 2001; Becker, 1999; Lynch et al., 2002; Carroll et al., 2004; Roth et al., 2004), this is the first study that has specifically shown that estrogen mediates cocaine-induced reinstatement of lever responding after an extended period of abstinence. Based on the relationship between sensitization and relapse, it is likely that estrogen is acting to enhance cocaine-induced reinstatement of lever responding by increasing the incentive-motivational properties of cocaine, presumably via an action in mesolimbic brain areas. Alterations in dopamine functioning in these areas are thought to mediate the acute rewarding effects, stimulus–reward associations, and incentive-motivational effects of psychostimulant drugs (reviewed in Spanagel and Weiss, 1999) and estrogen has been shown to directly modulate dopamine functioning in the striatum and nucleus accumbens (e.g., Thompson and Moss, 1994; Becker, 1990b; Disshon and Dluzen, 1999). However, more studies are needed to further understand the relationship between estrogen, dopamine, and reinstatement of cocaine-seeking behavior.

In humans, preventing relapse in detoxified individuals remains a persistent problem, and there are currently no medications that successfully treat cocaine use. The findings that females are more vulnerable to relapse than males and the implication that estrogen is a factor involved in relapse may facilitate sex-specific drug development efforts. The present results with reinstatement concur with previous findings indicating that estrogen is a main factor influencing the enhanced vulnerability to drug abuse in females (Roberts et al., 1989; Lynch et al., 2000, 2001, 2002; Carroll et al., 2004). In addition to being more vulnerable to drug abuse and relapse, females are more amenable (or sensitive) to behavioral (Cosgrove et al., 2002) and pharmacological (Carroll et al., 2001; Campbell et al., 2002; Cosgrove and Carroll, 2003) treatment interventions than males it and it is possible that estrogen mediates these differences as well. Therefore, understanding the role of estrogen in sex differences in reinstatement will aid in the development of targeted prevention and treatment strategies.

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