Female Gonadal Hormones Differentially Modulate Cocaine-Induced Behavioral Sensitization in Fischer, Lewis, and Sprague-Dawley Rats

RATNA SIRCAR and DAVID KIM

Laboratory for Developmental Neuroscience, Department of Psychiatry, Albert Einstein College of Medicine, Bronx, New York

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ABSTRACT

Evidence suggests the existence of genetic differences in cocaine sensitization in male rats. The present study was undertaken to investigate cocaine sensitization in female rats of genetically distinct inbred (Fischer 344 and Lewis) and outbred (Sprague-Dawley) strains. All female rats were bilaterally ovariectomized and randomly assigned to one of four experimental groups: 1) estradiol benzoate group, 2) progesterone group, 3) estradiol benzoate-plus-progesterone group, and 4) ovariectomized group. Additional controls included sham-operated female rats, female rats that received a single oil injection, and female rats that received repeated oil injections. To determine gender-related differences in the acute and chronic effects of cocaine, data obtained from female rats were compared with those from strain- and weight-matched male rats. Estradiol benzoate-plus-progesterone female rats showed greater locomotor effect in response to an acute dose of cocaine and had more robust sensitization in response to repeated cocaine than did male rats. The bilateral removal of ovaries abolished cocaine sensitization. In all strains of rats studied, progesterone alone did not alter the ovariectomy-induced attenuation of cocaine behavior, but estrogen alone restored cocaine-induced behavioral sensitization. There were significant strain effects on the degree of gonadal hormonal-induced modulation of cocaine sensitization in female rats. Female Lewis rats were extremely sensitive to repeated-cocaine effects, whereas the Fischer 344 female rats showed only marginal effects. The Sprague-Dawley rats ranked intermediate in their behavioral sensitivity. The present study strongly supports the hypothesis that female rats are more sensitive to both acute and chronic behavioral effects of cocaine than are male rats and that the effects are strain dependent. It also shows that estrogen plays an important role in the increased sensitivity of female rats to cocaine sensitization. Together, these data indicate significant interactions between ovarian steroid hormones and genetic factors in cocaine-induced behavioral effects.

Thirty percent of cocaine users in the United States are women. Although cocaine use among women is lower than that among men, according to the latest available census report (U.S. Department of Health and Human Services, 1993), 1.4 million women have said they used cocaine. Although men and women respond differently to substances of abuse, the literature on drug craving and withdrawal in women is limited and the interest in drug abuse in women has focused primarily on pregnancy-related issues (especially on the fetus). After similar alcohol intake, women become more intoxicated than men (Stein and Cyr, 1997). The pattern of marijuana smoking differs between males and females (Babor et al., 1984). Intermittent injections of amphetamine produce more robust behavioral sensitization in female rats than in male rats (Robinson, 1984). Amphetamine-induced rotational and stereotypic behavior is greatest during specific phases of the estrus cycle in female rats, and ovariectomy significantly diminishes the response (Becker and Beer, 1986; Camp et al., 1986). Sex differences in response to cocaine have also been reported in humans and animals. After the same dose of cocaine administration, male subjects achieved higher peak plasma cocaine levels and detected cocaine effects faster than female subjects (Lukas et al., 1996). In intact female rats, the dose of cocaine required to induce toxic reaction (circularly collapse) is almost 2-fold higher than that in male rats (Morishima et al., 1993). Gonadally intact female rats show higher levels of cocaine-induced locomotor activity than male rats (Haney et al., 1994). Female rats self-administer i.v. cocaine to higher breaking points than do male rats (Roberts et al., 1989). A single injection of cocaine augments behavioral response to a subsequent dose of cocaine in female rats but not in male rats (Glick and Hinds, 1984). Although sex differences have been demonstrated in cocaine self-adminis-
tion and locomotor activating effects by cocaine, gender-specific effects have not been seen in the discriminative stimulus effects of cocaine (Craft and Stratmann, 1996).

The increased sensitivity to cocaine in female rat fluctuates during different phases of the estrus cycle. Roberts et al. (1989) found that female rats reached their highest breaking point to cocaine during estrus. Dalton et al. (1986) reported that female rats showed haloperidol-induced increases in cocaine self-administration at diestrous. In rat, plasma estrogen levels peak during the proestrus phase of the estrus cycle and decline during the estrus and diestrous phases. Grimm and See (1997) studied the effects of estrogen on cocaine self-administration and reported that acute estrogen injection, but not chronic estrogen replacement, in ovariectomized (OVX) rats decreased cocaine self-administration on a progressive ratio schedule of reinforcement. Together, these studies suggest that female gonadal hormones, particularly estrogen, may affect the effects of cocaine in female rats.

The phenomenon of behavioral sensitization is the enhancement of responses after repeated exposure to stress or stimuli such as cocaine or amphetamine. Cocaine-induced behavioral activation becomes augmented after repeated administration of cocaine in rodents (Post et al., 1981; Karler et al., 1989; Kalivas and Alesdatter, 1993; Haracz et al., 1995). Analogously, human addicts become sensitized to cocaine-induced paranoia after repeated use of this drug. This generalization of cocaine sensitization across species suggests that the neurobiology of sensitization in animals may model brain mechanisms relevant to drug addiction in humans.

Several recent studies have suggested that genetic factors may be involved in the vulnerability to the effect of cocaine. Strain differences have been reported in the effect of cocaine on physiological (heart rate) and behavioral (Y-maze) activities (Ruth et al., 1988). Shuster et al. (1977) found C57BL/6J mice to be more sensitive to cocaine than the A/J mice. The two inbred strains of rat extensively used for testing of strains differences in cocaine-induced behavior have been the Fischer 344 (F344) and Lewis (LEW) rats. LEW rats show enhanced locomotor activity to repeated cocaine administration, an effect not seen in F344 rats (Kosten et al., 1994). In all these reports, male rats were used to study strain effects on cocaine sensitization. No report exists on cocaine sensitization in female F344 and LEW rats.

In the present study, strain differences in cocaine-induced behavior in female rats were studied using two inbred rat strains (F344 and LEW) and an outbred rat strain [Sprague-Dawley (SD)]. All rats were exposed repeatedly to cocaine for 5 days, and locomotor behavior was measured on the first day (acute effect), last day of injection (repeated cocaine effect), and on 2 days 1 drug-free week apart (expression of behavioral sensitization). To control for cyclic hormonal fluctuations, all female rats were OVX and given estrogen and progesterone sequentially. Acute and repeated cocaine-induced behavioral effects in female F344, LEW, and SD rats were compared with those in male rats of the same strain. To further investigate the role of female gonadal hormones on cocaine sensitization, OVX female rats were given either estrogen or progesterone alone. Here we report gender differences in cocaine-induced behavioral augmentation in three strains of rats with genetically distinct locomotor responses to acute and repeated exposure of cocaine.

### Materials and Methods

#### Drugs and Hormones.
Cocaine hydrochloride (cocaine HCl) was purchased from Sigma Chemical Co. (St. Louis, MO). Cocaine HCl was dissolved in 0.9% saline and injected i.p. at a dose of 15 mg/ml in a volume of 1 ml/kg. Progesterone (P) and estradiol benzoate (EB) were purchased from Steraloids, Inc. (Wilton, NH), dissolved in peanut oil, and injected s.c. in a volume of 0.1 ml. Earlier, we (Haracz et al., 1995, 1997) and others (Kalivas and Duffy, 1993) have shown that repeated injections with 15 mg/kg cocaine produce consistent and reproducible sensitization to a challenge dose of cocaine (15 mg/kg).

#### Subjects.
One hundred ninety-eight adult male and female F344, LEW, and SD rats, initially weighing 180 to 225 g, were housed in groups of four to six rats of the same sex and strain per cage. Rats were maintained on a 12-h light/dark lighting schedule and had free access to food and water. All animal protocols were approved by the Institute for Animal Care at the Albert Einstein College of Medicine, and principles of laboratory animal care as outlined in the National Research Council “Guide for the Care and Use of Laboratory Animals” were followed. Six to 12 rats were assigned to each of the hormone treatment groups (group sizes are given in the tables). All female rats were ovariehysterectomized (referred to as OVX here) while under anesthesia with Metophene (Mallinckrodt Veterinary, Inc., Mundelein, IL). A 1-week recovery period was given after ovarioectomy before rats were used for further experimentation.

#### Schedule of Cocaine Administration.
Rats were weighed daily before drug administration. All drug administration was performed once a day between 8:00 and 11:00 AM. The 19-day experimental protocol (Table 1) included a cocaine exposure period on days 1 to 5. Control rats received equivalent volumes of saline injections. Rats were housed in groups of four to six rats of the same sex and strain per cage. Rats remained drug-free for 1 week (no drug was given during this period). On day 12, rats were tested behaviorally after a challenge injection of saline or cocaine (15 mg/kg i.p.). Rats remained drug-free for another week and were given a saline or cocaine challenge on day 19, followed by behavioral testing. All cocaine injections were carried out in the behavioral testing room away from the home environment.

#### Schedule of Hormone Supplementation.
Female rats were randomly assigned to one of four groups: 1) sham group: rats were sham-operated; 2) OVX group: OVX rats were not given any hormonal supplementation; 3) EB group: rats were given EB injections (2 μg each) at 48 and 24 h before behavioral testing; 4) P group: rats received a single injection of P (500 μg) 4 h before testing; 5) EB + P group: rats received 2-μg injections of EB at 48 and 24 h before behavioral testing and, 4 h before testing, a single 500 μg injection of P; 6) oil controls: rats were given either a single (4 h before behavioral testing) or repeated oil injections at 48, 24, and 4 h before behavioral testing. Table 2 gives the time line for hormone treatment and behavioral testing.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Experimental time line showing 19-day experimental protocol</th>
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</thead>
<tbody>
<tr>
<td><strong>Day</strong></td>
<td>1</td>
</tr>
<tr>
<td>Injection</td>
<td>coc/sal</td>
</tr>
<tr>
<td>Behavior</td>
<td>+</td>
</tr>
</tbody>
</table>

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TABLE 2
Time line for hormone administration before behavioral testing
In OVX + EB/P rats, estrogen was injected at 48 and 24 h and progesterone was injected 4 h before cocaine injection. In OVX + EB rats, estrogen was injected 48 and 24 h before cocaine injection. In OVX + P rats, progesterone was injected 4 h before cocaine injection. OVX rats did not get any hormonal supplementation. Oil controls received one oil injection 4 h before behavioral testing. An additional oil control received three oil injections (48, 24, and 4 h) before behavioral scoring.

<table>
<thead>
<tr>
<th>Time</th>
<th>Injections</th>
<th>Behavior</th>
<th>Scoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>−48h</td>
<td>EB/oil</td>
<td>EB/oil</td>
<td>P/oil</td>
</tr>
<tr>
<td>−24h</td>
<td>EB/oil</td>
<td>P/oil</td>
<td>coc</td>
</tr>
<tr>
<td>−4h</td>
<td>coc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+1h</td>
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Behavioral Testing. Clear plastic test cages (28 × 18 × 20 cm) in a room separate from the animal colony room that contained the home cages were used for behavioral observations. Rats were given cocaine injections and placed immediately in the behavioral chamber for a 60-min session. To compare the acute behavioral effects of cocaine with those of repeated drug administration, each rat was behaviorally scored on day 1 and on day 5. Expression of cocaine-induced behavioral sensitization was done on day 12 (7 days after the last drug injection). Rats were again tested on day 19 (2 weeks after the last injection) to check for the long-term nature of cocaine-induced behavioral changes. For behavioral ratings, rats were placed in test cages and the scoring was performed by an observer blind to drug conditions. Ratings were assigned according to our 12-point scale (Table 3; Haracz et al., 1995, 1997), which was modified from the scale of King et al. (1993). Ratings were assigned during 30-s observation periods, which occurred at 5-min intervals for 60 min after the test injection of cocaine. A mean behavioral score was obtained for each rat by adding the ratings from all postinjection observation periods.

Statistics. One F344 female rat and one LEW male rat died from seizures after cocaine administration and one male SD rat died for no apparent reason. The behavioral score for each rat at each testing was averaged during the 60-min period and the mean behavioral score was determined. Behavioral scores were analyzed with a two-way, repeated measures ANOVA in the 12- and 19-day protocols (drug treatment × time). One-way ANOVA was used to compare behavioral scores across hormonal groups. Planned comparison t tests were paired or unpaired, respectively, for comparisons between or within days. InStat (GraphPAD, San Diego, CA) IBM-based computerized statistical software was used for data analysis.

Results

Behavioral sensitization was defined as the increase in cocaine behavioral score compared with day 1 behavioral score in rats of the same strain and same sex and with similar hormonal status.

Acute Cocaine Effects in Female versus Male F344, LEW, and SD Rats

The administration of a single injection of cocaine (15 mg/kg) significantly increased the locomotor behavioral score in all rats. The acute behavioral effect of cocaine was seen within 5 min of drug administration. Behavioral score reached a peak within 10 to 15 min and then started to decline (Fig. 1). Male rats showed no strain differences in the acute behavioral effects of cocaine (F_{2,29} = 1.071, p = .3557). Female OVX + EB/P rats were more sensitive to the acute behavioral effects of cocaine compared with male rats of the same strain (Table 4). Unlike male rats, female rats showed significant strain differences in the acute effect of cocaine (one-way ANOVA, F_{1,33} = 8.723, p < .0009). After a single cocaine administration, female F344 rats scored significantly higher than the LEW (p < .01) and SD (p < .01) female rats (Table 4). Female gonadal hormones affected acute cocaine behavior. F344 and SD OVX + EB/P rats scored significantly higher than OVX only, OVX + EB, and OVX + P rats (Table 4). LEW OVX + EB/P rats scored higher than the OVX only, OVX + EB, and OVX + P groups, but the differences were not statistically significant (Table 4).

This scale (Haracz et al., 1995, 1997) was modified from scale of King et al. (1993).
Effect of acute cocaine administration (15 mg/kg) on locomotor behavioral score in female estrogen-primed ovariectomized rats with or without progesterone and male rats

<table>
<thead>
<tr>
<th>Group</th>
<th>F344</th>
<th>LEW</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>6.99 ± 0.78 (n = 12)</td>
<td>5.59 ± 0.67 (n = 12)</td>
<td>5.82 ± 0.71 (n = 12)</td>
</tr>
<tr>
<td>O VX</td>
<td>7.35 ± 0.76 (n = 5)</td>
<td>6.69 ± 0.45 (n = 5)</td>
<td>5.73 ± 0.65 (n = 6)</td>
</tr>
<tr>
<td>O VX + P</td>
<td>7.73 ± 0.73 (n = 6)</td>
<td>6.21 ± 1.15 (n = 6)</td>
<td>4.97 ± 1.17 (n = 6)</td>
</tr>
<tr>
<td>O VX + EB</td>
<td>6.53 ± 1.03 (n = 6)</td>
<td>5.89 ± 0.72 (n = 6)</td>
<td>4.87 ± 0.89 (n = 6)</td>
</tr>
<tr>
<td>O VX + EB/P</td>
<td>9.29 ± 0.18 (n = 12)</td>
<td>7.30 ± 0.42 (n = 12)</td>
<td>7.56 ± 0.44 (n = 12)</td>
</tr>
</tbody>
</table>

*p < .05 between male and female O VX + EB/P LEW rats and between male and female O VX + EB/P SD rats.

**p < .01 between male and female O VX + EB/F344 rats.

*p < .05; significantly different from F344 female rats.

*p < .005 F344 O VX + EB/P versus O VX rats.

*p < .05 F344 O VX + EB/P and O VX rats.

*p < .01 F344 O VX + EB/P and LEW O VX + EB/P rats.

*p < .01 F344 O VX + EB/P and SD O VX + EB/P rats.

*p < .05 SD O VX + EB/P and O VX rats.

*p < .05 SD O VX + EB/P and P rats.

*p < .01 SD O VX + EB/P and O VX rats.

Repeated Cocaine Effects in Female versus Male F344, LEW, and SD Rats

The effects of repeated cocaine injections on the behavioral activity of male and female F344, LEW, and SD rats were next examined after 5 days of daily drug administration. In male rats, there were significant strain differences in the repeated cocaine-induced behavior. Compared with day 1, day 5 behavioral scores were higher in LEW and SD rats. In male F344 rats, repeated cocaine-induced behavioral scores did not differ from acute cocaine scores (Fig. 2).

Similar to the effects of acute cocaine, female rats were more sensitive to repeated cocaine than were male rats of the same strain (Table 5). All three strains of female O VX + EB/P rats showed marked behavioral augmentation after repeated cocaine administration (F5,65 = 12.723, p < .0001) compared with their day-1 scores. Both LEW and SD rats showed robust behavioral sensitization on day 5 of cocaine injection (Fig. 3). Female F344 rats scored higher on day 5 than on day 1, but the increase did not reach statistical significance.

Expression of Cocaine Sensitization in Female versus Male F344, LEW, and SD Rats

To study the expression of cocaine sensitization, male and female rats remained drug free for 1 week after the last daily cocaine administration, and then they were challenged with a single dose of cocaine (15 mg/kg) on day 12 followed by behavioral measurement. The long-term nature of cocaine-induced behavioral sensitization was studied by giving a second challenge injection on day 19, which was 1 week after the first cocaine challenge.

Behavioral scores of male LEW and SD rats on day 12 (as well as on day 19) were significantly higher than those on day 1; scores for male F344 rats remained unchanged (Fig. 2). Behavioral scores for female O VX + EB/P rats were higher than those for male rats of the same strain; in LEW rats, the difference between sexes was not statistically different (Table 6). All three strains of female (O VX + EB/P) rats showed significant augmentation in behavioral scores on day 12 compared with day 1 (Fig. 3), but the sensitization pattern differed between strains. In the LEW and SD rats, repeated cocaine-induced behavioral augmentation was long lasting (seen 1 and 2 weeks after cessation of repeated cocaine injection), but behavioral sensitization in F344 rats was present only on day 12 and had disappeared by day 19.

Effect of Estrogen and Progesterone on Repeated Cocaine Effects

To investigate further the role of female gonadal hormones on the expression of cocaine-induced behavioral sensitization, three groups of female rats were studied: O VX, O VX + EB, and O VX + P. O VX female F344 and SD rats that did not receive any exogenous gonadal hormones failed to show any behavioral sensitization, suggesting that female gonadal hormones play a facilitatory role in cocaine-induced behavioral effects.
sensitization (Fig. 4). LEW OVX rats showed a mild and transient increase in behavioral score only on day 19 \((p = .044)\). The administration of P alone did not alter OVX-induced cocaine behavioral score in F344, LEW, and SD rats (Fig. 5) again except in LEW rats on day 12. There was no difference in the behavioral scores between the OVX only and OVX + P rats of any strain. Figure 6 depicts data from OVX rats treated with estrogen only. Estrogen alone restored behavioral score to OVX only rats, but the difference was not statistically significant. Although progesterone alone did not alter the attenuated behavioral score seen in OVX only rats, there was a mild but statistically significant increase in scores between estrogen-primed only and estrogen-primed progesterone-treated rats (Table 6), particularly in the F344 and SD rats. LEW OVX + EB/P rats scored higher than the OVX + EB alone rats, but the difference was not statistically significant. These findings suggest that estrogen plays a major role in the robust cocaine sensitization seen in female rats and that P can modulate the effects of estrogen on acute and repeated cocaine behaviors.

**Repeated Injections Induced Stress and Cocaine Behavior**

**Repeated Daily Saline Injections followed by a Saline Challenge.** To investigate whether differences in stress responses due to repeated daily drug injections might be responsible for strain and sex differences in cocaine sensitization, male and female F344, LEW, and SD rats were given daily saline (1 ml/kg b.wt. i.p.) injections for 5 days, and behavioral testing was carried out on the first and last days of saline injections. One week after the last saline injection, a single challenge injection of saline was administered and behavior was scored for the next 60 min. There was no difference in saline challenge-induced behavior among the different female rat groups (Fig. 7) or the male rats (Fig. 8).

**Repeated Daily Saline Injections followed by a Cocaine Challenge.** To determine whether repeated drug/saline-induced stress produces cross-sensitization to cocaine, experiments were carried out in which F344, LEW, and SD female and male rats were given daily saline injections for 5 days and then challenged with a single cocaine injection (15 mg/kg). Female rats before saline injections were divided into six groups. Group 1 consisted of the sham group in which rats were sham-operated; group 2, one oil injection group in which OVX rats received a single oil injection (1 ml/kg s.c.) 4 h before cocaine injection; group 3, three oil injections group in which OVX rats received 3 oil injections 48, 24, and 4 h before cocaine injection; group 4, EB group in which OVX rats received one EB injection 48 h and a second EB injection 24 h before cocaine injection; group 5, P group in which OVX rats received a single P injection 4 h before cocaine injection; and group 6, EB + P group, in which OVX rats received EB injections 48 and 24 h and a P injection 4 h before cocaine injection. The results presented in Fig. 9 show that there was no difference in sensitivity to a single cocaine challenge between different hormone treatment groups. Also, there was no significant strain difference among the sham group, single oil injection, or three oil injection groups. These results indicate that stress due to single or repeated injections (oil or saline) did not produce any significant change in behavior in response to an acute cocaine injection. Among male rats, the SD rat was more sensitive to cocaine challenge than the F344 rat (Fig. 10). Male LEW and F344 rats did not differ from each other in their sensitivity to cocaine (Fig. 10).

**Repeated Cocaine Injections followed by Saline Challenge.** Behavioral sensitization has been associated with conditioning or context-dependent activity. Pairing of injection with a particular environment in which drug administration has been carried out is known to produce conditioning. To investigate whether different degrees of conditioning between strains and/or hormonal treatments could be responsible for altered responsiveness to cocaine, male and female rats were given daily cocaine injections (15 mg/kg) for 5 days followed by a saline challenge 1 week later. In both
female (Fig. 11) and male (Fig. 12) rats, there was no significant difference in the sensitivity to the saline challenge.

**Discussion**

The major findings of this study are that 1) significant sex differences exist in the behavioral effects of cocaine (female rats are more sensitive to both acute and repeated cocaine-induced behavior than are male rats), 2) bilateral ovariectomy attenuates cocaine-induced behavioral sensitization, 3) estrogen plays an important role in the hormonal modulation of cocaine-induced behavioral sensitization, and 4) genetic factors modify female gonadal hormone-dependent cocaine effects.

The term sensitization (augmentation of the locomotor activating effect of stimuli with repeated exposure) has been specifically used in this study to indicate the increase in behavioral score after repeated cocaine administrations compared with the day-1 (acute) cocaine effect in same-sex/hormonal status, same-strain rats. Repeated saline/oil injections produced some increase in locomotor behavior, especially in female rats, suggesting that perhaps the stress of these repeated injections may have contributed to the gender differences in the development of sensitization to the locomotor effects of cocaine. The ability of repeated stress exposure to sensitize animals to the stimulating effects of drugs (cocaine, amphetamine, and ethanol) has been extensively reported in the literature. Future studies will determine the effect of stress in the gender and strain differences in cocaine sensitization and attempt to determine the role of cross-sensitization in these differences.

Our data from repeated cocaine administration in male F344, LEW, and SD rats support the hypothesis that genetic background determines cocaine sensitization. LEW and F344 rats are two genetically inbred rat strains, whereas the SD rats are an outbred strain. Male LEW rats showed an enhanced behavioral response to repeated cocaine injections, whereas male F344 rats failed to have any behavioral enhancement on the fifth day of treatment or after challenge cocaine injections on day 12 or 19. These results are in accordance with earlier data showing increased cocaine-conditioned place preference and locomotor sensitization in LEW rats.

<table>
<thead>
<tr>
<th></th>
<th>F344</th>
<th>LEW</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>6.36 ± 0.80 (n = 12)</td>
<td>7.71 ± 0.85 (n = 12)</td>
<td>8.06 ± 0.38 (n = 12)</td>
</tr>
<tr>
<td>OVX + EB</td>
<td>8.37 ± 0.73 (n = 6)</td>
<td>8.92 ± 0.56 (n = 6)</td>
<td>8.18 ± 0.70 (n = 6)</td>
</tr>
<tr>
<td>OVX + EB/P</td>
<td>9.62 ± 0.13 (n = 12)</td>
<td>9.03 ± 0.22 (n = 11)</td>
<td>9.51 ± 0.10 (n = 12)</td>
</tr>
</tbody>
</table>

*p < .05 between male and female SD rats.

*b p < .05 between F344 OVX + EB and OVX + EB/P rats and between SD OVX + EB and OVX + EB/P rats.

*a p < .001 between male and female F344 rats.
60-min postinjection test session. A behavioral score on day 19 in F344 rats was not statistically significant. The increase in behavioral testing. Estrogen alone significantly restored OVX-induced abolition of cocaine sensitization in LEW and SD rats. The increase in male rats. Because the phase of the estrus cycle (Becker et al., 1982), Roberts et al. (1989) found increased self-administration of cocaine in female rats during the estrus phase of the cycle, whereas ovariectomy has been reported to decrease acute amphetamine-induced stereotypes in guinea pigs (Nausieda et al., 1979) and rats (Becker et al., 1982; Camp et al., 1986). Our data are in agreement with those by Haney et al. (1994) that gonadally intact female rats are more active in their response to a single cocaine administration than male rats and that removal of both ovaries significantly reduced the acute effects of cocaine. Glick and Hinds (1984) reported that female rats were more sensitive to repeated cocaine effects than were male rats. These authors observed severalfold increase in rotation behavior to a second cocaine injection in female rats but failed to see any increase in male rats. Because the phase of the estrus cycle was not monitored and the levels of estrogen and progesterone were not manipulated, the role of female gonadal hormones on cocaine sensitization could not be predicted in this study.

The present study provides further support to the hypothesis that ovarian hormones modulate the effects of cocaine. Combined EB + P treatment enhanced behavioral sensitization to repeated cocaine. The degree of cocaine-induced behavioral sensitization was greatest in the estrogen-primed progesterone-treated group (OVX + EB/P) compared with OVX + EB alone, OVX + P alone, OVX only, or male rats. These data are opposite to those by Peris et al. (1991), who reported that estrogen and progesterone together failed to have any effect on cocaine sensitization. Peris et al. (1991) exposed rats chronically (released from silastic implants) to high levels of both hormones simultaneously. Prolonged high constant levels of estrogen and progesterone used by these authors do not mimic the normal cyclic pattern of gonadal hormone levels seen in intact female rats. The sequential gonadal hormone injection protocol used in our experiments (estrogen injections given at 48 and 24 h before testing and a single progesterone injection 4 h before testing) mimics closely the hormonal profile during the proestrus phase of the cycle (Woolley et al., 1990; Berry et al., 1997).

Estrogen alone in OVX rats restored behavioral sensitization to repeated cocaine administration, suggesting that estrogen plays a major role in the enhanced behavioral sensitization seen in female rats. Although estrogen levels were

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**Fig. 6. Sensitization to repeated injections of cocaine in OVX female F344, LEW, and SD rats injected with estrogen 48 and 24 h before behavioral testing.**

Estrogen alone significantly restored OVX-induced abolition of cocaine sensitization in LEW and SD rats. The increase in male rats. Because the phase of the estrus cycle (Becker et al., 1982), Roberts et al. (1989) found increased self-administration of cocaine in female rats during the estrus phase of the cycle, whereas ovariectomy has been reported to decrease acute amphetamine-induced stereotypes in guinea pigs (Nausieda et al., 1979) and rats (Becker et al., 1982; Camp et al., 1986). Our data are in agreement with those by Haney et al. (1994) that gonadally intact female rats are more active in their response to a single cocaine administration than male rats and that removal of both ovaries significantly reduced the acute effects of cocaine. Glick and Hinds (1984) reported that female rats were more sensitive to repeated cocaine effects than were male rats. These authors observed severalfold increase in rotation behavior to a second cocaine injection in female rats but failed to see any increase in male rats. Because the phase of the estrus cycle was not monitored and the levels of estrogen and progesterone were not manipulated, the role of female gonadal hormones on cocaine sensitization could not be predicted in this study.

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Estrogen alone in OVX rats restored behavioral sensitization to repeated cocaine administration, suggesting that estrogen plays a major role in the enhanced behavioral sensitization seen in female rats. Although estrogen levels were
Fig. 7. Effect of a saline challenge injection on behavioral score in female rats. Rats were OVX (except the sham group) and received one of three hormonal treatments: EB, 48 and 24 h before testing; P, 4 h before testing; or both EB and P at same points. Control groups included sham-operated group, one oil injection group (one injection 4 h before testing), and three oil injections group (one oil injection each at 48, 24, and 4 h before behavioral testing). All rats received daily saline injection (i.p.) for 5 days. A saline challenge injection was given 1 week after the last daily saline injection. Behavior was scored immediately after injection for 60 min. Behavioral scores across different hormonal and control groups did not differ significantly from one another. One-way ANOVA yielded $F_{17,37} = 0.48, p = .95$.

Fig. 8. Effect of a saline challenge injection on behavioral score in male F344, LEW, and SD rats. All rats received daily saline injection (i.p.) for 5 days. A single saline injection was given 1 week after the last daily saline injection. There was no difference in behavioral scores between different rat strains after saline challenge. One-way ANOVA yielded $F_{2,15} = 0.65, p = .53$. 
Cocaine challenge 7 days after repeated saline injections in female rats

**Fig. 9.** Effect of a single cocaine challenge on behavioral score in female rats. Rats were OVX (except the sham group) and received one of three hormonal treatments: EB, 48 and 24 h before testing; P, 4 h before testing; or both EB (48 and 24 h) and P (4 h) before testing. Control groups included sham-operated group, one oil injection group, and three oil injections group. All rats received daily saline injection (i.p.) for 5 days. A single cocaine injection (15 mg/kg) was given 1 week after the last daily saline injection followed by behavioral testing. One-way ANOVA did not yield any significant hormone effect: \( F_{17,37} = 1.37, p = .21. \)

Cocaine challenge 7 days after repeated saline injections in male rats

**Fig. 10.** Effect of a single cocaine injection on behavioral score in male F344, LEW, and SD rats. All rats received daily saline (i.p.) injections from day 1 until day 5. A single cocaine (15 mg/kg) injection was given 1 week after the last daily saline injection. One-way ANOVA showed a strain effect: \( F_{2,15} = 3.86, p < .05. \) Behavioral scores of SD rats were significantly higher than those of the F344 male rats (\( p < .05 \)).
Saline challenge 7 days after repeated cocaine injections in female rats

Fig. 11. Effect of a saline challenge injection after repeated cocaine injections in female F344, LEW, and SD rats. Female F344, LEW, and SD rats were divided into six groups: EB only, P only, EB + P, sham-operated, one oil injection, and three oil injections (n = 3 or 4 rats per hormonal treatment group). All rats received daily cocaine injections for 5 days (15 mg/kg/day i.p.). One week after the last cocaine injection, all rats received a single saline injection. Behavioral score was measured after the saline injection. One-way ANOVA yielded $F_{17,37} = 2.44, p = .01$, but in the post-hoc Tukey-Kramer multiple-comparison test, none of the comparisons gave a $q$ value of >6.30 for the $p$ value to be <.05.

Saline challenge rats 7 days after repeated cocaine injections in male rats

Fig. 12. Effect of a single saline challenge injection after repeated cocaine injections in male F344, LEW, and SD rats. All rats were given cocaine (15 mg/kg/day i.p.) for 5 days. One week after the last cocaine injection, all rats received a single saline injection. Behavior was measured after the saline injection. There was no difference in scores between the rat strains: one-way ANOVA $F_{2,115} = 2.50, p = .12$. 

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not assessed in vivo after injection, the dose and injection regimens used were chosen to be physiologically relevant (Etgen and Pettiti, 1986). Because of the experimental design and estrogen dosage used, cocaine sensitization changes reported here can be attributed to an effect of estrogen. The only other study that examined the effect of estrogen on cocaine sensitization in female rats was that by Peris et al. (1991), who reported that estrogen significantly increased both locomotor and stereotypic behavior after the first cocaine administration and produced a greater degree of cocaine sensitization than control group after repeated cocaine injections.

Behavioral sensitization in female rat was attenuated after ovariectomy. Because ovariectomy lowers not only circulating estrogen level but also progesterone level, the present study looked at the effects of progesterone, alone and after estrogen priming, on cocaine sensitization. Progesterone alone did not have any significant effect on either the acute effect of cocaine or on repeated cocaine-induced behavior compared with OVX rats. Our finding that progesterone alone failed to enhance the acute effect of cocaine is supported by the work of Glantz and Woods (1994), who also reported that progesterone did not alter the acute effect of cocaine in female rats. When progesterone was administered in estrogen-primed female rats, it markedly potentiated the effect of estrogen on acute cocaine-induced behavior as well as cocaine sensitization. Together, these data indicate that although progesterone alone does not alter cocaine effects, it can modulate the regulatory role of estrogen in cocaine behavior.

The mechanism or mechanisms by which estrogen regulates cocaine-induced behavior are not known. Estrogen has been implicated in the neuroanatomic plasticity of the brain. Fluctuations in hippocampal morphology have been reported to coincide with phases of the estrus cycle (Woolley et al., 1990; Woolley and McEwen, 1992). The density of hippocampal CA1 apical dendritic spines have been shown to be significantly lower in OVX animals, whereas those receiving estrogen replacement maintained levels equivalent to those of intact subjects. In cycling rats, animals in estrus (when estrogen level is low) display significantly fewer hippocampal dendritic spines than animals in proestrus (when estrogen level is high) (Woolley et al., 1990). The density of axospinous synapses also shows fluctuations as a function of hormonal status (Woolley et al., 1990). Whether estrogen-induced neuroanatomic changes play any part in the facilitatory role of estrogen on cocaine behavior need to be investigated.

Classically, the psychomotor-stimulant effects of cocaine were considered to be mediated by the activation of the dopaminergic system. Recently, a significant amount of data have accumulated demonstrating that the glutamatergic system may also play an important role in the action of this drug. Anatomic evidence shows that the two transmitter systems (dopamine and glutamate) are in close proximity in the striatum. The corticostriatal glutamatergic fibers and the nigrostriatal dopaminergic system are the two major inputs into the striatum. Intrastriatal injections of cocaine and dopaminergic and glutamatergic agents, particularly of the N-methyl-D-aspartate type, induce similar stereotypic behavior, and the stimulant-induced stereotypy can be blocked by dopaminergic and glutamatergic antagonists, suggesting that activation of not only the dopaminergic system but also of the glutamatergic system is involved in cocaine-induced behavior (Pulvirenti et al., 1991; Kalivas and Alesdatter, 1993; Karler et al., 1993; Haracz et al., 1995). Dopaminergic/glutamatergic interaction occurs not only in the striatum but also in other brain regions, such as the nucleus accumbens. Several studies have shown that estrogen can modulate striatal dopamine levels, dopamine-induced adenylate cyclase activity, and striatal D2 receptor binding (van Hartesveldt and Joyce, 1986; Becker and Cha, 1989). Earlier reports have shown that some neuroendocrine and behavioral actions of estrogen may involve regulation of glutamatergic neurotransmission in the hypothalamic/prefrontal area. This hypothesis is based on 1) release of glutamate and aspartate from the preoptic area during sexual maturation and luteinizing hormone surge, 2) glutamate facilitates gonadotropin hormone-releasing hormone and luteinizing hormone release, 3) glutamate induces precocious puberty in female rats, and 4) glutamate agonist N-methyl-D-aspartate participates in the regulation of female sexual (lordosis) behavior. Glutamate may be a key neurochemical mediator of female gonadal hormone-modulated cocaine behavior. It will be interesting to see whether blocking glutamatergic pathways will attenuate hormone-dependent acute and repeated cocaine effects in female rats.

The neural substrates at which glutamate influences cocaine effects or what glutamate receptor type or types participate in the modulatory effects of estrogen on cocaine behavior remain to be determined. Understanding the molecular mechanisms underlying gonadal hormone-dependent changes in neuronal excitability and synaptic efficacy may provide novel prevention and treatment strategies for cocaine abuse in women.

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References


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Send reprint requests to: Ratna Sircar, Ph.D., Associate Professor of Psychiatry and Neurology, Director, Developmental Neuroscience Program, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461. E-mail sircar@aeom.yu.edu