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Acute and repeated intravenous cocaine-induced locomotor activity is altered as a function of sex and gonadectomy

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Abstract

The present experiment examined the effects of sex and gonadectomy on cocaine-induced locomotor activity via intravenous (IV) cocaine. Male, female, castrated (CAST), and ovariectomized (OVX) rats received daily IV cocaine injections (3.0 mg/kg/injection) for 13 consecutive days. Locomotor activity was measured in automated activity chambers for 60 min following the baseline-saline administration and after the 1st and 13th cocaine injections. Observational time sampling was also performed, and the observational data were grouped into locomotor and orofacial composite incidence scores. Females exhibited more cocaine-induced locomotor activity, rearing, and locomotor incidence compared to males. The orofacial data revealed a sex difference in the expression of behavioral sensitization: females exhibited more orofacial behaviors than males after repeated, but not acute, cocaine injection. Females exhibited more cocaine-induced locomotor activity, rearing, and locomotor incidence compared to OVX rats, but exhibited less orofacial incidence following acute cocaine administration. There were no differences between male and CAST rats. CAST rats showed more locomotor incidence than OVX after repeated, but not acute, cocaine injection. CAST rats exhibited behavioral sensitization, whereas OVX rats' locomotor incidence did not change with repeated cocaine injection. CAST rats showed less orofacial incidence than OVX after acute, but not repeated, cocaine injection. These findings demonstrate sex differences in response to IV cocaine and replicate earlier findings which show that OVX attenuates increased locomotor activity in females. Furthermore, these findings suggest that IV cocaine administration produces behavioral differences between male and female rats in the absence of circulating gonadal hormones.

Keywords: Cocaine; Ovariectomy; Castration; Locomotor activity; Rats

1. Introduction

Cocaine abuse represents a major public health problem in the United States. Recent estimates indicate that 1.2 million people initiated cocaine use in 2001 and that approximately 42% of these new cocaine users were women, reflecting the fact that women represent a large portion of this public health concern (NSDUH, 2002). Moreover, epidemiological studies suggest that there are gender differences in cocaine abuse, as negative health outcomes are more likely for women than men who abuse cocaine (Griffin et al., 1989; Kosten et al., 1993; Dudish and Hatsukami, 1996; Weiss et al., 1997). These data suggest that successful treatment of cocaine abuse for women will depend on a more accurate characterization of the drug's effects in females and that studies which elucidate the mechanisms that mediate cocaine-induced sex differences will be helpful in developing therapeutic interventions (Lynch et al., 2002).

Basic science research has demonstrated cocaine-induced sex differences in rats (Post et al., 1981; Glick et al., 1983; Glick and Hinds, 1984; van Haaren and Meyer, 1991; Bowman and Kuhn, 1996; Sircar and Kim, 1999; Chin et al., 2002). Behavioral sensitization refers to the augmenta-

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tion of a behavioral response, e.g. locomotor activity, following repeated administration of psychostimulant drugs (Downs and Eddy, 1932; Post, 1980; Post and Contel, 1983; Kalivas and Weber, 1988; Zahniser and Peris, 1992; Emmett-Olglesby, 1995). Studies investigating the neuropharmacological mechanisms that mediate cocaine-induced sex differences in rats' locomotor activity and in the expression of behavioral sensitization have revealed that ovariectomy (OVX) attenuates the hyperactivity produced by cocaine, suggesting that ovarian hormones may enhance cocaine's effects on activity (Sell et al., 2000). Normally cycling rats exhibited increased sensitivity to the locomotor effects of cocaine during estrus (Sell et al., 2000), and OVX rats that received estrogen replacement exhibited increased responsiveness to cocaine compared to OVX rats that received no estrogen treatment (Sell et al., 2000; Perrotti et al., 2001; Hu and Becker, 2003). Furthermore, estrogen is also important for the expression of behavioral sensitization to repeated cocaine exposure in female rats. Cocaine-treated, intact female rats exhibited more sensitized ambulatory activity compared to OVX, intact male, and castrated (CAST) rats (Chin et al., 2002). Moreover, OVX rats administered estrogen exhibited increased behavioral sensitization compared to OVX, CAST, and intact male rats that received cocaine but were not treated with estrogen (Peris et al., 1991; Hu and Becker, 2003). Collectively, these studies indicate that OVX attenuates the behavioral response to cocaine, presumably by preventing gonadal hormones from enhancing cocaine's locomotor activity effects.

Our laboratory has investigated cocaine-induced behavioral sensitization using the intravenous (IV) route of administration. In these studies, intact male rats received IV cocaine via a subcutaneous vascular access port (Mactutus et al., 1994). This research demonstrated that the kinetic profile observed using IV injection of cocaine in rats approximated the profile seen in human volunteers (Evans et al., 1996; Booze et al., 1997). Moreover, IV injection of cocaine produced robust behavioral sensitization in intact male (Wallace et al., 1996) and female rats (Booze et al., 1999b) and did not result in persistent vaginal estrus, estrus acyclicity, or changes in female body weight (Booze et al., 1999b).

The present experiment investigated the effects of IV cocaine administration on various behavioral changes in gonadectomized and intact male and female rats. Rats received acute and repeated IV cocaine injections and the subsequent behavioral response was measured on days 1 and 13, respectively, using automated activity chambers and a behavioral observation method previously described (Fray et al., 1980; Wallace et al., 1996; Booze et al., 1999a; Harrod et al., 2004). The aims of the present experiment were to (1) examine sex differences in the behavioral response to acute and repeated IV cocaine, and (2) examine the effects of gonadectomy on the behavioral response to acute and repeated IV cocaine. Although estrogen-mediated changes in cocaine-induced behaviors have been extensively reported, less is known about the role of androgens on cocaine-induced behavioral sensitization in males. This study also reports the effects of CAST on male behavioral changes following acute and repeated IV cocaine administration. Based on previous research (Glick et al., 1983; Glick and Hinds, 1984; van Haaren and Meyer, 1991; Perrotti et al., 2001; Chin et al., 2002), it was hypothesized that intact rats would demonstrate behavioral sensitization following repeated IV cocaine injection; that females would exhibit more cocaine-induced behavioral sensitization than males; and that gonadectomy would attenuate behavioral sensitization, relative to intact animals.

2. Method

2.1. Animals

Forty-eight adult male, female, CAST, and OVX Sprague–Dawley rats (70 days old) were obtained from Harlan Laboratories, Inc. (Indianapolis, IN). Upon arrival at the animal care facilities, rats were placed in quarantine for 7 days, and then transferred to the colony 14 days before catheter surgery. Animals were pair housed throughout the experiment. Rodent food (Pro-Lab Rat, Mouse Hamster Chow #3000) and water were provided ad libitum. The colony was maintained at 21±2 °C, 50±10% relative humidity and a 12L:12D cycle with lights on at 0700 h (EST). Daily injections and behavioral testing began at ~1400 h. The protocol for this research methodology was approved by the Institutional Animal Care and Use Committee (IACUC).

2.2. Catheter surgical procedure

An Intracath IV catheter (22 ga, Becton/Dickinson General Medical Corp., Grand Prairie, TX) was used as a subcutaneous dorsally implanted port for the chronic IV injections. Catheter implantation was performed as previously described (Mactutus et al., 1994). Briefly, rats were anesthetized using a mixture of ketamine hydrochloride and xylazine by intraperitoneal (IP) injection (7.5 mg ketamine/100 g b.wt. 30 mg xylazine/100 g b.wt.). Skin incisions were made on the dorsal surface of the rat, as well as on the ventral side of the neck to expose the jugular vein. The catheter was then inserted into the jugular vein and advanced toward the heart. After validation of patency, the catheter was secured with a suture. Then, neck and back skin were sutured closed, and triple antibiotic ointment was applied to both incision sites. The surgical procedure for each rat was completed in ~20 min. Rats were kept under periodic postoperative observation and returned to the vivarium upon recovery from anesthesia. The catheters were flushed daily with 0.2 ml of heparinized (2.5%) saline, and the animals were observed for any signs of discomfort or behavioral distress. Complete anesthesia, surgical recovery, and postoperative records were

maintained for each animal. Behavioral measures began one week after surgery. Previous research shows that injection of a ketamine/xylazine cocktail prior to cocaine may attenuate cocaine-induced behavioral sensitization when cocaine is delivered via the IP route of administration (Torres and Rivier, 1993). These interactions relate to the temporal occurrence of the two drugs or repeated daily injections of ketamine (Rofael and Abdel-Rahman, 2002). In the present experiment, the ketamine/xylazine cocktail was administered before surgery, one week prior to the beginning of cocaine injection. To the best of our knowledge, there is no evidence to support the contention that a single ketamine/xylazine injection preceding cocaine injection by one week could affect the induction of behavioral sensitization.

2.3. Experimental design and procedures

2.3.1. Locomotor activity

Rats in the male ($n=12$), female ($n=12$), CAST ($n=12$), and OVX ($n=12$) groups were habituated to the locomotor activity chambers on two consecutive days for two 60-min sessions, one/day. A baseline measure of activity was obtained on the day following the second habituation day. For the baseline activity test session, rats were injected with saline immediately prior to placement in the activity chambers. Rats remained in the activity chambers for 60 min. The activity monitors were square (40×40 cm) open-field chambers (Flex-Field, San Diego Instruments, San Diego, CA) that detected free movement of animals by infrared photocell interruptions. This equipment used an infrared photocell grid (32 emitter/detector pairs) to measure total locomotor activity. All activity monitors were located in an isolated room in our laboratory. Total activity, as well as rearing, was measured by assessing the number and type of photocell interruptions within a 60-min period. Photocell interruptions were collected in 10-min intervals. In addition to the automated monitoring, an observational time sampling procedure was employed. A trained observer, unaware of the treatment condition of the animal, observed and recorded the animal's behavior, using a modified version of a well-established protocol (Fray et al., 1980).

Each rat was observed for 10 s at six time periods (1, 5, 10, 15, 30, and 60 min). During each time-sampling period, behavior was recorded as present/absent. Booze et al. (1997) determined that 3.0 mg/kg/injection resulted in peak arterial plasma levels within one-minute of IV cocaine administration. Thus, five of the six observational time points occurred within the first 30 min of the session to insure that maximal amount of cocaine-induced stereotypy would be observed. The current procedure represents an adaptation of Fray et al. (1980). Briefly, the present procedure excluded the sway and miscellaneous categories and added scan, headbob, yawn, and lying down. Another modification included observations at 1, 5, 10, 15, 30, and 60 min, whereas Fray et al. (1980) observed behaviors at 10, 20, 30, 40, 50, and 60 min. Table 1 describes the behaviors recorded during the sampling period. The animals' locomotor activity response to cocaine was assessed on only two occasions: immediately after the first (day 1) and last (day 13) cocaine injections. This latter procedure is important to preclude the repeated pairing of cocaine injection and the testing environment that otherwise confounds the neural expression of sensitization with learning via classical conditioning (Anagnostaras and Robinson, 1996). Testing occurred in the absence of direct overhead lighting (<10 lx). Following completion of the experiment, CAST and OVX rats' circulating testosterone and estrogen levels were determined. Trunk blood was collected for steroid hormone determinations 24 h after the last behavioral testing was conducted. Intact females were sacrificed on the afternoon of proestrus. Serum levels of estradiol and testosterone were determined via standard RIA protocol (ICN Biochemicals, Costa Mesa, CA). The results confirmed the successful gonad removal in all operated animals.

Table 1
Descriptions of behaviors measured in the observational time sampling procedure

Behavior	Description
Still	Animal's body is completely still
Locomotion	Animal has moved all four legs from one location to another
Rearing	Animal has raised up on two hind legs
Head-up sniff	Animal's head is raised while sniffing
Head-down sniff	Animal's head is lowered while sniffing
Head bobbing	Animal rhythmically moving head up and down
Bite	Animal repeatedly bites anywhere on body
Grooming	Animal grooms body
Yawn	Animal yawns
Scanning	Animal visually searches while moving head to the side
Scratching	Animal scratches anywhere on body
Licking	Animal licks anywhere on body
Lying Down	Animal lies down, but is not asleep

2.4. Drug treatment

The cocaine treatment was always administered as a bolus injection delivered in a volume of 1 ml/kg body weight (15 s), and was followed by flushing (15 s) with 0.2 ml heparinized (2.5%) saline (i.e., the approximate volume of the catheter). The rate of infusion is an important factor that mediates the induction and expression of cocaine-induced sensitization. The injection rate used in the present experiment was used in the previous pharmacokinetic analysis (Booze et al., 1997) and is within the duration shown to produce moderate-to-robust behavioral sensitization (Wallace et al., 1996; Booze et al., 1999a,b; Samaha et al., 2002). The dose of cocaine hydrochloride (3.0 mg/kg/injection; Sigma, St. Louis, MO) was calculated on the weight of the salt and dissolved in saline for an injection volume of 1 ml/kg. The dose of IV cocaine was chosen based on the observations that (1) it produces peak arterial plasma levels in the male rat which are not significantly different from peak arterial levels in humans

administered 32 mg of IV cocaine (Booze et al., 1997; Evans et al., 1996); and (2) under experimental conditions, this dose is self-administered by "users" multiple times in a 2.5 h session (Fischman and Schuster, 1982), thus representing a low or recreational dose.

2.5. Data analysis

The automated data were analyzed using analysis of variance (ANOVA) techniques (BMDP Statistical Software, 1990; SPSS, 1989–2003; Winer, 1971). A mixed-model factorial ANOVA, with sex and gonadectomy as between-subjects factors and day and time as within-subjects factors, was conducted for the automated locomotor and rearing data. In the present study, sphericity was violated in the overall ANOVA for the measures of rearing (Mauchly's $W=0.22$ and 0.03 for time and day × time, $p<0.001$, respectively) and total locomotor activity (Mauchly's $W=0.01$ and 0.00 for time and day × time, $p<0.001$, respectively). To preclude any influence of violations in sphericity, the use of orthogonal decomposition was employed (Winer, 1971). Comparisons of main effects, simple main effects, and interactions were further analyzed where appropriate. Subsequent planned contrasts were conducted to determine differences between gonadectomized and intact male and female rats. Also, a significant main effect or interaction is described as linear or quadratic in nature.

Chi square (χ^2) tests were used to compare incidence scores between male and female, female and OVX, male and CAST, and CAST and OVX rats on the data derived from the observational time sampling procedure. Incidence refers to the occurrence or nonoccurrence, rather than the frequency, of a particular behavior. Incidence scores were derived by summing observations of a particular behavior, for example rearing, for a specific rat across six observations. Thus, the rearing incidence for each rat was summed across rats in a particular group. The incidence data are nominal and between subjects in nature and therefore were analyzed using the χ^2 test. Scores below 5 are not reliably measured with the χ^2 statistic (Siegel and Castellan, 1988). Therefore, composite scores were created to increase incidence scores, and therefore maintain statistical accuracy. Locomotor, head bobbing, and rearing incidence scores were combined for a locomotor composite, and grooming, licking, and scratching incidence were summed for the orofacial composite. To further ensure statistical accuracy, a Yates correction procedure was used with the χ^2 test.

3. Results

3.1. Automated locomotor activity

3.1.1. Rearing

The effects of acute and repeated IV cocaine injection on rearing behavior are illustrated in Fig. 1. Females exhibited more rearing than males on day 13, and more rearing than OVX rats on days 1 and 13. CAST exhibited similar rearing behavior as male and OVX rats following acute and repeated cocaine injection. ANOVA conducted for the automated

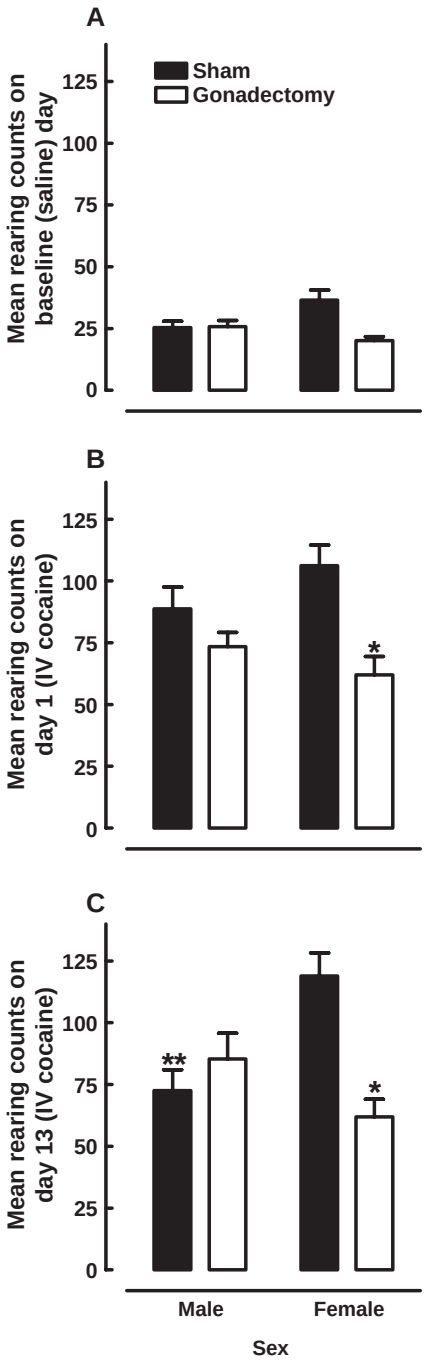

Fig. 1. Mean (±S.E.M.) number of rears during a 60-min session, as a function of day, sex, and gonadectomy. Rats were injected with either saline (baseline) or repeated IV cocaine (3.0 mg/kg/ml). All animals were habituated to the test environment for two sessions prior to baseline measurement. Panels A, B, and C represent data for baseline, day 1, and day 13, respectively. The day × sex × gonadectomy [$F(1,44)=9.95$, $p<0.01$] interaction indicates that, overall, females exhibited more rearing than male, CAST, and OVX rats. *Females and OVX $p<0.01$; **Females and males $p<0.01$; $n=12$ rats/group.

rearing data revealed main effects of day [$F(1,44)=186.76$, $p<0.001$; quadratic], time [$F(1,44)=112.01$, $p<0.001$; quadratic] and gonadectomy [$F(1,44)=14.42$, $p<0.001$]. The day × gonadectomy [$F(1,44)=5.27$, $p<0.05$; quadratic], day × time [$F(1,44)=27.57$, $p<0.001$; quadratic], sex × gonadectomy [$F(1,44)=5.27$, $p<0.05$], day × time × gonadectomy [$F(1,44)=9.21$, $p<0.01$; quadratic], and day × sex × gonadectomy [$F(1,44)=9.95$, $p<0.01$] interactions were significant. Planned contrasts indicate that females exhibited significantly more rears than males on day 13 [$F(1,22)=13.05$, $p<0.01$]. Moreover, females reared more than OVX rats on days 1 [$F(1,22)=11.83$, $p<0.01$] and 13 [$F(1,22)=19.60$, $p<0.001$]. There were no statistical differences between CAST and OVX rats [day 1: $F(1,22)=1.4$, $p>0.05$; day 13: [$F(1,22)=3.3$, $p>0.05$].

Time course data for the baseline day, day 1, and day 13 are presented in Fig. 2. Female rats exhibited a linear decrease in rearing behavior across the 60-min session on days 1 and 13, whereas male rats showed a linear decrease

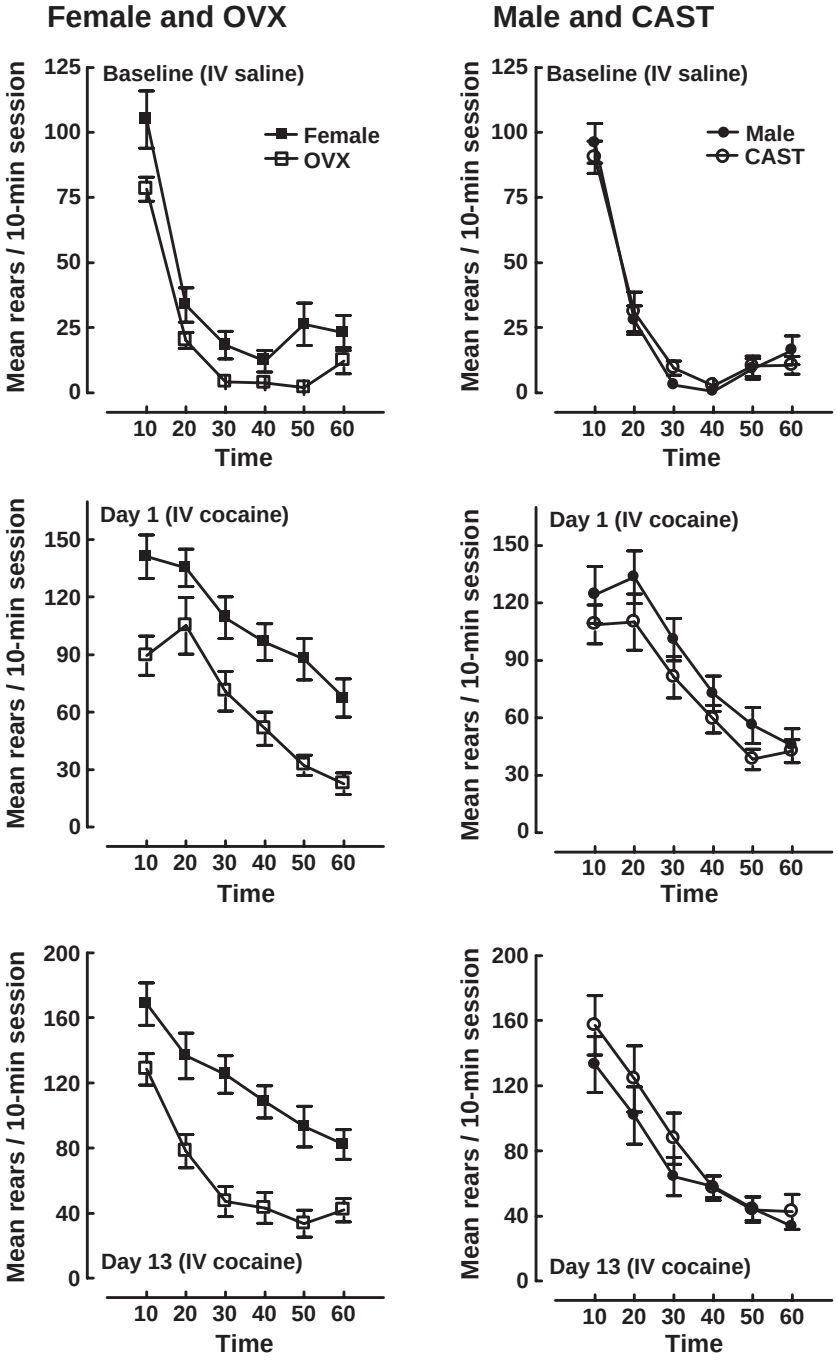

Fig. 2. Data are presented as mean (±S.E.M.) rearing as a function of group and time, for baseline (saline), day 1, and day 13 of IV cocaine (3.0 mg/kg/ml) injection. Columns represent data for female and OVX, and male and CAST, respectively. $n=12$ rats/group.

in rearing on day 1 and a quadratic decrease in rearing on day 13. These different patterns of behavior suggest that female rats exhibited enhanced cocaine-induced locomotor activity relative to male rats. OVX rats demonstrated a pronounced quadratic decrease in rearing behavior compared to females, suggesting that the enhanced cocaine-induced locomotor activity exhibited by females was attenuated by OVX. CAST rats exhibited a pattern of behavior similar to males and OVX. A simple main effects ANOVA, which used the error term from the overall ANOVA, was conducted for data from the male, CAST, female, and OVX groups as a function of time on days 1 and 13. The ANOVA revealed a main effect of time [$F(1,44)=17.80$, $p<0.001$; quadratic] and gonadectomy [$F(1,44)=16.20$, $p<0.001$]. The main effect of day was not significant. The day × time [$F(1,44)=34.98$, $p<0.001$; quadratic], sex × gonadectomy [$F(1,44)=14.73$, $p<0.01$], day × sex × gonadectomy [$F(1,44)=5.90$, $p<0.05$] and day × time × sex × gonadectomy [$F(1,44)=5.25$, $p<0.05$; quadratic] interactions were significant.

The time course data reveal the nature of the cocaine-induced potentiation of locomotor activity demonstrated by female rats. Although females exhibited a linear decrease in rearing across the session, male and OVX rats showed a quadratic decrease in rearing behavior. Thus, although females demonstrated a continuous decrease in rearing behavior, male and OVX rats' rearing behavior decreased early in the activity period and declined to asymptote toward the last half of the session.

3.1.2. Total automated activity

The effects of acute and repeated IV cocaine injection on total activity are illustrated in Fig. 3. Females showed more activity than males on day 13, and more activity than OVX on days 1 and 13. CAST exhibited similar activity as male and OVX rats following acute and repeated cocaine injection. ANOVA conducted for the total automated activity data revealed main effects of day [$F(1,44)=126.17$, $p<0.001$; quadratic], time [$F(1,44)=375.80$, $p<0.001$; quadratic] and gonadectomy [$F(1,44)=4.74$, $p<0.05$]. The day × time [$F(1,44)=33.72$, $p<0.001$; quadratic], sex × gonadectomy [$F(1,44)=11.87$, $p<0.01$], day × sex × gonadectomy [$F(1,44)=6.06$, $p<0.01$], and day × time × gonadectomy [$F(1,44)=5.92$, $p<0.01$; quadratic] interactions were significant. Planned contrasts indicate that females exhibited significantly more rears than males on day 13 [$F(1,22)=12.71$, $p<0.01$]. Moreover, females reared more than OVX rats on days 1 [$F(1,22)=5.23$, $p<0.05$] and 13 [$F(1,22)=11.38$, $p<0.01$]. There were no differences between CAST and OVX rats [Day 1: $F(1,22)=0.4$, $p>0.05$; Day 13: [$F(1,22)=1.2$, $p>0.05$].

Time course data for the baseline day, day 1, and day 13 are presented in Fig. 4. Female rats exhibited a linear decrease in rearing behavior across the 60-min session on days 1 and 13, whereas male rats showed a linear decrease in rearing on day 1 and a quadratic decrease in rearing on

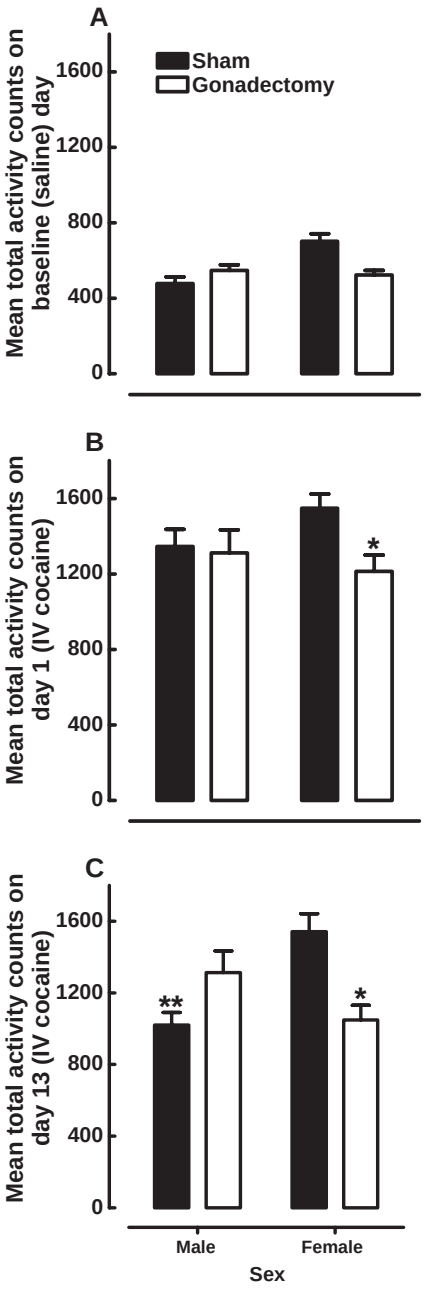

Fig. 3. Mean (±S.E.M.) total activity during a 60-min session, as a function of day, sex, and gonadectomy. Rats were injected with either saline (baseline) or repeated IV cocaine (3.0 mg/kg/ml). All animals were habituated to the test environment for two sessions prior to baseline measurement. Panels A, B, and C represent data for baseline, day 1, and day 13, respectively. The day × sex × gonadectomy [$F(1,44)=4.69$, $p<0.05$] interaction indicates that, overall, females exhibited more total activity than male, CAST, and OVX rats. *Females and OVX $p<0.01$; **Females and males $p<0.05$; $n=12$ rats/group.

day 13. These different patterns of behavior suggest that female rats exhibited enhanced cocaine-induced locomotor activity relative to male rats. OVX rats demonstrated a quadratic decrease in rearing behavior compared to females, suggesting that the potentiated response exhibited by females was attenuated by OVX. There were no statistical

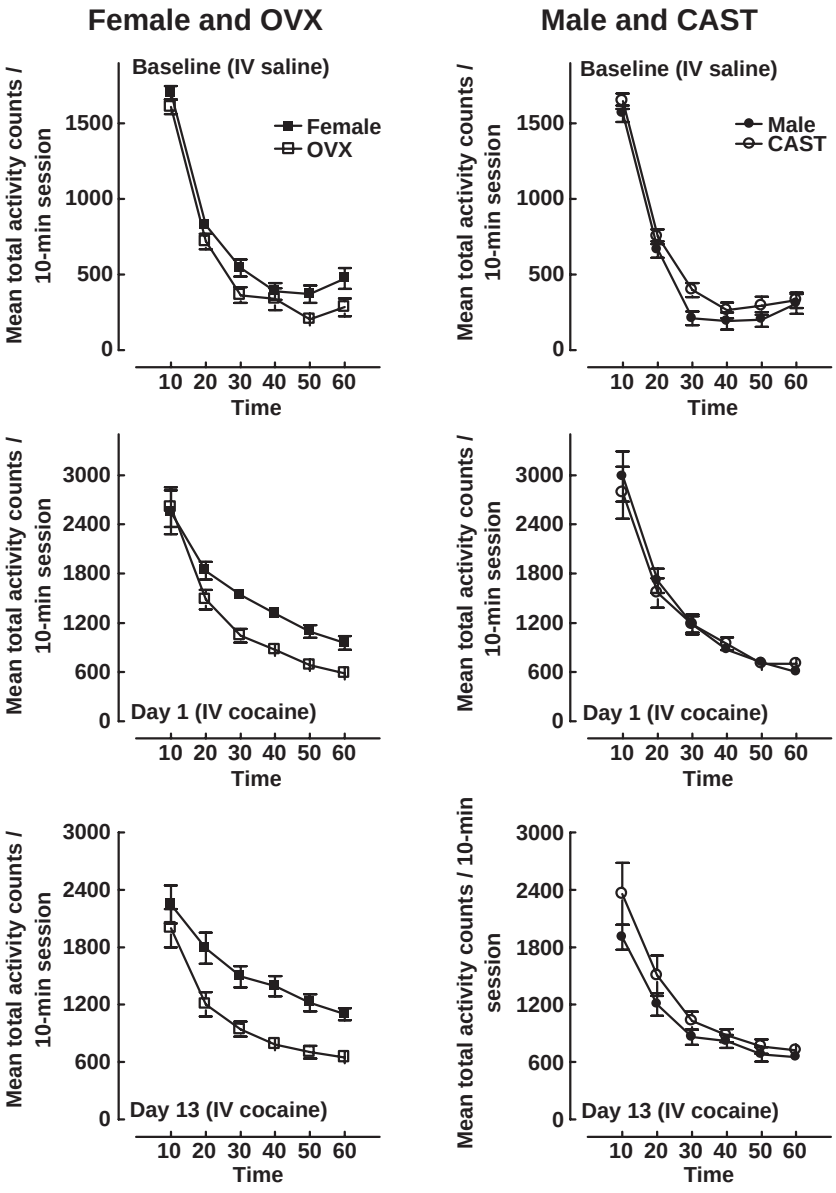

Fig. 4. Data are presented as mean (±S.E.M.) total activity as a function of group and session, for baseline (saline), day 1, and day 13 of IV cocaine (3.0 mg/kg/ml) injection. Columns represent data for female and OVX, and male and CAST, respectively. $n=12$ rats/group.

differences between CAST, males, and OVX rats. A simple main effects ANOVA, which used the error term from the overall ANOVA, was conducted on data from the male, CAST, female, and OVX groups as a function of time on days 1 and 13. The ANOVA revealed significant main effects of day [$F(1,44)=11.65$, $p<0.001$], time [$F(1,44)=201.07$, $p<0.001$; quadratic] and gonadectomy [$F(1,44)=5.28$, $p<0.05$]. The time × sex [$F(1,44)=7.69$, $p<0.05$; quadratic], time × treatment [$F(1,44)=4.49$, $p<0.05$], day × time [$F(1,44)=15.19$, $p<0.001$; quadratic], sex × gonadectomy [$F(1,44)=11.33$, $p<0.01$], day × sex × gonadectomy [$F(1,44)=4.69$, $p<0.05$], and day × time × sex × gonadectomy [$F(1,44)=4.41$, $p<0.05$] interactions were significant. CAST did not alter cocaine-induced activity relative to the intact males.

The time course data reveal the nature of the cocaine-induced enhancement of locomotor activity demonstrated by female rats. Although females exhibited a linear decrease in activity across the session, male and OVX rats showed a quadratic decrease in locomotor activity. Thus, while females demonstrated a continuous decrease in rearing behavior, male and OVX rats' rearing behavior decreased early in the activity period and declined to asymptote toward the last half of the session.

3.2. Observational time sampling of behavior

A modified version of the observational time sampling method first described by Fray et al. (1980) was used in the present experiment to examine the behaviors that rats

exhibited following acute and repeated IV cocaine administration.

3.2.1. Effects of sex, OVX, CAST, and IV cocaine on locomotor composite incidence scores

Fig. 5a illustrates the combined total activity, rearing, and head bobbing incidence observed during the acute (day 1) and repeated (day 13) administration of IV cocaine in male, female, CAST, and OVX rats. Female rats exhibited higher composite incidence scores compared to male rats on both days 1 and 13 (day 1: $\chi^2(1)=4.1$, $p<0.05$; day 13: $\chi^2(1)=5.4$, $p<0.05$). Neither the female nor male rats' composite incidence score changed across days 1 and 13 [$\chi^2(1)=0.61$, $p<0.05$, $\chi^2(1)=0.23$, $p<0.05$, respectively].

OVX rats exhibited significantly lower composite incidence scores than females in response to acute IV cocaine on both days 1 and 13 [$\chi^2(1)=8.9$, $p<0.01$, $\chi^2(1)=13.4$, $p<0.01$, respectively]. Furthermore, OVX rats' response to cocaine was similar on days 1 and 13 [$\chi^2(1)=0.01$, $p>0.05$]. CAST rats exhibited statistically similar composite incidence scores as male rats on days 1 and 13 [$\chi^2(1)=0.23$, $p>0.05$, $\chi^2(1)=1.33$, $p>0.05$, respectively]. CAST rats' composite incidence score increased across days 1 and 13 [$\chi^2(1)=4.1$, $p<0.05$], indicating that CAST rats exhibited behavioral sensitization following 13 IV cocaine injections when total activity, rearing, and head bobbing were combined as a composite incidence score. Although there were no differences between CAST and OVX rats following acute cocaine administration [$\chi^2(1)=0.2$, $p>0.05$], CAST rats exhibited more locomotor incidence than OVX following repeated cocaine injection [$\chi^2(1)=5.8$, $p<0.05$].

3.2.2. Effects of sex, OVX, CAST, and IV cocaine on orofacial composite incidence scores

Fig. 5b illustrates the orofacial composite incidence observed during the acute (day 1) and repeated (day 13) administration of IV cocaine in male, female, OVX, and CAST rats. There were no significant differences in the composite incidence scores as a function of sex in the animals' acute response to IV cocaine [$\chi^2(1)=0.42$, $p>0.05$]. However, the sensitized composite response on day 13, relative to day 1 [$\chi^2(1)=3.8$, $p<0.05$], displayed a pronounced sex difference in this effect (male vs. female on day 13; $\chi^2(1)=8.5$, $p<0.01$). Thus, females exhibited a 130% increase in grooming, licking, and scratching (day 1 vs. day 13: $\chi^2(1)=6.6$, $p<0.01$), whereas males' composite incidence score did not change across days (1 vs. 13: $\chi^2(1)=0.19$, $p>0.05$).

OVX rats exhibited significantly higher composite incidence scores than females in response to acute IV cocaine [$\chi^2(1)=5.9$, $p<0.01$]. In contrast to the female group which exhibited a significant increase in observed incidence across days 1 and 13 (see above), OVX rats' composite incidence did not change across days [$\chi^2(1)=0.17$, $p>0.05$]. CAST rats exhibited composite incidence scores statistically

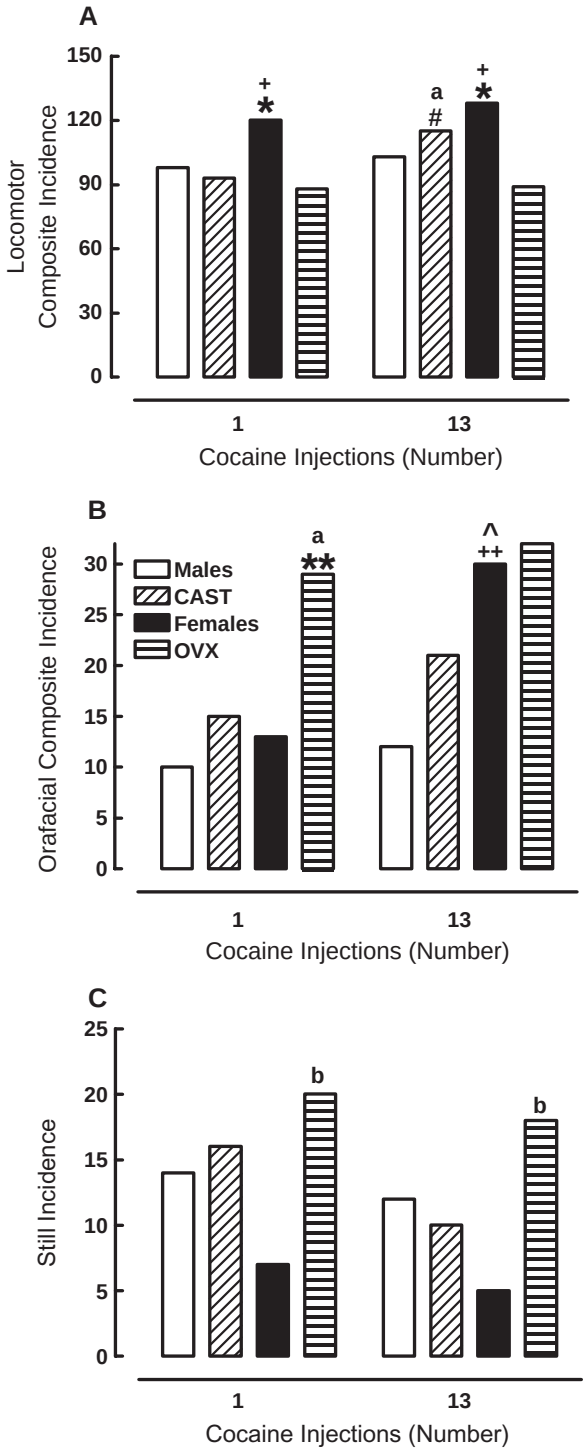

Fig. 5. Locomotor (A), orofacial (B), and still (C) composite incidence data following acute and repeated IV cocaine dosing (3.0 mg/kg 1/day for 1 and 13 days) are illustrated for the observational time sampling of behavior as a function of group. No measure of variance is indicated because the graph illustrates incidence (nominal) data. Data were analyzed using the χ^2 statistic. $^{+}$Female and male, $p<0.05$; *Female and OVX, $p<0.01$; $^{\#}$CAST, days 1 and 13, $p<0.05$; **Female and OVX, $p<0.01$; $^{++}$Female and males, $p<0.01$; $^{\wedge}$Female, days 1 and 13, $p<0.01$; ^{a}CAST and OVX, $p<0.05$; ^{b}Female and OVX, $p<0.05$. $n=12$ rats/group.

similar to the male rats on days 1 and 13 [$\chi^2(1)=0.68$, $p>0.05$, $\chi^2(1)=2.1$, $p>0.05$, respectively]. CAST rats' composite incidence score did not change across days 1 and 13 [$\chi^2(1)=1.1$, $p>0.05$]. Although CAST rats exhibited less orofacial behavior than OVX on day 1 [$\chi^2(1)=4.3$, $p<0.05$], there were no differences between CAST and OVX on day 13 [$\chi^2(1)=2.2$, $p>0.05$].

3.2.3. Effects of sex, OVX, CAST, and IV cocaine on still and sniffing incidence scores

Still, sniffing, yawn, scanning, lying down, and bite behaviors were not combined with other behaviors for composite incidence scores. Furthermore, χ^2 analyses were not conducted for the yawn, scanning, lying down, and bite incidence because there were fewer than 5 observations. Fig. 5c illustrates the still incidence observed during the acute (day 1) and repeated (day 13) administration of IV cocaine in male, female, CAST, and OVX rats. Female rats exhibited similar still incidence scores compared to male rats on days 1 [$\chi^2(1)=1.42$, $p>0.05$] and 13 [$\chi^2(1)=1.82$, $p>0.05$]. Female rats' still incidence scores differed from OVX rats on days 1 [$\chi^2(1)=4.38$, $p<0.05$] and 13 [$\chi^2(1)=5.29$, $p<0.05$]. Male rats did not differ from CAST rats on days 1 or 13 [$\chi^2(1)=0.02$, $p>0.05$; $\chi^2(1)=0.03$, $p>0.05$, respectively]. None of the groups exhibited a change in still incidence across 13 days (all $p>0.05$). There were no differences between CAST and OVX rats on days 1 or 13 [$\chi^2(1)=0.3$, $p>0.05$; $\chi^2(1)=1.9$, $p>0.05$, respectively]. Examination of the observed sniffing scores revealed that sniff behavior was observed regularly throughout the 60-min session, whereas other high occurrence behavior (e.g., rearing) was observed at the beginning of the session and subsided throughout the remainder of the activity test. Furthermore, the sniff observations were indistinguishable across groups, as well as consistent for each group across the session, and therefore do not appear to be sensitive to cocaine-induced stereotypy. These observations are in accord with the sniff findings from Fray et al. (1980) in that sniffing behavior occurred throughout the session on all doses of apomorphine tested. Based on these observations, the sniff scores were excluded from the orofacial composite and were analyzed separately. There were no differences in sniffing incidence between any of the intact or gonadectomized rats on days 1 or 13 (all $p>0.05$; data not shown). The sniffing incidence for male, CAST, female, and OVX was 76, 72, 75, and 67 on day 1, and 66, 57, 65, and 61 on day 13.

4. Discussion

The present experiment investigated the effects of acute and repeated IV cocaine administration on locomotor activity in intact and gonadectomized male and female rats. Behavior was measured by automated, photocell equipment (i.e., rearing and total activity) and by experimenter observation (i.e., orofacial and locomotor composite incidence). IV cocaine produced sex differences in rearing and total activity as females exhibited more activity following repeated, but not acute, IV cocaine administration. Moreover, a sex difference in the expression of behavioral sensitization of orofacial behaviors was observed: females exhibited more orofacial incidence following repeated IV cocaine injection relative to males. These results replicate previous findings which demonstrate that females exhibit more cocaine-induced locomotor activity and behavioral sensitization relative to males (Post et al., 1981; Glick et al., 1983; Glick and Hinds, 1984; van Haaren and Meyer, 1991; Bowman and Kuhn, 1996; Sircar and Kim, 1999; Chin et al., 2002).

Gonadectomy modulated the behavioral effects of IV cocaine. Thus, OVX attenuated cocaine-induced rearing, total activity, and locomotor incidence relative to intact female rats following acute and repeated cocaine injection. These results are consistent with previous research which shows that OVX decreases locomotor activity relative to intact female rats following repeated cocaine injection (Chin et al., 2002). Alternatively, CAST did not alter acute or repeated cocaine-induced rearing, total activity, or orofacial behaviors in the present experiment. Interestingly, CAST rats exhibited behavioral sensitization for locomotor incidence; however, neither the male nor OVX groups exhibited behavioral sensitization, suggesting that male, but not female, rats sensitize to the psychomotor effects of repeated IV cocaine in the absence of circulating gonadal hormones. These findings replicate previous research which show that CAST rats exhibited behavioral sensitization of rotational behavior (Hu and Becker, 2003), locomotor activity (Chin et al., 2002), and observed head bobbing (Becker et al., 2001) after repeated IP cocaine injections.

The present study also reports a novel effect of OVX on two measures of incidence behavior. In the present experiment, OVX rats exhibited a 123% increase in orofacial incidence and a 185% increase in still incidence following the acute administration of IV cocaine compared to intact females. This finding suggests that OVX increased female rats' sensitivity to the acute effects of cocaine. Repeated IV cocaine injections resulted in a high incidence of orofacial behavior in OVX and intact females; however, intact, but not OVX, females exhibited behavioral sensitization to repeated cocaine administration. This finding is consistent with previous results which showed that OVX rats did not express sensitization to repeated cocaine administration (Peris et al., 1991; van Haaren and Meyer, 1991; Sircar and Kim, 1999). The lack of sensitization in OVX rats was not due to a ceiling effect, as OVX rats groomed, licked, or scratched during 32 of 72 behavioral observations. Previous research which showed that estrogen-treated OVX rats exhibited enhanced cocaine-induced behavioral sensitization compared to OVX rats not treated with estrogen (Perrotti et al., 2001; Hu and Becker, 2003) suggest that the lack of behavioral sensitization by OVX rats in the

present experiment is due to the absence of circulating ovarian hormones during repeated IV cocaine injection. How the absence of ovarian hormones enhances the acute effects of IV cocaine is not clear from the present results.

Chin et al. (2002) examined if sex and gonadectomy altered the effects of cocaine-induced locomotor activity via the IP route of administration and did not report OVX-induced facilitation of cocaine's effects with either an automated or observational measure. Notably, the observational measures used in that study and in the present experiment are different. The observational measure used by Chin et al. (2002) measures activity with a rating scale, whereas the observed incidence procedure used in the present experiment measures whether discrete, stereotypic behaviors (e.g., grooming) are or are not expressed. Thus, the differences in observational techniques may account for discrepancies between the present findings and previously published research (Chin et al., 2002).

The mechanisms by which gonadal hormones alter the acute and repeated effects of IV cocaine cannot be determined from the present results. One mechanism may be related to estrogen-mediated changes in the expression of D_2-like DA receptors in the mesolimbic DA pathway. Indeed, gonadal hormones influence the expression of D_1 (Lee and Mouradian, 1999; Lee et al., 2001) and D_2-like (Bazzett and Becker, 1994; Zhou et al., 2002; Febo et al., 2003; see also Lammers et al., 1999 for exception) receptors, and alter functional activity in the striatum and nucleus accumbens of the rat brain (Thompson et al., 2001). If estrogen is important for regulating D_3 receptors as some investigators suggest (Zhou et al., 2002; Febo et al., 2003), then down-regulation of D_3 receptors in the VTA (Zhou et al., 2002) or nucleus accumbens (Wallace et al., 1996) may alter cocaine-related behaviors. This possibility is intriguing considering D_3 receptors have been implicated in the inhibition of dopamine D_2/D_3 related stereotypic behaviors. Thus, it has been suggested that cocaine-induced stereotypy reflects decreased inhibition, or a disinhibition, by D_3 receptors (Waters et al., 1994; Sautel et al., 1995; Menalled et al., 1999; Dall'Olio et al., 2002; Chiang et al., 2003; Richtand et al., 2003).

Consistent with these suggestions are findings which demonstrate that repeated IV cocaine (Wallace et al., 1996) or nicotine (Harrod et al., 2004)-induced alterations in the expression of striatal and accumbal D_3 receptors significantly predicted sex-dependent expression of behavioral sensitization using multiple regression analysis, suggesting that D_3 receptors contributed to IV psychostimulant-induced behaviors. Clearly, more studies need to be conducted to determine the role of gonadal hormones in psychostimulant-related sex differences in behavior, and furthermore, whether D_3 receptor density contributes to these sex differences. Although the direct influence of OVX on the expression of D_3 receptors—and thus cocaine-induced activity—cannot be determined from the present data, previous research which shows a relationship between behavioral sensitization, psychostimulant-induced changes in D_3 receptor density, and sex makes this an interesting question to pursue (Wallace et al., 1996; Harrod et al., 2004).

A caveat to our use of the term behavioral sensitization should be added as the present experiment did not include saline control groups that would otherwise permit statements about the absolute levels of behavioral sensitization observed. Using similar procedures, saline injected males displayed a linear increase in centrally directed locomotor activity across 14 days of injection with repeated testing (Wallace et al., 1996). Although that particular effect likely contains a component attributable to context conditioning due to the repeated pairing of saline injection and the test environment, the present lack of any statistically detectable increase in activity in injected males after 13 days of IV cocaine injection is particularly striking. Further, with similar procedures in the study of the effects of IV cocaine in females, saline injected animals displayed no significant increases in activity after 14 days of injection with repeated testing, even with the benefit of context conditioning (Booze et al., 1999b). Again, the contrast to the present study is particularly striking as the females displayed marked within subject increases across days 1 and 13, and moreover, exhibited significant differences in orofacial behavior compared to similarly injected males.

One important consideration with regard to studies of psychostimulant use is the route of administration. Administration of IV cocaine produces a rapidly peaking rise in plasma cocaine concentration followed by a precipitous clearance. The most common routes of cocaine administration for humans are smoking and injecting, and the pharmacokinetic profiles of these routes are similarly characterized by rapidly peaking plasma levels of cocaine (Evans et al., 1995). Following IV administration of 32 mg cocaine in humans, arterial sampling resulted in peak plasma levels (~2500 ng/mg) of cocaine by 30 s (Evans et al., 1995). In adult male rats, IV injection of 3.0 mg/kg/infusion cocaine increased arterial levels of cocaine by 30 s to ~2500 ng/mg (Booze and Wallace, 1995), suggesting that the IV route of administration in rats is preferable due to the similar kinetic profile compared to human studies.

Previous experiments from our laboratory demonstrated that repeated IV cocaine (3.0 mg/kg/infusion) produced behavioral sensitization in male (Wallace et al., 1996) and female rats (Booze et al., 1999b) when an automated measure of locomotor activity was used. In the present experiment, behavioral sensitization was reported for orofacial incidence but not for the automated measure of locomotor activity, regardless of sex or gonadectomy, following repeated IV cocaine administration. Although tolerance to repeated IP injection of cocaine has also been reported in OVX rats following repeated administration (Perrotti et al., 2001), it is not a common finding. One reason for the discrepancy between previous results from our lab (Wallace et al., 1996; Booze and Wallace, 1995) and

the present findings may be that the activity monitors used previously were different than the equipment used in the presently reported experiment. The locomotor equipment used in the past consisted of circular activity chambers whereas the present experiments used square chambers. Although we have not systematically compared the expression of psychostimulant-induced behavioral sensitization when circular versus square chambers are used, it appears that the former style of open field arena is more sensitive to an array of locomotor behaviors, and perhaps the expression of behavioral sensitization. Future studies will directly compare circular and square activity chambers to determine if there are parametric differences in the expression of psychostimulant-induced behavioral sensitization.

The present experiment demonstrates that the simultaneous use of both an observational and automated measure may be important for the detection of different psychostimulant-induced behaviors. For example, IV cocaine-induced behavioral sensitization and the sex difference thereof were reported for the orofacial incidence, but not the automated rearing or total activity measures. This finding suggests that the incidence measure is more sensitive to some behaviors compared to the automated measurement and that using both tests may increase the likelihood of detecting drug-induced changes in behavior.

It will be important for future research to elucidate the origin of estrogen-mediated cocaine-induced gender differences so that relevant pharmacotherapy which appropriately addresses women's treatment issues can be developed. Toward this end, further research in our laboratory will examine whether estrogen replacement enhances the acute and repeated locomotor effects of IV cocaine in OVX rats and determine the effect, if any, on the expression of DA receptors throughout the mesolimbic DA pathway.

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