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Estrous Cyclicity and Behavioral Sensitization in Female Rats Following Repeated Intravenous Cocaine Administration

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BOOZE, R. M., M. L. WOOD, M. A. WELCH, S. BERRY AND C. F. MACTUTUS. *Estrous cyclicity and behavioral sensitization in female rats following repeated intravenous cocaine administration.* PHARMACOL BIOCHEM BEHAV **64**(3) 605–610, 1999.—Repeated intermittent administration of cocaine is well known to produce behavioral sensitization in male animals. The present studies explored whether intact adult female rats maintained normal estrous patterns in response to repeated IV cocaine administration and whether behavioral sensitization occurred with this route of administration. Adult female Sprague–Dawley rats ($N = 48$) were surgically implanted with an intravenous access port. Animals received 3.0 mg/kg IV cocaine once/day for 14 days. Daily vaginal lavages indicated that female rats continued to cycle normally throughout the experiment. Estimates of statistical power for detecting alterations in estrous cycle length ranged from 0.61–0.95 for small (0.1) to large (0.4) effect sizes. Moreover, no cocaine-treated animals displayed persistent vaginal estrus or were acyclic and cocaine treatment did not decrease body weight. Immediately after the cocaine injection, animals were placed in IR photocell activity chambers for 60 min. Female rats displayed a significant 75% increase in locomotor activity across the 14-day time course of IV cocaine injections. These data indicate that 3.0 mg/kg of IV cocaine does not interfere with normal estrous cyclicity, and that behavioral sensitization occurs in female rats following repeated daily IV cocaine dosing. Collectively, these data suggest that the IV cocaine-dosing model may be particularly useful in exploring the gender-dependent effects of cocaine using intact female rats. © 1999 Elsevier Science Inc.

Cocaine Sensitization Tolerance Estrous cyclicity Intravenous administration Body weight Rats

WOMEN drug abusers represent a major public health problem. Current estimates are that women make up 37% of the illegal drug abusing population or over 4.4 million women (20). Moreover, recent epidemiological studies suggest that of women who abuse drugs, such as crack cocaine, many become more seriously addicted and spend a longer portion of their lives abusing illegal drugs relative to men (12,19). In addition, most of these women are of child-bearing age, placing future generations at risk. Nevertheless, most of the clinical treatment of drug abuse has focused on male abusers, and the basic sciences research effort has primarily used male subjects.

Repeated intermittent administration of psychoactive stimulants such as cocaine and amphetamine produces profound behavioral changes in humans as well as experimental animals (8,15,26,34,35). The augmentation of behavioral response following repeated cocaine administration (i.e., increased locomotor activity, and stereotypic behaviors) has been referred to as "behavioral sensitization" or "reversed tolerance" (13,14). The development and expression of behavioral sensitization appears dependent on the dose of cocaine, dosing regimen, and possibly, gender. In particular, there have been a small number of reports indicating that female rats are differentially sensitive to the effects of cocaine. The first report of sex differences in response to cocaine was by Selye (29). Subsequent work (11) confirmed that female rats demonstrated greater sensitization in response to cocaine relative to male rats.

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One complicating factor in evaluating the effects of cocaine in female rats is the ability of repeated dosing with cocaine to interfere with the normal estrous cycle of the rat. Cocaine, when administered subcutaneously to female rats (8–10 mg/kg/day), has been shown to produce persistent estrus, absence of proestrus, and prolonged diestrus (17,18). Thus, administration of cocaine via the subcutaneous route may interfere with experimental evaluations of intact female animals. The effect of cocaine administered via other routes on female rat estrous cyclicity is unknown.

In the current study, we have explored 1) whether estrous cyclicity is affected by repeated IV cocaine treatment, and 2) whether behavioral sensitization occurs in female rats in response to repeated daily IV cocaine administration. These results will indicate whether IV dosing may be used to study the effects of cocaine in intact female rats. The use of intact females would allow study of interactions between estrous cycle hormones and cocaine sensitivity.

METHOD

Animals

Female Sprague–Dawley rats (225–249 g) were obtained from Harlan Laboratories, Inc. (Indianapolis, IN). Upon arrival at the animal care facilities, rats were placed in quarantine for 7 days, then transferred to the colony. Animals were pair housed throughout the experiment. Rodent food (Pro-Lab Rat, Mouse Hamster Chow #3000) and water were provided ad lib. The colony was maintained at 21 ± 2°C, 50 ± 10% relative humidity, and a 12L:12D cycle with lights on at 0700 h (EST). The animal protocol for this research was approved by the IACUC of the University of Kentucky.

Surgical Procedure

An Intracath IV catheter (22 ga, Becton/Dickinson General Medical Corp., Grand Prarie, TX) was used as a SC dorsally implanted port for chronic IV injections. SC placement of the IV catheter eliminates the need for tethering and precludes catheter manipulation by the rat. Implantation of catheters was performed as previously described (22). Briefly, rats were anesthetized using a mixture of ketamine hydrochloride and xylazine by IP injection (7.5 mg ketamine/100 g body weight, 30 mg xylazine/100 g body weight). 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 inserted dorsally under the skin and threaded through to the ventral side. 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. Last, neck and back skin were sutured closed and triple antibiotic ointment [Triple Antibiotic Ointment by E. Fougera & Co. (Melville, NY), Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ointment USP] 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.

Experimental Design and Procedures

Two days after surgery, vaginal smears were taken on the rats to determine the stage of their estrous cycle. Vaginal smears were taken on these rats at the same time of day (between 0900–1000 h) every day thereafter throughout the experiment. An individual blind to both animal treatment group and cytology results of the previous day performed cytological assessments. Evaluation of vaginal smears was based upon the following cytological criteria for staging the estrous cycle: the predominance of pronucleated epithelial cells indicated proestrus, cornified epithelial cells indicated estrus, and leukocytes indicated diestrus I and II. Acyclicity and irregular cycling were defined by either persistent estrus, absence of proestrus, failure to progress from proestrus to estrus, or prolonged diestrus.

After the rats came into estrus for the second time, IV injections were initiated. All rats were injected between 1500–1700 h. One-half of the rats ($n = 24$) were injected with saline (0.1 ml/100 g). The remaining rats ($n = 24$) were injected with cocaine HCl (Lot #116H0368, Sigma Chemical Co., St. Louis, MO), 3.0 mg/kg/ml (0.1 ml/100 g). The 3-mg/kg IV dose was selected based on previous reports by Booze et al. (3) that this dose, when given to rats, mimics the pharmacokinetic profile seen in human cocaine users (i.e., transient levels of arterial cocaine peaking at ~2500 ng/ml within 30 s). The cocaine injections were followed with a catheter flush of 0.2 ml of heparinized (2.5%) saline. One squad of cocaine- and vehicle-treated rats was monitored for body weight and estrous cyclicity, whereas a second experiment was conducted with a squad of rats monitored for body weight and locomotor sensitization. Two separate experiments were performed because of the possibilities that 1) vaginal lavage prior to behavioral testing might alter subsequent locomotor activity, 2) repeated vaginal lavaging might interfere with assessment of cocaine's effects on sensitization, and 3) repeated behavioral testing might interfere with assessment of cocaine's effects on estrous cyclicity. Thus, to preclude any possible interaction of cocaine with these two dependent measures, particularly as the assessment of either one could confound the assessment of the other, two separate squads of animals (experiments) were used.

Animals in the second squad were habituated to the locomotor activity chamber for two 60-min sessions, one/day. Habituation sessions were employed to decrease the novelty of the test environment and, hence, baseline activity scores. Activity monitors were four circular, open-field chambers 0.46 m in diameter (San Diego Instruments, San Diego, CA) that detected free movement of animals by infrared photocell interruptions. Total activity, centrally directed (simultaneous breaking of ≥2 photocells), and peripherally directed locomotor activity were measured by assessing the number and type of photocell interruptions within a 60-min period. Photocell interruptions were collected in 10-min intervals. Locomotor activity monitoring was assessed every other day for 60 min, beginning immediately after cocaine or vehicle injections (33). The rationale for every other day testing was 1) to control for time of day effects, i.e., only half the animals needed to be tested on any one day, and 2) to reduce the number and predictability of "pairings" of environmental test cues with cocaine, i.e., cocaine was paired with both home cage and the test environment on alternate days. All behavioral testing was conducted between 1000–1800 h under dim light conditions, with direct overhead lighting turned off (<10 lx). Animals were sacrificed after 14–18 days of injections on the day of estrus.

Data Analysis

All behavioral data were analyzed using analysis of variance (ANOVA) techniques (BMDP Statistical Software, Los Angeles, CA), with test day as the repeated-measures factor. The Greenhouse-Geisser *df* correction factor was employed for those variables that violated compound-symmetry assumptions. The distribution of peripheral (single photocell interruption) and central (joint interruption of ⩾2 photocells) locomotor activity was also assessed. The SOLO power analysis module of the BMDP Statistical Package was used to compute power of the statistical analysis to determine the sensitivity of the experiment to detect the effects of cocaine on estrous cyclicity (5). An α level of $p \leqslant 0.05$ was the significance level for rejection of the null hypothesis.

RESULTS

Administration of 3.0 mg/kg/day IV cocaine over the 14-day test period did not significantly affect body weight (saline-treated controls vs. cocaine-treated animals) in the animals monitored for estrous cyclicity (saline 4% increase; cocaine 5% increase) or in the animals monitored for the development of behavioral sensitization (saline 6% increase; cocaine 7% increase). Data analysis failed to find a significant effect of treatment or an interaction of treatment × day. Power analysis estimates for a moderate (0.25) to large (0.4) effect size were in excess of 0.93 for detecting a potential effect of cocaine on growth (5).

Estrous cycle length was monitored throughout the repeated IV injections (Fig. 1). Two animals failed to cycle after surgery, and thus were removed from the analysis (i.e., they never received a cocaine injection). It was clear that there was no evidence of estrous cycle disruption, despite at least 14 days of IV cocaine treatment, $F(1, 20) = 0.98$, $p \leqslant 0.33$; treatment × time, $F(3, 60) = 1.3$, $p_{GG} \leqslant 0.28$. All cocaine-treated animals maintained the normal pattern of estrous cyclicity as determined by daily vaginal lavage. In addition, no cocaine-treated animal displayed persistent vaginal estrus or was acyclic.

Power analysis (5) estimates for a large (0.4) effect size of cocaine on estrous cyclicity, as would be expected based on previous work on cocaine-induced estrus disruption (17), were in excess of 0.95 for the repeated-measures terms. For the small to moderate effect sizes seen on the repeated measures terms (0.10–0.32), the power to detect alterations in estrous cycle length was 0.61–0.87. Collectively, these data indicate that if IV cocaine had a true effect on estrous cyclicity, it should have been detected.

Analysis of locomotor activity data indicated a significant main effect of cocaine treatment, $F(1, 22) = 102.0$, $p \leqslant 0.001$, as well as interactions of cocaine treatment with dependent measure (center vs. periphery), $F(1, 22) = 119.1$, $p \leqslant 0.001$, cocaine treatment with test day, $F(8, 176) = 27.7$, $p_{GG} \leqslant 0.001$, and cocaine treatment x dependent measure x test day $F(8,176) = 24.1$, $p_{GG} < 0.001$. Given the presence of this three-way interaction, tests of simple effects were subsequently conducted on each dependent measure.

As would be anticipated at pretreatment baseline (Figs. 2 and 3), there was no significant difference in overall locomotor activity between saline and cocaine-treated groups, $Fs(1, 22) < 1.0$. Also, not surprisingly, the initial (acute) response of drug-treated females indicated a large, significant, effect of cocaine on locomotor activity, $F(1, 22) = 50.4$, $p \leqslant 0.001$. The magnitude of the cocaine-induced increase was 184% (for centrally directed activity) and 106% (for peripherally directed activity) greater than the activity of saline-treated animals.

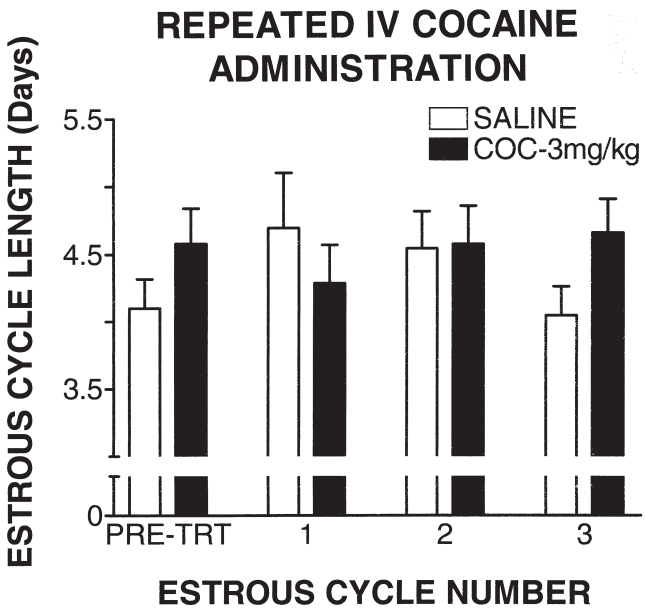

FIG. 1. Estrous cycle length is shown as a function of number of estrous cycles during the repeated IV injections. There was no evidence of estrous cycle disruption over 14 days of IV treatment, $F(1, 20) < 1.0$; treatment × time, $F(3, 60) = 1.3$, $p_{GG} \leqslant 0.28$. All cocaine-treated animals maintained the normal pattern of estrous cyclicity as determined by daily vaginal lavage. In particular, no cocaine-treated animals displayed persistent vaginal estrus or were acyclic.

Perhaps most importantly, drug-treated female rats displayed an overall increase in their locomotor activity response (i.e., behavioral sensitization) to repeated IV dosing (one/day for 14 days) with 3.0 mg/kg of cocaine, $F(8, 176) = 62.8$, $p_{GG} \leqslant 0.001$. In marked contrast, the locomotor activity of the saline-treated animals did not significantly change across test days, $F(8, 176) < 1.0$. Behavioral sensitization was clearly noted across the 7 behavioral test days on centrally directed locomotion in response to the cocaine treatment ($r = 0.91$, $p \leqslant 0.005$) with the increase in central activity scores over 75% greater on day 14 relative to that on day 2. With respect to peripherally directed locomotor activity, the drug-treated female rats failed to display any convincing increase in their response to the repeated IV cocaine dosing (3.0 mg/kg, one/day for 14 days) ($r = 0.70$, $p \leqslant 0.08$). Specifically, the magnitude of the increase in peripheral activity scores was only 31% greater on day 14 relative to that on day 2.

DISCUSSION

The present study found, first, that repeated IV cocaine administration (3.0 mg/kg/day) does not disrupt the estrous cycle. All cocaine-treated animals displayed normal estrous cyclicity over 14–18 days of IV treatment, as determined by daily vaginal lavage. Power analysis estimates further indicated that IV cocaine had no effect on estrous cyclicity. Second, these data also report that female rats display clear behavioral sensitization to repeated (14-day) IV cocaine administration. Females treated with cocaine displayed a 75% increase in their locomotor activity response from day 2 to day 14, whereas saline-treated animals did not display a signif-

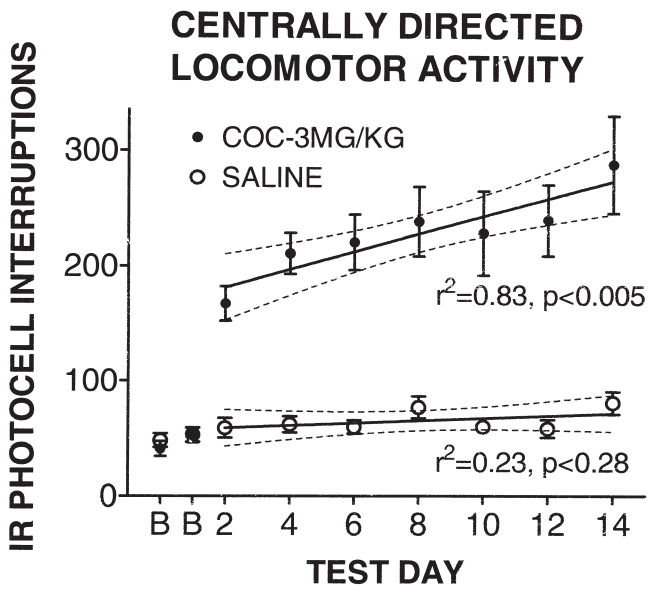

FIG. 2. Female rats displayed behavioral sensitization in response to repeated IV dosing (one/day for 14 days) with 3.0 mg/kg of cocaine. Locomotor activity was assessed under baseline conditions (B) and at 2-day intervals. Behavioral sensitization was clearly noted in the cocaine-treated females with the centrally directed locomotor activity response over 75% greater on day 14 relative to that on day 2. The centrally directed locomotor activity of the saline-treated animals did not significantly change across test days.

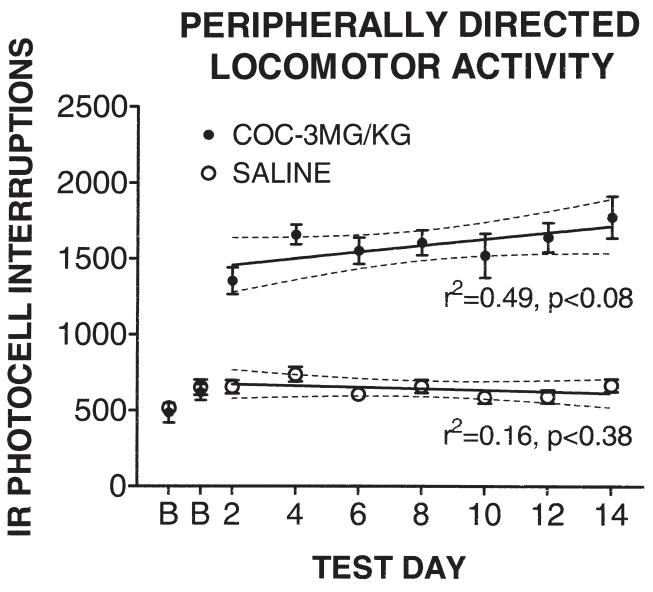

FIG. 3. Female rats displayed behavioral sensitization in response to repeated IV dosing (one/day for 14 days) with 3.0 mg/kg of cocaine. Locomotor activity was assessed under baseline conditions (B) and at 2-day intervals. Behavioral sensitization was not readily apparent in the cocaine-treated females on peripherally directed locomotion. The peripherally directed locomotor activity of the saline-treated animals did not significantly change across test days.

icant increase in locomotor activity. And third, these data report that administration of IV cocaine did not significantly affect body weight. Therefore, the drug administration model used in this study eliminates the need to yoke cocaine-treated rats with pair-fed controls as in other studies (26,34). This IV dosing model of 3.0 mg/kg/day also eliminates the influence of body weight on estrous cyclicity as a concern. Collectively, these body weight findings (lack of effect) are consistent with earlier findings in pregnant rats given the same dose of cocaine (22), and suggest that IV cocaine, when given at 3.0 mg/kg/day, is relatively nontoxic to female rats.

The IV bolus model of cocaine administration to unanesthetized, freely moving, rats produces rapidly peaking pharmacokinetic profile (3) characteristic of IV administration in large mammalian species and human male volunteers (9). Following a bolus injection of 3.0 mg/kg IV cocaine to male rats, noncompartmental pharmacokinetic analyses indicated a peak arterial plasma concentration of 2553 ± 898 ng/ml of cocaine 30 s after dosing and a $T1/2_{\beta}$ of 12.0 ± 2.0 min (3). Evans and colleagues (9) found in human males that plasma levels peaked rapidly following IV bolus injection (15–30 s), and that peak arterial plasma levels of approximately 2500 ng/ml were correlated with both euphoric and cardiovascular effects of cocaine. Thus, the rat 3.0-mg/kg IV bolus injection model offers the ability to reproduce the rapidly peaking pharmacokinetic profile of human IV cocaine abuse, an opportunity that is not present following SC, IP, or PO administration.

Some investigators (17,18) report that treatment with 10 and 20 mg/kg/day of cocaine HCl via SC injection produces significant estrous cycle irregularities within 7 days. Inhibition of mating-induced ovulation in rabbits treated daily with 40 mg/kg of cocaine HCl via SC injection has also been reported (16). However, in considering the disparities between these studies and the current study, it is important to note that these investigators (16–18) used the SC route of administration. Cocaine administered SC exhibits a significantly different kinetic profile than the IV route of administration. A sustained elevation of plasma cocaine (6) characterizes cocaine administration via SC injection. In contrast, IV cocaine administration is characterized by a rapid rise and fall of plasma cocaine levels. The rapid and transient peak cocaine levels in rats following IV bolus administration closely approximates the kinetic profile of that found in human cocaine IV or crack smoking abusers (3,9). Also noteworthy, these SC studies used cocaine doses of 10 and 20 mg/kg/day (17,18) and 30 mg/kg/day (16), compared to the 3.0 mg/kg/day IV used in this study. Consequently, the reported estrous cycle irregularities (12,13) and inhibition of ovulation (11) may be attributed to the use of much larger cocaine doses and/or sustained cocaine exposure following the SC dosing regimen.

There is one report in the literature (28) indicating that after 18 days of cocaine self-administration, female rats begin to display disruption of estrous cyclicity. Although self-administration paradigms deliver cocaine via an IV route, the self-administration model results in a much higher dose of cocaine, relative to the current study. Specifically, under the PR schedule, female rats (weighing 230 g) self-administered, on average, 17 injections of 0.6 mg/injection for a total dose of approximately 44 mg/kg/day. Of note is a calculation for the daily dose on an mg/kg basis; the magnitude of the self-administration dose was originally underreported as only 2.6 mg/kg/day. In addition, cocaine is delivered over a much longer time period (4 h/day) during self-administration, relative to the present experiment [one bolus injection/day with a $T1/2_{\beta}$ of 12 min (3)]. Therefore, animals are exposed to cocaine daily for a sustained time period during self-administration regimens.

Additionally, self-administering animals have exteriorized IV catheters, in contrast to the completely self-contained injection port system used in the current study (22). Thus, given the greater dose, the slower rate of delivery, and the longer period of exposure, it is not surprising that self-administration of cocaine might lead to estrous cycle irregularities, whereas a single bolus dose of 3.0 mg/kg/day for 14 days does not lead to cycle disruption.

A recent study found that daily follicular-phase IV cocaine administration of 4 mg/kg disrupted menstrual and ovarian cyclicity in rhesus monkeys (27). Five of six and six of six saline-treated control monkeys displayed normal cycle length and ovulation, respectively. Comparatively, only one of seven cocaine-treated monkeys displayed normal cyclicity and ovulation. The monkeys in this study were tethered to administer the cocaine intravenously. It is unknown whether tethering interacts with cocaine to affect cyclicity, but the rats in the present study were not tethered and were freely moving for behavioral assessments. In fact, disruption of cyclicity and ovulation could also be attributed to the interaction of tethering with the effects of 4 mg/kg/day IV cocaine administration. Finally, it is possible that there are species differences in sensitivity to cycle disruption between rats and rhesus monkeys. This study, however, does not address the issues of the dose-dependent effects of cocaine in rhesus monkeys. The use of lower doses of IV cocaine may not disrupt estrous cyclicity in primates.

Previous studies have shown that repeated administration of cocaine produces behavioral sensitization in male rats (8,9,26). Our prior work reports that male rats sensitized to 0.5, 1.0, and 3.0 mg/kg daily IV cocaine injections in a dose-dependent manner (33). The present data demonstrate that female rats display clear, even robust, behavioral sensitization to repeated (14-day) IV cocaine administration. However, this initial study did not distinguish between the roles of psychological (conditioning) and neurological components in the behavioral expression of the neuroadaptations that occur with repeated drug administration. Nevertheless, the use of habituation exposure would be expected to confer latent inhibitory and/or blocking effects with respect to the classical conditioning of the test environment and drug experience cues. When considered in conjunction with the fact that the "pairing" of environmental cues and cocaine involved pairing with both environments (home cage and test environment on alternating days), it is most likely that any significant conditioning occurred with respect to the home cage rather than to the test environment. Given the long-standing recognition of the roles of psychological factors in the neuroadaptations that occur with repeated drug administration—originating with the pioneering studies of Mitchell (1,10) and the subsequent elegant studies of Siegel (31,32)—our future studies will employ a paradigm that specifically precludes the repeated pairing of cocaine injections with the test environment.

Pharmacokinetics and dosing methodology factors may also account, at least in part, for some reports of behavioral sensitization to cocaine. Recent studies have shown that repeated IP dosing causes an increase in brain cocaine levels as much as 43%, whereas repeated IV dosing does not increase brain cocaine levels (23,24). This increase in brain cocaine can be attributed to absorption and distributional factors (24), and may account for the observed sensitization in cocaine-treated rats. The use of IV dosing eliminates absorption and distribution as pharmacokinetic factors that may influence behavioral sensitization (3). Therefore, IV dosing may be preferable to IP dosing in determining the neurobiological basis of behavioral sensitization.

Moreover, the neural basis of behavioral sensitization may depend on the route of cocaine administration. In male rats, withdrawal from IV binge cocaine produces a long-term decrease in dopamine transporter protein levels (25,30) and dopamine transporter mRNA levels (4). Interestingly, much higher doses of cocaine, when administered via the IP route, do not affect the density of dopamine transporters (2,21,25). Thus, it appears that the regulation of the dopamine transporter is dependent upon the route of cocaine administration, as well as hormonal status (7). However, interactions between cocaine and hormones following IV dosing remain to be explored.

In summary, the major finding of this study is the behavioral sensitization of female rats to 3.0 mg/kg/day IV cocaine without the disruption of estrous cyclicity. This finding is significant because it demonstrates the availability of an animal model that can be used to study the effects of cocaine on intact females without causing cycle disruption. Heretofore, using the SC or IP model of cocaine administration in rodents, it has not been possible to study behavioral sensitization in intact, cycling, female rats. Thus, this IV cocaine-dosing model may be particularly useful in exploring the gender-dependent effects of cocaine. The use of intact female rats allows study of the interaction between estrous cycle hormones (estrogen vs. progesterone) and drug sensitivity. It is possible that differing therapies based on stage of the hormonal cycle may be necessary to effectively treat women drug abusers.

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