Pharmacology Biochemistry and Behavior, Vol. 64, No. 4, pp. 827–839, 1999

0091-3057/99/$–see front matter

ELSEVIER

PII S0091-3057(99)00169-0

Behavioral Sensitization Following Repeated Intravenous Nicotine Administration: Gender Differences and Gonadal Hormones

ROSEMARIE M. BOOZE,*# MARIAN A. WELCH,# MARCIE L. WOOD,#
KATHARYN A. BILLINGS,¶ STEPHANIE R. APPLE¶ AND CHARLES F. MACTUTUS†‡§¶#

Department of Anatomy and Neurobiology, College of Medicine, †Division of Pharmaceutical Sciences, College of Pharmacy, ‡Tobacco and Health Research Institute, §Department of Psychology, ¶Department of Biology and #Graduate Center for Toxicology, University of Kentucky, Lexington, KY 40536-0084

Received 14 July 1999; Revised 14 July 1999; Accepted 14 July 1999

BOOZE, R. M., M. A. WELCH, M. L. WOOD, K. A. BILLINGS, S. R. APPLE AND C. F. MACTUTUS. *Behavioral sensitization following repeated intravenous nicotine administration: Gender differences and gonadal hormones.* PHARMACOL BIOCHEM BEHAV **64**(4) 827–839, 1999.—Repeated intermittent administration of stimulants is well known to produce behavioral sensitization in male animals. The present studies explored whether 1) behavioral sensitization occurred with the IV route of administration, 2) sensitization was greater in females than in males, 3) sensitization was modulated by gonadectomy, 4) intact adult female rats maintained normal estrous cytology patterns in response to repeated nicotine administration, and 5) the pharmacokinetics of IV nicotine dosing. Adult male, female, castrated, and ovariectomized Sprague–Dawley rats ($n = 48$) were surgically implanted with an intravenous access port. Animals received 50 μg/kg IV nicotine once/day for 14 days. Immediately after the initial nicotine injection and the final day 14 nicotine injection, animals were placed in IR photocell activity chambers for 60 min. Observational time sampling of behavior was also simultaneously performed by an observer blind to treatment condition. An increase in behavioral activity of greater than 120% occurred across the 14-day time course of IV nicotine injections. The magnitude of the increase, however, varied as a function of component of activity, gender, and gonadectomy. The behavioral observation data further suggested that the females demonstrated an increased sensitivity to repeated nicotine, as evidenced in a more rapid response, for example, grooming. These behavioral observations were associated with peak arterial levels of nicotine (~25 ng/ml) no greater than the average venous levels of nicotine commonly maintained by cigarette smokers. Repeated IV nicotine, at a dose of 50 μg/kg, did not interfere with intact female vaginal cytology or body weight; the failure to detect such alterations were not due to inadequate statistical power. Moreover, no nicotine-treated animals displayed persistent vaginal estrous or were acyclic. Collectively, these data suggest that the IV nicotine model may be particularly useful in exploring the gender-dependent effects of nicotine. © 1999 Elsevier Science Inc.

Tolerance Estrous cyclicity IV administration Rats

WOMEN tobacco smokers represent a major public health problem. Lung cancer, as a consequence of tobacco use, has surpassed breast cancer as the leading cause of cancer in US women (32). For U.S. teenagers, girls are now more likely than boys to have tried smoking a cigarette, and (overall) female teenagers are more likely than males to be smokers [for review, see (70)]. Robust genetic differences in the responses of different strains of mice to nicotine as reported by Collins and colleagues (21), strongly suggest that we should also consider the possibility of biologically based gender differences in response to nicotine. Nevertheless, most of the clinical treatment of tobacco smokers has focused on males, and the basic sciences research effort with nicotine has almost exclusively used male subjects.

Gender-dependent responses to nicotine have been little studied in experimental animal models. Nevertheless, there

Requests for reprints should be addressed to Rosemarie M. Booze, Ph.D., University of Kentucky College of Medicine, Department of Anatomy and Neurobiology, 800 Rose Street, Lexington, KY 40536-0084.

are a few reports in the literature indicating that female rats are differentially sensitive to the effects of nicotine. Nicotine has been found to stimulate locomotion more in female rats, relative to male rats (3). Rosecrans (61,62) also concluded that female rats are more chemically and behaviorally sensitive to nicotine, relative to males. In addition, numerous studies have found that nicotine affects body weight and eating behavior in female rats (30,31,49). Nicotine has greater effects in female rats, relative to males, in increased body weight following cessation of nicotine dosing (30,31). Thus, male and female rats have been found to be differentially sensitive to the effects of nicotine, with females displaying increased sensitivity to nicotine.

One mechanism that might be relevant to gender differences in sensitivity to nicotine would be gender-dependent differences in the pharmacokinetics and/or metabolism of nicotine. Male nonsmokers metabolize nicotine more quickly than do female nonsmokers (5). A similar gender difference has been reported for smokers (8). Injection of nicotine into male and female rats resulted in significantly higher levels of nicotine in the brains of female rats, relative to males (62). The metabolism and pharmacokinetics of nicotine are influenced by genetic (strain, gender) factors that can affect the induction status of nicotine metabolizing enzymes [for review, see (47)]. More recently, important sex differences were found for the urinary nicotine metabolite profile between male and female rats (64).

A primary goal for the development of an animal model of drug abuse is to select a route of administration that closely mimics the pharmacokinetics of the drug observed in humans. For stimulant drugs commonly abused by humans via smoking or IV injection, the often employed SC and PO routes of drug administration in rats consistently fail to mimic the rapidly peaking pharmacokinetic profile observed in humans (6,7,38,63). In marked contrast, the use of the IV route of administration in a rat model removes the process of drug absorption and provides near instantaneous distribution of nicotine through the vasculature, as well as 100% bioavailability of nicotine to the arterial side of the circulation.

Therefore, use of the IV route of nicotine administration in rats would be preferable due to the similar kinetic profile found in human studies with high arterial nicotine levels. Despite the fact that models for IV drug self-administration have been employed for a number of years, the general applicability of these models has been constrained by the use of chronic indwelling catheters that are exteriorized and tethered (73). A recent technical application is an SC implantable catheter as a port for the routine and repeated IV administration of drugs to group-housed rats (52). Using this implantable access port, locomotor activity can be evaluated in freely moving rats following IV drug injections [e.g., (71)].

Repeated intermittent administration of psychoactive stimulants such as nicotine and cocaine produces profound behavioral changes in humans as well as experimental animals (23,43,59,75,25,58). The augmentation of behavioral response following repeated nicotine administration (i.e., increased locomotor activity, stereotypic behaviors) has been referred to as "behavioral sensitization" or "reverse tolerance" (42). The development and expression of behavioral sensitization appears dependent on the dose of nicotine, 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 nicotine. Subsequent work in feeding behavior (31) and cognition (45) has found that female rats responded differentially to nicotine, relative to male rats. As the development of tolerance and/or sensitization has been reported following the repeated subcutaneous administration of nicotine to males [e.g., (46)], one purpose of the current study was to demonstrate that behavioral tolerance [e.g., (57)], and perhaps sensitization, would be observed following IV administration of nicotine.

One complicating factor in evaluating the effects of nicotine in female rats is the ability of repeating dosing with nicotine to interfere with the normal estrous cycle of the rat. Nicotine, when administered IP to female rats (8–10 mg/kg), has been shown to inhibit the proestrus surge of prolactin as well as the proestrus ovulatory surge of LH in the rat (11,12). Thus, administration of IP nicotine may interfere with experimental evaluations of intact female animals. The effect of nicotine administered via other routes on female rat estrous cyclicity is unknown.

Thus, in the current study we have explored 1) whether behavioral sensitization occurs with the IV route of administration, 2) whether sensitization is greater in females than in males, 3) whether sensitization is modulated by gonadectomy, 4) whether intact adult female rats maintained normal estrous cytology patterns in response to repeated nicotine administration, and 5) the pharmacokinetics of IV nicotine dosing. Collectively, these results will indicate whether IV dosing may be used to study the gender-dependent effects of nicotine in the brain.

METHOD

Animals

Adult male (M), female (F), castrated (CAST), and ovariectomized (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, 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 Institutional Animal Care and Use Committee (IACUC) of the University of Kentucky.

Catheter Surgical Procedure

An Intracath IV catheter (22 ga, Becton/Dickinson General Medical Corp., Grand Prarie, TX) was used as an SC dorsally implanted port for chronic IV injections for both the behavioral and pharmacokinetic studies. Implantation of catheters was performed as previously described (52). Briefly, rats were anesthetized using a mixture of ketamine hydrochloride and xylazine by 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, surgi-

cal, recovery, and postoperative records were maintained for each animal.

Experimental Design and Procedures—Behavioral Sensitization

The ovariectomized and castrate adults were given a 2-week recovery period to allow full depression of endogenous steroid levels. Rats were then anesthetized and implanted with the IV access port as described in our prior publications (14,52,71). Beginning 2 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 every day, at the same time (1000 h EST) thereafter throughout the experiment. An individual blind to both animal treatment group and cytology results of the previous day performed cytological assessments (16,18). 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 was 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 nicotine injections were initiated (50 μg/kg/ml). The nicotine injections were given during the afternoon (1500–1700 h EST), and were followed with a catheter flush of 0.2 ml of heparinized (2.5%) saline. One squad of rats was studied for the development of behavioral sensitization and potential disruption of estrous cyclicity, and the second squad of rats provided for pharmacokinetic analysis of arterial plasma levels of nicotine. The potential effects of IV nicotine on growth were assessed in both squads by daily measurement of body weights throughout the treatment period.

Animals in the first squad were habituated to the locomotor activity chambers for two 60-min sessions, one/day. A third activity test session was subsequently given and provided the measure of baseline activity, i.e., prior to any nicotine treatment. Activity monitors were 16-cm square, open-field chambers (Flex-Field, San Diego Instruments, San Diego, CA) that detected free movement of animals by infrared photocell interruptions. Total activity as well as rearing, centrally directed, 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. In addition to the automated monitoring, an observational time sampling procedure was employed. An observer, blind to the treatment condition of the animal, observed and recorded the animal's behavior, using a well-established protocol (26). 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 in the following categories: still, locomotion, rearing, head up sniff, head down sniff, head bobbing, swaying, biting, self-gnawing, licking, yawning, taffy pull, nonspecific mouth movements, and jaw tremor. The animals' locomotor activity response to nicotine was assessed on only two occasions: immediately after the initial nicotine injection (acute response) and immediately after the 14th injection. This latter procedure is critically important to preclude the repeated pairing of nicotine injection and the testing environment that otherwise confounds the neural expression of sensitization with learning via classical conditioning. Testing occurred between 1500–1700 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.

Drug Treatment

The nicotine 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 dose of nicotine bitartrate is calculated on the weight of the base and dissolved in saline for an injection volume of 1 ml/kg. Preliminary data demonstrated that an acute IV nicotine dose of 5, 10, or 30–300 μg/kg in adult male rats was behaviorally active producing a graded depression of locomotor activity of ~20, 40, and 75–80%.

Experimental Design and Procedures—Pharmacokinetics

Subsequent to the determination of behaviorally effective nicotine doses, and demonstrating that behavioral sensitization does occur with IV nicotine dosing over 14 days, we performed a pharmacokinetic study. The use of a conscious chronically catheterized model was chosen over an anesthetized preparation, as many common laboratory anesthetic agents may influence pharmacokinetic parameters as well as cytochrome P450 activity (33,34,48,51). All animals were surgically implanted with vascular catheters (as described above). All animals also had an arterial catheter implanted. Following at least 24 h recovery from surgery (33,34,39), the animals were randomly assigned to one of two groups (ns = 12) that received a daily IV injection of either saline or 50 μg/kg of nicotine for 13 days. On the 14th day, all animals received 50 μg/kg nicotine IV, thus forming an acute single injection group as well as a 14-day repeated injection group. The estrous status of the female animals was controlled by giving them up to four additional injections to allow blood sampling on a day of diestrus. Arterial withdrawals (400 μl) commenced immediately after injection and were withdrawn periodically thereafter (0.5, 1, 1.5, 2, 5, 10, 30, 60, 90, and 120 min after the initiation of nicotine injection). Thus, the earliest collection point was 30 s after the start of injection. The sampling intervals were chosen on the basis of previous reports (1,53) and the expected elimination half-life, as scaled from human data in the context of physiological time and metabolic rate (15,50,55). All samples were obtained with dry silanized syringes and transferred into dry silanized 1.5 ml microfuge tubes to prevent the absorption of nicotine, then centrifuged (2040 × g for 5 min), transferred to dry silanized tubes, and the plasma samples (200 μl) immediately frozen at −80°C until analysis. All animals were euthanatized after termination of the experiment.

GC/MS Methodology

In conjunction with Dr. Neil Benowitz and his colleagues, GC/MS analysis of the arterial plasma samples was performed using their published methodology (40). Initial analyses determined that HPLC did not have the sensitivity to detect the nicotine levels present after IV dosing. Thus, we then sought an initial comparison of the arterial plasma pharmacokinetics of nicotine between male and female animals (ns = 4–6) analyzed using GC/MS techniques (40). In brief, the extracted plasma samples were injected in a 0.2-μl volume onto a 30 m × 0.25 mm i.d. × 0.25 μm (film thickness) HP-5 MS column using helium as the carrier gas flowing at 1 ml/min (injection

temperature of 250°C, GC oven programmed from 70 to 280°C at 20°C/min). The GC effluent was ionized by electron bombardment at the standard 70 eV, and mass fragments separated by a quadropole filter and scanned from 70–360 mass units (electron multiplier set at 2200 V). A linear standard curve was generated based upon ion chromatograms for the parent 162 m/e ion and area response determination.

Pharmacokinetic Calculations

Initial curve fitting to a two-compartment open model was performed by derivative-free nonlinear regression with replicates ["repeated runs;" (24)] for the complete set of animals (Program AR, 13). This nonlinear regression analysis provided the parameters of $T_{1/2\alpha}$ and $T_{1/2\beta}$. Pharmacokinetic model parameters of AUC (0–∞) and AUMC (0–∞) were subsequently determined with the PKAnalyst program (MS Windows version of RSTRIP II, Micromath Inc, Salt Lake City, UT). Standard noncompartmental pharmacokinetic formulas were used to compute MRT (min), Vd_{ss} (1/kg),and Cl_{tot} (ml/min/kg) (27).

Data Analysis

Body weight, estrous cycle, and behavioral data were analyzed using analysis of variance (ANOVA) techniques (13,74), with test day, dependent measure, and/or time as repeated-measures factors. Violations of compound symmetry assumptions were either corrected with the Greenhouse-Geisser *df* correction factor (28) or precluded by the use of an orthogonal decomposition of the repeated-measure factor of interest (74). The distribution of peripheral (single photocell interruption) and central (joint interruption of ⩾2 photocells) locomotor activity as well as rearing activity were also assessed. The SOLO power analysis module of the BMDP Statistical Package (13) was used to compute power of the statistical analysis to determine the sensitivity of the experiment to detect the effects of nicotine on specific dependent measures. Specific linear contrasts were also employed to evaluate specific comparisons of interest: 1) is there a gender difference in the normal activity profile or in response to acute or repeated IV nicotine? 2) Does castration alter the normal male activity profile or the response to IV nicotine? 3) Does ovariectomy alter the normal female activity profile or the response to IV nicotine? The data from the observational time sampling procedure was analyzed by nonparametric methods (65). An α level of $p \leqslant 0.05$ was the significance level for rejection of the null hypothesis.

RESULTS

Body Weight

Administration of 50 μg/kg/day IV nicotine over the 14-day test period did not significantly affect body weight (saline-treated controls vs. nicotine-treated animals) in the animals studied for the pharmacokinetic analysis of plasma nicotine (Table 1). Specifically, the data analysis failed to find a significant effect of nicotine treatment or an interaction of nicotine treatment by test day. Power analysis estimates for detecting an effect of nicotine on growth, i.e., a significant interaction of drug and time, based on the actual effect size seen in our data, was in excess of 0.80 (0.92). In the animals examined for the development of behavioral sensitization and disruption of estrous cyclicity (Table 2, males, CAST, females, OVX), in which all animals received nicotine injections, the rate of increase in body weight was greater for the gonadectomized, relative to the intact, rats. Specifically, there was a significant effect of gonadectomy that interacted with growth rate, $F(13, 520) < 4.1$, $p_{GG} \leqslant 0.012$, with a significant linear component, $F(1, 40) < 6.3$, $p_{GG} \leqslant 0.016$.

Vaginal Cytology

Estrous cycle length is shown in Table 3 as a function of number of estrous cycles during the repeated IV injections relative to control data from an experiment conducted 2 months previously in which female animals had received repeated daily IV saline. Two animals failed to cycle after surgery and thus were removed from the analysis. All nicotine-

TABLE 1

EFFECTS ON IV NICOTINE ON ADULT BODY WEIGHT (MEAN ± SEM IN GRAMS) OF ANIMALS INJECTED FOR PLASMA PHARMACOKINETIC ANALYSIS

	Males				Females			
Days	Nicotine		Saline		Nicotine		Saline	
B	270.0	5.6	274.7	6.2	246.8	1.9	253.3	4.6
1	290.5	2.0	296.5	3.4	251.7	2.1	256.0	3.9
2	294.0	2.5	299.7	3.1	253.5	2.3	257.7	4.0
3	297.3	1.8	304.8	2.6	255.3	2.0	257.5	3.9
4	302.0	1.5	308.5	2.4	256.3	2.5	259.3	3.2
5	307.5	2.6	313.2	2.0	256.5	2.6	261.3	3.4
6	309.2	3.3	316.8	2.5	257.8	2.4	261.8	3.4
7	311.3	2.9	318.2	3.4	259.7	2.6	261.5	3.8
8	314.5	4.8	323.0	2.8	262.5	3.6	264.0	3.8
9	317.3	4.7	324.2	2.6	262.8	3.4	263.8	3.4
10	318.3	5.6	326.3	2.1	267.7	2.7	267.2	3.6
11	318.3	5.7	327.8	2.2	265.0	3.0	265.0	3.6
12	319.2	7.4	330.2	1.6	265.0	2.2	265.3	3.6
13	320.7	6.6	329.3	2.6	263.3	3.5	266.2	4.0
14	324.3	7.1	332.8	2.5	266.3	3.2	267.8	4.2

TABLE 2

EFFECTS ON IV NICOTINE ON ADULT BODY WEIGHT (MEAN ± SEM IN GRAMS) OF ANIMALS STUDIED FOR BEHAVIORAL SENSITIZATION

	Males				Females			
Days	Normal		Cast		Normal		OVX	
B	357.4	3.1	367.0	4.3	240.5	1.7	260.3	2.8
1	356.8	3.5	376.8	3.3	249.3	3.3	278.2	3.7
2	356.9	4.2	377.3	3.6	248.2	3.6	277.0	3.5
3	357.7	4.2	377.0	3.9	248.2	3.2	278.6	3.6
4	358.7	4.5	378.0	3.7	249.0	3.0	280.3	3.5
5	359.3	4.5	380.0	3.4	251.3	2.7	281.8	3.6
6	360.8	4.0	382.5	3.0	251.5	3.0	281.3	3.5
7	361.2	4.2	383.8	3.4	252.8	3.2	285.4	3.4
8	362.8	4.1	385.8	3.1	255.5	3.6	286.0	3.4
9	365.1	4.7	388.8	4.0	259.8	3.3	288.3	4.0
10	364.4	4.6	389.8	3.6	259.2	3.9	289.8	3.7
11	366.2	5.0	391.3	4.1	259.9	4.0	291.8	3.9
12	367.5	5.1	393.6	4.1	260.0	4.0	293.1	4.2
13	367.4	5.4	394.3	4.1	261.8	4.0	295.1	4.3
14	369.9	5.2	396.5	4.1	261.6	3.8	296.6	4.4

treated animals maintained the normal pattern of estrous cyclicity as determined by daily vaginal lavage; no nicotine-treated animals displayed persistent vaginal estrous or were acyclic. Of particular note, one of the two animals that failed to cycle after surgery began to cycle regularly after several days of nicotine treatment. Statistical analysis failed to find any evidence of alterations in vaginal cytology despite over 14 days of IV treatment, $F(1, 16) < 1.0$; treatment × time, $F(3, 48) < 1.0$, $p_{GG} \leqslant 0.69$).

Power analysis estimates (20) for a large effect size (0.6), as would be expected based on the above-cited literature on nicotine-induced disruption of LH (10-fold decrease) and PRL (10-fold decrease) secretion (11,12) were in excess of 0.95 for the repeated-measures interaction term. For the actual effect sizes seen in our data, the power to detect the development of drug-induced changes, i.e., a significant interaction of drug and time, was in excess of 0.80 (0.86). Although preliminary in the sense that we did not have a contemporaneous saline control, the data nevertheless suggest that if IV nicotine had a "true" effect on vaginal cytology, it should have been detected.

Locomotor Activity

Analysis of the automated behavioral activity data indicated significant main effects with females more active than males [gender, $F(1, 40) = 15.4$, $p \leqslant 0.001$], activity increasing as a function of nicotine injection [test day, $F(2,80) = 75.5$, $p \leqslant 0.001$], differences among the components of activity, $F(2, 80) = 1426.4$, $p \leqslant 0.001$, and habituation of activity across time, $F(5, 200) = 365.9$, $p \leqslant 0.001$. Also noted were significant interactions of gender with gonadectomy, $F(1, 40) = 4.2$, $p \leqslant 0.048$, gender with a dependent measure, $F(2, 80) = 34.8$, $p_{GG} \leqslant 0.001$, test day with gonadectomy, $F(2, 80) = 3.9$, $p_{GG} \leqslant 0.024$, marked by a quadratic test day component, $F(1, 40) = 6.2$, $p \leqslant 0.017$, test day (quadratic) with dependent measure (linear) and gonadectomy, $F(1, 40) = 8.0$, $p < 0.007$, and a dependent measure (linear) with gender and gonadectomy, $F(1, 40) = 4.6$, $p \leqslant 0.039$. Given the complexity and number of interactions observed as well as the planned comparisons sought, tests of simple effects were subsequently conducted on each dependent measure for each of the three test days (baseline, acute nicotine, repeated nicotine).

Baseline activity. For the habituated pretreatment baseline (Fig. 1, 2, and 3, left panel), although the female rats were generally more active than males, $F(1, 40) = 7.1$, $p \leqslant 0.011$, this effect was qualified by an interaction of gender with a dependent measure, $F(2, 80) = 18.8$, $p_{GG} \leqslant 0.001$. The gender difference varied (F ⩾ M) across the dependent measure for the intact animals, $F(2, 80) = 10.8$, $p_{GG} \leqslant 0.001$, and also varied (F =, <, > M) for the gonadectomized animals, $F(2, 80) = 8.4$, $p_{GG} \leqslant 0.003$. More specifically, a significant gender difference (F > M) was evident on the dependent measures of rearing and peripheral directed activity, $F(1, 40) = 4.1$, $p \leqslant 0.049$, $F(1, 40) = 14.7$, $p \leqslant 0.001$, but not on central directed activity. The gender effect on rearing (F > M) was present in the intact animals, $F(1, 40) = 5.5$, $p \leqslant 0.024$, but gonadectomy reduced this effect. Similarly, the gender effect on peripheral directed activity (F > M) was present in the intact animals, $F(1, 40) = 10.7$, $p \leqslant 0.002$, but was markedly reduced in the gonadectomized animals, however, the gender effect remained present, $F(1, 40) = 4.5$, $p \leqslant 0.041$. For centrally directed activity the significant main effect was of gonadectomy, $F(1, 40) = 6.1$, $p \leqslant 0.018$, with a pronounced effect on castration, $F(1, 40) = 9.5$, $p \leqslant 0.004$, but not of ovariectomy, $F(1, 40) < 1.0$. In sum, the profile of habituated baseline activity revealed gender differences (F >M) on two of the three dependent measures, with gonadectomy reducing the expression of this gender difference in both cases.

Acute effects of nicotine. The acute response to 50 μg/kg nicotine, delivered as an IV bolus, is illustrated in the center panel of Fig. 1, 2, and 2. There was an overall significant main effect of gender with the female rats generally more active than males, $F(1, 40) = 7.5$, $p \leqslant 0.009$, as well as an interaction of gender with a dependent measure, $F(2, 80) = 11.1$, $p_{GG} \leqslant 0.001$. Again, the gender difference (F > M) varied across the dependent measure for the intact animals, $F(2, 80) = 7.9$, $p_{GG} \leqslant 0.003$, as well as for the gonadectomized animals, $F(2, 80) = 3.7$, $p_{GG} \leqslant 0.047$. More specifically, a significant gender difference (F > M) was evident on the dependent measure of

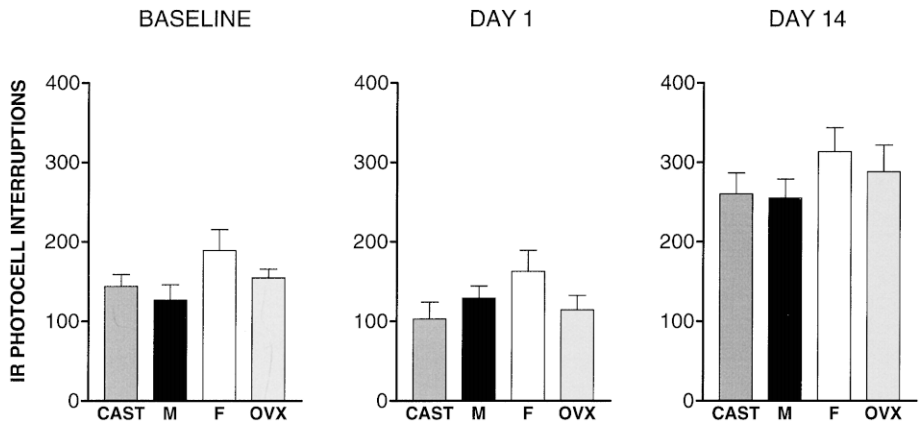

FIG. 1. Rearing behavior during a 60-min session, as a function of gender and gonadectomy, is shown under baseline conditions, following an acute IV injection of nicotine, and following the 14th IV injection of nicotine. All animals were previously habituated to the test environment for two prior sessions.

peripherally directed activity, $F(1, 40) = 11.6$, $p \leq 0.002$, but not on rearing or centrally directed activity. The gender effect on peripherally directed activity (F > M by 39%) was present in the intact animals, $F(1, 40) = 9.1$, $p \leq 0.004$, but was not markedly reduced in the gonadectomized animals such that the gender effect was not statistically significant. For centrally directed activity, although there was no significant gender effect present, ovariectomy reduced this component of activity relative to the intact females, $F(1, 40) = 4.3$, $p \leq 0.046$. In sum, the profile of activity following acute nicotine injection revealed a gender difference (F > M) on only the peripherally directed component of activity, with gonadectomy reducing the expression of this gender difference.

Explicit comparison of the activity profile following acute nicotine injection, relative to the baseline profile, indicated no main effect of nicotine treatment, but that nicotine treatment interacted with gonadectomy, $F(1, 40) = 8.1$, $p_{GG} \leq 0.007$, and that nicotine treatment also interacted with a dependent measure and gonadectomy, $F(2, 80) = 4.0$, $p_{GG} \leq 0.035$. Specifically, acute nicotine treatment produced an overall depression (18%) of rearing, $F(1, 40) = 8.6$, $p \leq 0.006$, that did not interact with gender or gonadectomy. Although nicotine had no overall significant effect on centrally or peripherally directed activity, both of these components of activity were altered as a function of gonadectomy, $F(1, 40) = 9.8$, $p \leq 0.003$; $F(1, 40) = 5.1$, $p \leq 0.03$, respectively. Nicotine primarily stimulated centrally directed activity of the intact animals by 25%, while depressing the centrally directed activity of the gonadectomized animals by 13%. Nicotine primarily depressed the peripherally directed activity of the gonadectomized animals by 11%, while stimulating peripherally directed activity of the intact animals by 6%.

An analysis of covariance was subsequently employed to evaluate the activity profile following acute nicotine injection independent of baseline behavioral differences. After removing the variance attributable to baseline differences, $F(1, 39) = 6.1$, $p \leq 0.018$, the behavioral response to acute nicotine was not significantly different between male and female rats, $F(1,$

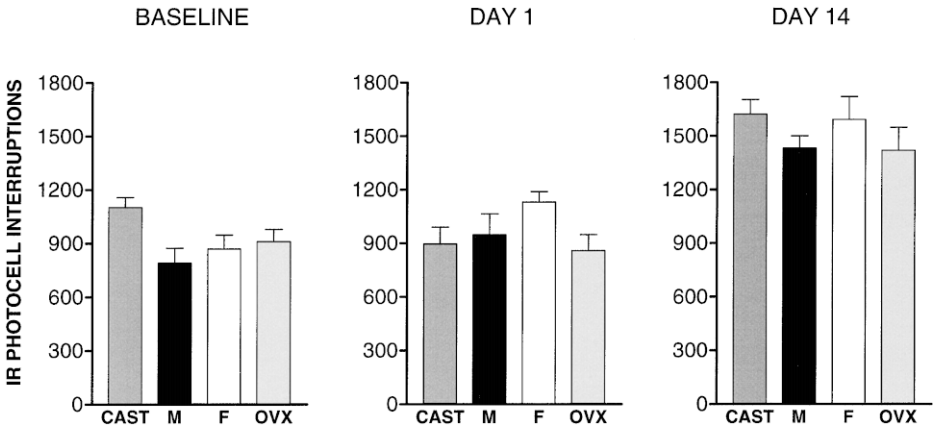

FIG. 2. Central directed locomotor activity during a 60-min session, as a function of gender and gonadectomy, is shown under baseline conditions, following an acute IV injection of nicotine, and following the 14th injection of nicotine (50 μg/kg, 1/day for 14 days). Animals were placed in the chamber immediately after the bolus IV injection. The repeated nicotine injections were explicitly unpaired with the test chambers, i.e., nicotine injections on days 2–13 were conducted in the colony.

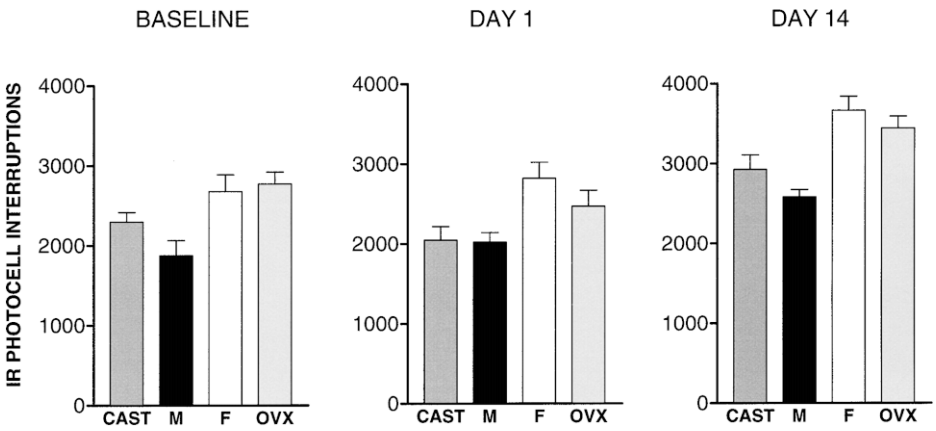

FIG. 3. Peripheral directed locomotor activity during a 60-min session, as a function of gender and gonadectomy, is shown under baseline conditions, following an acute IV injection of nicotine, and following the 14th IV injection of nicotine (50 μg/kg, 1/day for 14 days). Animals were placed in the chamber immediately after the bolus IV injection. The repeated nicotine injections were explicitly unpaired with the test chambers, i.e., nicotine injections on days 2–13 were conducted in the colony.

39) = 2.4, $p \leqslant 0.095$, but was reduced in gonadectomized relative to intact rats, $F(1, 39) = 4.5$, $p \leqslant 0.041$. This effect of gonadectomy was attributable to a significant effect of ovariectomy, $F(1, 39) = 4.3$, $p \leqslant 0.045$, but not of castration, $F(1, 39) < 1.0$.

Repeated effects of nicotine. The response to 14 repeated IV bolus injections of 50 μg/kg nicotine, delivered 1/day, is shown in the right panels of Figs. 1, 2, and 3. There was an overall significant main effect of gender, with the female rats generally more active than males, $F(1, 40) = 11.5$, $p \leqslant 0.002$, as well as an interaction of gender with a dependent measure, $F(2, 80) = 24.2$, $p_{GG} \leqslant 0.001$; the gender difference (F > M) varied across the dependent measure for the intact animals, $F(2, 80) = 16.9$, $p_{GG} \leqslant 0.001$, as well as for the gonadectomized animals, $F(2, 80) = 8.6$, $p_{GG} \leqslant 0.001$. More specifically, a significant gender difference (F > M) was evident on the dependent measure of peripherally directed activity, $F(1, 40) = 26.1$, $p \leqslant 0.001$, but not on rearing or centrally directed activity. The gender effect on peripherally directed activity (F > M by 42%) was present in the intact animals, $F(1, 40) = 21.9$, $p \leqslant 0.001$, and although reduced in the gonadectomized animals, $F(1, 40) = 3.2$, $p \leqslant 0.079$, a gender effect (F > M by 18%) remained present, $F(1, 40) = 6.0$, $p \leqslant 0.019$. In sum, the profile of activity following repeated nicotine injection revealed a gender difference on only the peripherally-directed component of activity, with gonadectomy reducing the expression of this gender difference.

Explicit comparison of the activity profile following repeated nicotine injection, relative to that following acute nicotine injection, indicated a main effect of nicotine with robust behavioral sensitization across the 14 days of treatment, $F(1, 40) = 114.6$, $p \leqslant 0.001$, and that the various measures of activity were differentially sensitive to nicotine sensitization, $F(2, 80) = 31.7$, $p_{GG} \leqslant 0.001$, rearing 222%↑, central directed 160%↑, peripheral directed, 135%↑. A main effect of gender with female rats generally more active than males was also present, $F(1, 40) = 13.8$, $p \leqslant 0.001$, with this gender effect pronounced in the intact, $F(1, 40) = 14.5$, $p \leqslant 0.001$, but not present in the gonadectomized rats. However, the failure to find an interaction of gender with nicotine treatment suggested that there was no gender difference in the expression of sensitization. That the overall gender difference in activity (F > M) may be modulated by gonadal hormones was suggested by an interaction of gender and gonadectomy that approached statistical significance, $F(1, 40) = 3.7$, $p \leqslant 0.062$. Although the three-way interaction with nicotine was not significant, the gender by gonadectomy interaction was suggested in the behavioral response to repeated nicotine treatment, $F(1, 40) = 3.9$, $p \leqslant 0.055$, but not acute nicotine treatment. Of the various activity components, only peripheral directed activity displayed and maintained a significant gender effect, $F(1, 40) = 29.1$, $p \leqslant 0.001$, and the suggestion of an interaction with gonadectomy animals, $F(1, 40) = 3.3$, $p \leqslant 0.079$; however, there was again no three-way interaction with nicotine treatment.

An analysis of covariance was subsequently employed to evaluate the activity profile following repeated nicotine injection independent of the acute response to nicotine. After removing the variance attributable to the acute response to nicotine, $F(1, 39) = 5.5$, $p \leqslant 0.024$, the behavioral response to repeated nicotine was significantly greater in female relative to male rats, $F(1, 39) = 5.5$, $p \leqslant 0.024$. Although the gender by gonadectomy interaction was not statistically significant, the gender difference (F > M) was found in the intact, $F(1, 39) = 6.9$, $p \leqslant 0.012$, but not in the gonadectomized, rats, $F(1, 39) < 1.0$. Further, this gender difference in the intact rats varied as a function of dependent measure, $F(1, 39) = 10.6$, $p \leqslant 0.001$, and was localized primarily to peripheral directed activity, $F(1, 39) = 15.2$, $p \leqslant 0.001$.

Observational Time Sampling of Behavior

To examine more specific components of this sensitized behavior, the data from the observational time sampling technique were examined. Figures 4 (D1 vs. D14 total response) and 5 (within-day time course) illustrates two of the behaviors that displayed the behavioral sensitization response via visual observation, rearing, and grooming. There were no significant alterations in either behavior within the test session as a function of gender/gonadectomy in the animals' acute response to nicotine (left panels of Fig. 5). However, the rearing response on day 14 demonstrated significant sensitization relative to day 1[Fig. 4, top panel, $\chi^2(1) = 8.8$, $p \leqslant 0.003$] and also displayed significant variation within the test session, $\chi^2(6) =$

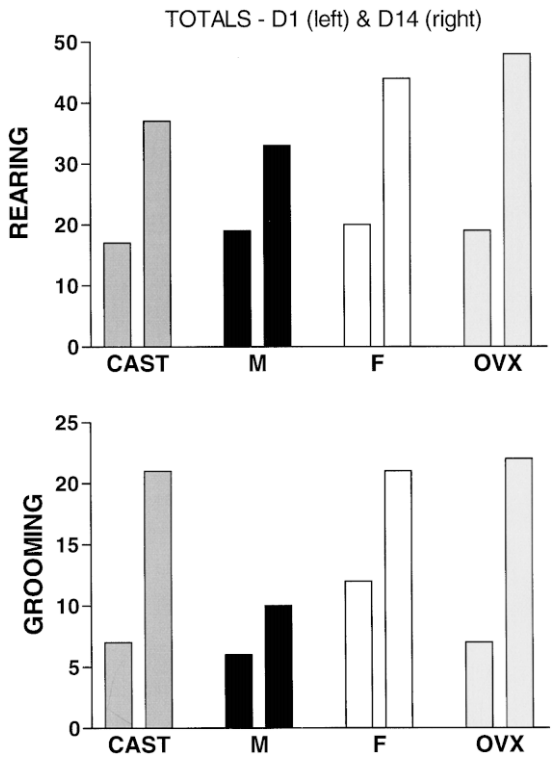

FIG. 4. The total rearing and grooming response to acute (left bars) and repeated (right bars) IV nicotine dosing (50 μg/kg 1/day for 1 or 14 days) is illustrated for the observational time sampling of behavior as a function of gender and gonadectomy nicotine. Behavioral sensitization was clearly noted after the 14th nicotine injection in both the male and female rats, and there was a significantly greater increase in the nicotine-treated females.

31.9, $p \leq 0.001$. Gender modulated the rearing response to repeated nicotine, $\chi^2(6) = 13.2$, $p \leq 0.003$, with females displaying a greater response throughout the test session. The grooming response on day 14 also displayed significant sensitization relative to day 1 [130%↑, $\chi^2(1) = 14.8$, $p \leq 0.001$], as well as significant modulation by gender/gonadectomy, $\chi^2(1) = 4.5$, $p \leq 0.034$; the intact males displayed the least grooming (Fig. 4, bottom panel). Significant variation as a function of gender/gonadectomy was also noted in grooming within the day 14 test session, $\chi^2(6) = 28.7$, $p \leq 0.001$; the greater and more rapid response of the females relative to the intact males was readily apparent. Locomotion (data not shown) also displayed a significantly greater response as a function of gender with females displaying a greater response across the test session, $\chi^2(6) = 38.8$, $p \leq 0.001$.

Nicotine Pharmacokinetics

As is evident in Fig. 6, the IV administration of nicotine with carotid artery sampling initiated immediately after termination of drug injection, produced a pronounced but transient increase in plasma concentrations (~25 ng/ml). The peak arterial concentrations of nicotine were obtained in all animals within 2 min after the start of the 30-s bolus injection. The disappearance of nicotine from plasma was biexponential, with distribution and elimination half-life (mean ± standard error) of 5 and 50 min, respectively. As derivative-free nonlinear regression found no significant lack of fit for an analysis based on replicates, $F(6, 89) < 1.0$, the curve fitting and noncompartmental analysis were accordingly determined across the entire set of animals. The trapezoidal AUC (T_0–T_{120}) represented 85% of the AUC (T_0–∞) and, thus, suggested that both our sampling intervals were appropriate, and extrapolation to infinity did not compromise our calculations. The pharmacokinetic parameters for the bolus IV injection of nicotine are shown in Table 4.

DISCUSSION

The present study found, first, that rats display clear behavioral sensitization to repeated (50 μg/kg, 14 days × 1/day) IV nicotine administration. Rats treated with nicotine displayed a robust increase in their behavioral response from day 1 to day 14, despite experiencing transient peak arterial levels of nicotine no greater than the average venous level of nicotine maintained by cigarette smokers (see pharmacokinetics below). Second, the magnitude of behavioral sensitization observed varies as a function of the component of activity measured, gender of the animal, gonadectomy, and whether a gross (automated) or fine grained (observational) analysis of behavior was conducted. Third, repeated IV nicotine administration does not disrupt body weight gain of male and female rats, nor the estrous cycle of the female rats. All nicotine-treated animals displayed normal estrous cyclicity over 14 days of IV nicotine treatment, as determine by daily vaginal lavage. And finally, these data report that administration of IV nicotine produces a transient rapidly peaking pharmacokinetic profile typical of drugs administered via the IV route.

Activity and Behavioral Sensitization

In these studies we have replicated the widely reported gender differences in locomotor activity. Specifically, female rats have most often been reported to have increased general locomotor activity, relative to males (4,17,22,60,69,72). In our IR photocell chambers, females demonstrated greater rearing activity than males. This sex difference was markedly reduced by ovariectomizing the female animals, suggesting a role for gonadal hormones, possibly estrogen, in mediating this behavior. Our automated locomotor activity chambers distinguish between central activity and activity localized to the periphery of the chamber. We have previously found that central and peripheral activity is differentially responsive to the effects of psychostimulants (71), and may represent different neural processes. In terms of baseline behavior, the central activity counts were not different between males and females, but castrated animals showed significantly higher levels of central activity. Peripheral activity was higher in females, relative to males, with gonadectomy reducing the magnitude of this effect. Thus, it appears that central and peripheral measures of activity may also be sensitive to hormonal effects.

Acute (day 1) administration of nicotine produced no overall depression in locomotor activity, but did selectively depress rearing behavior. The effect of acute nicotine on centrally and peripherally directed activity varied as a function of gonadectomy, but not gender. Specifically, in the presence of gonadal hormones acute nicotine stimulated locomotor activity, whereas in the absence of gonadal hormones nicotine depressed activity. One factor that likely contributed to the general lack of an initial depressant effect of nicotine, as often found in other studies with male rats (19,66), is that all ani-

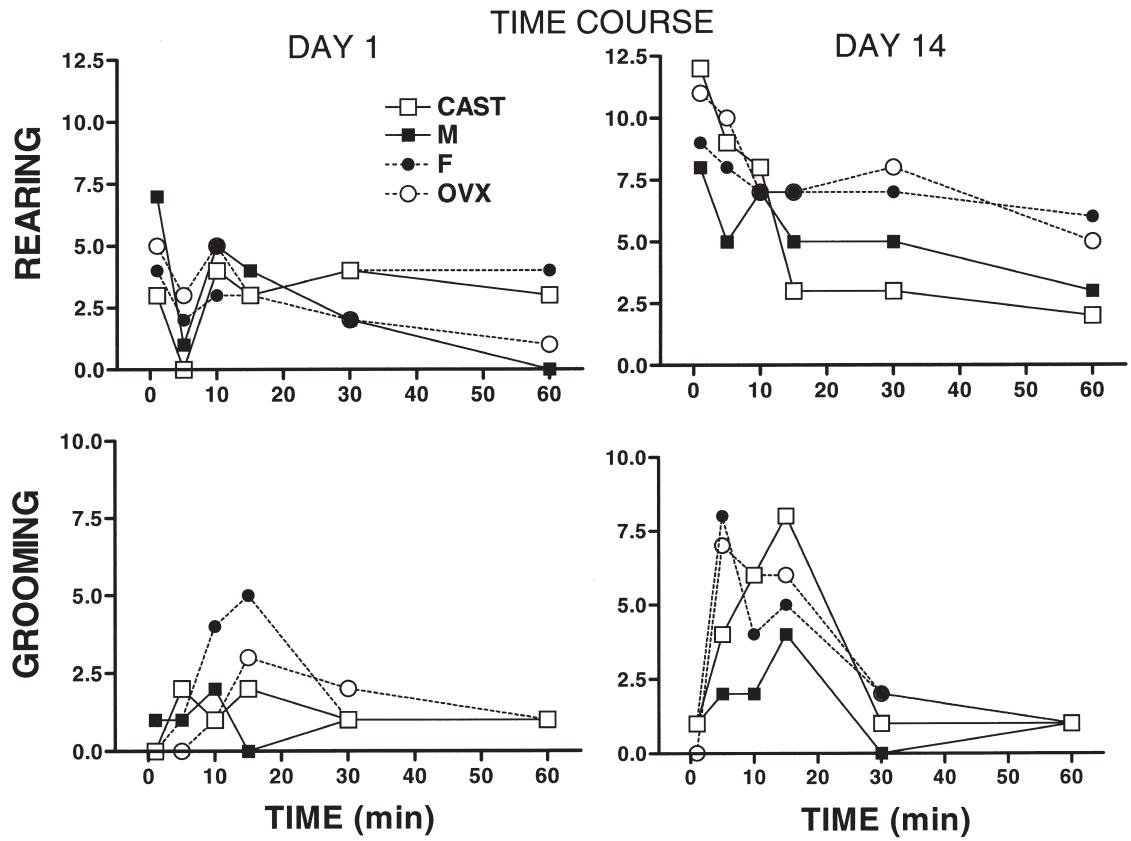

FIG. 5. The time course of the rearing and grooming response to acute (left panels) and repeated (right panels) IV nicotine dosing (50 μg/kg 1/day for 14 days) is illustrated for the observational time sampling of behavior as a function of gender and gonadectomy. nicotine. Gender modulated the rearing response to repeated nicotine with females displaying a greater response throughout the test session. With respect to sensitization of grooming behavior, the greater and more rapid response of the females relative to the intact males was readily apparent.

mals were well habituated to the test environment prior to receiving any nicotine.

With repeated administration of nicotine, the locomotor activating effect of nicotine becomes progressively greater, a phenomenon that may be related to the sensitization to other stimulants such as cocaine (41). Thus, after chronic exposure to nicotine, further doses of nicotine produce a substantial increase in locomotor activity (19,46,54). Our behavioral testing on day 14 confirms this finding using our IV dosing model. All groups of animals demonstrated a significant increase in each of the three components of activity (rearing, centrally directed, and peripherally directed) following 14 days of nicotine injection. Gonadectomy does play some role in the expression of behavioral sensitization, particularly in peripheral activity. Castration increased activity relative to intact males and ovariectomy decreased activity relative to intact females.

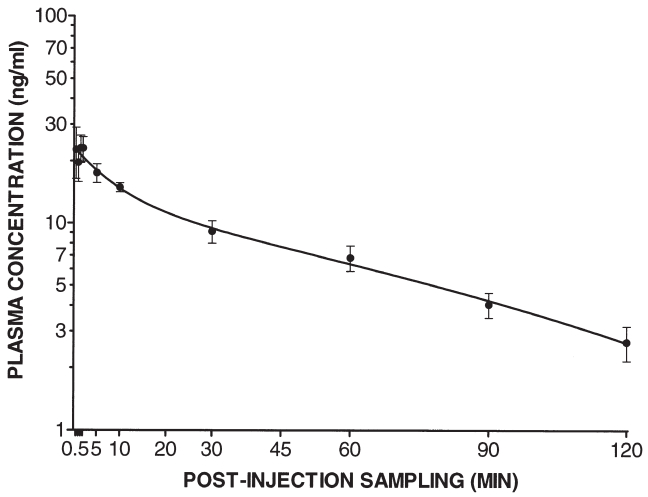

FIG. 6. The time vs. concentration curve for arterial plasma levels of nicotine (n = 10). An approximately equal number of male and female rats that had received either acute or repeated IV nicotine (50 μg/kg) injection were included.

To examine specific components of this peripheral behavior, we used an observational time-sampling technique. In this more fine-grained analysis of behavior, we observed the importance of gonadal hormones in nicotine sensitization. Castrates and ovariectomized animals display more rearing initially and a delayed appearance of grooming behavior in response to repeated nicotine. These differences in time course may result from hormonal modulation of brain "substrates" (2) or alternatively, from hormonally mediated differences in nicotine pharmacokinetics (47,64). The failure of others to detect robust effects of gender and gonadal hormones on nicotine induced changes in behavioral activity (44) may be related to any number of differences, for example, route of administration (SC vs. IV), low statistical power (ns = 6 vs. ns = 10–12), and/or level of behavioral analysis (automated photocells vs. behavioral observation).

TABLE 3

EFFECTS OF IV NICOTINE ON ADULT FEMALE ESTROUS CYCLICITY (MEAN ± SEM) OF ANIMALS MONITORED FOR THE DEVELOPMENT OF BEHAVIORAL SENSITIZATION

Cycle	Nicotine		Saline	
B	4.0	0	4.1	0.2
1	4.7	0.2	4.7	0.4
2	4.2	0.2	4.6	0.3
3	4.2	0.1	4.1	0.2

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 despite over 14 days of IV treatment, $F(1 / 16) < 1.0$; treatment × time, $F(3, 48) < 1.0$, $p_{GG} \leq 0.69$. All nicotine-treated animals maintained the normal pattern of estrous cyclicity as determined by daily vaginal lavage. In particular, no nicotine-treated animals displayed persistent vaginal estrus or were acyclic.

TABLE 4

PHARMACOKINETIC ANALYSIS SUMMARY FOR IV NICOTINE

Parameter	50 μg/kg/ml
n	10
Body weight (g)	297.8 ± 6.8
Pseudo r^2	0.99
$T_1/2\alpha$ (min)	5.1 ± 1.1
$T_1/2\beta$ (min)	49.7 ± 1.6
AUC (0–∞)	1096
AUMC (0–∞)	74411
MRT (min)	67.9
CL_{tot} (ml/min/kg)	45.6
Vd_{ss} (1/kg)	3.1

The lack of fit ANOVA based on replicates failed to achieve statistical significance, $F(6, 89) < 1.0$. All data are means; estimates of variability are SEM.

Estrous cyclicity. Sprague–Dawley female rats continue to cycle during repeated IV nicotine administration, as indicated by normal vaginal cytology. Although a disruption of estrous cytology may be observed at higher doses of SC nicotine, 14 daily injections of 50 μg/kg IV nicotine, one/day, did not induce persistent estrus in any animals, prolong diestrus, nor increase the length of the estrous cycle. Although preliminary in the sense that we did not have a contemporaneous saline control, the data nevertheless suggested that if IV nicotine had a "true" effect on estrous cyclicity, it should have been detected based on our power analyses. Moreover, the lack of effect of IV nicotine on bodyweight suggests that loss of bodyweight will not be a confounding factor in destabilizing estrous cyclicity in intact female animals. However, our observation of intact vaginal cytology does not address the issue of whether the female animals are, in fact, ovulating while being treated with nicotine. Further detailed studies using time sampling of hormone levels are necessary to address whether IV nicotine has more subtle, but nevertheless biologically significant, effects on endocrine parameters in females.

Pharmacokinetics. The most advantageous route of administration for animal models of nicotine abuse would be the one that closely mimics the pharmacokinetics of nicotine observed in humans. Cigarette smoking delivers nicotine in doses that produce rapid increase in plasma nicotine concentration. The levels of nicotine in the arterial circulation may be 10-fold greater than levels in the venous blood (6,7,38). The distinction between arterial and venous sampling is crucial in that arterial levels reflect the peak levels delivered to the brain following IV nicotine injection or cigarette smoking. This rapid delivery of nicotine achieved via smoking appears to enhance the addictive effects of nicotine (6,7,37). Nicotine delivered through cigarette smoking has a distribution half-life of 10–20 min in plasma, followed by an elimination half-life of 2–3 h (9,10). In chronic smokers, nicotine plasma levels reach a peak of ~40 ng/ml in the afternoons and decline to 5–10 ng/ml overnight (10). Nicotine administered by other delivery systems (i.e., oral or dermal) also has an elimination half-life of 2 h, and can mimic the average plasma nicotine levels, but cannot achieve the rapid and large increase in arterial nicotine levels associated with cigarette smoking and addiction. In addition, following oral administration of nicotine, about 70% of the absorbed nicotine is metabolized via first pass liver metabolism (68), further decreasing the nicotine levels delivered to the brain.

The disappearance of nicotine from plasma was biexponential with distribution and elimination half-life (mean ± standard error) of 5 and 50 min, respectively. The Vd_{ss} was large and consistent with the evidence for the widespread presence of nicotine in many tissues and organs [e.g., 35,67)]. The rate of Cl_{tot} is known to be dose dependent in the rate of 0.08 to 0.8 mg/kg (53); our observed value is similar to that observed at the low end of this range (45.6 vs. 42.2 ml/min/kg).

A primary goal for the development of an animal model of drug abuse is to select a route of administration that closely mimics the pharmacokinetics of the drug observed in humans. For stimulant drugs, such as nicotine, cocaine, and amphetamine, commonly abused by humans via smoking or IV injection, the often employed SC and PO routes of drug administration in rats consistently fail to mimic the rapidly peaking pharmacokinetic profile observed in humans. The bioavailability of nicotine administered by any non-IV route is a complex function of many factors that influence absorption and distribution processes. In marked contrast, the IV route of administration removes the process of absorption and provides near instantaneous distribution of nicotine through the vasculature. Although the rate of elimination as well as the rate of administration remain as factors that may alter the plasma nicotine pharmacokinetic profile, removing the influence of the absorptive process via IV administration guarantees 100% bioavailability of nicotine to the arterial side of the circulation. Accordingly, the use of an IV rodent model removes any variability due to absorptive processes.

The IV model of nicotine in unanesthetized, freely moving rats clearly produces a rapidly peaking pharmacokinetic profile characteristic of that route of administration in humans (6,7,36,63). Thus, the rat IV bolus injection model offers the ability to reproduce characteristics of human tobacco use not available via SC or PO rodent models. Furthermore, the peak arterial plasma levels provide a clinically relevant exposure; nicotine plasma levels of chronic smokers reach a peak of ~40 ng/ml in the afternoon, and decline to 5–10 ng/ml overnight (8,10). The pharmacokinetic parameters values obtained are also consistent with previously established values in male rats with methods that have used the quantification of

^{14}C-labeled nicotine (1,53). Our initial data of comparable peak arterial nicotine concentrations in the male and female rat following an IV nicotine bolus suggest that differential nicotine kinetics will not likely account for gender differences in nicotine responsiveness. The use of IV dosing method eliminates certain pharmacokinetic factors that may influence behavioral sensitization. Therefore, the IV dosing method is preferable to the IP dosing method in determining the neurobiological basis of behavioral sensitization.

SUMMARY

In summary, the major findings of this study are: 1) behavioral sensitization occurs following IV dosing with nicotine at a dose of 50 μg/kg/day, 2) females may display greater sensitization than males (at least by this route of administration), and 3) this dose level of nicotine dose not disrupt estrous cyclicity, as indicated by evaluations of vaginal cytology. These findings are significant because they demonstrate the availability of a low-dose nicotine animal model that may be used to study the effects of nicotine in intact female animals. Additionally, using time-sampling methods for assaying behavior, a role for androgens was apparent in the nicotine response of male animals. Thus, it will be possible to determine which gender-dependent behaviors are modified by gonadectomy and identify gender-dependent behaviors that are insensitive to loss of gonadal hormones in adulthood. Specifically, we can critically evaluate whether gonadectomized and hormone-replaced animals represent the full range of gender differences in behavioral responses to drugs. In the current study, the presence of gender differences in nicotine responsiveness that are not modulated by ovariectomy suggest that not all gender differences in drug sensitization are due to the direct effects of gonadal hormones. Thus, the experimental strategy of studying ovariectomized females with hormone replacement in assessing gender differences in drug response must be approached with caution.

These possibilities will be more directly addressed in further studies using hormone replacement strategies and perinatal modulation of brain development in this rodent model of nicotine use. In humans, it appears that smokers display gender-dependent responses to nicotine, in that men self-administer more of a nicotine nasal spray (56), whereas women may have greater difficulty in smoking cessation (29). Determining the involvement of gonadal hormones and brain adaptations to nicotine responses may, therefore, be important in developing therapeutically effective cessation strategies.

ACKNOWLEDGEMENTS

This work was supported by the National Institutes of Health (DA09160 and ES06259, C.F.M.; DA11337, R.M.B.), the UK Medical Center Women's Health Initiative, and the Tobacco and Health Research Institute (THRI) of the University of Kentucky, Lexington, KY. THRI is an administrative unit of the University of Kentucky, and is not affiliated with the Tobacco Research Council, nor does it receive any financial support from the Tobacco Institute or the tobacco industry. THRI does, however, receive support from the Commonwealth of Kentucky through a 0.5 cent tax/pack of cigarettes. A preliminary report of some of these findings was presented at the annual meeting of the International Behavioral Neuroscience Society, Nancy, France, June 22–26, 1999.

REFERENCES

1. Adir, J.; Miller, R. P.; Rotenberg, K. S.: Disposition of nicotine in the rat after intravenous administration. Res. Commun. Chem. Pathol. Pharmacol. 13:173–183; 1976.
2. Barber, P. V.; Arnold, A. G.; Evans, G.: Recurrent hormone dependent chorea: Effects of oestrogens and progestogens. Clin. Endocrinol. (Oxf.) 5:291–293; 1976.
3. Battig, K.: Smoking and the behavioral effects of nicotine. Trends Pharmacol. Sci. 2:145–147; 1981.
4. Beatty, W. W.: Gonadal hormones and sex differences in nonreproductive behaviors in rodents: Organizational and activational influences. Horm. Behav. 12:112–163; 1979.
5. Beckett, A. H.; Gorrod, J. W.; Jenner, P.: The effect of smoking on nicotine metabolism in vivo in man. J. Pharm. Pharmacol. 23:55S–61S; 1971.
6. Benowitz, N. L.: Pharmacokinetic considerations in understanding nicotine dependence. Ciba Found. Symp. 152:186–200; discussion 200–209; 1990.
7. Benowitz, N. L.: Clinical pharmacology of inhaled drugs of abuse: Implications in understanding nicotine dependence. NIDA Res. Monogr. 99:12–29; 1990.
8. Benowitz, N. L.; Jacob, P., III.: Daily intake of nicotine during cigarette smoking. Clin. Pharmacol. Ther. 234:153–155; 1984.
9. Benowitz, N. L.; Jacob, P., III,; Denaro, C.; Jenkins, R.: Stable isotope studies of nicotine kinetics and bioavailability. Clin. Pharmacol. Ther. 49:270–277; 1991.
10. Benowitz, N. L.; Kuyt, F.; Jacob, P., III.: Circadian blood nicotine concentrations during cigarette smoking. Clin. Pharmacol. Ther. 32:758–764; 1982.
11. Blake, C. A., Norman, R. L.; Scaramuzzi, R. J.; Sawyer, C. H.: Inhibition of the proestrous surge of prolactin in the rat by nicotine. Endocrinology 92:1334–1338; 1973.
12. Blake, C. A.; Scaramuzzi, R. J.; Norman, R. L.; Kanematsu, S.; Sawyer, C. H.: Effect of nicotine on the proestrous ovulatory surge of LH in the rat. Endocrinology 91:1253–1258; 1972.
13. BMDP Statistical Software: Berkley, CA: University of California Press; 1990.
14. Booze, R. M.; Lehner, A. F.; Wallace, D. R.; Welch, M. A.; Mactutus, C. F.: Dose-response cocaine pharmacokinetics and metabolite profile following intravenous administration and arterial sampling in unanesthetized rats. Neurotoxicol. Teratol. 19:7–15; 1997.
15. Boxenbaum, H.: Evolutionary biology, animal behavior, fourth dimensional space and the raison d'etre of drug metabolism and pharmacokinetics. Drug. Metab. Rev. 14:1057–1097; 1983.
16. Bridges, R. S.: A quantitative analysis of the roles of dosage, sequence, and duration of estradiol and progesterone exposure in the regulation of maternal behavior in the rat. Endocrinology 114:930–940; 1984.
17. Burke, A. W.; Broadhurst, P. L.; Behavioral correlates of oestrus cycle in the rat. Nature 209:223–224; 1966.
18. Butcher, R. L.; Collins, W. E.; Fugo, N. W.: Plasma concentration of LH, FSH, prolactin, progesterone and estradiol-17β throughout the 4-day estrous cycle of the rat. Endocrinology 94:1704–1708; 1974.
19. Clarke, P. B. S.; Kumar, R: The effects of nicotine on locomotor activity in non-tolerant and tolerant rats. Br. J. Pharmacol. 78: 329–337; 1983.
20. Cohen, J.: Statistical power analysis for the behavioral sciences. Hillsdale, NJ: Lawrence Erlbaum Associates; 1988.
21. Collins, A. C.; Miner, L. L.; Marks, M. J.: Genetic influences on acute responses to nicotine and nicotine tolerance in the mouse. Pharmacol. Biochem. Behav. 30:269–278; 1988.
22. Cronan, T.; Conrad, J.; Bryson, R.: Effects of chronically administered nicotine and saline on motor activity in rats. Pharmacol. Biochem. Behav. 22:897–899; 1985.

23. Downs, A. W.; Eddy, N. B.: The effect of repeated doses of cocaine on the rat. J. Pharmacol. Exp. Ther. 46:199–200; 1932.
24. Draper, N. R.; Smith, H.: Applied regression analysis, 2nd ed. New York: John Wiley & Sons; 1981.
25. Emmett-Oglesby, M. W.: Sensitization and tolerance to motivational and subjective effects of psychostimulants. In: Hammer, R. P., Jr., ed. The neurobiology of cocaine: Cellular and molecular mechanisms. Boca Raton: CRC Press; 1995:31–47.
26. Fray, P. J.; Sahakian, B. J.; Robbins, T. W.; Koob, G. F.; Iverson, S. D.: An observational method for quantifying the behavioral effects of dopamine agonists: Contrasting effects of *d*-amphetamine and apomorphine. Psychopharmacology (Berlin) 69:253–259; 1980.
27. Gibaldi, M.: Biopharmaceutics and clinical pharmacokinetics, 4th ed. Philadelphia: Lea & Febiger; 1991.
28. Greenhouse, S. W., Geisser, S.: On methods in the analysis of profile data. Psychometrika 24:95–112; 1959.
29. Gritz, E. R.; Nielsen, I. R., Brooks, L. A.: Smoking cessation and gender: The influence of physiological, psychological and behavioral factors. J. Am. Med. Womens Assoc. 51:35–42; 1996.
30. Grunberg, N. E.; Bowen, D. J.; Winders, S. E.: Effects of nicotine on body weight and food consumption in female rats. Psychopharmacology (Berlin) 90:101–105; 1986.
31. Grunberg, N. E.; Winders, S. E.; Popp, K. A.: Sex differences in nicotine's effects on consummatory behavior and body weight in rats. Psychopharmacology (Berlin) 91:221–225; 1987.
32. Grunberg, N. E.; Winders, S. E.; Wewers, M. E.: Gender differences in tobacco use. Health Psychol. 10:143–153; 1991.
33. Gumbelton, M.; Bent, L. Z.: Drug metabolism and laboratory anesthetic protocols in the rat: Antipyrine pharmacokinetics. Pharm. Res. 8:544–546; 1991.
34. Gumbelton, M.; Nicholls, P. J.; Taylor, G.: Differential effects of anesthesia regimens on gentamicin pharmacokinetics in the rat: A comparison with chronically catheterized conscious animals. Pharm. Res. 7:41–45; 1990.
35. Hansson, E.; Schmiterlow, C. G.: Physiological disposition and fate of ^{14}C-labeled nicotine in mice and rats. J. Pharmacol. Exp. Ther. 137:91–102; 1962.
36. Heishman, S. J.; Taylor, R. C.; Henningfield, J. E.: Nicotine and smoking: A review of effects on human performance. Exp. Clin. Psychopharmacol. 2:345–395; 1994.
37. Henningfield, J. E.; Keenan, R. M.: Nicotine delivery kinetics and abuse liability. J. Consult. Clin. Psychol. 61:743–750; 1993.
38. Henningfield, J. E.; Stapleton, J. M.; Benowitz, N. L.; Grayson, R. F.; London, E. D.: Higher levels of nicotine in arterial than in venous blood after cigarette smoking. Drug Alcohol Depend. 33:23–29; 1993.
39. Huang, C. S.-H.; Boudinot, F. D.; Feldman, S.: Effects of gender, pregnancy, and anesthesia on the pharmacokinetics of zidovudine in rats. Pharm. Res. 12:1647–1651; 1995.
40. Jacob, P., III; Yu, L.; Wilson, M.; Benowitz, N. L.: Selected ion monitoring method for determination of nicotine, cotinine and deuterium-labeled analogs: Absence of an isotope effect in the clearance of (S)-nicotine-3′,3′-d2 in humans. Biol. Mass Spectrom. 20:247–252; 1991.
41. Kalivas, P. W.: Neural basis of behavioral sensitization to cocaine. In: Hammer, R. P., Jr., ed. The neurobiology of cocaine: Cellular and molecular mechanisms. Boca Raton, FL: CRC Press; 1995:81–98.
42. Kalivas, P. W.; Stewart, J.: Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res. Rev. 16:223–244; 1991.
43. Kalivas, P. W.; Weber, B.: Amphetamine injection into the ventral mesencephalon sensitizes rats to peripheral amphetamine and cocaine. J. Pharmacol. Exp. Ther. 245:1095–1102; 1988.
44. Kanyt, L.: Stolerman, I. P.; Chandler, C. J.; Saigusa, T.; Pogun, S.: Influence of sex and female hormones on nicotine-induced changes in locomotor activity in rats. Pharmacol. Biochem. Behav. 62:179–187; 1999.
45. Kanyt, L.; Tapkfran, D.; Furedy, J. J.; Kulal, B.; McDonald, R.; Pogun, S.: Nicotine interacts with sex in affecting rat choice between "look-out" and "navigational" cognitive styles in the Morris Water Maze place learning task. Brain Res. Bull. (in press).
46. Ksir, C.; Hakan, R. L.; Keller, K. J.: Chronic nicotine and locomotor activity: Influences of exposure dose and test dose. Psychopharmacology (Berlin) 92:25–29; 1987.
47. Kyerematen, G. A.; Vesell, E. S.: Metabolism of nicotine. Drug. Metab. Rev. 23:3–41; 1991.
48. LaBella, F. S.; Queen, G.: General anesthetics inhibit cytochrome P450 monoxygen-ases and arachidonic acid metabolism. Can. J. Physiol. Pharmacol. 71:48–53; 1993.
49. Levin, E. D.; Morgan, M. M.; Galvez, C.; Ellison, G. D.: Chronic nicotine and withdrawal effects on body weight and food and water consumption in female rats. Physiol. Behav. 39:441–444; 1987.
50. Lin, J. H.: Species similarities and differences in pharmacokinetics. Drug Metab. Dispos. 23:1008–1021; 1995.
51. Loch, J. M.; Potter, J.; Bachmann, K. A.: The influence of anesthetic agents on rat hepatic cytochromes P450 in vivo. Pharmacology 50: 146–153; 1995.
52. Mactutus, C. F.; Herman, A. S.; Booze, R. M.: Chronic intravenous model for studies of drug (ab)use in the pregnant and/or group-housed rat: An initial study with cocaine. Neurotoxicol. Teratol. 16:183–191; 1994.
53. Miller, R. P.; Rotenberg, K. S.; Adir, J.: Effect of dose on the pharmacokinetics of intravenous nicotine in the rat. Drug. Metab. Dispos. 5:436–443; 1977.
54. Morrison, C. F.; Stephenson, J. A.: The occurrence of tolerance to a central depressing effect of nicotine. Br. J. Pharmacol. 45:151–156; 1972.
55. Nau, H.: Species differences in pharmacokinetics and drug teratogenesis. Environ. Health Perspect. 70:113–129; 1986.
56. Perkins, K. A.; Grobe, J. E.; D'Amico, D.; Fonte, C.; Wilson, A.; Stiller, R. L.: Low-dose nicotine spray use and effects during initial smoking cessation. Exp. Clin. Psychopharmacol. 2:157–165; 1996.
57. Porchet, H. C.; Benowitz, N. L.; Sheiner, L. B.: Pharmacodynamic model of tolerance: Application to nicotine. J. Pharmacol. Exp. Ther. 244:231–236; 1988.
58. Post, R. M.: Intermittent versus continuous stimulation: Effect of time interval on the development of sensitization or tolerance. Life Sci. 26:1275–1282; 1980.
59. Post, R. M.; Contel, N. R.: Human and animal studies of cocaine: Implications for development of behavioral pathology. In: Creese, I., ed. Stimulants: Neurochemical, behavioral and clinical perspectives. New York: Raven Press; 1983:169–203.
60. Rodier, W. T.: Progesterone–estrogen interactions in the control of activity-wheel running in the female rat. J. Comp. Physiol. Psychol. 74:365–373; 1971.
61. Rosecrans, J. A.: Effects of nicotine on behavioral arousal and brain 5-hydroxytryptamine function in female rats selected for differences in activity. Eur. J. Pharmacol. 14:29–37; 1971.
62. Rosecrans, J. A.: Brain area nicotine levels in male and female rats with different levels of spontaneous activity. Neuropharmacology 11:863–870; 1972.
63. Russell, M. A.; Feyerabend, C.: Cigarette smoking: A dependence on high-nicotine boli. Drug Metab. Rev. 8:29–57; 1978.
64. Schepers, G.; Rustemeir, K.; Walk, R.-A.; Hakenberg; U.: Metabolism of S-nicotine in noninduced and Aroclor-induced rats. Eur. J. Drug Metabol. Pharmacokinet. 18:187–197; 1993.
65. Siegel, S.: Nonparametric statistics for the behavioral sciences. New York: McGraw-Hill; 1956.
66. Stolerman, I. P.; Garcha, H. S.; Mirza, N. R.: Dissociations between the locomotor stimulant and depressant effects of nicotinic agonists in rats. Psychopharmacology (Berlin) 117:430–437; 1995.
67. Tsujimoto, A.; Nakashima, T.; Tanino, S.; Dohi, T.; Kurogochi, Y.: Tissue distribution of [3H] nicotine in dogs and rhesus monkeys. Toxicol. Appl. Pharmacol. 32:21–31; 1975.
68. U.S. Department of Health and Human Services: The health consequences of smoking: Nicotine addiction. A report of the Surgeon General. Rockville, MD: DHHS Publication No. (CDC) 88-8406, 1988.
69. Van Haaren, F.; Meyer, M. E.: Sex differences in locomotor activity after acute and chronic cocaine administration. Pharmacol. Biochem. Behav. 39:923–927; 1991.
70. Waldron, I.: Patterns and causes of gender differences in smoking. Soc. Sci. Med. 32:989–1005; 1991.

71. Wallace, D. R.; Mactutus, C. F.; Booze, R. M.: Repeated intravenous cocaine administration: Locomotor activity and dopamine D_2/D_3 receptors. Synapse. 23:152–163; 1996.
72. Wang, G. H.: The relation between spontaneous activity and oestrous cycle in the white rat. Comp. Psychol. Monogr. 2:1923.
73. Weeks, J. R.: Long-term intravenous infusion. In: Meyers, R. D., ed. Methods in Psychobiology, vol. 2. London: Academic Press; 1972:155–168.
74. Winer, B. J.: Statistical principles in experimental design. New York: McGraw-Hill; 1971.
75. Zahniser, N. R.; Peris, J.: Neurochemical mechanisms of cocaine-induced sensitization. In: Lakoski, J. M.; Galloway, M. P.; White, F. J., eds. Cocaine; Pharmacology, physiology, and clinical strategies. Boca Raton, FL: CRC Press; 1992:229–260.