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Influence of Sex and Female Hormones on Nicotine-Induced Changes in Locomotor Activity in Rats

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KANÝT, L., I. P. STOLERMAN, C. J. CHANDLER, T. SAIGUSA AND Ş. PÖĞÜN. *Influence of sex and female hormones on nicotine-induced changes in locomotor activity in rats.* PHARMACOL BIOCHEM BEHAV **62**(1) 179–187 1999.—The acute and chronic effects of nicotine (0.4 mg/kg SC) on locomotor activity in photocell cages have been compared in male, female, and ovariectomized hooded rats. In Experiment 1, female rats displayed higher locomotion than males (n = 12); acutely, nicotine-reduced locomotion, and this effect was slightly larger in females than males. Daily administration of nicotine for 21 days produced a similar, gradual increase in activity in both sexes. Tests then confirmed greater activity in females than males and as a function of previous chronic exposure to nicotine (n = 6); there was an activating effect of nicotine challenge but no interaction of nicotine effects with sex. In Experiment 2, ovariectomized rats were primed with 17-β-estradiol (50 μg/kg SC) and progesterone (2.5 mg/kg SC) or vehicle only. Acute administration of nicotine reduced activity in both groups similarly (n = 12). After nicotine daily for 21 days, there was increased activity as a function of both chronic nicotine and hormonal priming, and challenge with nicotine increased activity (n = 6). The effects of these challenges with nicotine were also slightly greater, as a function of previous nicotine exposure and priming. As a whole, these experiments showed robust effects of acute and chronic nicotine administration, sex, and hormonal priming; neither sex nor gonadal hormones had marked influences on changes in locomotor activity produced by nicotine. © 1998 Elsevier Science Inc.

Nicotine Estradiol Progesterone Ovariectomy Sex Locomotor activity

THE effects of nicotine on spontaneous locomotor activity in rats are complex, and include both stimulant and depressant actions (35). Several factors that influence these responses to nicotine have been identified in previous work, and the present study examines how sex and ovarian hormones may interact with the effects of nicotine on locomotion. In experimentally naive rats, nicotine can produce a short-lasting depression of locomotor activity; this depressant phase may be followed by a period in which activity is slightly increased (6). In contrast, in rats exposed previously to nicotine, tolerance developed rapidly to the initial depressant effect but the activating effects became more pronounced (6,22,30). Depression or stimulation of locomotor activity by nicotine is also dependent on the dose of nicotine and the time of testing after drug administration. Thus, large doses of nicotine administered acutely shortly before recording activity are likely to have depressant effects, whereas after chronic administration of nicotine, small doses administered at longer times before testing stimulate activity (6,30,36).

Several factors, including treatment paradigm, genotype, strain, and hormones, may regulate the rate and extent of the adaptations that occur when nicotine is administered repeatedly. For example, some strains of mice develop tolerance to the locomotor depressant effect that is dependent on the route

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and regimen (i.e., continuous infusion vs. pulses) of administration (19,20). However, some other mouse strains (C3H/2 and BUB) are relatively resistant to the development of tolerance, and in these animals tolerance can be seen only when large chronic doses of nicotine are used. In contrast to rats, there is little evidence for a sensitized locomotor activating effect of nicotine in mice.

There have been diverse explanations for adaptations to nicotine. Chronic treatment with agonists of other pharmacological classes often results in decreased numbers of neurotransmitter receptors and reduced sensitivity. However, chronic treatment with nicotine increases the number of neuronal nicotinic receptors in male rats (3,7,34) without changing the affinity or numbers of muscarinic receptors. Observations that the increased binding of radiolabeled nicotinic agonists does not always parallel sensitization to effects on locomotion suggest the operation of additional factors in adaptive processes (7,18). The widely discussed possibility that the increased numbers of receptors may be in a desensitized state might account for tolerance, but it cannot easily explain the complex pattern of tolerance to depressant and sensitization to stimulant actions of nicotine. Furthermore, it has been shown recently that in female rats, unlike males, nicotine (0.6 mg/kg SC daily for 15 days) does not upregulate neuronal nicotinic receptors, suggesting a sex difference in response (16). Different regimens of chronic exposure to nicotine may upregulate nicotinic receptors in female rats, as in mice.

Much evidence suggests that activation of the mesolimbic dopaminergic system mediates the locomotor stimulant effect produced by systemic administration of nicotine. When nicotine is administered by systemic injection, it acts in the ventral tegmental area to increase extracellular concentrations of dopamine in the nucleus accumbens (23). Infusions of nicotine into the ventral tegmental area produce locomotor activation (29), and the locomotor stimulant effect of nicotine administered systemically can be weakened by lesioning the ascending mesolimbic dopamine pathway (5). There has been some controversy over the effects of chronic treatment with nicotine on its ability to increase extracellular concentrations of dopamine in the nucleus accumbens. Studies have reported either sensitization, no change, or even tolerance with repeated administration of the drug (1). The role of the dopamine system in adaptations to nicotine, therefore, requires further elucidation.

Mechanisms other than receptor upregulation and the dopamine system may also be involved in adaptations to nicotine. Tolerance may be due, in part, to elevated plasma corticosterone levels, because repeated injections of nicotine are associated with elevated levels of corticosterone that can reduce sensitivity to nicotine (26,31). Acute stress (4) or administration of corticosterone (25,31) can reduce responsiveness to nicotine, and adrenalectomy can reverse tolerance induced by chronic injections (25,26). All of these studies suggest that adrenal corticoids may modulate tolerance to nicotine.

In several studies sex influences on responses to nicotine have been observed. Gilliam and Schlesinger (10) reported a nicotine-induced deficit in reacquisition of an active avoidance response in C57 mice that was more pronounced in females than in males. In rats of both sexes, small doses of nicotine improved performance of an active avoidance response, whereas a larger dose of nicotine had this effect in males only (43). Nicotine also had some sexually dimorphic effects in a Morris water maze task designed to screen differences in cognitive styles (15). Similarly, certain effects of nicotine on feeding behavior in rats are greater in female than in male rats (13).

As noted above, some effects of nicotine are mediated via the dopamine system; hormonal influences on this system may, therefore, be a mechanism underlying sex differences in response to nicotine. Estrogens and progesterone can modulate the function of dopamine systems in a complex manner (28,33,41). Estrogens can also enhance nicotine-induced dopamine release in striatal slices prepared from the brains of ovariectomized rats (9) and the binding of the nicotinic ligand [^{125}I]α-bungarotoxin in the suprachiasmatic nucleus (21). Thus, estrogens and progesterone may have effects on both dopaminergic and nicotinic cholinergic systems, leading to the possibility that sex and ovarian steroids may modulate the behavioral effects of nicotine. In other studies, it was found that progesterone directly inhibited nicotinic receptors expressed in *Xenopus* oocytes (2,39).

The aim of the study described here was to investigate sex differences and the effects of ovarian steroids on nicotine-induced changes in locomotor activity. In Experiment 1, the acute and chronic effects of nicotine on locomotor activity in photocell cages were compared in male and female rats. Experiment 2 used only ovariectomized female rats, and examined the effects of priming with estradiol and progesterone on nicotine-induced changes of locomotor activity; again, both the acute and chronic effects of nicotine were examined. In view of the number of other variables examined (sex, hormonal status, acute, and chronic administration of nicotine), the dose of nicotine was not varied in these studies. Instead, a single 0.4 mg/kg dose of nicotine was used because it was known to produce acute locomotor depressant and chronic locomotor stimulant effects under the conditions used (30,37). A preliminary account of these studies has been given (38).

METHOD

Animals

Rats were housed individually with free access to food and water, in rooms maintained at 20–22°C with a regular lighting cycle (lights from 0800–2000 h). A total of 12 male (230–270 g) and 36 female (190–220 g) Lister hooded rats purchased from Harlan Olac (Bicester, UK) were used in the two experiments (12 male and 12 female rats in Experiment 1, and 24 female ovariectomized rats in Experiment 2). All experiments complied with the UK Animals (Scientific Procedures) Act 1986.

Apparatus

Locomotor tests were done in photocell activity cages (30 × 30 × 30 cm) constructed from clear Perspex with wire mesh floors (30). Two parallel beams of infrared light were located 3 cm from the side walls and 4 cm above the floor. Beam breaks were recorded with the Arachnid system (Paul Fray Ltd, Cambridge, UK) running under RISC OS on an Acorn microcomputer in an adjoining room. Cage crosses indicated the number of times a rat moved from one beam to the other. Successive interruptions of the same beam were called "repeated moves"; this supplementary measure may reflect rearing onto the hind legs, grooming, and small locomotor movements falling short of crossing to the other side of the cage (plus other stereotyped behaviors).

EXPERIMENT 1: COMPARISON OF MALE AND FEMALE RATS

Acute Effects of Nicotine

The rats were naive to the drug and had no experience in the apparatus before these tests. Each rat was tested twice—

once with nicotine (0.4 mg/kg SC), and once with saline in random order. The second test took place 48 h after the first and, therefore, each rat served as its own control. Immediately following the injection of nicotine or saline, the rats were placed into the photocell cages and locomotor activity was assessed in six consecutive intervals of five min, for a total of 30 min.

Chronic Nicotine Treatment

The rats were divided into subgroups by a random method; thus, there were two subgroups of male rats and two subgroups of female rats ($n = 6$). One subgroup of each sex was treated chronically with nicotine (0.4 mg/kg SC) once per day for the next 21 days; the other subgroups received injections of vehicle. Test sessions began immediately after injections for each day and motor activity was recorded separately for six consecutive periods of 10 min each.

Tests After Chronic Nicotine Treatment

After the 21 days of chronic treatment, tests were carried out on 2 days. The second test day took place 72 h after the first. Each rat was tested once after injection of nicotine (0.4 mg/kg SC) and once after saline, in random order. After the injections, the rats were placed immediately into the photocell cages, and locomotor activity was recorded for 60 min. The regimen of chronic treatment was continued on the days between tests.

EXPERIMENT 2: HORMONE REPLACEMENT IN OVARIECTOMIZED RATS

Surgery and Hormone Replacement

Female rats were anesthetized with 0.5 ml/kg (IM) of a mixture of etorphine and methotrimeprazine (Small Animal Immobilon, Reckitt and Colman) and ovariectomized through incisions in the flank. After the wounds had been sutured, the rats were allowed to recover for a minimum of 2 weeks before commencing the experiments.

Hormone priming entailed administration of 17-β-estradiol (50 μg/kg SC in sesame oil) daily at 1700 h, starting 72 h before experiments and continuing throughout the period of chronic treatment. In addition, progesterone (2.5 mg/kg SC in sesame oil) was administered 5 h before tests of the acute effects of nicotine; control animals received sesame oil only when primed rats received hormone injections. Similar priming with progesterone (in addition to estradiol) took place 5 h before tests carried out after a 21-day period of chronic nicotine treatment. This priming regime would be expected to increase dendritic spine density in hippocampal pyramidal cells of ovariectomized rats (11) and to affect mesolimbic dopaminergic mechanisms (33).

Acute Effects of Nicotine

The procedures were very similar to those described above for acute tests in Experiment 1. However, instead of a comparison between the sexes, the experiment entailed comparisons between ovariectomized rats primed with hormones (estradiol + progesterone) and ovariectomized rats that received only the sesame oil vehicle ($n = 12$). As in Experiment 1, each rat was tested with nicotine (0.4 mg/kg SC) and saline in random order with 48 h between the two tests. Priming injections of progesterone were administered at 0800–1000 h, and the injections of nicotine or saline took place 5 h after priming; the rats were placed into the photocell cages immediately after the latter injections, and locomotor activity was assessed for a total of 60 min, followed by estradiol injection at 1700 h.

Chronic Nicotine Treatment

The groups of primed and control ovariectomized rats was divided into two subgroups by a random method. Primed animals received estradiol daily at 1700 h during this stage of the experiment, but did not receive progesterone. One subgroup of primed rats received chronic treatment with nicotine and the other subgroup received saline; similarly, the subgroups of control rats received treatments with either nicotine or saline. Chronic treatment with nicotine (0.4 mg/kg SC) or saline was continued for 21 days. Test sessions began immediately after the injections and data were recorded for 60 min.

Tests after chronic Nicotine Treatment

After completion of the 21 days of chronic treatment with nicotine, progesterone was administered to primed rats 5 h before testing, as described above for tests of the acute effects of nicotine. All animals (primed and controls) were tested twice—once with nicotine (0.4 mg/kg SC) and once with saline, in random order. The second test took place 72 h after the first, and the daily injections of nicotine, saline, estradiols and sesame oil vehicle continued during the 2-day interval. Therefore, on the test days, each animal was injected three times a day as follows: progesterone or sesame oil at 0800–1000 h, nicotine, or saline at 1300–1400 h, and estradiol or sesame oil at 1700 h. Following the injection of nicotine or saline, the rats were placed immediately into the photocell cages for 60 min.

Drugs

Nicotine bitartrate (BDH, Poole, Dorset) was dissolved in 0.9 % NaCl solution and the pH was adjusted to 7.2 ± 0.2 with dilute NaOH. Hormones (17-β-estradiol and progesterone, purchased from Sigma, Poole, Dorset) were dissolved in sesame oil. Nicotine (0.4 mg/kg, calculated as the free base), 17-β-estradiol (50 μg/kg), and progesterone (2.5 mg/kg) were administered subcutaneously, at the same time daily in a volume of 1 ml/kg; the saline and sesame oil vehicles were used for control injections.

Statistical Analysis

Data were subjected to two- and three-factor analysis of variance for repeated measures (ANOVA), followed by Tukey-B tests, with cage crosses and repeated moves examined separately as the dependent variables in each experiment (Unistat V.4, Unistat Ltd., London). The factors, some of which differed for each experiment, were as follows: sex (male or female) or hormonal manipulation (priming or control injections in ovariectomized rats), chronic drug treatment (nicotine or saline), days (1–21) within the chronic treatment periods, and test drug (nicotine or saline) administered prior to locomotor activity tests carried out before and after the periods of chronic treatment.

RESULTS

Experiment 1: Comparison of Male and Female Rats

Acute effects on locomotor activity. These data were examined with a two-factor repeated measures ANOVA, the factors being test drug (nicotine or saline) and sex (male or fe-

male). As Fig. 1 shows, nicotine (0.4 mg/kg) reduced cage crosses markedly, $F(1, 22) = 36.7$, $p < 0.001$, and female rats were generally more active than males, $F(1, 22) = 7.0$, $p < 0.05$. The effects of nicotine did not interact strongly with sex for the cage cross measure, $F(1, 22) = 3.30$, $p = 0.083$. As for the measure of repeated moves, the main effects of nicotine, $F(1, 22) = 102.9$, $p < 0.001$, and sex, $F(1, 22) = 12.7$, $p < 0.005$, were similar to those for cage crosses. However, with repeated moves, there was an interaction of test drug with sex, $F(1, 22) = 6.2$, $p < 0.05$. Inspection of Fig.1 suggests that the basis for this interaction was primarily the greater activity of females than males in the undrugged (saline) condition; this sex difference in activity levels was weaker after administration of nicotine.

Chronic nicotine treatment. During this stage of the experiment, nicotine or saline was administered daily to subgroups of male and female rats. The results were examined by means of a three-factor analysis of variance for repeated measures. The factors were chronic drug treatment (nicotine or saline), sex (male or female), and days of treatment (1–21).

As Fig. 2 shows, chronic nicotine treatment increased the number of cage crosses, $F(1, 20) = 84.0$, $p < 0.001$; females were more active than males, $F(1, 20) = 7.0$, $p < 0.05$, and activity increased across successive days of testing, $F(20, 400) = 13.0$, $p < 0.001$. The analysis also revealed an interaction between days of testing and chronic drug treatment, $F(20, 400) = 19.7$, $p < 0.001$; Fig. 2 suggests that this interaction arises from a progressive increase in the activity of the nicotine-treated animals while the saline-treated animals had a stable activity level throughout the testing period of 21 days. No other interactions were significant. Post hoc statistical tests were not carried out because there was no interaction between drug and sex, the factors of major interest. The results for the numbers of repeated moves were very similar to those for cage crosses, with significant effects for chronic nicotine treatment, $F(1, 20) = 20.6$, $p < 0.001$, sex, $F(1, 20) = 4.41$, $p < 0.05$, and nicotine $\times$ days of treatment interaction, $F(1, 20) = 11.9$, $p < 0.001$.

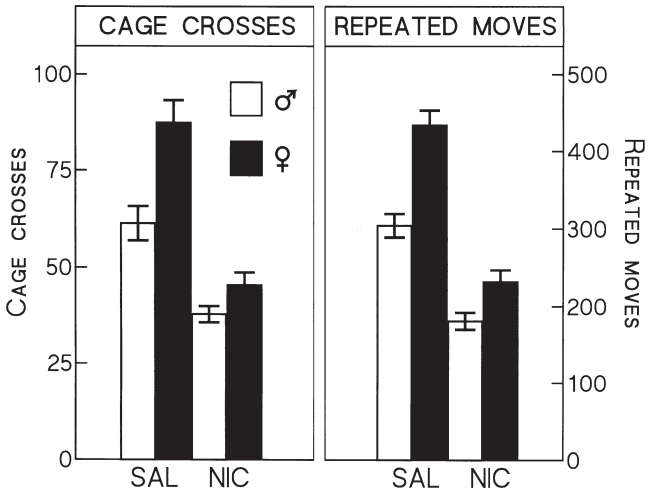

FIG. 1. Influence of nicotine on two measures of locomotor activity in male and female rats with no previous exposure to the drug (Experiment 1). Abscissae, administration of nicotine (0.4 mg/kg SC) or saline immediately prior to placing animals in photocell cages; ordinates, mean numbers of cage crosses or repeated moves (±SEM) for 30-min period of recording ($n = 12$).

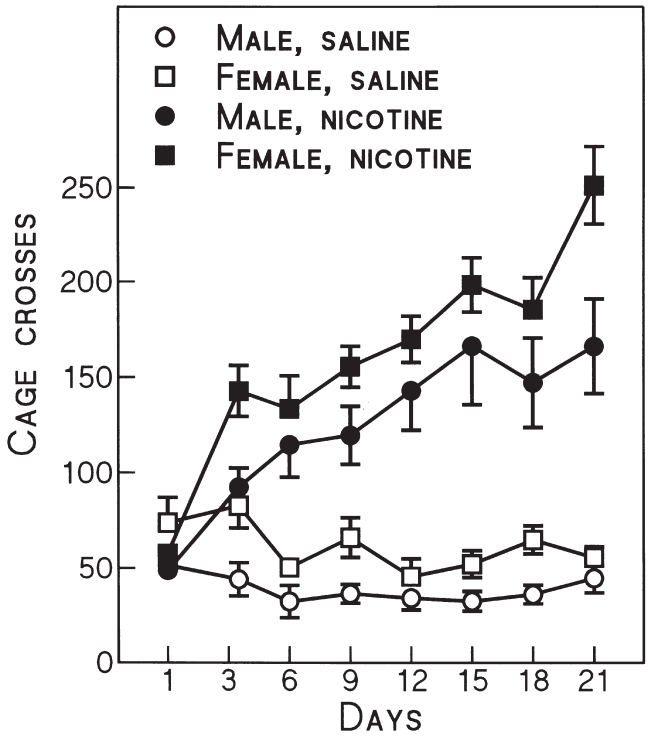

FIG. 2. Development of locomotor activation during chronic (daily) administration of nicotine (0.4 mg/kg SC) for 21 days (Experiment 1). Abscissa, days within chronic administration period; ordinate, mean number of cage crosses in 60 min (±SEM). Nicotine or saline was administered every day, but to maintain clarity, results are shown only for every third day ($n = 6$).

Tests after chronic nicotine treatment. In this final stage of the experiment, locomotor activity after administration of nicotine or saline was recorded in all rats after completion of the 21 days of chronic treatment with nicotine. It was, therefore, possible to examine the effects of sex and of prior treatment with nicotine on the response to the test dose of nicotine. Three-factor, repeated-measure analyses of variance were carried out, the factors being test drug, prior drug treatment, and sex.

The results showed that female and chronically nicotine-treated animals were more active as compared with males or saline-treated animals, respectively. The test dose of nicotine also increased the number of cage crosses in all groups compared with saline. Thus, the main effects due to sex, $F(1, 20) = 11.2$, $p < 0.005$, prior daily treatment with nicotine, $F(1, 20) = 33.9$, $p < 0.001$, and the test dose of nicotine, $F(1, 20) = 107.1$, $p < 0.001$, were all significant. Furthermore, there was a significant interaction of prior daily treatment with nicotine and the test dose of the drug, $F(1, 20) = 9.64$, $p < 0.01$; this interaction arose from the observation that the activating effect of the test dose of nicotine was more pronounced in animals previously treated with chronic nicotine than in those treated with saline (Fig. 3, upper panel). Although the female rats were consistently more active than the males, there was no interaction of sex with either prior daily treatment with nicotine or the test dose of nicotine, $F(1, 20) < 1$ and $F(1, 20) = 2.74$, respectively; the triple interaction was also not significant.

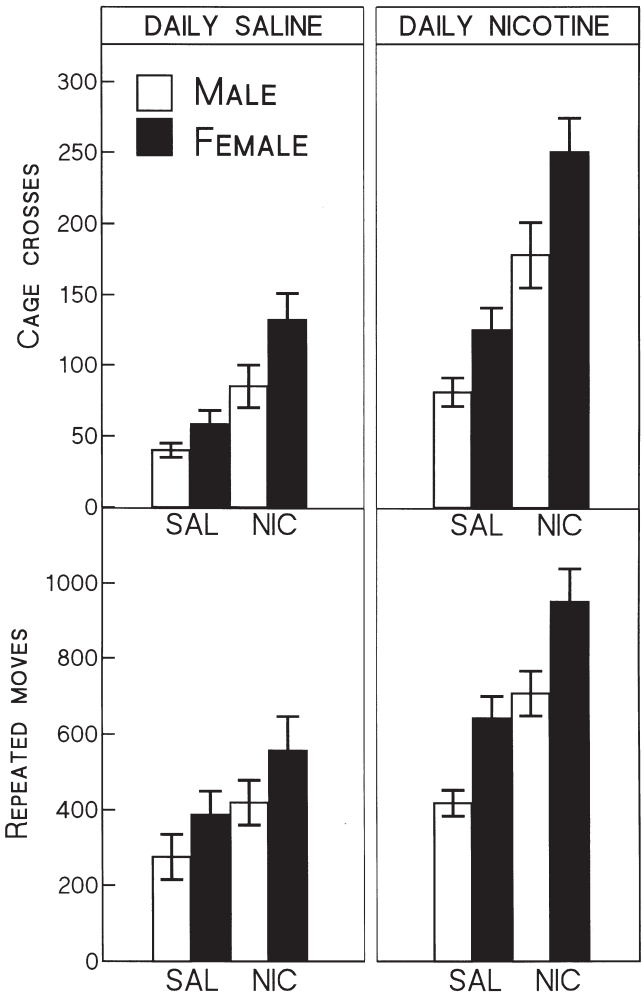

FIG. 3. Locomotor responses to nicotine after a 21-day period in which either saline (left panels) or 0.4 mg/kg of nicotine (right panels) was administered daily (Experiment 1). Abscissae, administration of nicotine (0.4 mg/kg SC) or saline prior to placing animals in photocell cages; ordinates, mean numbers (±SEM) of cage crosses (upper panels) or repeated moves (lower panels) for the 60-min period of recording ($n = 6$).

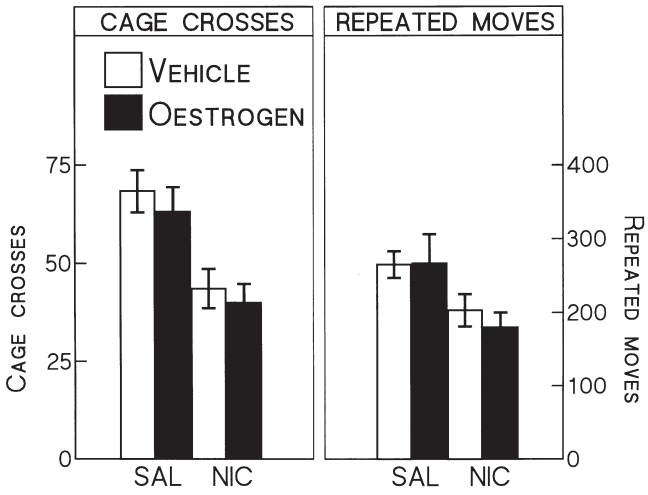

FIG. 4. Influence of nicotine on two measures of locomotor activity in two groups of ovariectomized rats with no previous exposure to the drug (Experiment 2). Rats were primed with estradiol plus progesterone ("estrogen") or with sesame oil ("vehicle"), as detailed in the Method section. Abscissae, administration of nicotine (0.4 mg/kg SC) or saline immediately prior to placing animals in photocell cages; ordinates, mean numbers of cage crosses or repeated moves (±SEM) in 30 min ($n = 12$).

Figure 3 (lower panel) shows that the results with the "repeated moves" measure were very similar to those for cage crosses. There were main effects of sex, $F(1, 20) = 10.3$, $p < 0.005$, of chronic nicotine treatment, $F(1, 20) = 22.8$, $p < 0.001$, and of the nicotine test dose, $F(1, 20) = 56.0$, $p < 0.001$. Prior daily treatment with nicotine interacted with the test dose of nicotine, $F(1, 20) = 5.5$, $p < 0.05$, but there were no interactions involving sex.

Experiment 2: Hormone Replacement in Ovariectomized Rats

Acute effects on locomotor activity. These data were examined by means of two-factor repeated measures ANOVA, the factors being test drug (nicotine or saline) and priming (vehicle or estradiol plus progesterone). As inspection of Fig. 4 suggests, nicotine depressed the activity levels in both the vehicle and the estradiol-primed groups, $F(1, 22) = 31.8$ for cage crosses, $F(1, 22) = 23.5$ for repeated moves; $p < 0.001$ in each case. Neither the effect of estradiol priming nor the interaction of nicotine with priming was significant.

Chronic treatment with nicotine. During this stage of the experiment, nicotine or saline was administered daily to subgroups of control and primed rats. The results were examined with a three-factor analysis of variance for repeated measures. The factors were chronic drug treatment (nicotine or saline), priming (vehicle or estradiol plus progesterone), and days of treatment (1–21). The results for cage crosses and repeated moves were very similar and Fig. 5 shows the data for cage crosses only.

Chronic nicotine treatment increased the number of cage crosses, $F(1, 20) = 59.6$, $p < 0.001$, estradiol-primed animals were more active than controls, $F(1, 20) = 14.28$, $p < 0.001$, and activity increased across successive days of testing, $F(20, 400) = 6.9$, $p < 0.001$. The analysis also revealed interactions between days of testing and chronic nicotine treatment, $F(20, 400) = 16.2$, $p < 0.001$, and between days and chronic hormone treatment, $F(20, 400) = 6.8$, $p < 0.001$; Fig. 5 suggests that these interactions arise from progressive increases in the activity of the nicotine- and estrogen-treated animals, while the saline-treated animals had a stable activity level throughout the testing period of 21 days. There were also a three-way interaction between days, estradiol, and nicotine, $F(20, 400) = 2.3$, $p < 0.002$; this interaction may be associated with slightly greater increase in cage crosses after chronic nicotine in the estradiol-primed rats, compared with chronic nicotine in the vehicle control rats (Fig. 5). No other interactions were significant. In view of the triple interaction involving the factors of major interest, nicotine, days, and hormone treatment, Fig. 3 shows the results of post hoc statistical tests that were carried out to define the days on which hormonal treatment had a significant effect.

The results for the numbers of repeated moves were very similar to those for cage crosses, with significant effects for chronic nicotine treatment, $F(1, 20) = 20.6$, $p < 0.001$, prim-

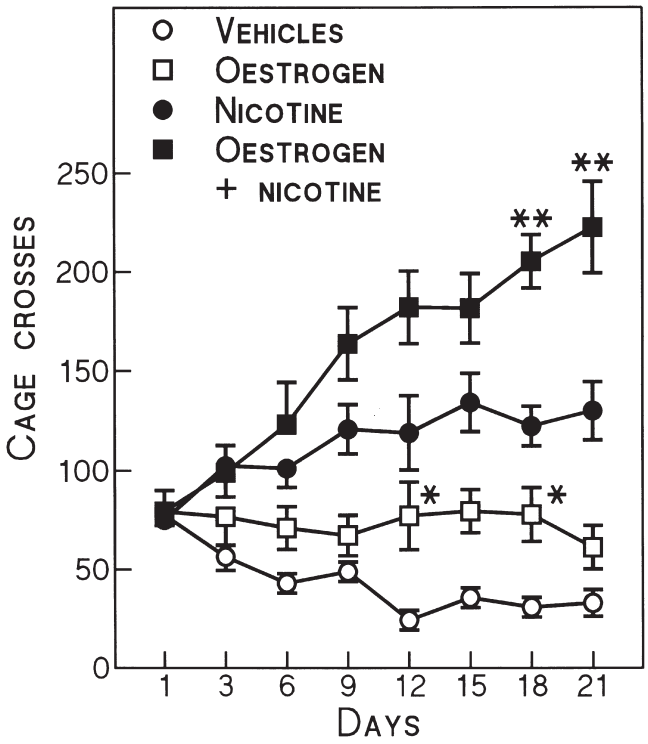

FIG. 5. Development of locomotor activation during chronic (daily) administration of nicotine (0.4 mg/kg SC) for 21 days (Experiment 2). Rats were divided into four groups receiving daily injections of estradiol, nicotine, nicotine and estradiol, or vehicles. Abscissa, days within chronic administration period; ordinate, mean number of cage crosses in 60 min (± SEM). Estradiol, nicotine and saline were administered every day but to maintain clarity, results are shown only for every third day ($n = 6$). Asterisks indicate where rats treated with estradiol showed significant differences from controls treated with vehicle ($p < 0.05$ for chronic saline rats, *; $p < 0.01$ for chronic nicotine rats, **, by Tukey-B tests).

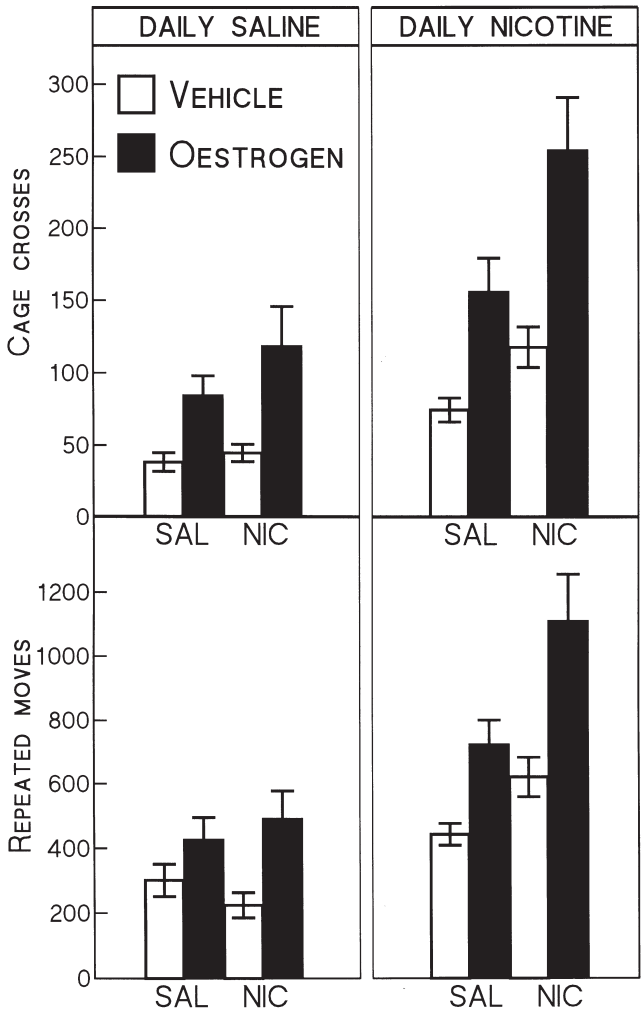

FIG. 6. Locomotor responses to nicotine after a 21-day period in which either saline (left panels) or 0.4 mg/kg of nicotine (right panels) was administered daily (Experiment 2). "Estrogen" indicates animals primed with estradiol plus progesterone; "Vehicle" indicates controls ($n = 6$). Abscissae, administration of nicotine (0.4 mg/kg SC) or saline prior to placing animals in photocell cages for 60 min; ordinates, mean numbers (± SEM) of cage crosses (upper panels) or repeated moves (lower panels).

ing, $F(1, 20) = 4.41$, $p < 0.05$, and for the nicotine × days interaction, $F(1, 20) = 11.9$, $p < 0.001$; as for cage crosses, there was a significant triple interaction between days, estradiol, and nicotine, $F(20, 400) = 2.03$, $p < 0.01$.

Tests after chronic nicotine treatment. In this final stage of the experiment, locomotor activity after administration of nicotine or saline was recorded in all rats after completion of the 21 days of chronic treatment with nicotine. It was, therefore, possible to examine the effects of priming and of prior treatment with nicotine on the response to the test dose of nicotine. Three-factor, repeated-measure analyses of variance were carried out, the factors being test drug (nicotine or saline), prior drug treatment (nicotine or saline), and priming (estradiol plus progesterone or vehicle).

For numbers of cage crosses, all main effects were significant. Nicotine at the time of testing, nicotine administered previously, and hormonal priming all increased activity, $F(1, 20) = 32.7$, 20.3, and 23.6, respectively; $p < 0.001$ in each case. In addition, there was an interaction of nicotine test with both prior exposure to nicotine, $F(1, 20) = 10.2$, $p < 0.005$, and with priming, $F(1, 20) = 6.8$, $p < 0.05$. As inspection of Fig. 6 (upper panel) indicates, the bases for these interactions were the slightly greater effects of the test doses of nicotine on the rats primed with estradiol plus progesterone than on control rats, and the slightly greater effects attributable to prior nicotine exposure. However, there was no interaction between priming and chronic exposure to nicotine, $F(1, 20) = 1.96$, and no triple interaction, $F(1, 20) = 0.79$.

For measurements of repeated moves, a very similar pattern was observed [Fig. 6, lower panel: nicotine test, $F(1, 20) = 13.2$, $p < 0.01$, chronic nicotine treatment, $F(1, 20) = 30.8$, $p < 0.001$, and the hormonal manipulation, $F(1, 20) = 20.0$, $p < 0.001$, all had significant effects. Similarly, there were interactions between nicotine test and both chronic nicotine treatment, $F(1, 20) = 14.3$, $p < 0.005$, and the hormonal manipulation, $F(1, 20) = 5.3$, $p < 0.05$. No other interaction was significant.

Experiments 1 and 2: Temporal Patterns of Locomotion

The preceding descriptions of results for cage crosses and repeated moves dealt only with the total scores for each session. Some findings might, therefore, have been different, depending upon the time within sessions. Time-course data for the second set of tests carried out in each experiment were analyzed by three-factor analyses of variance for repeated measures, the factors being test drug (nicotine or saline), time (successive 10-min periods), and either sex (Experiment 1) or hormone priming (Experiment 2). There was no interaction between the effect of nicotine and time within sessions in either Experiment 1 or Experiment 2, for either cage crosses or repeated moves [maximum, $F(5, 110) = 1.38$]. As noted previously, females were more active than males in Experiment 1, and primed animals were more active than controls in Experiment 2; however, neither of these effects interacted significantly with the time factor [maximum, $F(5, 110) = 1.09$]. There was also no triple interaction for either cage crosses or repeated moves in either experiment [maximum, $F(5, 110) = 1.43$]. Inspection of Figs. 3 and 6 suggests that the ovariectomized females in Experiment 2 had levels of activity approximately equal to those of the male rats in Experiment 1, and that hormonal priming reversed this possible effect of ovariectomy. However, statistical comparisons between these two studies have not been attempted.

DISCUSSION

The primary target of the present studies was the possible interaction of nicotine with sex or hormonal status; findings relating to these issues cannot be interpreted without an appreciation of basal differences in locomotor activity seen as a function of sex and ovariectomy or without understanding how the response to nicotine changes as a function of chronic treatment. Therefore, the findings will be considered from three points of view. First, the differences observed in the basal (undrugged) amounts of activity expressed by male and female rats, and by ovariectomized females, will be considered briefly. Second, the patterns of activity changes produced by acute and chronic exposure to nicotine in the male rats will be related to earlier observations in similar animals. Third, the question of whether sex, ovariectomy, and hormonal replacement influenced the responses to nicotine will be discussed in detail.

Main Effects of Sex and Hormonal Priming

In the present Experiment 1, intact female rats were more active than males, a finding that is in accordance with previous reports (8,40). Furthermore, the basal activity levels of the ovariectomized female rats in Experiment 2 were lower than those of the intact females in Experiment 1, and it appeared that this deficit was largely reversed by hormonal priming; although a direct statistical comparison between the two experiments would not be valid, such results are compatible with previous observations suggesting interactions between female hormones and the dopamine system (33,41).

Main Effects of Nicotine in Male Rats

The way in which previous exposure to nicotine alters responses to it has been examined in many earlier studies (6,22,30,37,42). The directly comparable studies of locomotor activity carried out previously in this laboratory used male rats exclusively, and this section will deal with studies in males. It was confirmed that, in experimentally naive rats with no previous exposure to nicotine or to the test apparatus, the 0.4-mg/kg test dose of nicotine profoundly depressed activity in both Experiments 1 and 2 (Figs. 1 and 4). Many other similar studies carried out in naive male rats have also demonstrated this phenomenon (6,30,37). Under some circumstances, including but not limited to cases where rats were exposed previously to the photocell cages used for recording activity, this acute depressant effect was not apparent (18,24,42). However, studies concur in finding that marked tolerance develops to the acute locomotor depressant effect of nicotine; this tolerance develops rapidly, sometimes as early as after one single exposure to the drug, and it can persist for weeks or even months in the aftermath of longer periods of chronic treatment (22,36). The difference between the initial depressant effect of nicotine seen in the initial stages of the present Experiments 1 and 2 and the absence of this effect in the subsequent tests may, therefore, be attributed at least partially to the development of tolerance; the additional exposures to the test apparatus may also have contributed to the changing reaction to nicotine, but no attempt was made to distinguish between these two factors because the experiments were intended to serve only as pilot investigations into sex and hormonal influences. Because of the known effects of chronic exposure to the test apparatus, it is not necessary to postulate an effect of repeated saline injections as a factor in the waning of the depressant effect of nicotine between the initial tests (Figs. 1 and 4) and the final tests (Figs. 3 and 6).

Concomitantly with the development of tolerance to the locomotor depressant effect of nicotine, it is often found that a locomotor activating effect of nicotine becomes progressively greater, a phenomenon that may be related to the sensitization to amphetamine and cocaine reviewed by Robinson and Berridge (32). Acutely, this activating effect of nicotine is typically seen 30–90 min after its administration to the rat, by which time the locomotor depressant effect has dissipated. Thus, after chronic exposure to nicotine, further doses of nicotine usually produce a marked increase in locomotor activity that can be detected within a few minutes of injection and that persists for 60–90 min (6,18,22). Both Experiments 1 and 2 show clear evidence for progressively increasing motor stimulant effects of nicotine during the stage of the studies where nicotine was administered daily for 21 days (Fig. 2 and Fig. 4), and these effects were confirmed in the final set of tests in each experiment (Fig. 3 and Fig. 6). Thus, the present findings for the acute and chronic effects of nicotine on locomotor activity in male rats are entirely consistent with earlier observations, and the scene is set for examining possible interactions with sex and hormonal status.

Interactions Between Effects of Sex and Nicotine

In the tests of the acute response to nicotine in Experiment 1, there was a significant sex $\times$ nicotine interaction for the measure of repeated moves only, and only a trend ($p = 0.083$) towards a similar interaction for cage crosses. Although this interaction may be attributed largely to the higher baseline of activity in the females than the males, it may indicate a slight difference between the sexes with respect to the locomotor depressant action of nicotine (0.4 mg/kg).

During the next stages of the study (when nicotine was administered daily), there was no significant interaction between sex and the locomotor stimulating effect of nicotine for either the cage cross or repeated moves measures. Finally, when responses to nicotine and saline were compared in each sex on

the sensitized locomotor response seen after the period of chronic treatment with nicotine, there were again no interactions of nicotine effects with the sex factor (Fig. 3), and this was also the case when data were examined in terms of responses during different periods of time within the 60-min test sessions. It can, therefore, be concluded that these pilot studies have not yielded any evidence that the chronic effects of nicotine on locomotor activity are substantially dependent upon the sex of rats. The main limitations of the study are the use of only one dose level of nicotine studied in animals of just one strain, and reliance upon a photocell apparatus which, although reliable and sensitive to effects of nicotine, does not permit as sophisticated an analysis as some newer devices [e.g., (14,17)].

Interactions Between Hormonal Status and Effects of Nicotine

The acute administration of nicotine decreased both cage cross and repeated moves measures of activity in the ovariectomized rats used in Experiment 2, and there was no interaction between this effect and the administration of replacement doses of estradiol plus progesterone.

During the 21 days of Experiment 2 in which nicotine was administered daily, there was no interaction between hormonal status and the main effect of the daily treatment with nicotine. However, the significant triple interaction of daily nicotine × daily hormone injections days of treatment (Fig. 5) was evidence for a modest potentiating effect of the hormone treatment on the sensitized response to nicotine. Furthermore, as described above, nicotine increased cage crosses and repeated moves in the final test phase of the study to a greater extent after priming than in control animals. These results suggest that the full expression of the sensitized locomotor response to nicotine in female rats may be dependent upon the presence of female sex hormones. This observation is compatible with the finding that estrogen may facilitate nicotine-induced dopamine release from striatal slices (9). However, it is emphasized that although there were significant interactions involving priming and locomotor responses to nicotine, effect sizes were small both in absolute terms and in comparison with the very pronounced main effects of both nicotine and priming. The data described above indicate that these conclusions would not be altered substantially by further analyses of results for separate periods of time within the total 60-min tests sessions. In addition, priming did not influence the development of sensitization to nicotine (as contrasted with its expression), because there was no significant interaction between the effects of prior hormonal priming and prior daily nicotine exposure on the response to the test doses of nicotine (prior in this context refers to the treatments given in the 21-day chronic treatment phase).

CONCLUSIONS

Differences between the basal levels of locomotor activity of male and female rats (Experiment 1), the possible effects of ovariectomy (comparison between female rats in Experiments 1 and 2), and the effects of hormonal priming in ovariectomized rats (Experiment 2), all supported previous observations. This agreement strengthens the case that the findings with nicotine are reliable, although they cannot be extrapolated to doses other than that used. Earlier work also suggested that sex was an important factor that influences certain behavioral effects of nicotine, such as those on feeding behavior (13) and cognition (15). In contrast, the present results do not suggest that there are marked and robust interactions between sex and the acute and chronic effects of nicotine on locomotor activity in rats; there was some suggestion of greater acute effects in female than in male rats, but this preliminary finding requires confirmation. The clearest evidence for an influence on responses to nicotine came from the study in ovariectomized rats, where hormonal replacement enhanced the chronic locomotor activating effect of nicotine. The most likely mechanism for this effect involves an interaction of female hormones with the mesolimbic dopamine system through which the locomotor activation is mediated.

There are reports of a complex pattern of differences in responses to nicotine in male and female smokers, including more self-administration of a nicotine nasal spray by men (27) and greater difficulty in cessation attempts by female smokers (12). As in studies with rats, there does not seem to be a generally lower or higher sensitivity to nicotine in females, but rather, different relative sensitivities for different effects. It follows that there is a need for more detailed and comprehensive studies of sex and hormonal influences on responses to nicotine in animal models for dependence-related behaviors. For example, the positive reinforcing effect of nicotine in the male rat depends upon an intact mesolimbic dopamine system, but there are no studies of sex differences in nicotine reinforcement in rats, or of the possible modulating influence that female hormones may have upon this effect that is thought to play a central role in tobacco use.

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