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Sex Differences in Amphetamine-Induced Locomotor Activity in Adult Rats: Role of Testosterone Exposure in the Neonatal Period

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FORGIE, M. L. AND J. STEWART. *Sex differences in amphetamine-induced locomotor activity in adult rats: Role of testosterone exposure in the neonatal period.* PHARMACOL BIOCHEM BEHAV **46**(3) 637–645, 1993. — The present studies assessed the extent to which adult sex differences in responsiveness to both acute and repeated amphetamine (AMPH) treatment can be attributed to differential exposure to testosterone (T) during the early critical period for sexual differentiation. At birth, male pups were sham-operated or gonadectomized, whereas female pups were given T or an oil injection. In adulthood, all animals were gonadectomized or sham-operated. Locomotor activity in response to either 1.5 mg/kg AMPH (IP) or the saline vehicle was measured for 2 h every third day, on five occasions. On the sixth occasion, all animals received 0.75 mg/kg AMPH (IP) in a test for sensitization. In Experiment 1, animals were tested in the absence of circulating gonadal hormones, whereas in Experiment 2, all animals received 5.0 μg estradiol benzoate (SC), 30–35 min prior to each behavioral test. Results indicate that neonatal exposure to T suppresses responsiveness to AMPH in adulthood. The differences between neonatal T-exposure groups were magnified in the presence of circulating estradiol. The fact that female animals were more responsive to AMPH regardless of neonatal T exposure suggests that lifetime exposure to estradiol alters responsiveness to this hormone, and to AMPH, in adult animals and/or that exposure to T both pre- and postnatally is necessary for the full suppression of responsiveness seen in adult male animals.

Sexual differentiation Locomotor activity Amphetamine Development Sex differences
Testosterone Estradiol Stereotypy

INDIVIDUAL animals vary in their responsiveness to the psychomotor stimulant amphetamine (AMPH), both with respect to its acute behavioral activating effects and to the sensitization of these effects produced after repeated, intermittent exposure to the drug (28,29,31). One of the factors that influences the level of responsiveness to AMPH is sex; it has been found repeatedly that female rats are more responsive to this drug than are males (8,10–12,27,30). In addition to the sex difference in response to the acute effects of AMPH [e.g., (8,10)], sex differences in the degree or rate of sensitization to AMPH have been reported (11,12,27,28,30).

These sex differences have been attributed, in part, to differences in circulating levels of gonadal hormones at the time of testing (8,10–12,27–30). For example, it has been found that castration of adult males increases their responsiveness to AMPH [(12,27,30), but see (10)], whereas in adult females, AMPH-induced behaviors have been found to fluctuate with the estrous cycle (5,8). The increase in AMPH-induced behavior at estrus is accompanied by increases in extracellular levels of dopamine (DA) in the striatum (5). Further, estradiol administered to ovariectomized female rats facilitates AMPH-induced behaviors (1,3), enhances AMPH-stimulated release of DA from striatal slices (2,3), and increases extracellular levels of DA in the striatum as measured by in vivo microdialysis (1). Other ovarian hormones (e.g., progesterone) may also influence the effects of AMPH (4,7). Thus, the differences between adult male and female animals can be attributed in part to these "activational" effects of their circulating gonadal hormones.

These studies do not take into account, however, either the perinatal organizational actions of testicular hormones or the differential history of hormonal exposure over the life span

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of the two sexes. Both of these factors could be expected to have considerable impact on the behaviors exhibited by intact, adult animals, either by affecting directly the response to AMPH, or by affecting the response of adult animals to circulating gonadal hormones. Male animals are exposed to testosterone (T) during the early critical period for sexual differentiation of the brain. This exposure is known to affect the development of brain regions involved in both reproductive and nonreproductive behaviors, and has been shown to alter responsiveness to circulating gonadal hormones in the adult animal [see (37) for review]. Although it has been reported that neonatal exposure to gonadal hormones does not influence the sexual dimorphism in the effects of AMPH on striatal DA release, in vitro, exposure of female animals to ovarian hormones in the peripubertal period may contribute to their greater responsiveness to AMPH as adults (6).

The present studies were undertaken to investigate the contribution of differential neonatal exposure to T to the adult sex difference in the development of sensitization to repeated administrations of AMPH. The development of sensitization can be measured by an increase in behavior over repeated administrations of the drug, increased responsiveness to a challenge injection of the drug following repeated administration, or by comparing the behavior of animals previously exposed to the drug with the behavior of animals receiving the drug for the first time. In the present experiments, locomotor activity was measured in response to repeated treatments with either a moderate dose of AMPH or the saline vehicle. Following these repeated treatments, all animals were challenged with a low dose of AMPH in a test for sensitization. In Experiment 1, the responsiveness to AMPH was tested, in the absence of circulating gonadal hormones, in adult male and female animals that were or were not exposed to T at birth. Experiment 2 was similar except that estradiol was administered to all the adult animals at the time of testing.

EXPERIMENT 1

METHOD

Subjects

Subjects were 36 male and 36 female New Colony Wistar rats obtained from 10 litters born in the laboratory at Concordia University. All animals were housed in a temperature- and humidity-controlled room, under a 12L : 12D cycle, with lights on between 0800 and 2000 h. All testing took place during the light cycle. Food and water were available ad lib throughout the course of the experiment.

Breeding. Adult male and female rats obtained from Charles River Breeding Farms (St. Constant, Québec) were mated in groups of three or four females to one male. Successful mating was assessed by noting the presence of sperm in daily vaginal smears, and this day was defined as embryonic day zero (E0). Females were removed from the mating cage and individually housed in standard, wire-mesh cages. On E17 to E19, pregnant females were moved to wire-topped, polypropylene shoebox cages with hardwood chip bedding.

Neonatal hormonal manipulations. Beginning on E21, the breeding cages were checked every few hours for the presence of pups. When pups were found (designated postnatal day zero; PN0), they were removed from the cage and transported to another room. There the litter was sexed and culled to a maximum of 12 pups and the following manipulations were made. Male animals were either gonadectomized under hypothermic anesthesia (GDXMALE) or were subjected to the anesthetic procedure alone (MALE). Female animals received a subcutaneous (SC) injection of either 200 μg testosterone propionate (Sigma; TPFEMALE) or the peanut oil vehicle (0.1 ml; FEMALE) on both this and the following day. The site of the injection was sealed with Collodion (Fisher). Approximately half of the litters contained MALE, GDXMALE, and FEMALE animals, whereas the remainder contained only MALE and TPFEMALE animals. This was done to avoid the possibility of TP exposure to the FEMALE and GDXMALE groups. Each litter contained at least two pups of each sex condition. Following these manipulations, the pups were returned to the nest, and after a few minutes, the mother was reintroduced. Pups were removed briefly from the nest approximately 24 h later on PN1, to administer a second treatment to the female animals.

The litters remained undisturbed except for weekly cage cleaning procedures until PN21, when weaning took place. On PN25 to 26, the animals were housed in same-condition pairs in standard wire-mesh hanging cages. Animals remained in these pairs until they were 63 to 66 days of age, at which time they were individually housed.

Adult surgical procedures. When animals were 84 to 87 days of age, they were either gonadectomized (MALE, FEMALE, TPFEMALE) or sham-operated (GDXMALE) under methoxyflurane (Metofane; Pitman-Moore) anesthesia. Animals received an intramuscular injection of 0.1 ml penicillin G (Ayerst) at the time of this surgery.

Apparatus

Locomotor activity was measured in a bank of 12 activity boxes, each box measuring 20 (width) × 40 (length) × 25 cm (height). Each box was constructed of pressed wood on the sides and back, with a hinged Plexiglas wall on the front. The floor of the cage consisted of stainless steel rods set 1 cm apart and the top was of wire-mesh screen. Four photocells were located around the perimeter of the box. Two were located at a height of 3.5 cm above the floor along the front and rear walls and spaced 20 cm apart to measure horizontal activity, and two were located in the side walls, 20 cm above the floor and spaced evenly apart to measure vertical movements. Each time the animal crossed a photocell beam two activity counts were recorded. The room in which the boxes were located was illuminated by the red photocell lights. A 75-dB white noise generator was used to mask extraneous sounds.

Procedure

Subject assignment. Within each litter, each member of a pair of animals of each sex type was randomly assigned to receive either D-amphetamine sulphate (AMPH; Smith Kline & French) or the 0.9% sterile saline vehicle (SAL), creating eight experimental groups with nine subjects each: FEMALE-AMPH, TPFEMALE-AMPH, GDXMALE-AMPH, MALE-AMPH, FEMALE-SAL, TPFEMALE-SAL, GDXMALE-SAL, MALE-SAL. Animals were assigned to activity boxes such that no two members of the same experimental group were tested in the same box more than once, and the order of the groups in the 12 activity boxes was counterbalanced across the six testing squads.

Preexposure period. Activity testing began 15 to 16 days following the adult surgeries. Animals received an intraperitoneal (IP) injection of either 1.5 mg/kg AMPH or 1.0 ml/kg SAL just prior to being placed into the activity boxes. Collection of activity data commenced a few minutes after injection

of the last rat and continued for 2 h. Following the activity session, animals were returned to their home cage. Animals were tested every third day for a total of 5 preexposure days.

Test for sensitization. Three days following the last preexposure day, all animals received 0.75 mg/kg AMPH (IP) prior to being placed into the activity box. Because sensitized responding to AMPH can be manifested by a decrease in locomotor activity due to an increase in focused stereotyped behaviors (24,31,32,34), a low challenge dose was used. This dose would be expected to produce mainly locomotor activity, even in sensitized animals.

Scoring of stereotypy. In an earlier pilot study (19), it was noted that several of the animals receiving the 1.5 mg/kg dose of AMPH showed stereotyped behaviors, consisting mainly of sniffing and rearing in one place, often for extended periods of time. To quantify these behaviors, the activity sessions were videotaped on the last day of the preexposure period. The behavior of the animals in the AMPH groups was then observed for 2 min, every 15 min, beginning 13 min after the start of the 2-h session, by a rater who was blind to group membership (i.e., sex and T-exposure condition). Each animal was assigned a single stereotypy rating, ranging from 0 to 6 during two, 10-s scoring periods (spaced 50 s apart), within each of the 2-min observation periods. The scale used was modified from two sources (14,18): 0 = asleep; 1 = stationary, normal in place activity such as grooming; 2 = increased locomotor activity; 3 = predominately active with bursts of stereotyped sniffing or rearing; 4 = predominately stereotyped sniffing or rearing with bursts of locomotor activity; 5 = continuous stereotyped behavior such as sniffing over a wide area; 6 = continuous focused stereotyped sniffing or rearing in one location without locomotion. It should be noted that, in the presence of AMPH, the range of obtained ratings will be restricted to the high end of this scale, insofar as AMPH-treated animals would be expected to obtain a rating of at least 2 or 3 throughout the session.

Data analyses. Total photocell counts recorded for each animal on each day of the preexposure period were analyzed with a four-way repeated measures analysis of variance (ANOVA) with T exposure (T vs. NO-T), sex (male vs. female), and drug (AMPH vs. SAL) as the between-subjects factors and preexposure day as the within-subjects factor. Total photocell counts recorded for each animal for each 30-min time block across the 2-h session on the test day for sensitization were analyzed by means of a four-way repeated measures ANOVA with T exposure, sex, and preexposure group as the between-subjects factors and time block as the within-subjects factor (significance level for ANOVA effects, $p = 0.05$). Post hoc analyses of significant interaction effects were made using simple main effects and Tukey tests where appropriate [(23); significance level for post hoc comparisons, $p = 0.01$].

To obtain a single stereotypy score for each animal, the median score was calculated based on the 16 individual ratings made across the 2-h test session. These median scores were then subjected to a Kruskal–Wallis ANOVA to detect overall between-group differences. Subsequent pairwise comparisons were then made using the Mann–Whitney U-test ($p = 0.05$).

RESULTS

Preexposure Period

The mean total locomotor scores for each group on each day of the preexposure period are shown in Fig. 1. The data for one GDXMALE-SAL animal were discarded from the experiment when the animal received the incorrect injection on day 3. AMPH significantly increased activity for all groups [drug: $F(1, 63) = 107$, $p < 0.0005$]. Animals that had been exposed to T at birth were significantly less active overall than animals that did not receive such exposure [T exposure: $F(1, 63) = 5.40$, $p < 0.05$]. This effect did not vary as a function of drug group; thus, T exposure decreased both baseline and AMPH-induced locomotor activity [T exposure × drug: $F(1, 63) < 1$]. These data are similar to those obtained in an earlier pilot experiment [conducted with the same eight experimental groups and a total of 62 subjects (19)]. In that experiment, however, there appeared to be a larger effect of neonatal T exposure on responsiveness to AMPH.

There were also significant differences between sex groups, with female animals (FEMALE + TPFEMALE) showing

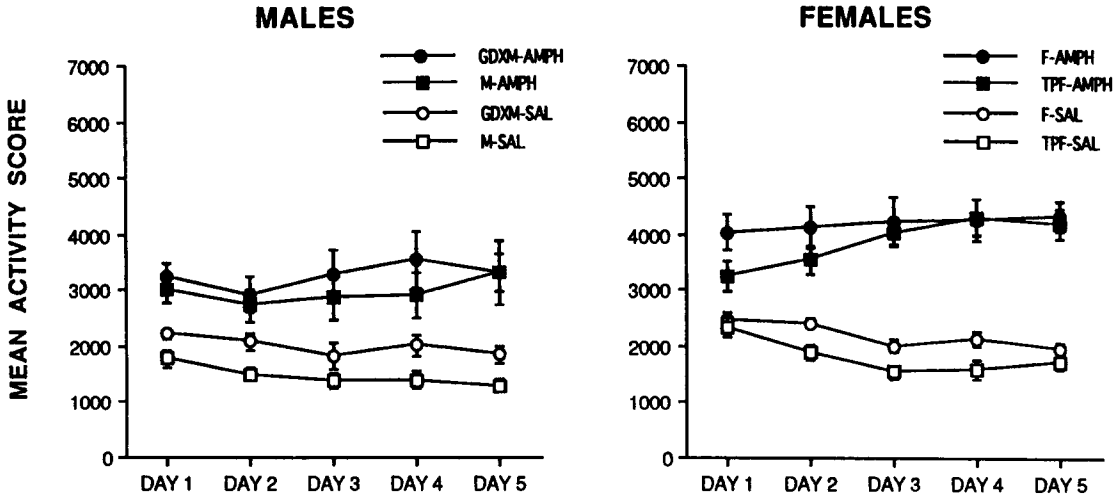

FIG. 1. Mean total locomotor activity scores (± SEM) in response to either 1.5 mg/kg AMPH or SAL for the 2-h activity session on each day of the preexposure period. Data for male (groups MALE and GDXMALE) and female animals (FEMALE and TPFEMALE) are shown on the left and right sides of the figure, respectively.

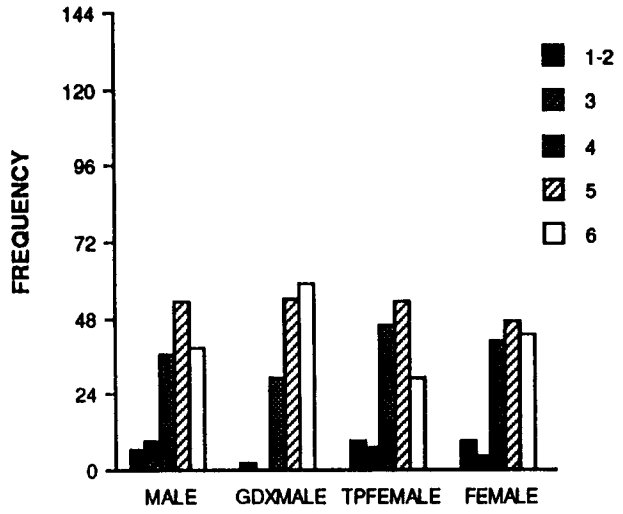

FIG. 2. Frequency distribution of stereotypy ratings on preexposure day 5 for AMPH-treated animals.

greater activity than male animals [MALE + GDXMALE; sex: $F(1, 63) = 13.14, p < 0.001$]. This sex difference tended to depend on drug condition [sex × drug: $F(1, 63) = 3.95$, $p = 0.051$]. Post hoc analyses revealed that there was a significant sex difference only for AMPH-treated animals ($p < 0.01$). The three-way interaction of T exposure × sex × drug was not significant, $F(1, 63) < 1$, and therefore, this effect did not depend on neonatal exposure to T (thus, TPFEMALE > MALE and FEMALE > GDXMALE; see Fig. 1). In the previous pilot experiment, there was no significant effect of the sex of the animal, although the pattern of results was similar.

There was a small increase in AMPH-induced locomotor activity across the 5 days of the preexposure period. Visual inspection of the locomotor scores for individual animals made it clear that whereas some animals showed an increase in locomotor activity in response to AMPH with successive injections, other animals did not, and several showed decreasing activity scores over days of testing. Thus, although there is a tendency for some groups to show an enhancement of AMPH-induced locomotor activity with days of testing (e.g., group TPFEMALE), the effects of day in the ANOVA were not significant, except by drug [day × drug: $F(4, 252) = 11.87, p < 0.0005$]. This lack of change in behavior might be due to the development of stereotypy in some animals, which would in turn reduce the levels of activity (24,31,32,34).

Figure 2 shows the frequency distribution of stereotypy ratings for the four groups of AMPH-treated animals on day 5 of the preexposure period. A Kruskal-Wallis ANOVA on the median stereotypy scores revealed a significant effect of group, $H(3) = 8.59$, $p < 0.05$, with group GDXMALE showing the highest median stereotypy scores and group TPFEMALE the lowest median stereotypy scores. Subsequent analyses revealed a significant effect of T-exposure group ($U = 90.5$, $p < 0.05$); NO-T animals showed higher median stereotypy scores than T animals. There was no difference as a function of sex.

Test for Sensitization

In response to a low-dose AMPH challenge, those animals previously exposed to AMPH showed significantly higher levels of activity than those receiving the drug for the first time [preexposure group: $F(1, 63) = 13.99$, $p < 0.001$; see Fig. 3]. Thus, sensitization was observed for all groups previously exposed to AMPH. Animals that had been exposed to T neonatally showed suppressed activity scores in comparison to those animals that did not [T exposure: $F(1, 63) = 14.19$, $p < 0.001$], but this effect did not differ as a function of drug preexposure group [T exposure × drug: $F(1, 63) < 1$]. Therefore, although neonatal T exposure decreased both the acute (i.e., among the SAL preexposed animals) and the sensitized (i.e., among AMPH preexposed animals) response to the challenge injection of AMPH, the magnitude of sensitization

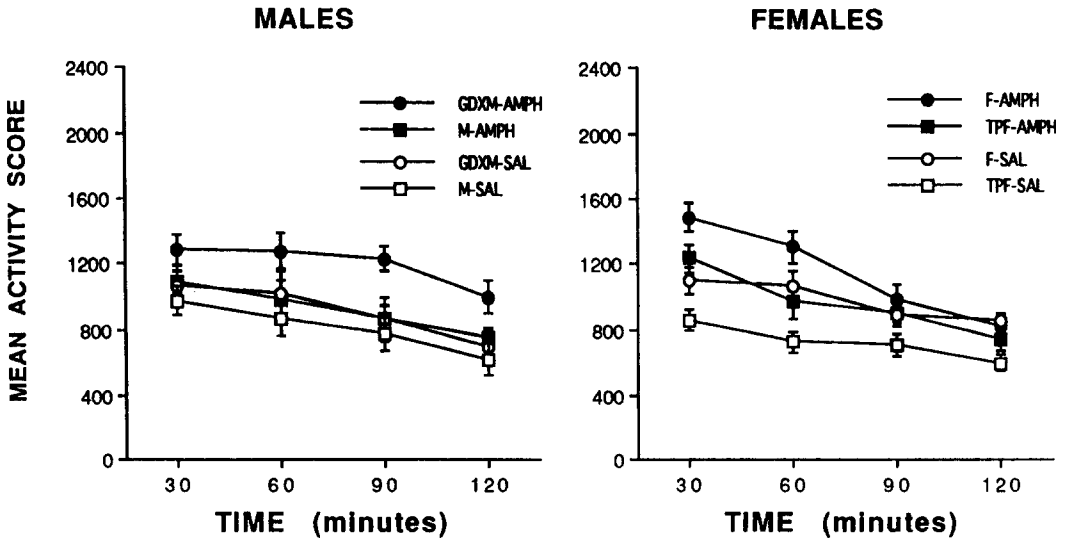

FIG. 3. Mean total locomotor activity scores (±SEM) for each 30-min time block on the test day for sensitization, when all animals received 0.75 mg/kg AMPH. The left and right panels show the data for male and female animals, respectively.

(as defined by the difference in activity scores between those animals preexposed to AMPH and those preexposed to SAL) did not differ as a function of neonatal exposure to T. These data are virtually identical to those obtained in the earlier pilot experiment [(19); preexposure group: $F(1, 54) = 13.39, p < 0.005$; T exposure: $F(1, 54) = 9.28, p < 0.004$; data not shown]. The finding that NO-T animals (FEMALE + GDXMALE) showed enhanced activity in comparison to T animals (TPFEMALE + MALE) on the test day in response to a low dose of AMPH supports the hypothesis that these animals were responding with greater stereotypy to the 1.5 mg/kg dose of AMPH used in the preexposure period.

Activity scores declined over the 2-h session for all groups [time block: $F(3, 189) = 76.39, p < 0.0005$; see Fig. 3]. This effect did not vary as a function of T-exposure group, but there was a significant interaction of sex × preexposure group × time block, $F(3, 189) = 4.91, p < 0.01$. Activity scores of females (FEMALE + TPFEMALE) preexposed to AMPH showed a greater decline across the test session than did the activity scores of males (MALE + GDXMALE) (all $p < 0.01$). There were no other significant effects of sex.

EXPERIMENT 2

The results of Experiment 1 indicate that at least part of the adult sex difference in responsiveness to AMPH can be attributed to an underlying sexual dimorphism produced by exposure of the male animal to T during the early neonatal period, critical for sexual differentiation. When animals were tested with a low dose of AMPH, in the absence of circulating gonadal hormones, animals exposed to T at birth (MALE + TPFEMALE) were less active than animals not so exposed (FEMALE + GDXMALE). There is, however, ample evidence that circulating gonadal hormones at the time of testing contribute to the sex difference in responsiveness to AMPH (12). Both testicular and ovarian hormones have been implicated in this effect. Experiment 2 was carried out to determine the extent to which the facilitatory effect of estradiol on responsiveness to AMPH in the adult female (1,3) is influenced by neonatal exposure to T.

METHOD

Subjects

Subjects were 36 male and 36 female New Colony Wistar rats obtained from eight litters born at Concordia University. Details of housing conditions, breeding, neonatal hormonal manipulations, and adult surgeries were similar to those described for Experiment 1 with some exceptions. The animals were moved from one housing facility to another when they were 59 to 63 days of age. Up to the time of the transfer, animals were housed in same-condition groups of two to three rats, and, upon arrival in the new facility, animals were housed individually. Adult surgical procedures were undertaken when the animals were 81 to 86 days of age.

Apparatus

The apparatus used in this experiment was identical to that described for Experiment 1 except that the testing room had additional illumination provided by two, overhead red light bulbs (25 W each).

Procedure

Locomotor testing began 15 to 17 days following the adult surgeries. The procedures for subject assignment and testing of locomotor activity for the preexposure period were similar to those described for Experiment 1 except that 30 to 35 min prior to receiving the AMPH (1.5 mg/kg, IP) or SAL (1.0 ml/kg, IP) injection and being placed into the activity boxes, all animals received 5.0 μg estradiol benzoate (EB; Sigma) in 0.1 ml peanut oil (SC) as they were removed from the home cage. This dose of EB has been shown to facilitate AMPH-induced rotational behavior and to produce an increase in extracellular levels of striatal DA in ovariectomized female animals when AMPH is administered 30 min after the hormone injection (1). On the test day, all animals again received EB 30 to 35 min prior to receiving 0.75 mg/kg AMPH (IP).

Once again, the last day of the preexposure period was videotaped, and stereotypy was scored and analyzed as described in Experiment 1.

RESULTS

Preexposure Period

Total locomotor activity scores for each animal on each day of the preexposure period were analyzed as described for Experiment 1. Due to a mechanical failure of the horizontal photocells during the second hour of one activity session on preexposure day 5, some locomotor data for three AMPH subjects (one GDXMALE, one TPFEMALE, and one MALE animal) were lost. The missing data for each of the three subjects were estimated with the mean horizontal photocell counts recorded by the other eight members of their respective groups during this time period.

As in Experiment 1, AMPH significantly increased activity for all groups [drug: $F(1, 64) = 180, p < 0.0005$], and neonatally T-exposed animals showed lower activity scores than did animals not exposed to T [T exposure: $F(1, 64) = 14.17$, $p < 0.001$]. There was a tendency for the effect of T exposure to be larger for the animals receiving AMPH [T exposure × drug: $F(1, 64) = 2.91, p = 0.09$].

There were again significant differences in activity between female (FEMALE + TPFEMALE) and male (MALE + GDXMALE) animals [sex: $F(1, 64) = 10.67, p < 0.005$; sex × drug: $F(1, 64) = 6.40, p < 0.02$]. Post hoc analyses revealed that the sex difference was significant only for animals treated with AMPH ($p < 0.01$). The three-way interaction was not significant [T exposure × sex × drug: $F(1, 64) < 1$; again, FEMALE > GDXMALE and TPFEMALE > MALE].

Animals treated with AMPH showed sensitization in the preexposure period as evidenced by an increase in activity across days (see Fig. 4). This change in activity was greater in NO-T (GDXMALE + FEMALE) animals than in T (MALE + TPFEMALE) animals [day × T exposure × drug: $F(4, 256) = 3.17, p < 0.05$]. Although the groups did not differ in AMPH-induced locomotor activity on the first day of testing ($p > 0.01$), on day 5 the NO-T animals showed significantly greater AMPH-induced activity than the T animals ($p < 0.01$). There were no differences as a function of T-exposure group or days in the SAL-treated groups (all $p > 0.01$). The change in activity across days for AMPH-treated animals was also greater in females (FEMALE + TPFEMALE) than in males [MALE + GDXMALE; day × sex × drug: $F(4, 256) = 2.49, p = 0.05$]. Although there was no significant sex difference on the first day of testing, on day 5 female animals showed significantly greater AMPH-induced activity than males ($p < 0.01$). Again, there were no differences in the SAL-treated groups (all $p > 0.01$). The four-way interaction was not significant, $F(4, 256) < 1$.

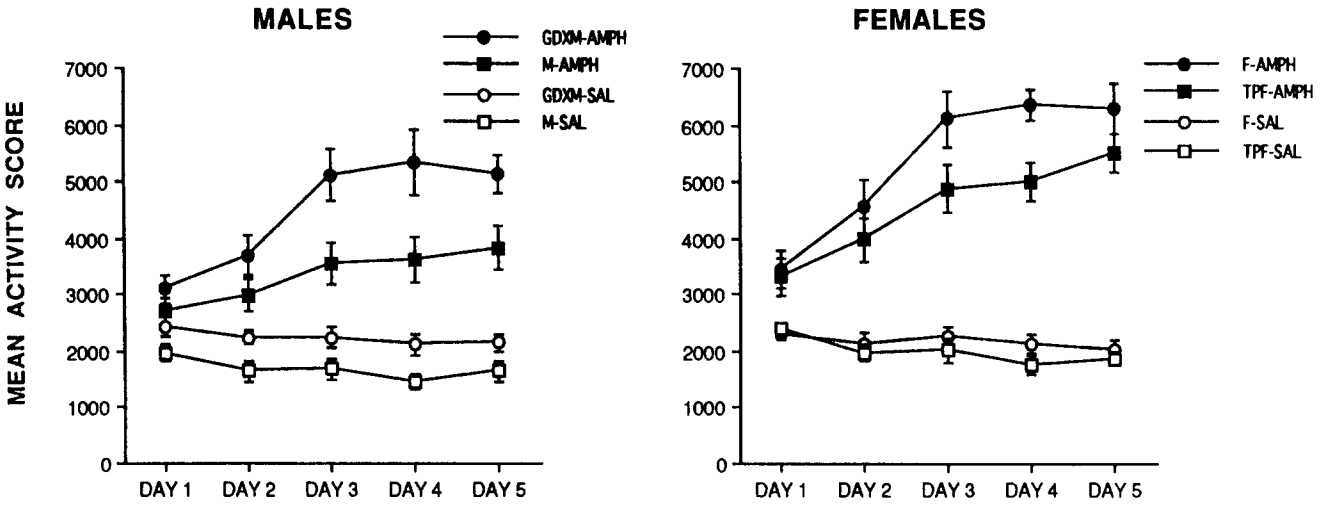

FIG. 4. Mean total locomotor activity scores (±SEM) in response to either 1.5 mg/kg AMPH or SAL, for the 2-h activity session on each day of the preexposure period for animals receiving EB at the time of each test. Data for male (groups MALE and GDXMALE) and female (FEMALE and TPFEMALE) animals are shown on the left and right sides of the figure, respectively.

This increase in activity across days may be due to lower stereotypy scores for animals tested with AMPH in the presence of circulating estradiol. Figure 5 shows the frequency distribution of stereotypy ratings for the four groups of AMPH-treated animals on the last day of the preexposure period. The median scores of the four groups did not differ significantly from each other [Kruskal-Wallis ANOVA: $H(3) = 3.67, p = 0.299$].

Test for Sensitization

When animals preexposed to AMPH or SAL in the presence of EB were challenged with AMPH in the presence of EB, animals preexposed to AMPH again showed higher levels of activity than did animals receiving the drug for the first time [preexposure group: $F(1, 64) = 13.97$, $p < 0.001$; see Fig. 6]. Animals exposed to T neonatally showed lower locomotor activity scores in comparison to those animals that did not [T exposure: $F(1, 64) = 42.22, p < 0.0005$]. As in Experiment 1, neonatal T exposure reduced both the acute and sensitized response to the challenge injection of AMPH, but did not reduce the degree of sensitization as measured by the difference between AMPH- and SAL-preexposed animals [T exposure × drug: $F(1, 64) < 1$].

The main effect of time block was significant, $F(3, 192) = 128$, $p < 0.0005$, and thus activity scores again declined across the 2-h test session for all groups. This effect varied by T-exposure group [T exposure × time block: $F(3, 192) = 7.3$, $p < 0.0005$] and sex [sex × time block: $F(3, 192) = 3.03$, $p < 0.05$]. NO-T animals and females showed a greater decline in activity scores across the session than did T animals and males, respectively (all $p < 0.01$).

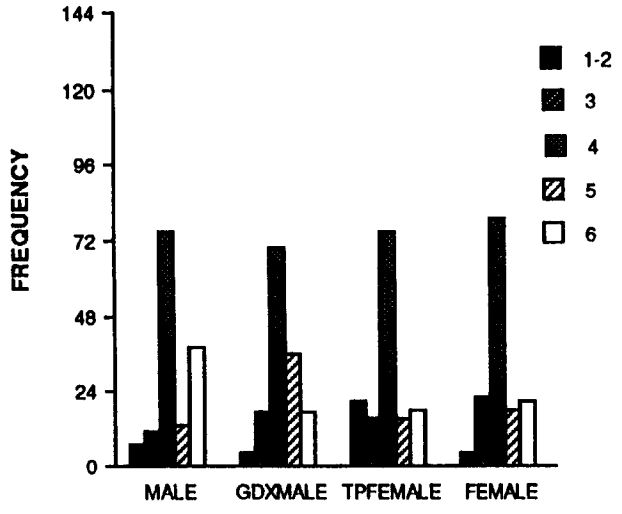

FIG. 5. Frequency distribution of stereotypy ratings on preexposure day 5 for animals receiving AMPH in the presence of EB.

DISCUSSION

Several findings emerge from these experiments. First, animals exposed to T in the neonatal period (MALE + TPFEMALE) were less responsive to the effects of AMPH on locomotor activity than were those not exposed to T (FEMALE + GDXMALE).

Second, it is apparent from the data in both experiments that the manipulation of T in the neonatal period in both males and females was not sufficient to eliminate the sex difference in responsiveness to AMPH. GDXMALE animals were not as active after AMPH as FEMALE animals, nor were TPFEMALE animals as inactive as MALE animals; throughout the experiments the mean activity scores of groups GDXMALE and TPFEMALE tended to fall between those of groups FEMALE and MALE. One explanation of the finding that T exposure in the neonatal period was not sufficient to completely eliminate the sex difference in adult animals is that the timing and/or length of exposure to T during the critical period is an important contributing factor to adult responsiveness to AMPH. Normal male rats received exposure to T both pre- and postnatally. Both TPFEMALE and GDXMALE animals, by virtue of the manipulations made, received exposure

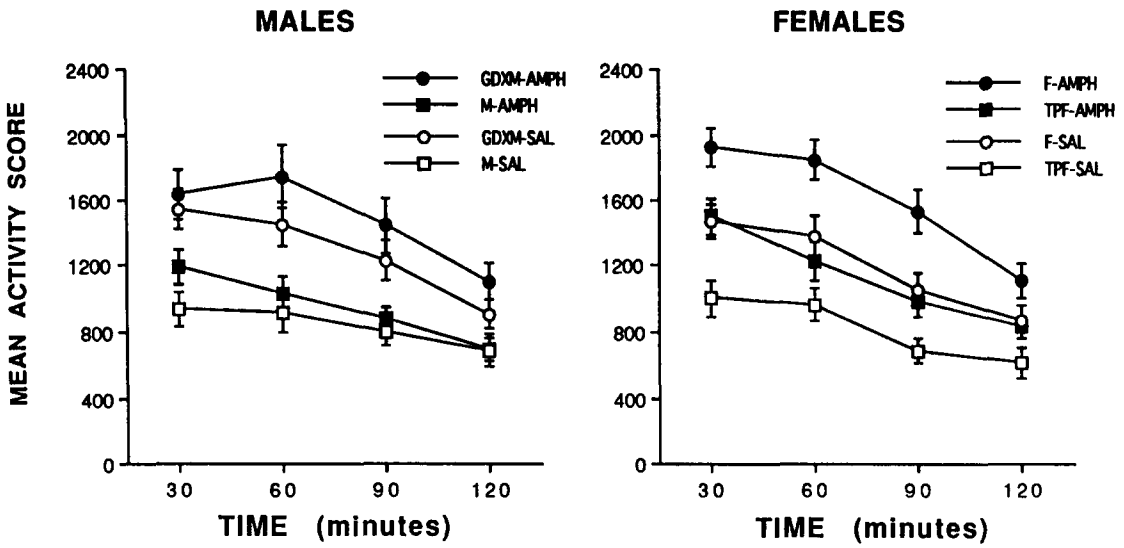

FIG. 6. Mean total locomotor activity scores (±SEM) for each 30-min time block on the test day for sensitization, when all animals received 0.75 mg/kg AMPH in the presence of EB. The left and right panels show the data for male and female animals, respectively.

during only one of these periods. Therefore, exposure to T during only the postnatal period, for example, is not equivalent to the exposure to T experienced by normal male animals, even though it results in the masculinization/defeminization of certain behavior patterns. Moreover, neither the TPFEMALE nor GDXMALE animals received subsequent exposure to T during later developmental time points that may also be important for development of the adult behavior pattern (during puberty, for example). Thus, it is possible that more than either pre- or postnatal exposure to T is necessary to produce the full suppression of responsiveness seen in the normal adult male animal.

The sex of the animal was also important in determining the response of the animal to AMPH, both in the absence and presence of EB. Female animals, regardless of neonatal T exposure, showed increased locomotor scores in response to AMPH in comparison to male animals during the preexposure period. This suggests that factors other than neonatal T exposure contribute to their greater locomotor responsiveness to AMPH. One possibility is that the exposure of the female animal to estradiol over the life span influences the subsequent responsiveness of the animal to AMPH and/or estradiol. Although it has been commonly assumed that ovarian secretions do not participate in the sexual differentiation of the female phenotype, this idea is challenged by several findings (15,17, 20,33,35). Furthermore, there is some evidence to suggest that the critical period for these effects may not be the same as that for the effects of T exposure (20,33), in particular, the time of puberty may be important (6,20). Thus, it is possible that female animals remain differentially responsive to AMPH, and to estradiol, because of prior exposure to this hormone.

A fourth finding is that the effect of neonatal exposure to T appeared to be magnified when, as in Experiment 2, the animals were tested with AMPH in the presence of EB. This magnification of the difference between T and NO-T groups was due to the increased effectiveness of AMPH in the NO-T groups treated with EB both during preexposure period (compare Figs. 1 and 4) and during the test for sensitization when all animals were treated with AMPH (compare Figs. 3 and 6). This effect is specific to the AMPH groups; note that in the SAL groups, levels of activity are similar in both experiments. Clearly, neonatal T exposure rendered the animals less sensitive to the effects of estradiol as adults.

Estradiol has been shown to have a variety of both short- and long-term effects on the midbrain DA systems, affecting the neurochemical response to DA agonists and the behaviors known to depend on them [e.g., (9,16,21,22,25,26,36)]. There is, however, no clear consensus on the direction of the effects produced by estradiol; they have been shown to vary as a function of the dose of the hormone used and the time after administration that the effect is measured [see (36) for review]. Furthermore, with some exceptions, most previous research has employed high doses of estradiol that might be expected to have effects very different from those of low doses in the physiological range. The acute administration of low doses of estradiol increases the turnover of DA in the striatum (16,26), alters the affinity state of certain DA receptors (25), and enhances the effect of AMPH on striatal DA release, both in vivo and in vitro (1,2). These effects appear to be very short acting, beginning within 30 min after administration of the hormone. Given these data, it might be supposed that EB in combination with the intermediate dose of AMPH used in the preexposure period would act like a higher dose of AMPH to induce greater stereotypy, and concomitantly, decrease locomotor activity [cf. (24,32)]. This would also be expected given the evidence that intact, adult females show greater levels of stereotypy than intact, adult males (11,12) in response to high doses of AMPH. Instead, in the present experiments, in which a relatively moderate dose of AMPH was used, locomotor activity was increased and stereotypy decreased, in the presence of EB. This finding is currently under investigation.

Estradiol has also been shown to have longer-term effects on the midbrain DA systems and on behaviors dependent on these systems [e.g., (3,9,13,21)]. Therefore, although a single, low dose of estradiol may either enhance or suppress DA-

dependent behaviors, acutely, long-term effects induced by each injection of estradiol may have contributed to the results obtained in Experiment 2, in that the animals were receiving multiple exposures to a low dose of the hormone throughout the preexposure period.

Finally, in these experiments, there was little evidence that either neonatal exposure to T, or sex, increased the magnitude of sensitization to AMPH when the measure of sensitization was the difference in response to AMPH between animals preexposed to AMPH and those receiving the drug for the first time. This was true whether animals received EB (Experiment 2) or not (Experiment 1). Previous studies investigating sex differences in sensitization to AMPH have measured sensitization as a change in behavior with repeated treatment with the drug [e.g., (11,12)]. If we view the data from the present experiments in this way, evidence for a differential effect of T-exposure group on the development of sensitization comes from Experiment 2; the NO-T animals showed greater increases in AMPH-induced activity over the preexposure days than did the T animals. The NO-T animals were also more active in response to AMPH than the T animals on the last day of the preexposure period, when sensitization to the drug would be at its maximum. The direction of this effect can be compared to the data of Camp and Robinson (11,12), who found, using high doses of AMPH (3.0 and 2.6 mg/kg), that intact females showed greater increases in stereotypy with repeated injections than did intact males.

Sensitization has also been measured by the response of AMPH-preexposed animals to a subsequent challenge injection of the drug. Camp and Robinson (11,12) report a sex difference in response to a challenge with AMPH in intact animals after they had been preexposed to AMPH. A similar finding was found in AMPH-preexposed animals in these experiments on the test for sensitization; T animals were less active in response to the low-dose challenge injection of AMPH than were NO-T animals.

In conclusion, the present experiments demonstrate that at least part of the sex difference in responsiveness to AMPH in adult animals results from the exposure of the male animal to T during the early neonatal period for sexual differentiation. This exposure is not, however, sufficient to explain entirely the sex difference. It is probable that exposure of both sexes to gonadal hormones at other time points in development is also involved. Finally, the presence of, and the pattern of, circulating estradiol in the intact female animal undoubtedly contributes to the greater activity exhibited by this group.

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