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Environmental Enrichment Attenuates Locomotor Sensitization, But Not In Vitro Dopamine Release, Induced by Amphetamine

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BARDO, M. T., S. L. BOWLING, J. K. ROWLETT, P. MANDERSCHEID, S. T. BUXTON AND L. P. DWOSKIN. *Environmental enrichment attenuates locomotor sensitization, but not in vitro dopamine release, induced by amphetamine.* PHARMACOL BIOCHEM BEHAV 51(2/3) 397–405, 1995.—Rats were raised from weanling until young adulthood in either an enriched condition (EC) or isolated condition (IC). Following this, the locomotor and rewarding effects of amphetamine were determined using the conditioned place preference (CPP) paradigm. EC rats were more sensitive to the acute locomotor stimulant effect and rewarding effect of amphetamine relative to IC rats. In contrast, EC rats were less sensitive than IC rats to the locomotor sensitization effect obtained across repeated amphetamine injections. To determine the effect of environmental enrichment on alteration of brain dopamine (DA) function induced by amphetamine, the effect of amphetamine on electrically evoked release of DA and dihydroxyphenylacetic acid (DOPAC) was determined in vitro using tissue slices from the nucleus accumbens and striatum of EC and IC rats. No differences between EC and IC rats in release of DA or DOPAC were evident, suggesting that the environmentally induced difference in sensitivity to the behavioral effects of amphetamine involves a neural mechanism extrinsic to the mesolimbic and nigrostriatal terminal field regions.

Amphetamine Dopamine Dihydroxyphenylacetic acid Nucleus accumbens Striatum Environmental enrichment

INDIVIDUAL differences in sensitivity to drugs of abuse have been the focus of much research and attempts have been made to determine what variables may be critical predictors of these individual differences (15,25,26). Using various animal models, it has been demonstrated that both genetic and environmental factors contribute to individual differences in drug sensitivity. The influence of genetics has been studied largely using inbred strains of mice, whereas the role of the environment has been studied primarily using rats raised in different housing conditions.

Although numerous studies have found differences in sensitivity to psychostimulant drugs induced by raising rats in either a group-cage or single-cage housing condition (1,8,16, 30,31), perhaps the most profound environmentally induced differences are produced by raising rats in either an enriched or isolated housing condition (10,13,35). In an enriched condition, animals are maintained in social groups with novel objects that are rearranged daily to maintain a complex, interactive sensory experience. In contrast, isolated animals are raised alone, without objects for interaction. After being raised in these conditions from weanling to early adulthood, the animals are tested for their sensitivity to the behavioral effects of psychostimulant drugs.

Recent research from our laboratory has shown that environmental enrichment alters the effect of psychostimulant drugs on dopamine (DA)-mediated behaviors. In particular, we have focused on amphetamine-induced locomotor activity and conditioned place preference, as both of these behaviors have been shown previously to be blocked by either dopamine antagonists (14,23) or by 6-hydroxydopamine lesions in the nucleus accumbens (33). Rats raised in an enriched environment have been found to be more sensitive to the acute locomotor stimulant and rewarding effects of amphetamine relative to isolate-reared rats (6,7). Concomitant with these behavioral differences, enrich-reared rats are also more sensitive than isolate-reared rats to the amphetamine-induced

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decrease in the DA metabolite dihydroxyphenylacetic acid (DOPAC) content within the nucleus accumbens (6). When assessed using an in vitro slice preparation, however, we have found no differences between enrich- and isolate-reared rats in basal or amphetamine-induced release of DA from the nucleus accumbens (6,9). Taken together, these results suggest that the enhanced behavioral effects of acute amphetamine administration observed in enrich-reared animals may reflect either a pharmacokinetic difference in drug bioavailability or a pharmacodynamic difference in some brain region extrinsic to the nucleus accumbens.

At present, little is known about the influence of environmental enrichment on the effect of amphetamine administered chronically. It is well known that chronic administration of psychostimulant drugs enhances the locomotor stimulant effect, a phenomenon referred to as behavioral sensitization (19,28). In addition, it is also thought that chronic exposure to psychostimulants may enhance their rewarding effect as measured by the conditioned place preference paradigm (21). Because chronic drug effects need to be studied to understand the mechanisms involved in drug addiction, it seems important to examine the influence of environmental factors that may alter this process. Thus, the primary purpose of the present study was to examine the effect of repeated administration of amphetamine on locomotor activity and conditioned place preference in differentially reared animals.

A secondary purpose of the present study was to assess the effect of environmental enrichment on amphetamine-stimulated release of dopamine (DA) in the nucleus accumbens, as well as in the striatum. Previous work from our laboratory failed to find a difference in DA release evoked by amphetamine in vitro from accumbens slices taken from enrich-reared and isolate-reared rats (6). However, in that study we used a static in vitro preparation that measured DA release at only one time point (15 min). To increase the sensitivity of our assay procedure, in the present study we used a dynamic in vitro superfusion preparation that measured DA release in a minute-by-minute time frame.

METHOD

Subjects and Housing Conditions

The subjects were male Sprague–Dawley rats (Harlan Industries, Indianapolis, IN) received at 21 days of age. For each experiment, animals were randomly placed in either an enriched or isolated environmental condition immediately upon arrival in the laboratory. The enriched condition (EC) consisted of a large box, 94 × 94 × 51 cm high, made of plywood and painted grey. A 19 × 12 cm opening was made at the bottom center of each side and was covered with wire mesh. Through two of these openings, water was continuously provided, and laboratory rodent chow (Purina) was continuously provided in food hoppers hung inside the box on the other two openings. The floor was covered with pine chip bedding, and the lid to the box consisted of wire mesh tacked to a wooden frame to provide maximum ventilation. Various objects made of metal or hard plastic were provided in the box. These objects consisted of various plastic "toys" (e.g., rattles, buckets, blocks, trucks, ladders) purchased from a local store, as well as junk objects such as discarded milk jugs, tubing, and old cage materials. These objects were rearranged daily, with new objects being introduced each day. The rats were removed from their box so that the objects could be replaced and moved. Each EC box housed 12–14 rats. The floor area per animal exceeded the minimum space recommendations for rats outlined in the Guide for the Care and Use of Laboratory Animals (NIH, 1985, Table 2-1).

The isolated condition (IC) consisted of an individual hanging metal cage (17 × 24 × 20 cm high) constructed of three stainless steel solid walls, a wire mesh front wall, and a wire mesh floor. Water and chow were available continuously and each rat was housed singly. The IC rats were handled on three different occasions just prior to the beginning of conditioning to habituate them to handling (EC rats were handled daily when the objects were rearranged).

Drug

Amphetamine sulfate (Sigma) was dissolved in saline (0.9% NaCl), with dosages calculated based on the salt form of the drug. Amphetamine was injected subcutaneously at a volume of 1 ml/kg.

Behavioral Apparatus

The CPP apparatus consisted of a rectangular box with three compartments made of ½ in. plywood. The end compartments measured 24 × 30 × 45 cm high, with the smaller middle compartment measuring 24 × 10 × 45 cm high. One end compartment was painted white, had a wire mesh floor, and pine bedding beneath it. The other end compartment was painted black and had a rod floor with cedar chips beneath it. The middle compartment was painted grey and had a solid wood floor. Partitions separating the end compartments were replaceable with partitions that contained a 10 × 10 cm opening that would allow the rat free access to all three compartments. A white noise generator (ambient background of 70 dB) was also located in the same room. The CPP box was located in a room separate from the colony room. A video camera was hung directly over the apparatus to record behavior using a video monitor in an adjacent room.

Procedure for Locomotor Activity and Conditioned Place Preference

Previous work in our laboratory indicated that naive rats tend to display a slight preference for the black compartment of the CPP apparatus. Thus, in the first behavioral experiment, amphetamine was paired with the normally nonpreferred white compartment starting at 53 days of age. Rats from each environmental condition (EC or IC) were assigned randomly to one of the treatment groups (0, 0.1, 0.3, or 1 mg/kg amphetamine, n = 6–8 per group). Each conditioning trial took place over 2 days. On the first day, half of the rats from each environmental condition were injected with their respective drug dose and were placed individually in holding cages (individual standard hanging cages) for 10 min, then were placed in the white compartment for 20 min. The other half were injected with saline and were placed in a holding cage for 10 min, then were placed in the black compartment for 20 min. On the second day, rats that received a drug–white pairing were given a saline–black pairing, and rats previously given a saline–black pairing were given a drug–white pairing. This conditioning was continued for 8 consecutive days (four drug conditioning trials total).

To assess the acute and chronic locomotor effects of amphetamine during conditioning, horizontal activity (line crosses) and vertical activity (rears) were recorded for the first and fourth drug pairing in white for each rat. An observer, unaware of each rat's treatment, recorded the activity, with a line cross being defined as two front paws crossing a line

bisecting the compartment, and a rear being defined as two front paws leaving the floor, excluding grooming behavior. Activity was recorded according to a time sampling procedure in which data were recorded from minutes 0–4, 8–12, and 16–20 of the 20 min conditioning trial.

On the day immediately following the last conditioning day, each rat received a 10-min preference test while in a drug-free state. Partitions in the apparatus were replaced with partitions containing an opening to allow free access to the entire apparatus. The rat was placed in the middle grey compartment to begin the test. An observer, unaware of treatment, recorded the duration spent in the white and black compartments, as well as the number of entries into white and black. An entry was defined as two front paws crossing into the respective compartment. To assess extinction of the preference behavior, the test procedure was repeated on the next day for each animal.

A second behavioral experiment was conducted to determine if pretreating animals with amphetamine differentially affects the locomotor and rewarding effects of amphetamine in EC and IC animals. Beginning at 50 days of age, EC and IC rats received either amphetamine (1 mg/kg) or saline once daily for 6 consecutive days. All rats then received no injections for the next 6 days, as amphetamine-induced locomotor sensitization is generally more robust following a "washout" period (20,28,29); IC rats were not handled during this "washout" period, but EC rats were handled during the daily rearrangement of objects in the home cage. After the "washout" period, beginning at 63 days of age, rats were then conditioned with either amphetamine (1 mg/kg) or saline as described previously. Thus, there were four drug treatment groups ($n = 7$ per group) within each environmental conditioning: amphetamine pretreated/amphetamine conditioned (group AA); amphetamine pretreated/saline conditioned (group AS); saline pretreated/amphetamine conditioned (group SA); and saline pretreated/saline conditioned (group SS). For each of these groups, locomotor behavior and place preference behavior were measured as described previously.

Procedure for In Vitro DA Release

Rats were killed by rapid decapitation and the brains were removed and placed onto an ice-cold dissection plate. Using a McIlwain tissue chopper, 500-μm slices of tissue from the nucleus accumbens and striatum were dissected for each experiment. Slices were incubated for 60 min in Kreb's buffer containing, in mM: 118 NaCl, 4.7 KCl, 1.2 $MgCl_2$, 1.0 NaH_2PO_4, 1.3 $CaCl_2$, 11.1 glucose, 25 $NaHCO_3$, 0.11 L-ascorbic acid, and 0.004 EDTA (pH 7.4 and saturated with 95% O_2/5% CO_2) in a metabolic shaker at 34°C to allow for recovery of responsiveness and energy stores. After rinsing in fresh buffer, one striatal slice (9 mg/slice) or two nucleus accumbens slices (4 mg/slice) were transferred to a glass superfusion chamber containing two platinum electrodes. Slices were superfused at the rate of 1 ml/min with Krebs buffer maintained at 34°C and pH 7.4 by continual aeration. Sample collection began after 60 min of superfusion when the rate of basal outflow was stabilized.

Experiments were performed to determine the effect of amphetamine on stimulation-evoked overflow from nucleus accumbens and striatal slices from EC and IC rats. After 60 min of superfusion, three samples (1 ml each) were collected to determine basal outflow of DA and DOPAC. Slices were then superfused for 5 min prior to the electrical stimulation with buffer containing amphetamine (0.1, 1.0, or 10 μM for accumbens slices and 1.0 or 10 μM for striatal slices). During the 5-min period of superfusion with amphetamine, five 1-ml samples were collected at 1-min intervals. Subsequently, slices were depolarized for a 1-min period (60 pulses, 1 Hz, unipolar rectangular pulses, 20 mA, 2 ms duration) with a Grass stimulator, model SD9. Following stimulation, slices were superfused for an additional 45 min with either buffer alone or buffer containing amphetamine. Fifteen consecutive 1-ml samples followed by three 1-ml samples at 5-min intervals were collected to determine the effect of amphetamine on stimulation-evoked overflow. In each experiment, one chamber containing EC nucleus accumbens or striatal slices and one chamber containing IC slices were superfused for the entire experiment in the absence of amphetamine and served as controls.

The concentration of DA and DOPAC in superfusate was determined using an HPLC-EC methodology described previously (11). Superfusate samples were collected on ice and stored at −70°C until assay. Ascorbic acid oxidase (10 μl of 0.5 mg/ml, pH 5 acetate buffer) was added to each thawed sample; 50 μl of the resulting solution was injected onto the HPLC. Chromatograms were recorded using a dual pen recorder. Retention times of standards were used to identify all peaks. Peak heights were used to calculate the detected amounts of each compound. The HPLC-EC system consisted of a Beckman model 116 pump, Beckman model 507 autosampler, ESA 3-μm (4.6 × 75 mm) ODS ultrasphere reverse-phase column, and an ESA 5100-A Coulochem electrochemical detector with a model 5011 dual detector analytical cell (E1 and E2 set at oxidation potentials of 0.05 and 0.32 V, respectively). The eluent was 0.07 M citrate/0.1 M acetate buffer, pH 4.0, containing 50 mg/l disodium EDTA, 100 mg/l octylsulfonic acid-sodium salt, and 7% methanol. All separations were performed at room temperature using a flow rate of 2 ml/min. Complete separations of DA and DOPAC and reequilibration of the system took 5 min. The detection limit of the system was typically 1 pg/ml superfusate for both DA and DOPAC.

Data Analysis

In the first behavioral experiment, line crosses and rears were analyzed separately using a 2 × 4 × 2 mixed factor analysis of variance (ANOVA), with two levels of environment (EC or IC), four levels of conditioning drug (0, 0.1, 0.3, or 1.0 mg/kg amphetamine), and two levels of conditioning day (first or last). On the two place preference tests days, the data from each animal were transformed into a place preference ratio score in which the duration spent in the drug-paired white compartment was divided by the duration spent in both the white and black compartments. Preference ratios from each test day were then analyzed separately using a 2 × 4 factorial ANOVA, with two levels of environment and four levels of conditioning drug. Where significant interactions occurred, the overall analysis was broken down to one-way ANOVAs with subsequent post hocs using either Tukey's HSD test to make comparisons across environments or Dunnett's test to compare drug groups to saline control. Where significant interactions were not obtained, Tukey's and Dunnett's tests were performed using the overall ANOVA mean square error. In those cases where conditioning day was a repeated measure, Bonferroni's t statistic was used to make comparisons across days. The data from the second behavioral experiment were analyzed similarly, except that the between-subject factors in the overall ANOVAs consisted of two levels of envi-

ronment (EC or IC), two levels of pretreatment drug (amphetamine or saline), and two levels of conditioning drug (amphetamine or saline).

The neurochemical data were analyzed using a mixed factor ANOVA for DA and DOPAC values from the striatum and nucleus accumbens, using the environmental condition as a between-subject factor and amphetamine concentration as a within-subject factor.

RESULTS

Amphetamine-Induced Locomotion and CPP in EC and IC Rats

Figure 1 summarizes the amphetamine dose-effect curves for horizontal and vertical activity in EC and IC rats on the first and last conditioning days. The overall ANOVA for line crosses revealed significant main effects of environment, $F(1, 108) = 43.11$, $p < 0.0001$, drug, $F(3, 108) = 160.80$, $p < 0.0001$, and conditioning day, $F(1, 108) = 10.06$, $p < 0.01$, as well as significant interactions of environment × drug, $F(3, 108) = 3.15$, $p < 0.05$, environment × conditioning day, $F(1, 108) = 15.22$, $p < 0.001$, and drug × conditioning day, $F(3, 108) = 19.10$, $p < 0.0001$. The overall ANOVA for rears revealed significant main effects of drug, $F(3, 108) = 113.20$, $p < 0.0001$, and conditioning day, $F(1, 108) = 22.90$, $p < 0.0001$, as well as significant interactions of environment × drug, $F(3, 108) = 11.73$, $p < 0.0001$, environment × conditioning day, $F(1, 108) = 32.30$, $p < 0.0001$, drug × conditioning day, $F(3, 108) = 40.62$, $p < 0.0001$, and environment × drug × conditioning day, $F(3, 108) = 2.94$, $p < 0.05$.

Post hoc statistical comparisons for activity measures in saline-injected control animals revealed that EC rats were less active than IC rats (Fig. 1). This difference in saline-injected controls was statistically significant on both the first and last conditioning days, except for rearing on conditioning day 1. Also, as expected, acute administration of amphetamine produced a dose-dependent increase in line crosses and rears in both EC and IC rats. That is, on conditioning day 1, post hoc analyses revealed that both EC and IC rats given 0.3 or 1.0 mg/kg amphetamine displayed increased line crosses and rears relative to saline controls. Additionally, the EC rats given 1 mg/kg amphetamine on conditioning day 1 showed a significant increase in rearing compared to IC rats given 1 mg/kg amphetamine, indicating that EC rats were more sensitive to the acute stimulant effect of amphetamine, at least for vertical activity.

Post hoc statistical comparisons between the first and last conditioning days also revealed that repeated amphetamine injections produced dose-dependent locomotor sensitization in both EC and IC rats (Fig. 1). At the highest dose of amphetamine tested (1 mg/kg), both EC and IC rats displayed a

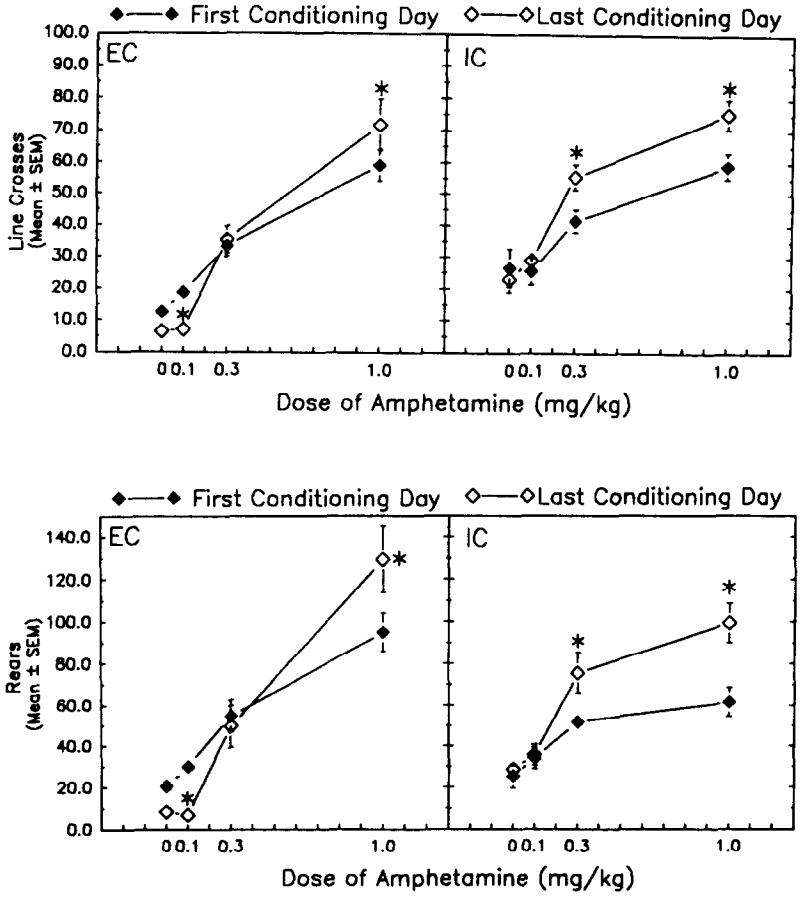

FIG. 1. Horizontal and vertical activity on the first and last conditioning trial from EC and IC groups administered either 0, 0.1, 0.3, or 1.0 mg/kg amphetamine. Asterisks (*) represent a significant difference from the first conditioning trial, $p < 0.05$.

significant increase in line crosses and rears from the first to the last conditioning day. In contrast, the 0.3-mg/kg dose of amphetamine increased line crosses and rears from the first to the last conditioning day in IC rats, but not in EC rats. Direct comparisons between EC and IC rats on the last conditioning day revealed that EC rats line crossed and reared significantly less than IC rats following either 0.1 or 0.3 mg/kg amphetamine, indicating that EC rats were less sensitive to the chronic stimulant effect of amphetamine. Additionally, there was a significant decrease in line crosses and rears from the first to the last conditioning day following 0.1 mg/kg amphetamine in EC rats, but not IC rats.

Figure 2 summarizes the place preference results from EC and IC rats on the 2 test days. The overall ANOVA for preference ratio scores from test day 1 revealed significant main effects of environment, $F(1, 50) = 27.06$, $p < 0.0001$, and drug, $F(3, 50) = 3.27$, $p < 0.05$, whereas the overall ANOVA for preference ratio scores from test day 2 revealed only a significant main effect of environment, $F(1, 50) = 12.34$, $p < 0.001$. Post hoc statistical comparisons on test day 1 revealed that EC rats conditioned with saline displayed lower ratio scores than IC rats conditioned with saline. More important, post hoc comparisons also revealed that EC rats were more sensitive than IC rats to amphetamine-induced CPP. That is, relative to their saline-injected control groups on test day 1, amphetamine increased preference ratios at all doses tested in EC rats. In contrast, there was no significant difference between any dose of amphetamine and the saline control group in IC rats. On test day 2, the only group that maintained a significant CPP was the EC group that was conditioned with 0.3 mg/kg amphetamine.

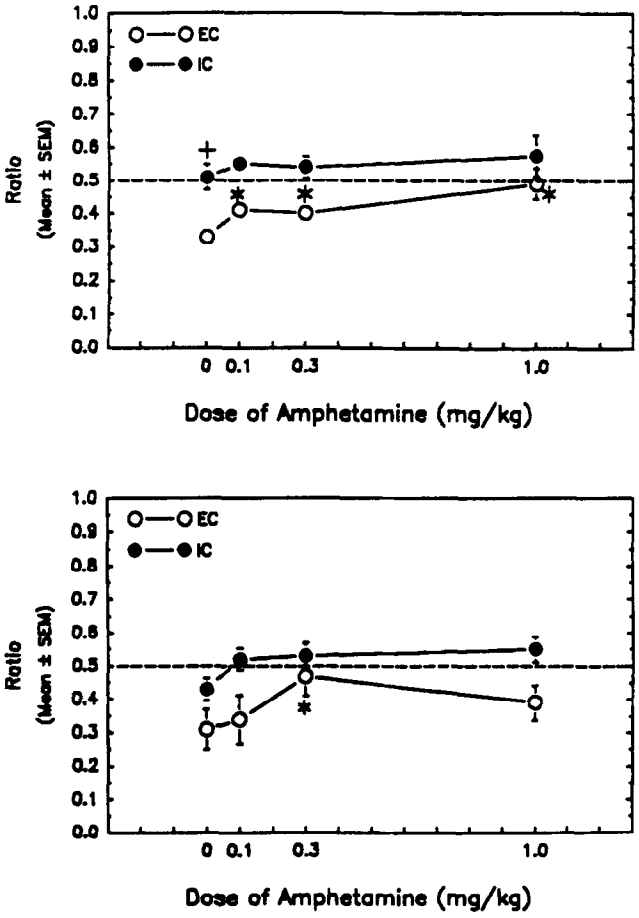

FIG. 2. Preference ratio on the CPP test day from EC and IC groups conditioned with either 0, 0.1, 0.3, or 1.0 mg/kg amphetamine on test day 1 (top panel) and test day 2 (bottom panel). Ratio score was calculated as duration in white compartment divided by duration in white and black compartments. Asterisks (*) represent a significant difference from the saline control, $p < 0.05$ and plus sign (+) represents a significant difference from EC group, $p < 0.05$.

Effect of Amphetamine Pretreatment on Amphetamine-Induced Locomotion and CPP in EC and IC Rats

Figure 3 summarizes the effect of amphetamine pretreatment on amphetamine-induced horizontal and vertical activity in EC and IC rats on the first and last conditioning days. The overall ANOVA for line crosses revealed a significant main effect of conditioning drug, $F(1, 104) = 814.17$, $p < 0.0001$, as well as significant interactions of environment × conditioning drug, $F(1, 104) = 50.25$, $p < 0.0001$, environment × conditioning day, $F(1, 104) = 21.82$, $p < 0.0001$, conditioning drug × conditioning day, $F(1, 104) = 40.09$, $p < 0.0001$, and environment × conditioning drug × conditioning day, $F(1, 104) = 17.24$, $p < 0.0001$. The overall ANOVA for rears revealed significant main effects of environment, $F(1, 104) = 20.31$, $p < 0.0001$, conditioning drug, $F(1, 104) = 362.49$, $p < 0.0001$, and conditioning day, $F(1, 104) = 8.08$, $p < 0.01$, as well as significant interactions of environment × conditioning day, $F(1, 104) = 38.72$, $p < 0.0001$, conditioning drug × conditioning day, $F(1, 104) = 30.17$, $p < 0.0001$, and environment × conditioning drug × conditioning day, $F(1, 104) = 26.49$, $p < 0.0001$. There was no main effect of pretreatment drug on either line crosses or rears.

Similar to the previous behavioral experiment, activity measures in saline-injected control animals revealed that EC rats tended to be less active than IC rats (Fig. 3). However, post hoc statistical comparisons showed no significant differences in line crosses or rears between EC and IC saline-controls (groups SS) on the first or last conditioning days. As expected, acute administration of amphetamine produced an increase in line crosses and rears in both EC and IC rats. That is, on conditioning day 1, post hoc analyses revealed that both EC and IC rats conditioned with amphetamine (groups SA and AA) displayed increased line crosses and rears relative to rats conditioned with saline (groups SS and AS). Additionally, EC rats given amphetamine on conditioning day 1 showed a significant increase in line crosses compared to IC rats given amphetamine, indicating that EC rats were more sensitive to the acute stimulant effect of amphetamine, at least for horizontal activity.

Post hoc statistical comparisons between the first and last conditioning days revealed that repeated amphetamine injections across the four conditioning days produced locomotor sensitization in IC rats, but not EC rats (Fig. 3). That is, only IC rats conditioned with amphetamine (groups SA and AA) displayed a significant increase in line crosses and rears from the first to the last conditioning day. Pretreatment with amphetamine prior to conditioning had no significant effect on line crosses or rears in either EC or IC rats conditioned with amphetamine or saline (i.e., AA group is not significantly different from SA group).

Figure 4 summarizes the place preference results from EC

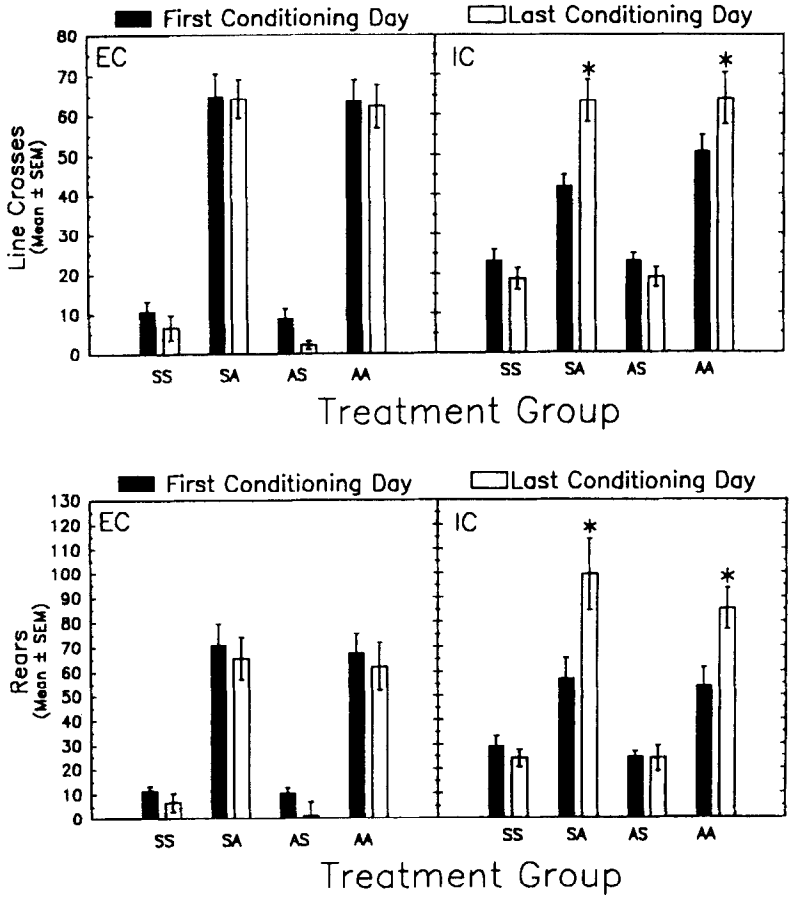

FIG. 3. Horizontal and vertical activity on the first and last conditioning trial from EC and IC groups pretreated with amphetamine (1 mg/kg) or saline and then conditioned with amphetamine (1 mg/kg) or saline. Group SS was pretreated with saline and conditioned with saline; group SA was pretreated with saline and conditioned with amphetamine; group AS was pretreated with amphetamine and conditioned with saline; and group AA was pretreated with amphetamine and conditioned with amphetamine. Asterisks (*) represent a significant difference from the first conditioning trial, $p < 0.05$.

and IC rats on the 2 test days. The overall ANOVA for preference ratio scores from test day 1 revealed only a significant main effect of conditioning drug, $F(1, 48) = 65.02$, $p < 0.0001$, whereas the overall ANOVA for preference ratio scores from test day 2 revealed significant main effects of environment, $F(1, 48) = 4.34$, $p < 0.05$, and conditioning drug, $F(1, 48) = 18.54$, $p < 0.0001$, as well as a significant interaction of pretreatment drug × conditioning drug, $F(1, 48) = 7.02$, $p < 0.01$. Post hoc statistical comparisons on test day 1 revealed that, regardless of environmental condition, rats conditioned with amphetamine (groups SA and AA) displayed significantly higher ratio scores than rats conditioned with saline (groups SS and AS), indicating that CPP was obtained. On test day 2, only the rats that were pretreated with saline and conditioned with amphetamine (group SA) displayed a significant CPP.

Amphetamine-Induced DA Overflow in Nucleus Accumbens and Striatal Slices From EC and IC Rats

The results shown in Table 1 revealed a significant main effect of amphetamine concentration on DA overflow in the nucleus accumbens, $F(3, 69) = 6.91$, $p < 0.001$, and striatum, $F(2, 45) = 54.31$, $p < 0.0001$. In both brain regions amphetamine increased DA overflow in a concentration-dependent manner. However, there was no significant main effect of environmental condition nor any significant interaction between environmental condition and amphetamine concentration in the ANOVAs from nucleus accumbens or striatum. These latter results indicate that the effect of amphetamine on DA overflow was similar in tissue slices taken from EC and IC rats.

Analysis of DOPAC overflow revealed a significant main effect of amphetamine in the nucleus accumbens, $F(3, 83) = 37.34$, $p < 0.0001$, and striatum, $F(2, 59) = 103.06$, $p < 0.0001$ (Table 1). In both brain regions amphetamine decreased DOPAC overflow in a concentration-dependent manner. However, there was no significant main effect of environmental condition nor any significant interaction between environmental condition and amphetamine concentration in the ANOVAs from nucleus accumbens or striatum. These latter results indicate that the effect of amphetamine on DOPAC overflow was similar in tissue slices taken from EC and IC rats.

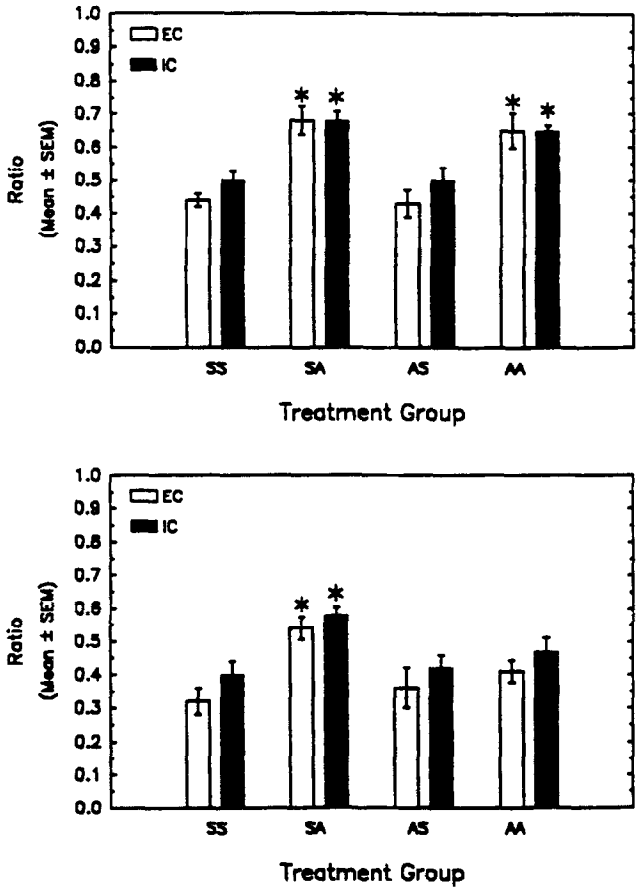

FIG. 4. Preference ratio on the CPP test day from EC and IC groups pretreated with amphetamine (1 mg/kg) or saline and then conditioned with either amphetamine (1 mg/kg) or saline on test day 1 (top panel) and test day 2 (bottom panel). Ratio score was calculated as duration in white compartment divided by duration in white and black compartments. Treatment group designations are identical to those described in the legend of Fig. 3. Asterisks (*) represent a significant difference from the SS control group, $p < 0.05$.

DISCUSSION

The present results show that the acute locomotor stimulant effect of amphetamine, expressed by vertical rearing, is greater in EC rats that in IC rats. In the absence of amphetamine, the level of activity in EC rats was found to be lower than in IC rats, a finding that corroborates several previous studies (6,7,22). However, when challenged with amphetamine (0.1–1 mg/kg), EC rats were more active than IC rats, a finding that also corroborates previous research (6,7). This environmentally induced difference in activity was found to be dose dependent, as it was evident at 1 mg/kg amphetamine, but not at either 0.1 or 0.3 mg/kg amphetamine.

In contrast to the acute stimulant effect of amphetamine, the present study found that behavioral sensitization produced by chronic amphetamine, expressed by either horizontal or vertical activity, is less in EC rats than in IC rats. This environmentally induced difference in response to amphetamine across four injections (conditioning trials) was found to be dose dependent. That is, whereas 1 mg/kg amphetamine produced sensitization in both EC and IC rats, only IC rats given 0.3 mg/kg amphetamine displayed sensitization. At the lowest dose tested (0.1 mg/kg), neither EC or IC rats displayed sensitization to amphetamine; to the contrary, there was a decrease in activity in EC rats, but not in IC rats, across four repeated conditioning trials at this ineffective dose. This latter finding likely reflects a greater rate of habituation to novelty in EC rats compared to IC rats across four repeated conditioning trials. Consistent with this, other research has shown that IC rats show a greater degree of "behavioral rigidity" than socially reared rats (18).

EC and IC rats also appeared to differ in their sensitivity to amphetamine-induced CPP, at least with the lower doses of amphetamine tested. That is, when compared to their respective saline-injected control groups, EC rats showed a greater preference for the amphetamine-paired compartment than IC rats. This environmental difference in CPP was evident using 0.1, 0.3, or 1 mg/kg amphetamine. However, in a follow-up experiment, no environmental difference in CPP was evident using 1 mg/kg amphetamine. We have no cogent explanation for the failure to replicate the environmental difference in CPP induced by 1 mg/kg amphetamine across the two behavioral experiments of the present study. Despite this discrepancy at the highest dose of amphetamine tested, however, the conclusion that EC rats are more sensitive to amphetamine-induced CPP than IC rats is most consistent with our previous results (7).

It is interesting to note the apparent paradox that, relative to IC rats, EC rats appear to be more sensitive to amphetamine-induced CPP but less sensitive to the locomotor-stimulant effect of amphetamine across repeated conditioning trials. One explanation of this apparent paradox is that the locomotor-stimulant and rewarding effects of amphetamine represent dissociable phenomena, perhaps with different neural substrates. However, this explanation is not consistent with current theoretical formulations about the neurobiology of drug abuse that link locomotor behavior and reward (34).

TABLE 1

EFFECT OF AMPHETAMINE ON STIMULATION-EVOKED DA AND DOPAC OVERFLOW

Dopamine Overflow			
Brain Region	Amphetamine (μM)	EC Rats (N = 13)	IC Rats (N = 13)
N. accumbens	0	4.3 ± 2.4	12.6 ± 5.1
	0.1	12.4 ± 6.0	31.0 ± 14.6
	1.0	57.6 ± 20.9	117.6 ± 40.9
	10.0	188.8 ± 88.5	162.9 ± 52.6
Striatum	0	11.1 ± 5.3	20.6 ± 6.8
	1.0	75.3 ± 25.9	50.7 ± 14.1
	10.0	447.6 ± 67.1	328.0 ± 72.0
Dopac Overflow			
Brain Region	Amphetamine (μM)	EC Rats (N = 19)	IC Rats (N = 15)
N. accumbens	0	236.3 ± 23.3	224.4 ± 25.1
	0.1	156.9 ± 39.1	126.5 ± 29.4
	1.0	64.7 ± 13.9	33.2 ± 11.5
	10.0	40.2 ± 8.0	14.9 ± 4.1
Striatum	0	161.3 ± 14.3	173.3 ± 22.6
	1.0	50.5 ± 10.2	31.1 ± 8.6
	10.0	20.9 ± 4.3	16.5 ± 4.4

DA and DOPAC overflow (bottom panel) from nucleus accumbens and striatal slices are expressed as mean ± SEM pg/mg/min.

An alternative explanation of this apparent paradox is that amphetamine-induced CPP may be primarily established during the first conditioning trial, rather than during later conditioning trials. Consistent with this notion, drug-induced CPP has been demonstrated using a single trial (2,3,24). Because EC rats are more sensitive than IC rats to the locomotor stimulant effect of an acute injection of amphetamine, they may also be sensitive to the rewarding effects of amphetamine administered on the first conditioning trial. This enhancement of reward in EC rats on trial 1 might potentiate the establishment of CPP across repeated trials.

One unexpected finding in the present study was that pretreating animals with amphetamine prior to conditioning did not produce sensitization to amphetamine-induced locomotor activity or CPP. With locomotor behavior, amphetamine pretreatment had no significant effect in either EC or IC rats across conditioning trials 1 and 4. With CPP, amphetamine pretreatment appeared to decrease the strength of reward in both EC and IC rats. That is, on test day 2, amphetamine-pretreated rats showed a diminished amphetamine-induced CPP relative to rats pretreated with saline (cf. AA and SA groups in Fig. 4). Because CPP is generally thought to reflect a form of classical conditioning, these data are consistent with the literature suggesting that preexposure to an unconditioned stimulus (i.e., amphetamine) prior to conditioning can weaken the strength of the conditioned response (27). However, they are contrary to a previous report that found pretreating rats with amphetamine enhances amphetamine-induced CPP (21).

In the present report, amphetamine pretreatment consisted of six daily injections of 1 mg/kg amphetamine in the home cage environments, with the sixth pretreatment injection occurring 4 days prior to the first amphetamine conditioning trial. This pretreatment regimen was chosen based upon a previous report showing sensitization to amphetamine-induced CPP (21). However, the failure to obtain sensitization with amphetamine pretreatment in the present study may have occurred because of a number of procedural reasons. For instance, the previous study (21) used a slightly higher dose of amphetamine (1.5 mg/kg) than in the present study. Also, in contrast to the present study, the previous study (21) included a procedure in which rats were given amphetamine-induced taste aversion training prior to CPP training to reduce the aversive aspect of amphetamine. This latter procedure may be necessary to produce sensitization to amphetamine-induced CPP. In any case, because repeated amphetamine injections failed to produce sensitization when administered prior to CPP conditioning in the present report, but did produce sensitization when administered during CPP conditioning, the type of sensitization obtained here from the first to the last conditioning trial appears to be context specific [see (4,12)].

Finally, the present study found no difference between EC and IC rats in the effect of amphetamine on in vitro stimulation-evoked release of DA or DOPAC in either the nucleus accumbens or striatum. This finding, which was obtained using a superfusate methodology, corroborates previous results obtained with a static (no superfusion) methodology (6). When viewed together with the behavioral data, these results suggest that the critical change underlying the environmentally induced alteration in sensitivity to amphetamine may occur in brain areas extrinsic to the DA terminal fields of the nucleus accumbens and striatum. For example, it may be that neural changes in dopaminergic cell bodies within the midbrain ventral tegmental area may be involved, as this somatodendritic region has been implicated in mediating the changes induced by chronic exposure to amphetamine (19). Alternatively, we cannot rule out the possibility that environmentally induced differences in sensitivity to the behavioral effects of amphetamine may be mediated by pharmacokinetic factors related to the bioavailability of amphetamine to the brain when administered in vivo. For example, EC rats have a larger capillary blood volume in brain (32) and smaller livers (5) compared to IC rats. These pharmacokinetic factors may explain the enhanced locomotor effect (6,7) and enhanced neurotransmission of DA (6,17) in EC rats following an acute injection of amphetamine in vivo. With repeated injections, however, EC rats may metabolize amphetamine more rapidly than IC rats, thus attenuating the process of locomotor sensitization.

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