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Early Handling and Maternal Behavior: Effect on d-Amphetamine Responsiveness in Rats

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SCHREIBER, H., R. BELL, G. WOOD, R. CARLSON, L. WRIGHT, M. KUFNER AND R. VILLESCAS. *Early handling and maternal behavior: Effect on d-amphetamine responsiveness in rats.* PHARMAC. BIOCHEM. BEHAV. 9(6) 785–789, 1978.—This study determined whether early handling and maternal behavior could influence the behavioral changes associated with chronic amphetamine administration. In Experiment 1, seven litters received early handling (rat pups were removed from the nest for 3 min daily during the first week following parturition) and 7 litters were undisturbed. At 105 days of age, male animals from non-handled litters showed a decline in rearings induced by 2.0 mg/kg d-amphetamine across 12 test days in a Y-maze but handled animals showed no such pattern. Handling had no effect on entries. In Experiment 2, four litters were handled and returned to a mother-present nest (H), 4 litters were handled and returned to a mother-absent nest 1 hr prior to reunion with the rat mother (HMS), 4 litters were separated from mothers for 1 hr (MS), and 4 litters were undisturbed (C). At 55 days of age, a handling (Groups H and HMS) effect on open-field activity was observed. At 75 days of age, male animals were injected with 0.0, 2.5 or 10.0 mg/kg d-amphetamine and tested in a Y-maze. At 2.5 mg/kg handled animals, whether maternally separated or not (H and HMS) showed no decline in drug-induced rearings; maternally separated animals whether handled or not (MS and HMS) showed fewer entries and increased body weight. However, only animals at 2.5 mg/kg from handled, mother-present litters (H) showed a retarded increase in stereotypy across 8 test days. These results indicated that behavioral manipulations early in life may influence responsivness to d-amphetamine in adulthood, either directly or through associated changes in maternal behavior.

Early handling Maternal behavior Chronic drug administration d-Amphetamine Stereotypy
Tolerance Infantile stimulation Locomotor activity Catecholamines Rats

EVENTS experienced in infancy can reduce the toxicity associated with a single high dose of amphetamine in adulthood. In mice, early handling reduced latency to convulse and percent mortality [14]. In rats, early handling plus return to a mother present nest increased latency to death following d-amphetamine overdose, whereas early handling plus return to a mother absent nest did not, thus implying a crucial maternal component [11]. Conceivably, these early life experiences might influence the behaviors induced by chronic low dose amphetamine administration as well. Therefore, Experiment 1 of the present study investigated the effect of early handling on d-amphetamine-induced locomotor activity, whereas Experiment 2 investigated the effect of early handling plus maternal presence or absence on sterotypy and locomotor activity.

METHOD

Animals and Housing

In Experiment 1, fourteen Sprague-Dawley rats and their litters (ranging from 5 to 13 pups per litter) were placed in standard hanging wire-mesh cages modified with hardboard panels to form nesting areas. At 28 days of age pups were weaned (1 cage per same sexed littermates). At 40 days of age males were placed in individual cages. Females were discarded.

In Experiment 2, sixteen mothers and litters (reduced to 8 pups per litter on the first day after parturition) were housed in opaque plastic maternity cages with cedar shavings bedding. At 21 days of age, litters were weaned and placed in individual wire-mesh cages.

Manipulations

In Experiment 1, half of the litters were handled according to typical handling procedures [3,7] except that the mothers were removed to a holding area during the handling episode. That is, from their second through their seventh day after birth, pups were removed from the nest and singly

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placed in shavings filled containers for 3 timed minutes daily. Control litters and mothers were not disturbed.

In Experiment 2, four litters were randomly assigned to each of the following 4 treatments for the second to the sixth day after parturition: (1) handled litters (H) were removed for 3 min and returned to the mother present nest, (2) the handled and mother-separated litters (HMS) were removed for 3 min and when returned to the nest, the mother rats were separated from them for 1 hr by a solid or perforated hardboard partition. The long hour period of separation for group HMS was to allow handled pups to return to "normal", (3) as a control for the hour long period of separation, mother-separated litters (MS) were undisturbed, but the mother rat was separated from the pups by a solid or perforated hardboard partition for 1 hr, (4) control litters (C) were undisturbed. Following reunion of Groups H, HMS and MS mothers with pups, observations of maternal behavior were recorded at 15 sec intervals for 20 min. At 55 days of age the offspring received 4 daily 4 min tests in an open field. At 60 days of age, the offspring were observed during a single 10 min trial in an exploratory maze with 4 arms providing differing amounts of tactile stimulation.

Drug Trials

In Experiment 1, animals received 5 consecutive days of saline (0.10 cc, SC) injections at 105 days of age (± 5). Then, animals (one from each litter) were assigned to a d-amphetamine (2 mg/kg, 2 mg/cc, SC) group or saline group (comparable volume, SC), Ns=7 per cell. At the seventh hour of light ± 2 hr, animals were injected daily in random order 30 (± 5) minutes prior to testing. Rearing and entries were recorded for 12 consecutive test days with a single daily 6 min trial in a Y-maze with dark gray arms 18 wide×45 long×30 cm high, marked at the beginning of each arm by a red line. Six days of post-test, when the saline groups as well as the amphetamine groups received amphetamine, followed the test period. A "rearing" was defined as the animal standing on its hind limbs with neither forepaw touching the Y-maze floor; the rearing was terminated when a single forepaw touched the floor. An "entry" occurred when the base of the animal's tail passed beyond the red line marking the entrance to the arm of the Y-maze. In Experiment 2, twelve male animals (75 ± 5 days of age) from each group (MS, H, HMS, C) were injected (30 min ± 5 min prior to testing) with one of 3 treatments, 0.0 mg/kg, 2.5 mg/kg, or 10.0 mg/kg d-amphetamine (comparable volume of saline vehicle, SC), Ns=4 per cell. Female animals were used in other experiments [11]. Two observers independently recorded rearings, entries, and stereotypy for 3 min trials for 8 days with each observer seeing half of the animals per cell on one day and the other half on the following day. On the ninth day, all animals received d-amphetamine at 2.5 mg/kg, SC. Entries and rearings were defined as in Experiment 1. "Stereotypy" was defined as seconds spent in repetitive head bobbing and sniffing.

Statistical Analysis

Experiment 1 scores were summed across two-day blocks, minimizing day-to-day variation, in order to meet the homogeneity of variance requirements of analysis of variance. Experiment 2 scores were summed across two-day blocks to eliminate observer differences and, further, were transformed with a square root transformation $(x+1)^{1/2}$ to meet homogeneity of variance requirements.

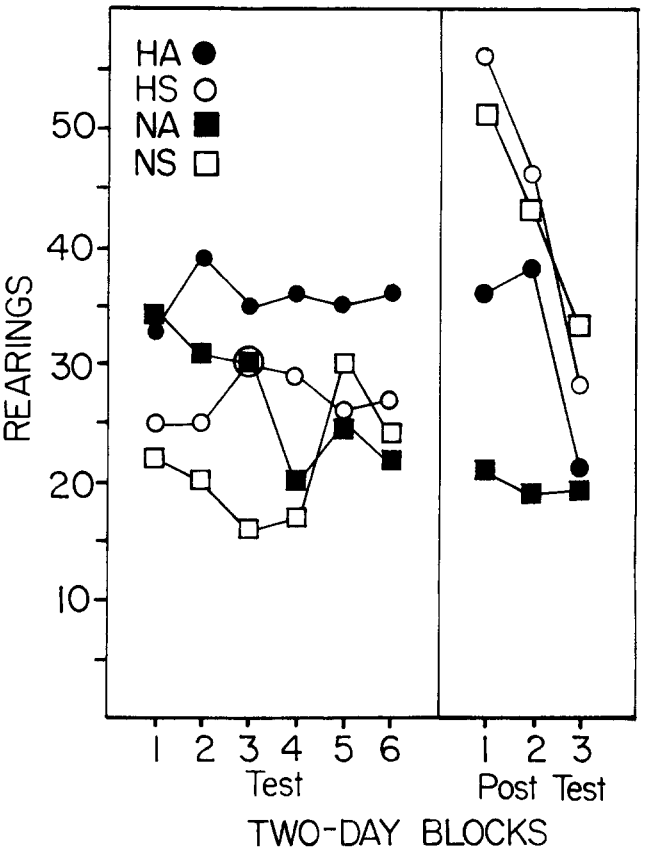


FIG. 1. Mean number of rearings per two-day block for test and post-test periods for the handled/amphetamine (HA), non-handled/amphetamine (NA), handled/saline (HS), and non-handled/saline (NS) groups (all Ns=7).

RESULTS

Experiment 1

Rearings. The rearings of the non-handled/amphetamine group declined significantly from block 1 to block 6, $F(1,44)=8.025$, $p<0.01$, as illustrated in Fig. 1. No other group showed a comparable decline during the test period.

On the first two-day block of post-test (when all animals received d-amphetamine 2.0 mg/kg), animals with a dose history of saline showed significantly more rearings than animals with a dose history of d-amphetamine, $F(1,72)=6.095$, $p<0.05$. Also during post-test, there was a significant decline over blocks for the amphetamine groups, $F(1,48)=5.814$, $p<0.05$, and the saline groups, $F(1,48)=33.305$, $p<0.01$, with the saline groups showing the greatest rate of decline. While the non-handled/amphetamine continued their slow rate of decline (from mean=20.5, SD=16.1 on block 1 post-test to mean=19.2, SD=15.6 on block 3 post-test), the handled/amphetamine group showed a much faster rate of decline (from mean=36.1, SD=13.1 on block 1 post-test to mean=20.6, SD=9.8 on block 3 post-test). Handled groups showed the greatest decline in post-test rearings, $F(1,48)=33.258$, $p<0.01$.

Entries. All groups showed equivalent patterns of entries

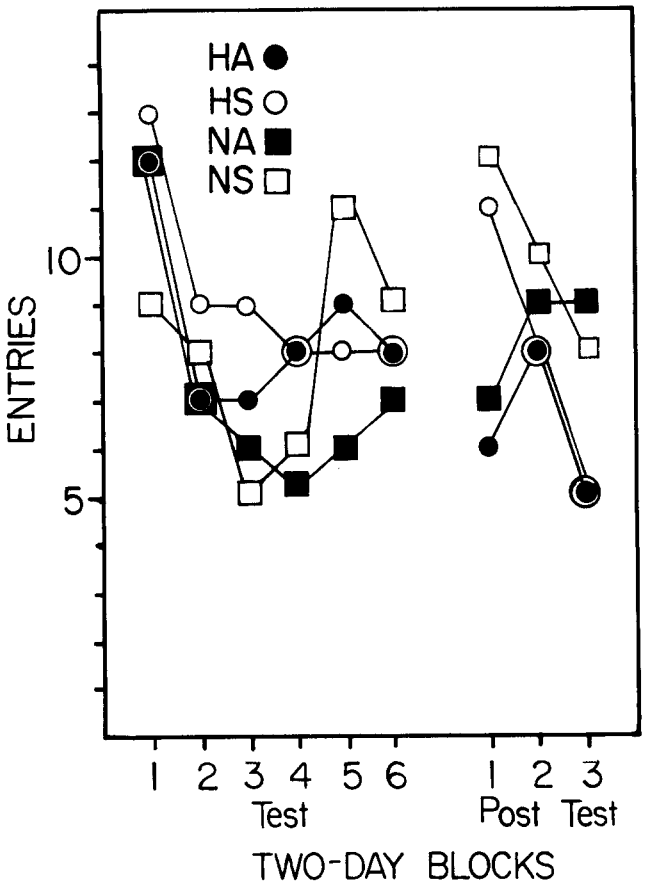


FIG. 2. Mean number of entries per two-day block for test and post-test periods for the handled/amphetamine (HA), non-handled/amphetamine (NA), handled/saline (HS) and non-handled/saline (NS) groups (all Ns=7).

which varied curvilinearly over 2 day blocks, as illustrated in Fig. 2.

Animals with a dose history of saline showed the greatest rate of decline in entries over blocks during the post-test amphetamine injection period, $F(2,48)=8.713$, $p<0.01$. However, despite the observed difference in entries, there was no significant difference between saline dose history and amphetamine dose history animals on any single block.

Experiment 2

Although a synopsis is included here, maternal behavior and offspring open-field behavior are presented elsewhere [16]. No significant differences in maternal behavior were detected between groups. Handled pups, regardless of whether they had been returned to a mother present or a mother absent nest, showed more rearings in the open-field. Handled, mother absent (Group HMS) males exhibited less defecation than males simply maternally-separated (Group MS). No significant differences between groups were found in the exploratory-maze results.

Rearings. As may be seen in Fig. 3A, only groups receiving 2.5 mg/kg d-amphetamine showed differences between Groups H, HMS, MS and C over the four two-day blocks.

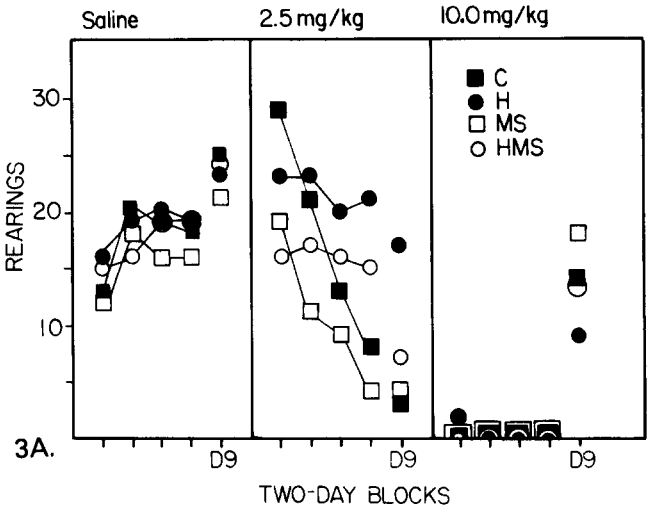
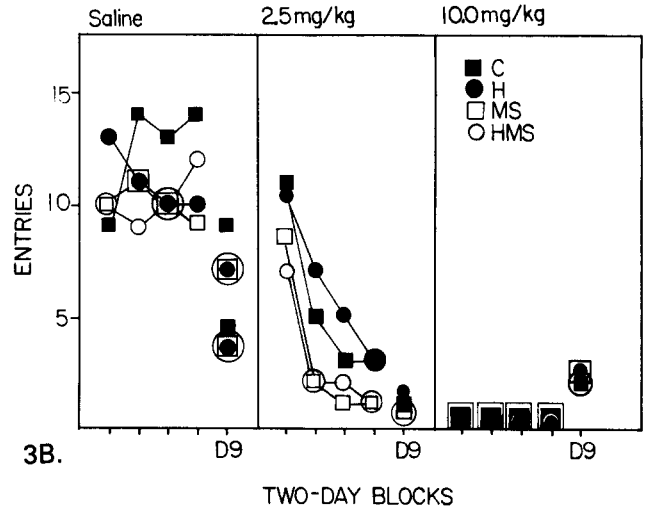
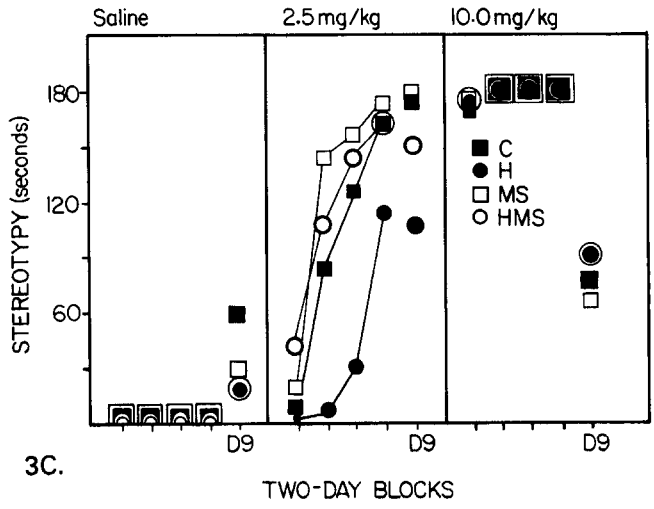


FIG. 3A. Mean number of rearings for the control (C), handled, mother present (H), maternally separated (MS), and handled, mother absent (HMS) subjects at 3 d-amphetamine levels (saline, 2.5 mg/kg, 10.0 mg/kg) over 4 two-day blocks and Day 9.





The control group (C), and the maternally-separated group (MS), showed a significant decline in rearings across two-day blocks, $F(3,108)=19.934$, $p<0.01$ and $F(3,108)=10.9802$, $p<0.01$, respectively. Neither the handled, mother-separated group (HMS), nor the handled, mother-present group (H), showed a significant decline in rearings, $F(3,108)=2.1358$, $0.25>p>0.10$ and $F(3,108)=0.36775$, $p>0.25$, respectively. On Day 9 post-test (when all animals received 2.5 mg/kg d-amphetamine), there was a handling effect only among the animals which had received 2.5 mg/kg d-amphetamine during the 8 day test period, $F(1,36)=4.9049$, $p<0.05$, with the handled animals (Groups H and HMS) showing more rearings that non-handled animals (Groups C and MS). Also on Day 9 post-test, there was a significant effect of dose history with the saline groups showing significantly more rearings than the 2.5 mg/kg groups $q(3,45)=7.514$, $p<0.01$, or the 10.0 mg/kg groups $q(3,45)=4.343$, $p<0.01$.

Entries. Maternally separated animals (Groups MS and HMS) showed significantly fewer entries than non-maternally separated animals (Groups C and H) during the test period. During the test period, only the 2.5 mg/kg groups showed a decline across blocks, $F(3,144)=21.783$, $p<0.01$, as illustrated in Fig. 3B. There was a dose history effect on Day 9 post-test, $F(2,36)=12.193$, $p<0.001$, with the saline groups showing significantly more activity than 2.5 mg/kg $q(3,45)=6.981$, $p<0.01$. Also, the 10 mg/kg groups showed more activity than the 2.5 mg/kg groups, $q(3,45)=3.642$, $p<0.05$.

Stereotypy. The dose of amphetamine significantly influenced the amount of stereotypy observed over the test period. The saline groups showed virtually no stereotypy; the 2.5 mg/kg groups showed an increasing pattern of stereotypy; and, the 10.0 mg/kg groups showed virtually the maximum amount of stereotypy. The following pattern of stereotypy arose for Group H: Although handled, mother-present animals (Group H) were not significantly different from the other groups on the first two-day block (H vs. C: $q=0.5397$, H vs. MS: $q=1.327$, H vs. HMS: $q=3.206$; $df=4,36$; $p>0.05$), they showed significantly less stereotypy than the other groups on the second two-day block (H vs. C: $q=4.972$; H vs MS: $q=8.934$; H vs HMS: $q=6.732$; $df=4,36$; $p<0.01$), as well as on the third 2-day block, (H vs C: $q=6.114$, H vs MS: $q=7.299$, H vs HMS: $q=6.558$; $df=4,36$; $p<0.01$). However, by the fourth two-day block, Group H did not significantly differ from the other groups (H vs. C: $q=2.568$, H vs. MS: $q=2.977$, H vs. HMS: $q=2.366$; $df=4,36$; $p>0.05$). Thus, handled, mother-present animals showed a slower onset of amphetamine-induced stereotypy. On Day 9 post-test (when all animals were injected with 2.5 mg/kg d-amphetamine), the 2.5 mg/kg groups and the 10.0 mg/kg groups exhibited more stereotypy than the saline groups, $qs(3,45)=10.244$, 6,153, respectively, $p<0.01$. Also, the 2.5 mg/kg groups exhibited more stereotypy than the 10.0 mg/kg groups, $q(3,45)=4,091$, $p<0.05$.

Body weight. The maternally separated animals (Groups MS and HMS) were significantly heavier than the non-maternally separated animals (Groups C and H) (mean=326.35, SD=26.6 vs. mean=310.35, SD=31.9) ($F(1,36)=4.586$, $p=0.0368$. Saline animals showed a significant increase in weight, $F(2,180)=3.392$, $p<0.01$ whereas the increases in body weight shown by both dose levels of amphetamine subjects were not significant.

DISCUSSION

The results clearly indicated that certain early life experiences influence the adult response to chronically-administered amphetamine. Experiment 1 showed that early handling delayed the decline in rearings which ordinarily occurs over days with repeated amphetamine injections at this dosage. Experiment 2 replicated the early handling effect on rearings seen in Experiment 1, and also showed that maternal presence moderated the decline in amphetamine-induced entries. More importantly, however, Experiment 2 showed that early handling plus return to a mother present nest ameliorated the increase over days in amphetamine-induced stereotypy, implying a crucial maternal component in this early handling effect. Even though qualified by the absence of observable differences in maternal behavior, this conclusion remains the most likely explanation for two reasons. First, similar maternal behaviors may have differential effects on stressed (handled) pups (Group H) versus pups recovered from stress (Group HMS). Second, the important changes in the maternal behavior of Group H mothers may have occurred at times remote from the period of observation [6].

In fact, the early handling-plus-maternal-presence effect on amphetamine-induced stereotypy may have produced both the early handling effect on rearings seen in Experiments 1 and 2 and the maternal presence effect on entries seen in Experiment 2. Stereotypy and locomotor activity come to have a reciprocal relationship during chronic amphetamine administration of this dosage [12,17]. Similarly, the effect on amphetamine responsiveness, in the present study, was not prepotent but required repeated injections of d-amphetamine in order to be manifested. Thus, rather than singular effects on rearings, entries and stereotypy, the present results may have reflected a change in sensitization. An apparent qualification of this conclusion involves the brevity of the test period; a 3-min Y-maze observation may not adequately characterize amphetamine sensitization because extraneous factors such as drug-conditioned activity [5,15] may have contaminated the results. However, why only Group H animals should show, for example, drug conditioned stereotypy is not easily explained.

The mechanism by which early handling plus maternal behavior influenced the response to amphetamine is unclear. However, because dopamine is purported to be integral to amphetamine-induced stereotypy [2,10], some neurochemical system which influences dopamine would be expected to be involved. Conceivably, maternal behavior elicited by early handling may have influenced the stress response which, in turn, may have affected some aspect of catecholamine biosynthesis or release during the stress produced by repeated amphetamine injections. This notion is supported by two well-established findings: (a) infantile stimulation alters the efficiency of the stress response [4,8], and (b) chronic stress affects the biosynthesis and release of catecholamines [13]. Alternatively, early handling and the resultant changes in maternal behavior may directly influence neural development. The neonatal period is one of rapid developmental change in aminergic neurochemical systems [1,9] and thus may be sensitive to environmental input.

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