Pharmacology Biochemistry & Behavior, Vol. 19, pp. 487–496, 1983. Ankho International Inc. Printed in the U.S.A.

Acute and Chronic Amphetamine Treatment: Differential Modification of Exploratory Behavior in a Radial Maze[1]

VENERA BRUTO AND HYMIE ANISMAN

Department of Psychology, Carleton University, Ottawa, Ontario, K1S 5B6, Canada

Received 23 June 1981

BRUTO, V. AND H. ANISMAN. *Acute and chronic amphetamine treatment: Differential modification of exploratory behavior in a radial maze.* PHARMACOL BIOCHEM BEHAV **19**(3) 487–496, 1983.—Mice permitted to explore an 8-arm radial maze displayed high levels of spontaneous alternation as measured by the frequency of visiting (a) the 4 least recently entered arms, (b) the 2 least recently visited arms, and (c) sequences of arms which are adjacent to one another. Acute treatment with low doses of amphetamine (1.0 mg/kg) eliminated the alternation tendency. Higher doses (5.0–7.0 mg/kg) also produced marked stimulus perseveration, such that mice tended to revisit the two arms that had been most recently entered. With repeated amphetamine treatment the perseveration tendency was attenuated. The abatement of perseveration in the radial maze did not appear to reflect simply the reduction in the potency of the drug. That is, the reduction of perseveration after chronic exposure to amphetamine was not accompanied by recovery of normal exploratory patterns. In fact, the alternation and adjacent alternation patterns typical of naive animals were absent in mice chronically treated with amphetamine even when tested in the nondrug state. It was suggested that the attenuation of amphetamine induced perseveration after chronic amphetamine administration may reflect a breakdown of normal behavior patterns rather than the development of a genuine tolerance.

Amphetamine Acute Chronic Exploration Perseveration Alternation

WHEN permitted to explore a Y- or T-maze, both rats and mice exhibit a preference for the arm of the maze that was least recently visited [2,3]. This behavior, termed spontaneous alternation, is assumed to reflect habituation to the most recently visited arms of the maze, resulting in a preference for the more novel arm [3]. Treatments believed to disrupt habituation, such as anticholinergics, provoke random response patterns, such that chance levels of alternation are typically observed [5, 7, 23]. Treatment with amphetamine will also disrupt alternation, but this effect can be distinguished from the behavioral effects of anticholinergics. Unlike anticholinergic agents, treatment with intermediate doses of amphetamine will elicit a marked perseverative tendency, i.e., animals successively visit two arms of the maze [1, 11, 12, 13]. It has been suggested that amphetamine-induced stimulus perseveration may be dependent on the functional integrity of the noradrenergic system [13] and may reflect enhanced attention to selective aspects of the environment [15].

In contrast to behaviors such as stereotypy, which are enhanced with repeated amphetamine treatment [20,21], the perseverative tendency is reduced with chronic administration of amphetamine [10, 13, 14]. Typically, among animals that received injections of amphetamine, performance is neither characteristic of saline-treated animals nor of animals that received acute injection of the drug [10, 13, 14]. That is to say, the attenuation of the perseverative tendency in mice chronically treated with amphetamine did not result in behavior which resembled that of pharmacologically naive animals. Whereas animals treated with saline exhibited above chance alternation scores (between 65 and 70%), animals with a history of 5 to 30 days of amphetamine treatment exhibited levels of alternation which did not deviate from chance (i.e., alternation scores between 50 and 55%).

It was recently proposed that the reduction of the perseverative tendency with chronic amphetamine treatment may not be a consequence of drug tolerance, but rather may be reflective of the intrusion of other drug-induced tendencies that are antagonistic with the perseveration [15]. It was suggested that chronic amphetamine treatment may provoke disruption of attentional abilities that may be fundamental for the execution of both perseverative sequences and alternation behavior. Unfortunately, this conclusion is tempered owing to the lack of sensitivity of the alternation measure employed. More specifically, the limited number of possible responses available, and hence the limited types of response profiles that can be expected in a Y-maze, often makes it difficult to distinguish between animals exploring in a random fashion (i.e., alternation scores between 50 and 55%) and animals exploring the maze systematically (e.g., alternation scores between 65 and 70%). The purpose of the present investigation was to assess the acute and chronic effects

[1]Supported by Grant A9845 from the Natural Sciences and Engineering Research Council to Hymie Anisman.

of d-amphetamine in an 8-arm radial maze which permitted identification of a broad range of response profiles.

EXPERIMENT 1

In the Y-maze, entering a given arm may be designated as either an alternation or a perseveration response. For instance, if an animal had previously visited arms 1 and 2, a subsequent entry into arm 3 is defined as an alternation, while entry into arm 1 is defined as a perseveration response. In the 8-arm radial maze it is not only possible to distinguish between alternation and perseveration, but degrees of these responses can be determined. That is, if an animal had shown a series of arm entries such as 1, 2, 3, 4, 5, 6, 7, on the next arm entry it may choose to alternate (i.e., visit arm 8), or perseverate (i.e., visit arm 6); the probability associated with the occurrence of either an alternation or a perseveration response is 0.125. In contrast to the Y-maze, however, a nonalternation does not necessarily constitute a perseveration. For instance, the animal may choose to revisit either arms 1, 2, 3, 4, or 5, which are less novel than arm 8, but more novel than arm 6. Accordingly, entries to these arms might be considered weaker alternation responses. In a similar fashion, a break in a perseverative tendency does not necessarily constitute an alternation. If an animal had entered arms 1, 2, 3, 4, 3, 4, 3, 4, on the next entry it may choose to revisit arm 3 (i.e., perseverate) or enter one of arms 5, 6, 7, or 8 (i.e., alternate). Alternatively, the animal may choose to revisit either arm 1 or 2, which are relatively more novel than either arm 3 or 4, and as such might be viewed as weaker perseveration.

The intensity of the alternation tendency may be assessed by comparing the proportion of visits to one of the two (2-arm alternation), or to one of the four (4-arm alternation) least recently entered arms; the probability of visiting one of the two least recently entered arms being much less (0.25) than the probability of visiting one of the 4 least recently entered arms (0.50). Likewise, the intensity of the perseverative tendency might be examined by comparing the proportion of visits to one of the two, or to one of the four, most recently entered arms (2-arm and 4-arm perseveration, respectively).

Finally, the use of the radial maze might allow for clarification as to whether alternation reflects a preference for the most temporally novel arms, or whether the alternation tendency simply involves a sequential pattern of stimulus sampling. For example, mice may respond to novelty and may also be most inclined to visit arms that are adjacent to one another. In the Y-maze every perseveration or alternation response must involve entry into an immediately adjacent arm. In the 8-arm radial maze alternation and perseveration responses need not be directed to adjacent arms of the maze.

The preceding discussion suggests that the radial maze offers advantages over the Y-maze in assessing patterns of exploration. Not only is it possible to assess adjacent alternation, but degrees of alternation and perseveration can be detected. Assessment of these tendencies may provide a method with which to differentiate the behavior of animals treated with saline from that of mice treated acutely or chronically with amphetamine. The purpose of the first experiment was simply to examine the exploratory response patterns of mice in a radial maze, and to compare the behavior of saline-treated animals with that of animals acutely treated with amphetamine.

METHOD

Subjects

A total of 60 male, Swiss Webster mice, obtained from Biobreeding Laboratories, Ottawa, Ontario, at 50 to 65 days of age served as subjects. Animals were acclimatized to the laboratory for a period of seven days prior to behavioral testing. Mice were housed in groups of five in opaque polypropylene cages, and provided access to lab chow and water ad lib.

Apparatus

An elevated, eight-arm wooden radial maze similar to that described by Olton [16, 17, 18] was used to measure spontaneous alternation, perseveration, and locomotor activity. Each of the arms (55.9 cm by 11.4 cm) radiated from a central octagonal area (25.4 cm in diameter), thus requiring the animal to enter the central area in order to gain access to another arm. The surface of the maze was sand-colored grain.

Procedure

Animals were randomly assigned to one of six groups and injected intraperitoneally (IP) with either physiological saline (10.0 ml/kg) or one of five doses of d-amphetamine sulfate (1.0, 3.0, 5.0, 7.0 or 9.0 mg/kg) salt weight, in a volume of 10.0 ml/kg) and placed in individual holding cages to await behavioral testing. Fifteen minutes after IP injection, the subject was placed in the center of the 8-arm maze and allowed to explore freely for a period of fifteen minutes. The sequence and number of arm entries was recorded. The maze surface was cleaned between tests to minimize the odor cues of previously tested animals. All testing was conducted during the light portion of the light-dark cycle.

Behavioral Scoring

Arm entries were analyzed in terms of: (a) locomotor activity: total number of arm entries; (b) 2-arm alternation: the proportion of entries to one of the two least recently visited arms. The proportion of such responses was calculated by dividing the total number of 2-arm alternations (i.e., number of visits to one of the 2 least recently entered arms) by the total number of arm entries minus 6. By chance the probability of such responses occurring is 0.25. In this context, a perseveration response would be a return to one of the 6 most recently entered arms. (c) 4-Arm alternation: the proportion of visits to one of the four least recently entered arms. In this case, the proportion of alternation responses was determined by dividing the total number of 4-arm alternations (i.e., number of entries to one of the 4 least recently visited arms) by the number of arm entries minus 4. The probability of such an alternation response is 0.50, and as such this measure may be considered a more lax form of the alternation tendency. A return to one of the four most recently entered arms would constitute a 4-arm perseveration. The probability of such a response occurring would be 0.50. In effect, the probability of a non-alternation in the 4-arm alternation measure is equivalent to that of a perseveration; thus these measures are analogous to the alternation and perseveration scores seen in the Y-maze task. (d) Perseveration: the proportion of entries to the most recently visited arms. The proportion of such responses was determined by dividing the number of perseverations by the total number of

TABLE 1

MEAN NUMBER OF ARM ENTRIES (+S.E.M.) AS A FUNCTION OF DRUG TREATMENT

Saline	1.0 mg/kg	3.0 mg/kg	5.0 mg/kg	7.0 mg/kg	9.0 mg/kg
56.8 + 5.3	89.9 + 14.1	126.8 + 5.0	118.3 + 19.1	46.4 + 10.0	39.1 + 12.8

arm entries minus two. The probability of a perseveration is 0.125 and thus provides a more stringent index of the perseverative tendency than the 4-arm perseveration measure. (e) Sequential/adjacent alternation: the proportion of alternation responses in which three arm entries occurred in immediately adjacent arms. For instance, if the arms of the maze were labelled 1 through 8, a pattern of entries such as 2, 3, *4, 5, 6, 5, 7, 8, 7, 6,* would receive a score of four adjacent alternations (the italic entries would be considered adjacent alternation responses). The proportion of adjacent alternation responses was calculated by dividing the number of such responses by the total number of arm entries minus two.

RESULTS AND DISCUSSION

Locomotor Activity

As shown in Table 1, amphetamine administration produced a dose-dependent change of the number of arms visited, $F(5,54)=9.64$, $p<0.001$. Newman-Keuls multiple comparisons ($\alpha=0.05$) indicated that the 3.0 and 5.0 mg/kg doses of amphetamine significantly increased the number of arm entries relative to saline-treated mice; the 3.0 mg/kg dosage was found to be maximally effective in this respect. In contrast, neither 1.0, 7.0, nor 9.0 mg/kg of amphetamine significantly enhanced locomotor activity relative to saline-treated controls. In fact, five of the ten mice tested with 9.0 mg/kg showed a decline in activity; these animals exhibited fewer than 8 arm entries during the 15-minute test period, and this group was consequently not included in the analyses of alternation and perseveration.

Spontaneous Alternation

Independent analyses of variance were conducted for each of the alternation measures. In addition, χ^2 analyses of each of the group scores were conducted in order to determine whether performance deviated from chance. As typically seen in the Y-maze [11,12], saline-treated animals permitted to explore the radial maze freely exhibited a pattern of exploration characterized by a marked preference for the least recently visited arms of the maze. In fact, χ^2 analyses of the total group proportion of alternation responses indicated that saline-treated animals exhibited levels of alternation which exceeded chance, irrespective of whether the 2- or 4-arm alternation measure was considered. In contrast, as seen in Table 2, treatment with amphetamine was found to reduce alternation to chance or below chance levels. Not unexpectedly, Analysis of Variance revealed that acute injection of amphetamine reduced the proportion of responses to the least recently entered arms, $F(4,45)=9.41$, 9.44, $p<0.05$ for the 2-arm and 4-arm alternation measures, respectively (see panels B and C of Fig. 1). Newman-Keuls multiple comparisons revealed that relative to saline treated subjects, acute treatment with all doses of amphetamine effectively reduced the proportion of 2-arm alternation responses. Predictably, when the less stringent or less sensitive 4-arm alternation measure was considered, Newman-Keuls multiple comparisons ($\alpha=0.05$) indicated that only the 3.0, 5.0 and 7.0 mg/kg dosages significantly reduced the proportion of alternation responses.

In addition to a preference for the temporally most novel arms of the maze, saline-treated mice showed a marked tendency to sample their environment in a systematic fashion. As seen in Panel A of Fig. 1, saline-treated mice exhibited sequences of at least 3 arm entries in which they successively entered adjacent arms (adjacent alternation). The frequency of such responses exceeded chance (see Table 2). Acute amphetamine treatment influenced the proportion of adjacent alternation responses, $F(4,45)=7.3$, $p<0.05$. Newman-Keuls multiple comparisons ($\alpha=0.05$) revealed that all doses of amphetamine significantly reduced the proportion of such responses relative to saline-treated controls. Moreover, among amphetamine-treated mice adjacent alternation levels either did not differ from chance or were lower than chance levels (see Table 2).

Perseveration

The frequency of 2-arm perseveration responses as a function of drug treatment is shown in Panel D of Fig. 1. As seen in the Y-maze exploratory task [11, 12, 13, 14], acute amphetamine treatment influenced the occurrence of stimulus perseveration, $F(4,45)=6.68$, $p<0.05$. Subsequent Newman-Keuls multiple comparisons ($\alpha=0.05$) revealed that, relative to saline-treated animals, 5.0 and 7.0 mg/kg of amphetamine increased the frequency of perseverative responses. χ^2 Analyses (see Table 2) indicated that perseveration scores in these two groups significantly differed from chance values. It will be recalled that analysis of the 4-arm alternation (which is the reciprocal of 4-arm perseveration) indicated that 3.0, 5.0 and 7.0 mg/kg of amphetamine significantly reduced 4-arm perseveration. These data are consistent with the supposition that 2-arm perseveration is a more stringent indicator of the perseverative tendency than 4-arm perseveration and hence changes are detected within a more limited range of doses. Moreover, examination of the effects of amphetamine on alternation and perseveration in the radial maze suggests that amphetamine-induced decrements in alternation (2- and 4-arm) may be distinguished from amphetamine-induced perseveration. For example, acute treatment with 1.0 mg/kg produced a significant decrease in the proportion of 2-arm alternation responses but the reduction in the alternation tendency was not accompanied by any appreciable increase in 2-arm perseveration. In higher doses, of course, the reduced alternation was accompanied by marked perseveration.

The effects of amphetamine on perseverative behavior

TABLE 2

χ^2 ANALYSIS OF THE TOTAL GROUP PROPORTION OF RESPONSE PATTERNS

	Treatment				
	SAL	1.0 mg/kg	3.0 mg/kg	5.0 mg/kg	7.0 mg/kg
Adjacent Alternation	16.7*	0.21	6.60*	2.77	6.60*
2-arm Alternation	19.2*	0.85	4.32*	0.05	6.47*
4-arm Alternation	21.1*	4.00*	3.24	2.56	6.76*
Perseveration	1.12	0.57	1.85	50.5*	128.6*

In each case, df 1, χ^2 value necessary for $p < 0.05 = 3.86$.
*Deviates from chance performance.

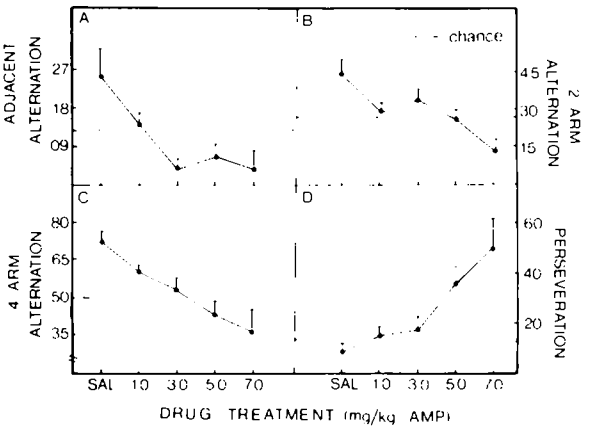

FIG. 1. Mean proportion (%) ±S.E.M. of adjacent alternation, 2-arm alternation, 4-arm alternation and perseveration responses as a function of Drug Treatment (10.0 ml/kg saline or 1.0, 3.0, 5.0 or 7.0 mg/kg d-amphetamine). In each case, the dotted lines designate chance levels of responding.

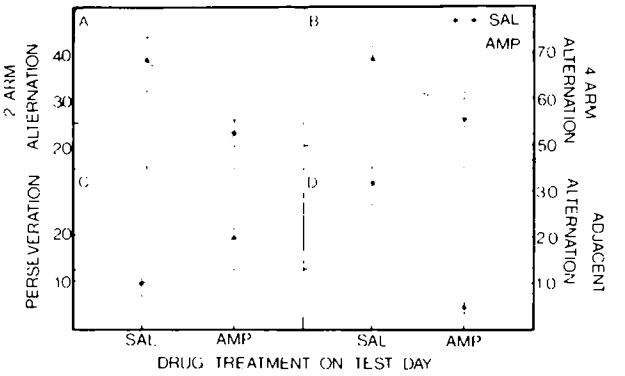

FIG. 2. Mean proportion (%) ± S.E.M. of 2-arm alternation, 4-arm alternation, perseveration and adjacent alternation responding as a function of Drug History (10.0 ml/kg saline or 10.0 mg/kg d-amphetamine for 5 consecutive days) and Drug Treatment on Test Day (10.0 ml/kg saline or 3.0 mg/kg d-amphetamine). The dotted lines denote chance response rates.

appeared to be independent of changes in locomotor activity. As seen in Fig. 1, maximal perseveration was observed following 5.0 and 7.0 mg/kg of amphetamine. In contrast, the peak drug effect on locomotor activity occurred after 3.0 mg/kg, and the 7.0 mg/kg dose did not increase activity at all (see Table 1).

EXPERIMENTS 2a–2c

It will be recalled that the perseverative response pattern induced by amphetamine is absent following repeated exposure to the drug. Kokkinidis and Anisman [15] suggested that this may have been due to a breakdown in normal behavior patterns, possibly owing to attentional deficits, rather than a reflection of drug tolerance. The purpose of Experiment 2 was twofold: first, to establish the parameters necessary to provoke the decline in the perseverative response to amphetamine in the 8-arm radial maze; and, second, to compare the response patterns of saline-treated animals to those of animals that had received chronic amphetamine treatment. More specifically, would the decline in perseveration in the latter condition be associated with an increase in the frequency of one of the forms of alternation or would the response pattern appear haphazard?

METHOD

Subjects and Apparatus

Experiments 2a, b, and c each involved 40 naive Swiss-Webster mice. All particulars regarding subjects and apparatus were identical to those outlined in Experiment 1, with the qualification that animals were individually housed from commencement of the chronic drug or saline regimen.

Procedure

In Experiment 2a, mice received daily IP injections of either d-amphetamine sulfate (10.0 mg/kg, salt weight, in a 10.0 ml/kg volume) or physiological saline (10.0 ml/kg) on 5 consecutive days. On the sixth day, these groups were subdivided such that one-half the animals received a 3.0 mg/kg test dose of d-amphetamine sulfate (saline-amphetamine,

TABLE 3

MEAN NUMBER OF ARM ENTRIES (±S.E.M.) AS A FUNCTION OF DRUG HISTORY AND DRUG TREATMENT ON DAY OF TESTING

Drug History	Drug Treatment on Day of Testing	
	Saline	3.0 mg/kg amphetamine
10.0 ml/kg saline day for five days	54.3 ±6.2	118.8 ±10.0
10.0 mg/kg d-amphetamine day for five days	60.7 ±6.3	141.3 ±8.0

amphetamine-amphetamine), while the remaining mice received saline (saline-saline, amphetamine-saline). This procedure was replicated in Experiment 2b, with the exception that a challenge dose of 5.0 mg/kg was administered prior to testing. In Experiment 2c, animals received daily IP injections of 10.0 mg/kg d-amphetamine sulfate or saline for ten consecutive days and received either a challenge dose of 5.0 mg/kg or saline prior to testing on the eleventh day. The behavioral testing and scoring procedures were identical to those previously described in Experiment 1.

RESULTS AND DISCUSSION

Experiment 2a

Locomotor activity. As seen in Table 3, and confirmed by Analysis of Variance, amphetamine administered on test day significantly increased locomotor activity, $F(1,36)=86.98$, $p<0.05$. Several investigators [20,21] previously reported chronic amphetamine treatment to enhance the motor excitation induced by subsequent amphetamine treatment. As evident in Table 3, suggestion of such an effect was evident in Experiment 2a. However, in the absence of a detailed analysis of locomotor and stereotypic changes that occur over various time intervals following amphetamine treatment, it is difficult to determine whether the activity changes that occurred in the present investigation were comparable to those previously reported.

Spontaneous alternation. As evident in Panels A and B of Fig. 2, the degree of preference which animals demonstrated for the relatively novel arms of the maze was only influenced by Drug Treatment on Day of Testing, $F's(1,36)=10.12$, 16.60, $p<0.05$, for the 4-arm and 2-arm alternation measures, respectively. Irrespective of drug history, treatment with 3.0 mg/kg of amphetamine on the day of testing reduced the proportion of alternation responses. Animals treated only with saline exhibited alternation scores which were significantly above chance, whereas chance or below chance level alternation was characteristic of those mice tested in the drug state. Moreover, while injection of 10.0 mg/kg/day for 5 consecutive days did not significantly influence alternation, it is noteworthy that the 4-arm alternation scores of AMP/SAL mice did not deviate from chance levels. Likewise, six of the ten mice chronically treated with amphetamine and tested with saline exhibited 2-arm alternation scores which did not deviate from chance (see Table 4).

Analysis of Variance of the proportion of adjacent alternation responses indicated a significant interaction between Drug Treatment on day of testing and Drug History, $F(1,36)=5.02$, $p<0.05$ (see Fig. 2). Newman-Keuls multiple comparisons of the simple main effects comprising the interaction ($\alpha=0.05$) revealed that, among saline-pretreated mice, 3.0 mg/kg of amphetamine significantly reduced the proportion of adjacent alternation responses relative to mice that received saline throughout. Chronic treatment with amphetamine did not modify the degree of adjacent alternation responses produced by a 3.0 mg/kg challenge dose of the drug. Regardless of prior drug history, mice tested in the amphetamine condition exhibited below chance levels of adjacent alternation (see Table 4). Interestingly, adjacent alternation behavior was modified by chronic amphetamine among mice subsequently tested in the saline condition. That is, the adjacent alternation scores of amphetamine-saline treated mice were significantly lower than those of mice that were repeatedly treated with saline. In fact, whereas the adjacent alternation scores of the latter group exceeded chance, those of the former group did not deviate from chance (see Table 4).

Perseveration. The proportion of perseveration responses as a function of the Drug Treatment is shown in Panel C of Fig. 2. Analysis of Variance indicated that 3.0 mg/kg of amphetamine significantly increased the proportion of perseveration responses relative to saline-treated controls, $F(1,36)=17.00$, $p<0.05$. Pre-exposure to amphetamine (10.0 mg/kg) for 5 consecutive days was ineffective in reducing the perseveration tendency. In spite of this, however, the perseveration scores of animals pretreated and tested with amphetamine may be differentiated from those of the acute drug group in that scores of the latter exceeded chance whereas those of the former did not deviate from chance (see Table 4).

Experiment 2b

Locomotor activity. As in Experiment 2a, treatment with amphetamine on test day enhanced locomotor activity, as reflected by the number of arm entries, $F(1,36)=21.25$, $p<0.05$ (see Table 5). Repeated treatment with amphetamine for five consecutive days did not significantly influence subsequent activity (see also the Results and Discussion of Experiment 2c).

Spontaneous alternation. Consistent with Experiment 1, administration of the 5.0 mg/kg dosage of amphetamine on test day reduced the proportion of both alternation and adjacent alternation responses, $F's(1,36)=19.83$, 35.08, 28.67, $p's<0.05$ for the 4-arm, 2-arm and adjacent alternation measures, respectively (see Fig. 3). Amphetamine pretreatment was without effect on any of these measures.

Perseveration. Analysis of Variance of the proportion of perseveration responses indicated that mice treated with amphetamine on the day of testing showed a significantly greater proportion of perseveration responses than did animals that received saline, $F(1,36)=9.04$, $p<0.05$. Although the Analysis of Variance indicated that prior drug history did not influence perseveration, χ^2 analyses (see Table 6) indicated that amphetamine pretreatment significantly affected the degree of perseveration observed after a 5.0 mg/kg challenge dose. Whereas mice in the acute amphetamine group displayed perseveration scores which exceeded chance, mice chronically treated with amphetamine and then tested in the drug state exhibited perseveration scores which did not deviate from chance. It might also be noted that although the analysis of variance indicated that five days of pre-exposure to amphetamine did not signifi-

TABLE 4

χ^2 ANALYSIS OF THE TOTAL GROUP PROPORTION OF RESPONSE PATTERNS

Treatments	Adjacent Alternation	2-arm Alternation	4-arm Alternation	Perseveration
saline-saline	34.8*	10.45*	21.2*	0.57
saline-amphetamine	5.23*	0.21	4.0*	5.14*
amphetamine-saline	0.21	5.33*	0.36	1.12
amphetamine-amphetamine	7.83*	0.21	2.56	1.12

In each case, $df=1$, χ^2 value necessary for $p<0.05=3.86$.
*Deviates from chance performance.

TABLE 5

MEAN NUMBER OF ARM ENTRIES (±S.E.M.) AS A FUNCTION OF DRUG HISTORY AND DRUG TREATMENT ON DAY OF TESTING

Drug History	Drug Treatment on Day of Testing: Saline	Drug Treatment on Day of Testing: 5.0 mg/kg amphetamine
10.0 ml/kg saline/day	56 ± 6	115 ± 15
10.0 mg/kg d-amphetamine/day	47 ± 4	85–14

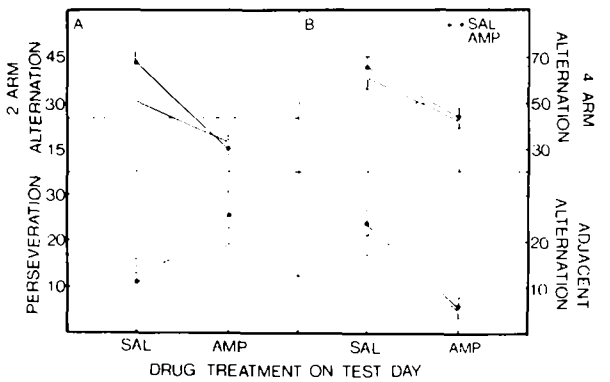

FIG. 3. Mean proportion (%) +S.E.M. of 2-arm alternation, 4-arm alternation, perseveration and adjacent alternation responses as a function of Chronic Drug Pretreatment (10.0 ml/kg saline or 10.0 mg/kg d-amphetamine for 5 consecutive days) and Drug Treatment on Day of Testing (10.0 ml/kg saline or 5.0 mg/kg d-amphetamine).

cantly alter perseveration, half the mice in the amphetamine-amphetamine group showed an attenuation of the perseverative responding normally observed after 5.0 mg/kg of the drug.

Taken together, the results of Experiments 2a and 2b indicate that daily injections of 10 mg/kg for 5 consecutive days are ineffective in altering perseveration after subsequent challenge doses of 3.0 or 5.0 mg/kg of amphetamine. However, when tested after injection of saline, animals with a history of repeated amphetamine treatment do not display patterns of adjacent alternation ordinarily observed in saline-treated mice.

Experiment 2c

Locomotor activity. As seen in Table 7, animals treated with 5.0 mg/kg of amphetamine exhibited higher levels of locomotor activity than did mice tested with saline, $F(1,36)=27.15$, $p<0.05$. Consistent with the results of Experiment 2a and those of other investigators [20,21] mice chronically treated with amphetamine exhibited a greater number of arm entries than saline-pretreated mice, $F(1,36)=7.46$, $p<0.05$.

Spontaneous alternation. Panels A and B of Fig. 4 illustrate the proportion of alternation responses as a function of drug treatments. The degree of preference animals demonstrated for the 4 more novel arms of the maze varied as a function of Drug History × Drug Treatment on Day of Testing, $F(1,36)=7.90$, $p<0.01$. Newman-Keuls multiple comparisons ($\alpha=0.05$) indicated that treatment with amphetamine on test day significantly reduced the proportion of alternation responses relative to mice pretreated and tested with saline; this reduction was to a lesser degree among mice pretreated with amphetamine. Moreover, alternation scores of the saline-saline group were higher than those of animals in the amphetamine-saline group. It will be recalled that the 4-arm alternation measure is, in effect, analogous to the alternation/perseveration index of the Y-maze (where the probability of both an alternation and a perseveration is 0.50). As such the slight increase in 4-arm alternation (and thus slight decrease in amphetamine induced 4-arm perseveration) in the chronic amphetamine group tested with the drug is consistent with earlier reports using a Y-maze exploratory task [10,13]. However, it may be noteworthy that, while the acute amphetamine group displayed levels of 4-arm alternation which fell significantly below chance, the 4-arm perseveration scores of the chronic amphetamine group did not deviate from chance (see Table 8).

As evident in Panel A of Fig. 4 and substantiated by Analysis of Variance, 2-arm alternation performance varied as a function of Drug History × Drug Treatment on Test Day, $F(1,36)=8.43$, $p<0.05$. Newman-Keuls multiple comparisons ($\alpha=0.05$) indicated that irrespective of chronic drug history, injection of 5.0 mg/kg of amphetamine prior to testing reduced the proportion of 2-arm alternation responses relative to saline-treated controls. That is, irrespective of drug history, mice tested following injection of 5.0 mg/kg exhibited 2-arm alternation scores which did not deviate from, or fell significantly below, chance (Table 8).

TABLE 6

χ^2 ANALYSIS OF TOTAL GROUP PROPORTION OF RESPONSE PATTERNS

Treatment	Adjacent Alternation	2-arm Alternation	4-arm Alternation	Perseveration
saline-saline	12.09*	17.28*	10.2*	0.21
saline-amphetamine	3.86*	4.32*	1.44	16.7*
amphetamine-saline	8.25*	3.41	3.24	0.21
amphetamine-amphetamine	5.14*	1.92	1.44	3.85

In each case, $df = 1$, χ^2 value necessary for $p < 0.05 = 3.86$.
*Deviates from chance performance.

TABLE 7

MEAN NUMBER OF ARM ENTRIES (±S.E.M.) AS A FUNCTION OF DRUG HISTORY AND DRUG TREATMENT ON DAY OF TESTING

	Drug Treatment on Day of Testing	
Drug History	Saline	5.0 mg/kg amphetamine
10.0 ml/kg saline/day	42 ± 4	101 ± 17
10.0 mg/kg d-amphetamine/day	71 ± 8	140 ± 15

TABLE 8

χ^2 ANALYSIS OF TOTAL GROUP PROPORTION OF RESPONSE PATTERNS

Treatment	Adjacent Alternation	2-arm Alternation	4-arm Alternation	Perseveration
saline-saline	28.0*	23.52*	29.2*	1.12
saline-amphetamine	1.12	5.33*	12.96*	64.2*
amphetamine-saline	0.02	3.41	9.0*	2.77
amphetamine-amphetamine	3.86	1.92	1.0	16.7*

In each case, $df = 1$, χ^2 value necessary for $p < 0.05 = 3.86$.
*Deviates from chance performance.

Moreover, amphetamine pretreatment was found to influence alternation behavior even when testing was conducted in the saline condition. That is, mice repeatedly injected with amphetamine and tested with saline exhibited significantly lower 2-arm alternation scores than did mice that were both pretreated and tested with saline . Indeed, whereas the performance of the latter group exceeded chance, that of the former group did not deviate from chance (see Table 8). Thus, there appears to be a breakdown of the alternation tendency following chronic amphetamine treatment, which is evident both after a challenge dose of amphetamine and after saline.

As in the case of 2-arm alternation, Analysis of Variance indicated that adjacent alternation performance varied as a function of the interaction between Drug History and Drug Treatment on Day of Testing, $F(1,36) = 7.46$, $p < 0.05$ (see Fig. 4). Pairwise comparisons of the simple effects revealed that, relative to mice in the saline-saline condition, mice pretreated with saline and tested with amphetamine displayed a marked reduction of adjacent alternation, such that the proportion of these responses did not deviate from chance (see Table 8). Amphetamine administration prior to testing among mice that had been chronically treated with amphetamine resulted in adjacent alternation scores which did not differ from those of animals in the acute amphetamine condition. Indeed, as seen in Table 8, χ^2 tests indicated that levels of adjacent alternation in the amphetamine-amphetamine group did not deviate from chance. Moreover, adjacent alternation responses were less frequent among mice that received chronic amphetamine treatment and were tested in the saline condition than among animals that received saline throughout both pretreatment and testing.

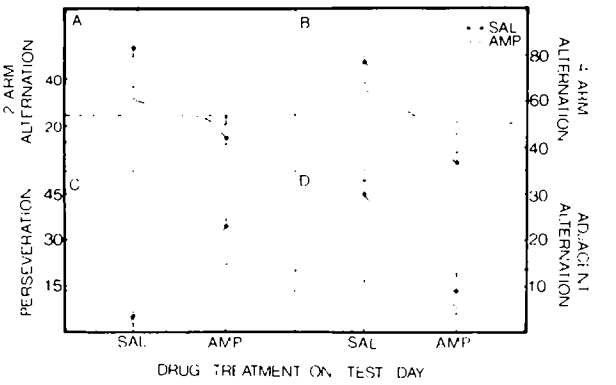

FIG. 4. Mean proportion (%) ± S.E.M. of 2-arm alternation, 4-arm alternation, perseveration and adjacent alternation responding as a function of Drug History (10.0 ml/kg saline or 10.0 mg/kg d-amphetamine for 10 consecutive days) and Drug Treatment on Test Day (10.0 ml/kg saline or 5.0 mg/kg d-amphetamine). The dotted lines symbolize chance response rates.

Whereas mice in the latter group exhibited adjacent alternation levels that exceeded chance, animals in the former group displayed adjacent alternation scores that did not deviate from chance. Thus, regardless of treatment on day of testing, animals with a history of chronic amphetamine administration did not exhibit the above chance levels of 2-arm or adjacent alternation typical of saline-saline mice.

Perseveration. Amphetamine treatment on test day increased the frequency of perseverative responses, $F(1,36)=49.77$, $p<0.05$ (see Fig. 4, Panel C). Contrary to Experiments 2a and 2b, however, chronic treatment with amphetamine was found to influence perseveration significantly, $F(1,36)=4.92$, $p<0.05$. Despite the fact that the interaction between these two factors did not reach statistical significance, $F(1,36)=3.01$, $p=0.09$, Newman-Keuls multiple comparisons ($\alpha=0.05$) of the simple main effects were conducted on the basis of a priori predictions. These tests indicated that among mice pretreated with saline, a 5.0 mg/kg challenge dose significantly increased the proportion of perseveration responses compared with mice that were tested with saline. Pretreatment with amphetamine did not influence perseverative responding among mice that were tested in the saline condition. Among mice chronically treated with amphetamine, a significant attenuation of the perseverative tendency was seen following administration of the 5.0 mg/kg challenge dose. Despite the reduction in amphetamine-induced perseveration, χ^2 analyses revealed that among both groups tested with amphetamine (i.e., saline-amphetamine and amphetamine-amphetamine) group perseveration scores exceeded chance. Regardless of the pretreatment condition, animals tested with saline demonstrated levels of perseveration which did not deviate from chance (see Table 8). Summarizing, inasmuch as the alternation of amphetamine-induced perseveration was not associated with an increase in the frequency of alternation (2-arm or adjacent alternation), the results of Experiment 2c suggest that the decline in perseveration following chronic amphetamine treatment may reflect a breakdown in normal behavior patterns. Indeed, the profile of 2-arm and adjacent alternation responses suggest that the exploratory patterns typical of normal animals are not evident among mice chronically treated with amphetamine even if tested in the non-drug state.

GENERAL DISCUSSION

As previously observed in the free running Y-maze exploratory task [11,12], animals treated with saline tend to visit those arms of a radial maze that were least recently explored (alternation). Moreover, mice frequently exhibited response patterns, of at least 3 arm entries in length, in which they sequentially visited immediately adjacent novel arms (adjacent alternation). As in the Y-maze [11,12], as well as other tests of exploration [8,19], acute treatment with amphetamine produced a dose-dependent change of exploratory patterns. Acute administration of low dosages of amphetamine eliminated both alternation and adjacent alternation tendencies. In addition to a disruption of alternation tendencies, higher doses of amphetamine produced a marked perseverative tendency, such that mice frequently re-entered the two arms that had been most recently visited. The effects of amphetamine on exploratory patterns were apparently unrelated to the motor changes induced by the drug. Treatment with 7.0 mg/kg, which did not enhance motor activity, and 5.0 mg/kg which augmented locomotor activity, both produced significant reductions in alternation and adjacent alternation coupled with marked perseveration.

Several of the behavioral changes ordinarily induced by acute amphetamine treatment were modified among mice with a history of chronic amphetamine administration (10.0 mg/kg of amphetamine for 10 consecutive days). As in the Y-maze, the perseverative tendency typically induced by 5.0 mg/kg of amphetamine was reduced if mice had received chronic exposure to amphetamine. The behavior of those animals chronically treated with amphetamine and given a challenge dose of 5.0 mg/kg did not approach the levels of alternation or adjacent alternation typically seen in animals that had received saline throughout the experiment. In effect, while apparent "tolerance" to amphetamine was seen on measures of perseveration, the level of performance on the alternation measures was not increased by repeatedly exposing animals to the drug. In fact, it appeared to be the case that repeated amphetamine treatment influenced the behavior of animals even when subsequently tested in the nondrug state. Both the 2-arm and adjacent alternation scores of the amphetamine-saline group were reduced relative to animals that had received only saline throughout the experiment. As such, the behavior of animals chronically treated with amphetamine was clearly distinguishable from that of mice treated after acute injection with amphetamine or after saline.

Several possible interpretations of these data are available. For example, amphetamine could have been stored in adipose tissue [22], thereby having long-lasting effects which would have been apparent when mice were subsequently tested in the amphetamine condition. However, it would follow from such an interpretation that perseverative tendencies would be enhanced after chronic drug treatment when mice were tested with a dosage that ordinarily did not elicit the perseveration (e.g., 3.0 mg/kg). The fact that no such effect was evident in Experiment 2a, where a low dosage was used in the test, is inconsistent with such an interpretation.

An alternative accounting for the effects of chronic amphetamine treatment, previously offered by Kokkinidis and Anisman [15], is that such a treatment either directly or indirectly results in disruption of attentional processes. Thus, it would follow from this hypothesis that, after chronic amphetamine treatment, not only would the perseverative effects normally observed after acute treatment be eliminated,

but the alternation tendency ordinarily observed after saline administration would also be antagonized. In effect, the behavior of animals chronically pretreated with amphetamine could be distinguished from that of acutely treated mice or saline controls in that their behavior would appear haphazard and low levels of both alternation and perseveration would ensue. In previous Y-maze studies [10, 13, 14] where the measurement of alternation and perseveration were not independent of one another, the reduction of perseveration and hence the increase of alternation after chronic treatment could have been interpreted as a reduction in the potency of amphetamine. However, in the 8-arm radial maze, adjacent and 2-arm alternation measures may be dissociated from changes in perseveration. As expected, amphetamine-induced perseveration was eliminated following repeated amphetamine treatment without any indication of recovery of the alternation patterns. Indeed, even when mice were tested with saline after a chronic amphetamine regimen, the exploratory patterns ordinarily seen in pharmacologically naive mice were absent. Thus the data provisionally suggest that repeated exposure to amphetamine provoked changes beyond simply reducing the potency of the drug.

It is noteworthy at this juncture that behavior of animals in a radial maze has been used to assess spatial memory [16, 17, 18]. However, it should be emphasized that the way in which the radial maze is utilized in the spatial memory experiments is fundamentally different from its use in the present studies. As such, direct comparisons between these two series of experiments may be tenuous at best. In the case of spatial memory studies, the particulars of the test situation were designed to maximize the animals' use of spatial cues relative to intra-maze cues. In addition, animals were food deprived and the arms of the maze baited with food. As such, the animals' use of spatial information to minimize visits to already entered arms, to attain food reinforcement, was maximized. In contrast, mice in the present experiments were neither food deprived nor was any food placed in the maze; mice were simply allowed the opportunity to explore. It cannot be determined, on the basis of the present data, whether the alternation and perseveration tendencies were directed by spatial cues or by intra-maze contextual cues. It has been demonstrated that in both Y-maze and T-maze exploratory tasks, intra- and extra-maze cues can be effective in influencing alternation/perseveration behavior [4, 9, 14]. In the context of the present investigation, the question of the relative importance of spatial and/or intra-maze cues in influencing exploratory patterns was of secondary importance. The critical concern was to compare the behavior of animals chronically treated with amphetamine with that of saline-treated animals. The 8-arm radial maze, by virtue of its many arms, simply provided a more sensitive instrument than the Y-maze, with which to assess/gauge the strength of the spontaneous alternation and perseveration tendencies (see Introduction).

Inasmuch as mice exhibit a tendency to enter sequentially a series of immediately adjacent arms, it is possible that the adjacent alternation tendency reflects a response bias quite apart from cue utilization processes, i.e., a series of consistent right or left turns will produce adjacent alternation responses; see analogous discussion of response sequences and spatial abilities in Eckerman, Gordon, Edwards, MacPhail, and Gage [6], and in Watts, Stevens, and Robinson [24]. In addition, it is not clear whether the adjacent alternation tendency acts to minimize entries to recently entered arms. As such it might be argued that chronic amphetamine treatment may disrupt animals' preference for the novel arms by virtue of its elimination of adjacent alternation quite apart of any change in stimulus sampling. The observations that alternation and adjacent alternation tendencies can be distinguished from one another argue against such an interpretation. Disruption of the adjacent alternation tendency can occur in the absence of a concomitant disruption of 4-arm alternation (see Experiment 2).

REFERENCES

1. Adkins, J., J. W. Packwood and G. L. Marshall, Jr. Spontaneous alternation and d-amphetamine. *Psychonom Sci* **17:** 167–168, 1969.
2. Anisman, H. and L. Kokkinidis. Effects of scopolamine, d-amphetamine and other drugs affecting catecholamines on spontaneous alternation and locomotor activity in mice. *Psychopharmacologia* **45:** 55–63, 1975.
3. Carlton, P. L. Cholinergic mechanisms in the control of behavior by the brain. *Psychol Rev* **70:** 19–39, 1963.
4. Douglas, R. J. Cues for spontaneous alternation. *J Comp Physiol Psychol* **62:** 171–183, 1966.
5. Douglas, R. J. and R. L. Isaacson. Spontaneous alternation and scopolamine. *Psychonom Sci* **4:** 283–284, 1966.
6. Eckerman, D. A., W. A. Gordon, J. D. Edwards, R. C. MacPhail and M. I. Gage. Effects of scopolamine, phenobarbital and amphetamine on radial arm maze performance in the rat. *Pharmacol Biochem Behav* **12:** 595–602, 1980.
7. Egger, G. H., P. Livesey and R. G. Dawson. Ontogenetic aspects of central cholinergic involvement in spontaneous alternation behavior. *Dev Psychol* **6:** 289–299, 1973.
8. File, S. E. and A. G. Wardell. Validity of head-dipping as a measure of exploration in a modified holeboard. *Psychopharmacologia* **44:** 53–59, 1975.
9. Glanzer, M. Stimulus satiation: An explanation of spontaneous alternation and related phenomena. *Psychol Rev* **60:** 257–268, 1953.
10. Kokkinidis, L., D. Walsh, R. Lahue and H. Anisman. Tolerance to d-amphetamine behavioral sepcificity. *Life Sci* **18:** 913–918, 1976.
11. Kokkinidis, L. and H. Anisman. Dissociation of the effects of scopolamine and d-amphetamine on a spontaneous alternation task. *Pharmacol Biochem Behav* **5:** 293–297, 1976.
12. Kokkinidis, L. and H. Anisman. Interaction between cholinergic and catecholaminergic agents in a spontaneous alternation task. *Psychopharmacology (Berlin)* **48:** 261–270, 1976.
13. Kokkinidis, L. and H. Anisman. Behavior specific tolerance following chronic d- or l-amphetamine treatment: Lack of involvement of p-hydroxynorephedrine. *Neuropharmacology* **17:** 95–102, 1978.
14. Kokkinidis, L. and H. Anisman. Abatement of stimulus perseveration following chronic d-amphetamine treatment: Absence of behaviorally augmented tolerance. *Pharmacol Biochem Behav* **8:** 557–563, 1978.
15. Kokkinidis, L. and H. Anisman. Amphetamine models of paranoid schizophrenia: An overview and elaboration of animal experimentation. *Psychol Bull* **88:** 551–596, 1980.
16. Olton, D. S. Mazes, maps and memory. *Am Psychol* **34:** 583–596, 1979.
17. Olton, D. S. and R. J. Samuelson. Remembrance of places passed: Spatial memory in rats. *J Exp Psychol (Anim Behav Proc)* **2:** 97–115, 1976.

18. Olton, D. S., J. A. Walker and F. Gage. Hippocampal connections and spatial discrimination. *Brain Res* **139:** 295–308, 1978.
19. Robbins, T. W. and S. D. Iversen. A dissociation of the effects of d-amphetamine on locomotor activity and exploration in rats. *Psychopharmacologia* **28:** 155–164, 1973.
20. Segal, D. S. Behavioral and neurochemical correlates of repeated d-amphetamine administration. In: *Neurobiological Mechanisms of Adaptation and Behavior*, edited by A. J. Mandell. New York: Raven Press, 1975, pp. 247–262.
21. Segal, D. S. and A. J. Mandell. Long-term administration of amphetamines: Progressive augmentation of motor activity and stereotypy. *Pharmacol Biochem Behav* **2:** 249–255, 1974.
22. Sparber, S. B., S. Nagasawa and K. E. A. Burkland. A mobilizable pool of d-amphetamine in adipose after daily administration in rats. *Res Comp Chem Pathol Pharmacol* **18:** 423–431, 1977.
23. Swonger, A. K. and R. H. Rech. Serotonergic and cholinergic involvement in habituation of activity and spontaneous alternation of rats in a Y-maze. *J Comp Physiol Psychol* **81:** 509–522, 1972.
24. Watts, J., R. Stevens and C. Robinson. Effects of scopolamine on radial maze performance in rats. *Physiol Behav* **26:** 845–851, 1981.