Pharmacology Biochemistry & Behavior, Vol. 8, pp. 557–563. Printed in the U.S.A.

Abatement of Stimulus Perseveration Following Repeated d-Amphetamine Treatment: Absence of Behaviorally Augmented Tolerance[1]

LARRY KOKKINIDIS AND HYMIE ANISMAN

Carleton University, Ottawa, Ontario, K1S 5B6, Canada

(Received 31 October 1977)

KOKKINIDIS, L. AND H. ANISMAN. *Abatement of stimulus perseveration following repeated d-amphetamine treatment: Absence of behaviorally augmented tolerance.* PHARMAC. BIOCHEM. BEHAV. 8(5) 557–563, 1978. – Acute administration of d-amphetamine results in animals perseverating between two compartments when placed in a free running Y-maze exploratory situation. Experiment 1 indicated that perseverative behavior was attenuated by making the arms of the maze distinctively different. Experiments 2 and 3 demonstrated that repeated amphetamine treatment reduced stimulus perseveration. Drug-induced locomotor activity and stereotypy were not affected by chronic drug administration. The course of the tolerance effect was not altered by pairing the repeated drug experience with Y-maze exposure. It was concluded that although stimulus factors are involved in the perseverative response, conditioning factors are not of primary relevance in determining the tolerance. It was also suggested that the mechanisms which subserve stimulus perseveration are different from those which mediate locomotor activity and stereotypy.

d–Amphetamine Perseveration Locomotor activity Stereotypy Stimulus factors Tolerance Conditioning

THE DEVELOPMENT of tolerance to the repeated administration of amphetamine appears to be dependent upon the behavioral or physiological index under consideration. Whereas some drug effects are attenuated following repeated exposure to d-amphetamine, e.g., anorexia [12, 13, 24], stimulus perseveration [14, 15, 19], facilitation of self-stimulation [21], as well as the disruption of time dependent schedules of reinforcement [3,26], the intensity of other behaviors (e.g., locomotor activity, stereotypy) are not diminished, and in fact, have been observed to increase following protracted drug treatments [11, 15, 22, 27, 28].

Several investigators have proposed that neurochemical and physiological changes may be responsible for the diverse behavioral effects associated with repeated drug exposure [2, 27, 28]. Other investigators maintain that in addition to the neurochemical effects associated with chronic amphetamine administration, environmental influences or conditioning factors also play an important role in this respect [3, 5, 24, 32, 33]. That is, tolerance may reflect adaptation or accommodation to the behavioral effects of the drug through conditioning [32] or habituation processes [25]. For example, Carlton and Wolgin [5] found that tolerance to the anorexigenic effects of d-amphetamine developed if the drug preceded presentation of sweetened milk, but not if the animals received post trial injections of the drug. Similar findings have been reported with respect to the disruptive effects of d-amphetamine on a DRL schedule for appetitive reinforcement [3]. Conditioning factors have also been proposed as a determinant for the increased locomotor excitation seen after chronic drug treatment [25,33]. It has been argued, however, that this factor cannot entirely account for the observed variance [27].

An alternative, behaviorally-based approach, to explain tolerance phenomena has recently been proposed [29,30]. According to this view, a compensatory antagonistic physiological reaction is triggered by drug treatment. This compensatory reaction is stimulus bound, and as a result the apparent strength of the primary drug reaction decreases with successive treatments. In support of this position, it has been demonstrated that following tolerance to a particular drug, a single saline injection produces a behavioral response opposite in direction to that typically observed after acute drug administration [30]. Presumably, this reflects the elicitation of the conditioned compensatory reaction in the absence of the primary systemic effects of the drug.

Recent reports from this laboratory have indicated that stimulus perseveration induced by d-amphetamine is attenuated following chronic drug treatment [14,19]. In particular, when animals are given d-amphetamine and per-

[1] Supported by Grant A9845 from the National Research Council of Canada. Appreciation is extended to Smith, Kline and French for the gift of d-amphetamine. Requests for reprints should be sent to L. Kokkinidis, Department of Psychology, Carleton University, Ottawa, Ontario, K1S 5B6, Canada.

mitted to explore freely in a symmetrical Y-maze, they exhibit trains of responses in which only two arms of the maze are visited (perseveration) [1, 16, 17, 18]. This is in marked contrast to the behavior of non-drugged animals which, more often than not, successively visit the arm least recently entered i.e., animals do not return to the arm visited in the previous trial (spontaneous alternation) [1, 17, 31]. Following repeated d-amphetamine administration the drug-induced perseverative response is substantially reduced [14,19]. Since stimulus factors appear to be involved in the perseverative effects of d-amphetamine [16,17], it would be of considerable interest to determine whether the development of tolerance to perseveration following chronic drug treatment involves conditioning factors.

EXPERIMENT 1

The influence of stimulus factors on the amphetamine-induced perseverative response was examined in Experiment 1. It was previously demonstrated that prior exposure to the Y-maze resulted in a marked enhancement of perseverative behavior [17]. Presumably, habituation to the apparatus attenuated the alternation tendency [31], which ordinarily competes with perseveration, thus maximizing the perseverative response [17]. Accordingly, it would be expected that changing the stimulus array of the apparatus, by making the arms of the Y-maze distinctively different from one another, would result in the course of habituation being attenuated [9], and thus the intensity of the perseverative response should decline.

METHOD

Animals

A total of 36 male and 36 female Swiss Webster mice procured from the Bio-Breeding Laboratories served in this experiment. Mice were housed in groups of six in standard polypropylene cages and allowed free access to food and water. Animals were approximately 55–65 days of age at time of testing.

Apparatus

The apparatus used to measure spontaneous alternation/perseveration and locomotor activity consisted of a symmetrical black Plexiglas Y-maze with arms 9.0 cm wide and 7.0 cm high and covered with a clear Plexiglas roof. The floor of the apparatus consisted of removable black or white Plexiglas sections which completely covered the arms and choice area of the maze. Each arm of the Y-maze had two sets of infrared photo-electric relays mounted in the side walls 1.0 cm above the Plexiglas floor. The first set of photocells was positioned at the entrance of the arm, while the second set was placed within the arm spaced 7.60 cm from the first. The photocells were wired such that a count, as measured by a deflection of one of three pens of an Esterline Angus recorder, was triggered only after both beams were broken. Once a count was recorded the second beam could not be triggered until the beam at the arm entrance was again broken. Thus, the animals was required to enter the chamber at least half-way into the arm in order for an arm entry to be recorded. The apparatus was housed in an illuminated room.

Procedure

Experiment 1 involved a 3(drug) × 4(floor arrangement) factorial design. Mice (N = 6/cell) received intraperitoneal (IP) injections of either saline or d-amphetamine sulfate (3.0 or 5.0 mg/kg salt weight). Drugs were administered in a 10 ml/kg volume. Fifteen min following injection mice were placed in the Y-maze and allowed to explore freely. The floor of the apparatus was manipulated such that four possible color arrangements existed. All arms were either a single color (i.e., all white or all black) or one arm was an odd color (i.e., 1 black, 2 white or 1 white, 2 black). The position of the odd color was counterbalanced across arms.

The sequence and number of arm entries were recorded over a 15 min period. Scoring of alternation/perseveration consisted of the evaluation of response sequences in which entering into the arm least recently visited was considered an alternation response (e.g., 1, 2, 3 or 1, 3, 2). Non-alternation was considered to be the case when animals returned to the compartment they had been in most recently (e.g., 1, 2, 1 or 1, 3, 1). The proportion of alternation was computed by dividing the number of alternations by the total number of alternations plus non-alternations. Perseverative behavior was defined as subjects making significantly fewer alternations than would be expected at a chance level (50%). The data were analyzed by analysis of variance of the individual alternation scores, and by χ^2 analysis of the proportion of total alternations for a given group of animals. The latter measure is weighted in favor of more active animals since each arm entry is weighted equally. That is, animals which make 30 arm entries influence the χ^2 analysis to a greater degree than animals which make only five arm entries. The former measure, on the other hand, weights the score of each animal equally regardless of the number of arms entered. In each of the experiments reported, the proportion of alternations were comparable regardless of the method of computation, suggesting that alternation scores were not biased by the levels of activity demonstrated within groups. Activity levels were determined on the basis of the number of arm entries.

RESULTS AND DISCUSSION

The mean proportion of alternation scores as a function of drug treatment and floor arrangement are shown in Table 1. Since alternation performance was comparable among mice when the floor of the apparatus was a single color (i.e., all white or all black), and performance among mice did not vary as a function of the position of the odd color, an analysis of variance was conducted on a 3(drug) × 2(floor color; same vs different) design (N = 12/cell).

This analysis revealed a significant main effect of drug treatment only, $F(2,66) = 32.0$, $p<0.001$. Newman-Keuls multiple comparisons of the simple main effects were carried out since an *a priori* prediction concerning the interaction was made [34]. Together with χ^2 analyses of the total proportion data, these comparisons revealed that regardless of the floor arrangement mice in the saline groups alternated at levels which exceeded chance, $\chi^2(1) = 51.10, 51.06$, $p<0.01$ (for mice treated with saline and assigned to the same and different conditions, respectively). In contrast treatment with d-amphetamine (5.0 mg/kg) significantly reduced spontaneous alternation to levels which were well below chance (perseveration), $\chi^2(1) = 225.3, 19.68$, $p<0.01$ (for mice treated with d-am-

TABLE 1

MEAN PROPORTION (± SEM) OF SPONTANEOUS ALTERNATION AS A FUNCTION OF DRUG TREATMENT AND FLOOR ARRANGEMENT

	Saline	d-Amphetamine 3.0 mg/kg	d-Amphetamine 5.0 mg/kg
Same	.635	.593	.291
	±.015	±.021	±.046
Different	.621	.568	.402
	±.020	±.024	±.069

phetamine 5.0 mg/kg and assigned to the same and different conditions, respectively). As predicted perseveration was more pronounced when the floor of the apparatus was a single color than when a compartment had a floor of an odd color (see Table 1).

Owing to the relatively large variability of the 5.0 mg/kg group tested with the odd floor arrangement, a partial replication of the experiment was undertaken. Consistent with Experiment 1 it was found that the effect of d-amphetamine (5.0 mg/kg) on perseverative behavior was dependent on the stimulus aspects of the maze, $t(18) = 2.17$, $p<0.05$. When the floor of the maze was homogeneous in color marked perseveration was noted ($\bar{X}$ ± SEM = .324 ± .058; $\chi^2(1) = 77.16$, $p<0.01$), whereas chance level performance was observed when the floor was heterogeneous in color ($\bar{X}$ ± SEM = .484 ± .048; $\chi^2(1) = 0.16$, $p>0.10$).

Analysis of variance of the mean number of arm entries revealed that all arms were frequented equally, regardless of drug treatment or floor arrangement. Consistent with previous reports [1,17], treatment with d-amphetamine (3.0 or 5.0 mg/kg) produced a marked increase in the number of arms entered, $F(2,66) = 11.34$, $p<0.001$ (means for saline, 3.0 and 5.0 mg/kg groups, respectively, were 63.96, 98.46, 139.02). In contrast to the data involving perseveration, changes in floor color had no effect on the locomotor excitation induced by d-amphetamine. Taken together, the results of Experiment 1 provide further evidence as to the distinct nature of these two amphetamine-induced behaviors. Whereas, perseverative behavior is dependent upon stimulus factors, changes in the stimulus array of the apparatus did not alter the locomotor excitation produced by drug treatment.

EXPERIMENT 2

As previously discussed, conditioning factors have been found to play an important role in the development of tolerance [3,5]. Since stimulus factors are involved in perseveration, it would be of considerable interest to determine whether conditioning factors play a role in the development of tolerance to amphetamine-induced perseverative behavior. Accordingly, mice were treated repeatedly with d-amphetamine such that the systemic effects of the drug were either congruent with experience in the Y-maze, or occurred following exposure to the Y-maze. If conditioning factors influence the development of tolerance, then it would be expected that a greater attenuation of amphetamine-induced stimulus perseveration would occur when the drug and testing experience were temporally congruent.

The notion of conditioned tolerance [29,30] can be tested readily using the same paradigm. If the observed tolerance to stimulus perseveration is the result of an antagonistic conditioned compensatory reaction, then a single saline injection to tolerant animals should produce a marked increase in alternation levels relative to mice injected with d-amphetamine. Experiment 2 was designed to evaluate these possibilities. In addition, two other amphetamine-induced behaviors were assessed (i.e., locomotor activity, and stereotypy). It should be noted that experiments involving these behaviors typically utilize relatively long observation periods [26,27]. In the present experiments, mice were observed only for the period corresponding to spontaneous alternation testing, in order to determine whether the development of tolerance to the perseverative effects of d-amphetamine is related to changes in locomotor activity or stereotypy following chronic drug treatment.

METHOD

Animals and Apparatus

A total of 48 (24 female and 24 male) Swiss Webster mice served in this experiment. All specifications concerning subjects were the same as described in Experiment 1, with the exception that mice were housed individually. The apparatus was identical to that used in Experiment 1, except that the Plexiglas floors were removed exposing the grid floor. The grid floor was made up of 0.25 cm stainless steel rods spaced 1.0 cm apart.

Procedure

Mice were randomly assigned to one of three groups (N = 16/cell), and tested in the alternation task for 15 min on three consecutive days. On Days 4–8, mice received IP injections of either saline or d-amphetamine sulfate (10.0 mg/kg) and were subsequently tested in the Y-maze 15 min following injection (pretrial). Mice in the third group received an IP injection of 10 mg/kg d-amphetamine immediately following testing (posttrial). The number and sequence of arm entries were recorded throughout training.

In addition to monitoring levels of spontaneous alternation and locomotor activity, mice were rated for stereotypy throughout the 15 min test period. The ratings were taken at 5 min intervals using the rating scale described by Ellinwood and Balster [10]. Briefly the scale consisted of the following criteria: (1) Lying down – eyes closed; (2) Lying down – eyes open; (3) Normal groominng; (4) Exploration, sniffing, rearing; (5) Running movement characterized by rapid changes in position (jerky); (6) Repetitive exploration – hyperactivity; (8) Remaining in the same place with fast repetitive head and/or foreleg movement, licking, chewing gnawing stereotypies; (9) Backing up, jumping, seizures, dyskinetic movements, abnormally maintained postures. The posttrial group was rated twice, once during behavioral testing and 15 min after drug injection in the home cage. On test day (Day 9) mice were subdivided such that half the animals in each group (N = 8/cell) received an IP injection of either saline or 5 mg/kg of d-amphetamine. Fifteen min after injection

mice were tested for spontaneous alternation, locomotor activity and stereotypy in the Y-maze for a 15 min period.

It is noteworthy that the behavioral consequences of d-amphetamine are non-linear and dose dependent [27]. That is, high dosages of the drug typically elicit a marked stereotypic response, whereas lower dosage of d-amphetamine produce locomotor excitation accompanied by only minimal stereotypy. As is the case with locomotor activity and stereotypy, the elicitation of perseverative behavior is dependent upon the dose and is most reliably observed after treatment with 5 mg/kg of the drug [14]. The dosage of amphetamine used during the chronic phase of Experment 2 (10.0 mg/kg, administered over five successive days) was previously found to produce tolerance to the perserverative behavior ordinarily observed after an acute injection of 5.0 mg/kg of the drug [14].

RESULTS AND DISCUSSION

Pre-habituation – Phase 1

The mean proportion of spontaneous alternation, the mean number of arm entries as well as the mean rating score for stereotypy during the three phases of Experiment 2 are shown in Fig. 1. Analyses of variance of the alternation scores, arm entries, and stereotypy scores of the first phase of Experiment 2 (i.e., no-drug treatment) yielded a significant days main effect, F's (2,90) = 16.34, 25.36, 10.43, $p<0.001$ (for spontaneous alternation, locomotor activity and stereotypy, respectively). Subsequent Newman-Keuls multiple comparisons ($\alpha = 0.05$) revealed that animals showed a decline in frequency of the three behaviors over successive days.

Spontaneous Alternation/Perseveration – Phase 2

Analysis of variance of the sequence of arm entries yielded a significant main effect for drug treatment, F(2,45) = 8.33, $p<0.001$. Newman-Keuls multiple comparisons ($\alpha = 0.05$) together with χ^2 analysis revealed that performance was at chance or just above chance levels throughout Phase 2 (Days 4–8) among mice treated with saline prior to testing, or injected with amphetamine following testing (posttrial). In contrast, treatment with d-amphetamine prior to testing significantly reduced alternation, such that performance was below chance levels $\chi^2(1) = 144.2, 39.8, 27.4, 11.0, 17.42$, $p<0.01$. As seen in Fig. 1, alternation increased from Day 4 to Day 5. It is not entirely clear whether this increase represents tolerance, or whether the particularly low perseveration scores observed on Day 4 were spurious. The latter possibility is not unlikely since previous work from this laboratory [14], as well as the results of Experiment 3, indicated that perseverative behavior is maximal after 5.0 mg/kg and typically decreases to approximately 40% following 10 mg/kg of d-amphetamine [1, 16, 17].

Locomotor Activity and Stereotypy

Analysis of variance of the number of arm entries yielded a significant day of testing, F(4,180) = 3.45, $p<0.01$, as well as drug treatment, F(2,45) = 17.46, $p<0.001$, main effect. Subsequent Newman-Keuls multiple comparisons ($\alpha = 0.05$) revealed that regardless of the drug treatment locomotor activity decreased with repeated testing. Treatment with d-amphetamine prior to behavioral testing significantly increased the number of arms entered (see Fig. 1).

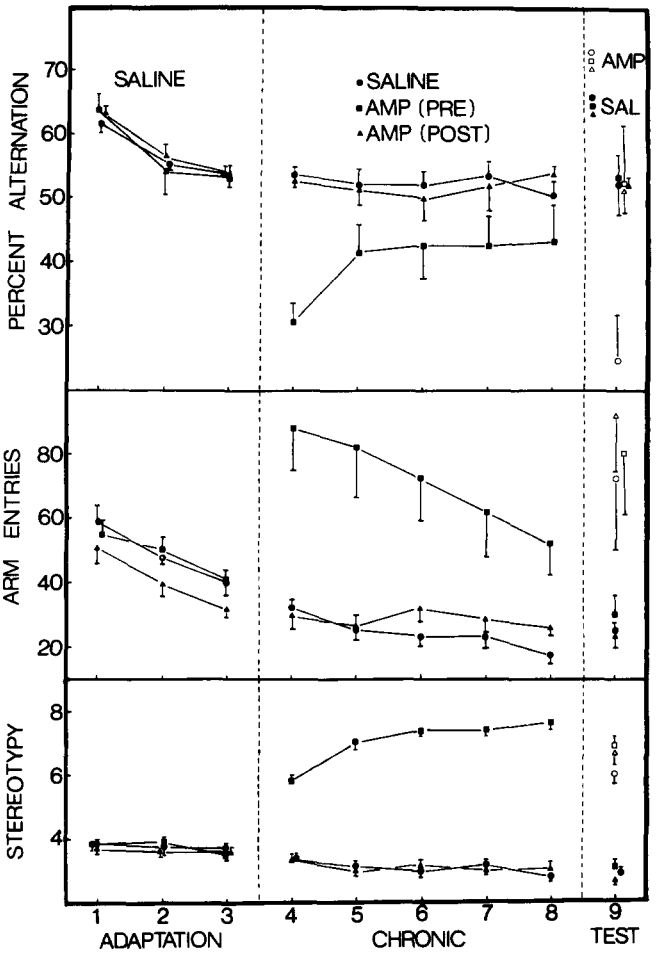

FIG. 1. Mean proportion of alternation responses (± SEM), mean number of arm entries (± SEM), as well as mean stereotypy scores (± SEM) as a function of prehabituation (Phase 1, no-drug), drug treatment during the chronic phase (Phase 2, pretrial injection of saline or d-amphetamine 10.0 mg/kg or posttrial injection of d-amphetamine 10.0 mg/kg), and drug treatment on test day (saline or 5.0 mg/kg of d-amphetamine).

With respect to the stereotypy ratings, a significant Day of Testing × Drug Treatment interaction was observed, F(8,108) = 11.81, $p<0.001$. Newman-Keuls multiple comparisons ($\alpha = 0.05$) showed that stereotypy ratings of mice in the saline and posttrial amphetamine groups did not change with repeated testing. In contrast, pretrial injection of d-amphetamine produced stereotypy which significantly exceeded that seen in the remaining groups, and the stereotypy was further enhanced with repeated Drug-Test pairings. Moreover, the observed augmentation of stereotypy following successive drug treatments was not situation specific. Specifically, a separate analysis carried out on the ratings of mice in the pretrial amphetamine condition (rated in the Y-maze), and mice in the posttrial amphetamine group (rated in the home cage following drug injection), revealed that in both groups stereotypy increased over days, F(1,120) = 38.66, $p<0.001$, and performance was comparable between groups.

Spontaneous Alternation/Perseveration – Test Day

Analysis of variance of the alternation data on test day yielded a significant Chronic x Acute Drug Treatment interaction, $F(2,42) = 3.45$, $p<0.05$. As seen in Fig. 1, mice pretreated with saline and tested with d-amphetamine (5 mg/kg) exhibited marked perseverative behavior, $\chi^2(1) = 67.8$, $p<0.01$. In contrast, mice which received repeated d-amphetamine treatment and were treated with d-amphetamine alternated at significantly higher rates, which reached or exceeded chance levels, $\chi^2(1) = 4.8$, 1.3 (for pretrial and posttrial groups, respectively). The finding that this was the case regardless of whether animals received drug injection prior to or following Y-maze exposure during Phase 2, suggests that conditioning factors are not critical for development of the observed tolerance. Moreover, administration of saline to animals chronically treated with d-amphetamine did not affect subsequent performance, suggesting that a conditioned compensatory reaction is not involved in the observed tolerance (see Fig. 1).

Locomotor Activity and Stereotypy

In contrast to perseveration induced by d-amphetamine tolerance was not observed to the locomotor excitation and stereotypy produced by d-amphetamine. As seen in Fig. 1 mice treated with d-amphetamine on test day were more active and had higher stereotypy ratings than did saline treated animal, regardless of the prior drug history or testing schedule, $F's(1,42) = 20.39$, 405.40, $p<0.001$, for locomotor activity and stereotypy, respectively. In addition to the observed main effect for drug treatment on test day, a significant chronic drug treatment main effect was observed with respect to the stereotypy ratings, $F(2,42) = 4.48$, $p<0.05$. Subsequent Newman-Keuls multiple comparisons ($\alpha = 0.05$) revealed that when testing occurred with d-amphetamine, mice in the chronic pretrial amphetamine group displayed a small but significant increase in stereotypy relative to the pretrial saline controls. However, the source for this effect is not well understood given that stereotypic behavior of mice in the posttrial amphetamine group was not different from that observed among mice in the pretrial saline or amphetamine groups.

Summarizing, consistent with previous reports, chronic treatment with d-amphetamine attenuated the perseverative response elicited by the drug, whereas tolerance was not observed to amphetamine-induced locomotor excitation and stereotypy [10, 14, 19, 27, 28]. Taken together, these findings suggest that the development of tolerance to the perseverative effects of the drug is not related to changes in locomotor activity or stereotypic behaviors produced by repeated drug administration. Furthermore, since pairing of drug treatment with the apparatus cues did not influence the extent of the tolerance in the case of perseveration, it is unlikely that conditioning factors play a prominent role in the occurrence of tolerance.

EXPERIMENT 3

Experiment 2 revealed that both chronic pre- and posttrial drug treatment produced tolerance to the perseveration elicited by d-amphetamine. These results argue against the involvement of conditioning factors in the development of tolerance. The absence of behaviorally augmented tolerance, however, may have resulted from the rapid development of tolerance observed with repeated drug treatment, i.e., ceiling effects prevented the influence of conditioning factors from becoming apparent. Since conditioning factors did not affect performance under conditions where tolerance was apparent it might be expected that behaviorally augmented tolerance would appear when the treatment conditions were such that only limited tolerance would develop. Experiment 3 was designed to examine this possibility. Specifically, work from this laboratory demonstrated that chronic drug treatment for three consecutive days was not sufficient to produce tolerance [14]. If conditioning factors affect the rate at which tolerance develops, then tolerance to the perseverative effects of amphetamine might be expected to develop after only three daily drug-test pairings.

METHOD

Animals

A total of 20 male and 20 female Swiss Webster mice procured from the Bio-Breeding Laboratories served in this experiment. Mice were individually housed and received ad lib access to food and water.

Apparatus and Procedure

The apparatus was identical to that described in Experiment 2. Mice were randomly assigned to one of five treatment conditions (N = 8/cell) and were tested in the spontaneous alternation task for a 15 min period on three consecutive days. On Days 4 through 6 the injection and testing procedure for three of the groups (i.e., pretrial saline, pretrial amphetamine, posttrial amphetamine) was identical to that described in Phase 2 of Experiment 2. In addition, two separate groups were treated with three daily injections of either saline or d-amphetamine (10 mg/kg), and placed in a carrying cage for a 15 min period following injections. The latter two groups did not receive behavioral testing until test day (Day 7). On Day 7 all mice were tested in the Y-maze after a single IP injection of d-amphetamine (5.0 mg/kg). The testing procedure was identical to that observed in the previous experiments.

RESULTS AND DISCUSSION

As observed in Experiment 2 alternation during the nondrug phase was comparable in all groups and declined over the three test sessions, $F(2,70) = 25.01$, $p<0.001$. Analysis of variance of the sequence of arm entries from Day 4 through 6 yielded a significant main effect for drug treatment only, $F(2,21) = 8.74$, $p<0.005$. As seen in Table 2, treatment with d-amphetamine prior to testing produced significantly lower alternation in relation to performance of mice tested in the nondrug state. Moreover performance was observed to be stable over the three drug/test pairings (see Table 2).

Consistent with previous observations [14] tolerance was not evident after three daily injections of amphetamine. In particular, mice assigned to the five experimental conditions exhibited marked perseverative behavior when 5 mg/kg of d-amphetamine was administered on test day, $\chi^2(1) = 34.64$, 89.2, 32.2, 30.4, 80.4, for pretrial saline and amphetamine, posttrial amphetamine, and no-test saline and amphetamine groups, respectively. Since behaviorally augmented tolerance was not observed (see Table 2), it is likely that conditioning factors do not

TABLE 2

MEAN PROPORTION OF SPONTANEOUS ALTERNATION (± SEM) AS A FUNCTION OF PRIOR HABITUATION (PHASE 1, NO DRUG), CHRONIC DRUG TREATMENT (SALINE OR 10 MG/KG OF D-AMPHETAMINE) DURING PHASE 2, (PRETRIAL, POSTTRIAL, NO TEST), AS WELL AS ACUTE DRUG TREATMENT (5 MG/KG) ON TEST DAY

	Days						
	Phase 1 (No Drug)			Phase 2			Test (d-Amphetamine) 5.0 mg/kg
	1	2	3	4	5	6	7
Saline	.658 ±.019	.583 ±.018	.587 ±.018	.530 ±.047	.522 ±.026	.563 ±.040	.349 ±.058
d-Amphetamine (pretrial)	.702 ±.036	.636 ±.035	.570 ±.017	.426 ±.065	.411 ±.047	.446 ±.043	.362 ±.050
d-Amphetamine (post trial)	.685 ±.026	.563 ±.027	.562 ±.015	.576 ±.021	.562 ±.016	.565 ±.021	.373 ±.044
Saline (no test)	.640 ±.033	.556 ±.036	.563 ±.031				.351 ±.044
d-Amphetamine (no test)	.690 ±.036	.632 ±.019	.571 ±.023				.389 ±.045

play a major role in the development of tolerance to amphetamine-induced stimulus perseveration.

GENERAL DISCUSSION

The results of the present investigation extend previous observations concerning the effects of d-amphetamine on perseverative behavior in a Y-maze exploratory task [1, 17, 18]. The fact that differentiating the arms of the Y-maze disrupted perseveration is congruent with the notion that stimulus factors play an integral role in the elicitation of the perseverative response [17]. It is not clear, however, whether the attenuation of perseveration was secondary to decreased rates of habituation (see [17]) or whether stimulus factors directly influence the perseveration.

Although stimulus factors are apparently involved in amphetamine-induced perseveration, conditioning factors probably do not play a major role in the development of tolerance. Specifically, the development of tolerance was not augmented by pairing the chronic drug experience with Y-maze exposure, nor was the magnitude of the tolerance effect altered by prior drug-test pairings. Furthermore, the fact that repeated amphetamine treatment did not enhance alternation when animals were tested with saline, suggests that conditioned rebound effects [29,30] likewise do not play a major role in the development of tolerance. It must be emphasized that although the data of the present report do not support a role for conditioning in the development of tolerance to amphetamine-induced perseveration, this is not to say that such processes are not involved in tolerance to other amphetamine-induced behaviors. Moreover, the possibility also exists that the occurrence of perseveration and its subsequent disruption with repeated drug treatments involves variations in the response to selective stimuli. For example, amphetamine-induced perseveration may well reflect increased attention and responsivity to stimuli which are high in the organisms repertoire. The reduction in perseveration may be a result of a break-down of attentional processes, rather than development of genuine tolerance. While admittedly speculative, this notion warrants further consideration.

In contrast to stimulus perseveration, tolerance was not observed to develop to the locomotor or stereotypic effects of the drug. Taken together with previous findings [1,17], these results suggest that the perseverative response is not related to drug-induced locomotor excitation or stereotypy. Similarly, although the competitive relationship between stereotypy and locomotor activity is apparent after chronic drug treatment [27], it is likely that the tolerance observed to stimulus perseveration is independent of changes in these behaviors following repeated drug administration.

Whether the tolerance obtained in the present investigation is primarily mediated by physiological factors is not entirely clear at this point. However, recent work from this laboratory has provided *prima facie* evidence indicating that the false neurotransmitter, p-hydroxynorephendrine [20] which has been implicated in the development of tolerance to other amphetamine-induced effects [2], is not involved in the tolerance observed to stimulus perseveration [14]. However, a role for other false transmitters cannot be excluded.

The fact that norepinephrine has been implicated in subserving the perseverative effects of amphetamine [1], whereas dopaminergic activity primarily is involved in mediating the locomotor and stereotypic effects of the drug [4, 6, 7, 8], raises the possibility that tolerance may occur exclusively to those behaviors which involve norepinephrine [14,19]. Recent data from this laboratory have, in fact, revealed that other behaviors which involve noradrenergic activity (e.g., startle reflex, circling after systemic amphetamine injection) are subject to tolerance effects [15]. Moreover, when these data are coupled with those showing development of tolerance to the facilitative effects of d-amphetamine on self-stimulation from the medial forebrain bundle [21], but not from the substantia nigra [23], the possibility is raised that there is a neurochemical specificity in the development of tolerance.

REFERENCES

1. Anisman, H. and L. Kokkinidis. Effects of scopalamine, d-amphetamine and other drugs affecting catecholamines on spontaneous alternation and locomotor activity in mice. *Psychopharmacologia* **45:** 55–64, 1975.
2. Brodie, B. B., A. K. Cho and G. L. Gessa. Possible role of p-hydroxynorephedrine in the depletion of norepinephrine induced by d-amphetamine and in tolerance to this drug. In: *Amphetamines and Related Compounds,* edited by E. Costa and S. Garattini. New York: Raven Press, 1970.
3. Campbell, J. C. and L. S. Seiden. Performance influence on the development of tolerance to amphetamine. *Pharmac. Biochem. Behav.* **1:** 703–708, 1973.
4. Carlsson, A. Amphetamine and brain catecholamines. In: *Amphetamines and Related Compounds,* edited by E. Costa and S. Garattini. New York: Raven Press, 1970.
5. Carlton, P. L. and D. L. Wolgin. Contingent tolerance to the anorexigenic effects of amphetamine. *Physiol. Behav.* **7:** 221–223, 1971.
6. Creese, I. and S. D. Iversen. Amphetamine response in rat after dopamine neuron destruction. *Nature, New Biol.* **238:** 247–248, 1972.
7. Creese, I. and S. D. Iversen. The role of forebrain dopamine systems in amphetamine induced stereotyped behaviour in the rat. *Psychopharmacologia* **39:** 345–357, 1974.
8. Creese, I. and S. D. Iversen. The pharmacological and anatomical substrates of the amphetamine response in the rat. *Brain Res.* **83:** 419–436, 1975.
9. Egger, G. J. The relevance of memory, arousal and cue factors to developmental changes in spontaneous alternation by rats. *Devl Psychobiol.* **6:** 459–468, 1973.
10. Ellinwood, E. H. Jr. and R. L. Balster. Rating the behavioural effects of amphetamine. *Eur. J. Pharmac.* **28:** 35–41, 1974.
11. Ellinwood, E. H. Jr. and O. Escalante. Behavior and histopathalogical findings during chronic methedrine intoxication. *Biol. Psychiat.* **2:** 27–39, 1970.
12. Gotestam, K. G. Acute tolerance to amphetamine in rats. *Psychopharmacology* **49:** 113–116, 1976.
13. Gotestam, K. G. and T. Lawander. The duration of tolerance to the anorexigenic effect of amphetamine rats. *Psychopharmacologia* **42:** 41–46, 1975.
14. 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.
15. Kokkinidis, L. and H. Anisman. Acute and chronic effects of d-amphetamine: Behavioral and neurochemical specificity. *Soci. Neurosci.* (Abstr.) **3:** 443, 1977.
16. Kokkinidis, L. and H. Anisman. Perseveration and rotational behavior elicited by d-amphetamine in a Y-maze exploratory task: Differential effects of intraperitoneal and unilateral intraventricular administration. *Psychopharmacology* **52:** 123–128, 1977.
17. Kokkinidis, L. and H. Anisman. Interaction between cholinergic and catecholaminergic agents in a spontaneous alternation task. *Psychopharmacology* **48:** 261–270, 1976.
18. Kokkinidis, L. and H. Anisman. Dissociation of the effects of scopolamine and d-amphetamine on spontaneous alternation: Genuine perseveration or dishabituation. *Pharmac. Biochem. Behav.* **5:** 293–297, 1976.
19. Kokkinidis, L., M. D. Walsh, R. Lahue and H. Anisman. Tolerance to d-amphetamine: Behavioral specificity. *Life Sci.* **18:** 913–918, 1976.
20. Kopin, I. J. False adrenergic transmitters. *Ann. Rev. Pharmac.* **8:** 377–394, 1968.
21. Leith, N. J. and R. J. Barrett. Amphetamine and the reward system: Evidence for tolerance and post-drug depression. *Psychopharmacologia* **46:** 19–26, 1976.
22. Lewander, T. A mechanism for the development of tolerance to amphetamine in rats. *Psychopharmacologia* **21:** 17–31, 1971.
23. Liebman, J. M. and D. S. Segal. Lack of tolerance or sensitization to the effects of chronic d-amphetamine on substantia nigra self stimulation. *Behav. Biol.* **16:** 211–220, 1975.
24. Magour, S., H. Coper and C. Fandrich. The effects of chronic treatment with d-amphetamine on food intake, body weight, locomotor activity and subcellular distribution of the drug. *Psychopharmacologia* **34:** 45–62, 1974.
25. Rech, R. H., H. A. Tilson and W. J. Marquis. Adaptive changes in behavior after repeated administration of various psychoactive drugs. In: *Neurobiological Mechanisms of Adaptation and Behavior,* edited by A. J. Mandell. New York: Raven Press, 1975, pp. 263–286.
26. Schuster, C. R., W. S. Dockens and J. H. Woods. Behavioral variables affecting the development of amphetamine tolerance. *Psychopharmacologia* **9:** 170–182, 1966.
27. 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.
28. Segal, D. S. and A. J. Mandell. Long-term administration of d-amphetamine: progressive augmentation of motor activity and stereotypy. *Pharmac. Biochem. Behav.* **2:** 249–255, 1974.
29. Siegel, S. Morphine analgesic tolerance: its situation specificity supports a Pavlovian conditioning model. *Science* **193:** 323–325, 1976.
30. Siegel, S. Morphine tolerance acquisition as an associative process. *J. exp. Psychol.* **3:** 1–13, 1977.
31. 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.
32. Thompson, T. and C. Schuster. *Behavioral Pharmacology.* Englewood Cliffs, New Jersey: Prentice-Hall, 1968.
33. Tilson, H. A. and R. H. Rech. Conditioned drug effects and absence of tolerance to d-amphetamine induced motor activity. *Pharmac. Biochem. Behav.* **1:** 149–153, 1973.
34. Winer, B. J. *Statistical Principles in Experimental Design.* New York: McGraw-Hill, 1971.