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d-Amphetamine: Disruptive Effects on the Long-Term Store of Memory and Proactive Facilitatory Effects on Learning in Inbred Mice[1]

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CRABBE, J. C. AND H. P. ALPERN. *d-Amphetamine: disruptive effects on the long-term store of memory and proactive facilitatory effects on learning in inbred mice.* PHARMAC. BIOCHEM. BEHAV. 3(4) 647–652, 1975. – Male, C57BL/6J mice were given two daily trials on an appetitively-motivated successive brightness discrimination maze problem; they then received daily intraperitoneal injections of saline or d-amphetamine for 5 days. When trained again in the maze, mice in all d-amphetamine groups tended to display impaired retention: retention was significantly impaired in the 2.0 mg/kg group. Naive mice were treated exactly as were the pretrained mice except that they received no initial maze training prior to drug treatments. Mice in all naive d-amphetamine groups tended to display enhanced acquisition of the maze problem: acquisition was significantly enhanced in the 1.0 mg/kg group. These results could not be explained as effects of d-amphetamine on attentional, motivational or other performance factors.

d-Amphetamine Long-term store of memory Memory Learning Inbred mice
Facilitation of learning Proactive facilitation Memory disruption

NEURAL excitants have both facilitatory and disruptive influences on learning and memory processes. Administration of d-amphetamine shortly before training has been demonstrated to affect discrimination learning [12, 13, 18, 19]. When administered shortly after training, amphetamine can also affect the consolidation of memory [5, 11, 12, 14, 17, 18]: as McGaugh has argued, effects of agents administered after training cannot easily be attributed to effects on performance, motivation, or other factors unrelated to memory [21]. The effects of d-amphetamine on both acquisition and memory consolidation are time-dependent and have usually been reported to be limited to four hours [11, 12, 18]. However, Bauer and Duncan have reported that repeated administration of d-amphetamine to rats could proactively facilitate learning when training was begun 24 hours after the last drug injection [4]. Bauer has subsequently reported similar enhancement with pentylenetetrazol [3]. With repeated administration, d-amphetamine can, therefore, exert proactive effects on acquisition that persist beyond the usual temporal limits of the effects of the drug.

Recently, neural excitants have been shown to affect the long-term store of memory itself, as distinguished from memory consolidation processes [1, 8, 30]. Alpern and Crabbe found that, after exposure to a maze, 10 daily administrations of a low dosage of strychnine sulphate facilitated retention of the maze problem in mice [1]. The first injection was administered 24 hr after initial training, well beyond the temporal limits for retrograde facilitation of the consolidation process with strychnine [26]. In addition, when the same injection series was administered to animals that had not been partially trained in the maze, no enhancement of acquisition was observed. Thus, the enhancement seen in pretrained animals could not have resulted from proactive facilitation of learning ability, and the results were interpreted to indicate an effect of the drug on the

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long-term store of memory [1,2]. A subsequent study extended the range of enhancing treatments to include pentylenetetrazol and again found no proactive facilitation of learning ability in naive mice given either strychnine or pentylenetetrazol [8]. Furthermore, d-amphetamine (1.0 mg/kg) administered daily for 5 days significantly disrupted the long-term store of memory; however, naive animals treated with this dosage of d-amphetamine tended to show proactive facilitation.

In the research reported here, the dose-response relationships of the paradoxical effects of repeated administrations of d-amphetamine on acquisition and on the long-term store of memory were examined. Specificially, the effects on maze acquisition of 5 daily administrations of d-amphetamine were studied in naive mice and in mice that had previously been exposed to the maze. It should be pointed out that single injections of d-amphetamine at the intervals selected would have neither proactive effects on acquisition nor retroactive influences on the consolidation process.

METHOD

Animals

One hundred fifty-five male mice of the C57BL/6J strain were purchased from the Jackson Laboratories and were received in our colony at the age of 50 ± 4 days. Mice were housed 6 to a cage (20 cm × 28 cm × 10 cm) and had Wayne Laboratory Chow available ad lib. The colony room was maintained on a 12 hr on/12 hr off light-dark cycle. Training commenced approximately one week after arrival of the mice.

Apparatus

The maze consisted of 10 brightness discrimination units, linearly arranged, in addition to a start and goal box (see Fig. 1). Each unit consisted of a short entryway, painted flat gray, which led to the choice point. The floor of the choice point was painted flat white on one side and flat black on the other, as was a discrimination panel directly in front of the mouse entering the choice point. Two parallel alleys 23 cm long and 2.9 cm wide led from the choice point to the entryway of the next unit; they were each obstructed at the distal end by a solid Plexiglas barrier, also painted black, which could not be seen from the choice point (see Fig. 1). Sliding doors, painted flat gray, prevented retracing into previous maze units or exit from the goal box. The righthand alley was white in Units 1, 4, 6, 7 and 10, preventing solution of the discrimination problem by position or alternation preferences.

The goal box, painted flat white, had a 1/4-teaspoon mounted at drinking height at the distal end. The start box had a rectangular-shaped funnel attached to its top so that a mouse could be introduced into the maze by dropping it directly into the start box. The maze units and start and goal boxes were 12.7 cm high and open at the top. All maze parts were constructed from 1/8 in. Plexiglas; the floor of the maze was constructed from 1/4 in. Plexiglas. Background illumination was from overhead fluorescent fixtures; the light radiating from the black and white sides of the discrimination panel was 0.5 foot-lamberts and 7.7 foot-lamberts, respectively. The task consisted of the elimination of incorrect entries into black alleys, since male C57BL/6J mice display a marked preference for black alleys [7].

Procedure

Pretrained groups. Seventy-two mice were deprived of water 24 hr before the first day of training. On Day 1, each mouse was dropped into the start box of the maze and was allowed to drink a 0.3 percent saccharin in tapwater solution for 20 sec upon reaching the goal box. No animal required more than 60 sec to find the reinforcing solution after entering the goal box on the first day of training; thereafter, all animals drank immediately upon entering the goal box. After 20 sec, the mouse was removed, placed in a holding cage and returned to its home cage. One hr after the last mouse had been trained, all mice received access to water in their home cages for 30 min. On the second day of initial training, this procedure was repeated, with the exception that 1 hr after all mice had completed training, water was restored ad lib.

Latency (in minutes) to reach the goal box, Initial Errors (first entry into the incorrect alley in any maze unit) and Total Errors (first and all subsequent reentries into incorrect alleys) were scored on each daily trial. An animal could commit a maximum of 10 Initial Errors on each trial (one per unit) while the number of Total Errors was not constrained. An error was scored if more than one-half of the mouse's body crossed an imaginary line perpendicular to the alleys across the proximal end (see Fig. 1).

Seventy mice were allocated to 5 experimental groups. At least one mouse from each cage was included in each group, and the ordinal position of mice within cages was also reasonably distributed across groups. This was done to eliminate confounding treatment differences with any possible difference between mice that were the first, or last, in their cages to be run in the maze. The mean numbers of Initial and Total Errors for each group on each day of initial training were then calculated. The assignments to conditions for a few mice were then switched to eliminate differences in mean error performance during initial training. These exchanges were also employed to distribute mice with extremely high latencies (over 5 min) evenly across groups; however, the representation of all cages in all groups and the control for cage-order effects were maintained. Mice were thus partitioned into 5 groups, matched as nearly as possible for training (errors), performance (latency), and order in cage, following initial quasi-random assignment.

Each of the 5 groups of mice then received a daily intraperitoneal injection for 5 successive days, starting on the third day (that is, 24 hr after the second training trial). The 5 solutions were coded so that injections could be administered blind. The 5 groups were: (1) saline; (2) 0.25 mg/kg; (3) 0.50 mg/kg; (4) 1.00 mg/kg; and (5) 2.00 mg/kg of d-amphetamine sulphate. Mice were weighed daily before injection and were injected at the same time each day. Injections were administered in the latter half of the light cycle.

Twenty-four hr after the last (fifth) injection, all mice were again water deprived. Twenty-four hr later, each mouse was again given a training trial in the maze according to the aforementioned procedure. One hr after all mice had completed their maze trials, they were allowed access to water for one-half hour. All training sessions were in the latter half of the light cycle, and training for each mouse occurred at nearly the same time each day. All mice were trained until they had reached the criterion of learning, or for 8 days. The learning criterion was two Initial Errors or less summed over 2 days. If an animal reached criterion, it

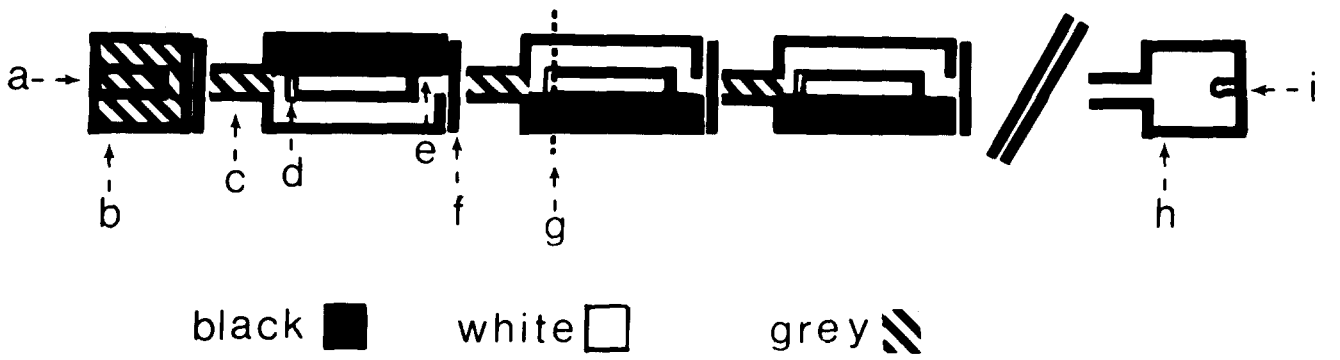

FIG. 1. Discrimination Maze: (a) start box (b) rectangular funnel (c) entryway (d) discrimination panel at choice point (e) barrier (f) sliding door (g) error line (h) goal box (i) drinking spoon.

was not trained further in the maze, but was allowed to remain undisturbed in its home cage.

Naive groups. Eighty-three mice were allocated to 1 of 5 groups on the basis of order in cage and cage number. In other words, 5 groups were created by balancing ordinal position in cage and insuring that each cage was represented in each group. Since these animals were assigned to groups before any treatment was applied, it was not possible to balance groups for maze performance. These groups were treated exactly the same as the groups described above with the important exception that they received no initial exposure to the maze before drug treatment. Accordingly, all water was removed for 24 hr, and on the next day, one-half hour of water was allowed in home cages. On the third day, water was restored ad lib. Twenty-four hr later, the first of 5 daily intraperitoneal injections was administered. Groups received either saline, 0.25 mg/kg, 0.50 mg/kg, 1.00 mg/kg, or 2.00 mg/kg of d-amphetamine sulphate. Twenty-four hr after the last injection, all mice were again water-deprived, and 24 hr after that, they were given their first trial in the maze for saccharin reinforcement. Training continued for 8 days, or until criterion was reached; mice received one-half hour of access to water in their home cages, one hour after each daily maze session was completed. The naive mice, therefore, were treated exactly as were the pretrained mice, including being water-deprived before the drug injection series, except for not receiving training in the maze before drug treatment.

All groups. According to our previously established standard experimental procedure [1, 7, 8], mice were eliminated from the experiment for any of the following reasons: (a) Failure to run in the maze; (b) Administration of the wrong drug dosage during the injection series; (c) If an individual mouse's score on any measure was more than three standard deviations above the mean for its group; or (d) If all animals in a cage performed extremely poorly, all mice from that cage were eliminated from the experiment. Thirteen individual mice and one cage (6 mice) were eliminated for the above reasons: of these, 6 were in the pretrained groups and 13 were in the naive groups.

RESULTS

Pretrained Groups

Data from 66 mice receiving initial training in the maze indicated that the level of initial training was essentially similar for all groups. To confirm this observation, Dunnett's t statistic [31] compared the number of Initial and Total Errors on Days 1 and 2 of initial training for each drug group with the saline group. No group differed significantly from the saline group on either measure. Data from Days 1 and 2 of initial training were not considered further; all data reported below represent retention testing Days 1 to 8. Comparisons of Trials, Initial Errors, and Total Errors to Criterion for each amphetamine group with the saline group were also made with Dunnett's t statistic. The 2.0 mg/kg group required significantly more Trials to Criterion than the control group ($p<0.05$): this difference was reflected more strongly in each of the error measures (p's<0.025) (see Fig. 2). Overall, every group receiving amphetamine was poorer upon retention testing than the group receiving saline.

Performance effects of amphetamine treatment were examined by analysis of latency on the first retention trial and on the trial on which criterion was attained (Criterion Trial). Comparisons of the mean latencies of each drug group with the control group mean by Dunnett's t revealed no significant differences in latency on either trial. It was concluded that the effects of amphetamine on re-acquisition of the discrimination problem were not due to performance decrement caused by drug treatment. Further, no observable differences in the maze behavior of mice treated with amphetamine as opposed to those given saline were evident.

Naive Groups

As discussed elsewhere [1, 2, 8] it cannot be concluded from the pretrained groups alone that amphetamine treatment altered memory processes. Since proactive effects of repeated amphetamine treatment on learning ability have been reported [4,8], the naive groups were necessary to control for effects on problem acquisition rather than the long-term store of memory.

Data for 70 naive mice were analyzed. The mean of each amphetamine group was compared with the control group mean by Dunnett's t for Trials, Initial, and Total Errors to Criterion. The 1.0 mg/kg amphetamine group showed significant facilitation (p's<0.05) for Trials and Total Errors to Criterion; the t value for Initial Errors to Criterion approached significance ($t = 2.44$, $df = 4,65$, $p<0.06$). It is clear that pretreatment with all dosages of amphetamine

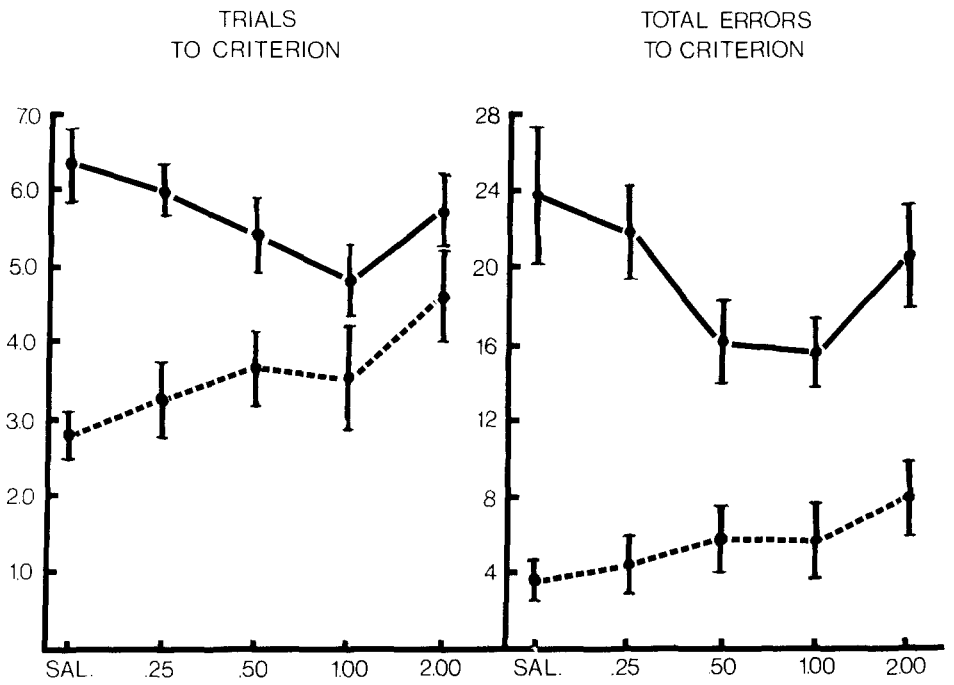

FIG. 2. Means and standard errors for trials and total errors to criterion. Solid lines are naive groups: dashed lines are pretrained groups. Abscissa shows dosages of d-amphetamine sulphate in mg/kg and saline on logarithmic scale. Ordinate shows trials or errors. N = 13–16 per group.

employed tended to improve acquisition (see Fig. 2). Latencies on the first training trial and on the criterion trial were also compared with the control group by Dunnett's *t* statistic. No amphetamine group differed significantly from the control group on either measure.

The preliminary findings of Crabbe and Alpern [8] were thus confirmed in this experiment. All moderate dosages of amphetamine tended to disrupt memory in previously trained mice, while the same dosages facilitated acquisition in naive mice. These differences were not attributable to performance differences, because the latencies on first and last retest trials bore no illuminating relationship to drug dosage or drug effect.

DISCUSSION

In contradistinction to the results reported here, moderate dosages of d-amphetamine have usually been reported to facilitate retention of a discrimination problem, whether given either shortly before or after training [5, 11–14, 17–19]. Why, then, did we find disruption of retention in our pretrained groups? The answer to this question depends upon two points of contrast between previous studies and our own: the time at which amphetamine was first administered, and the number of times that it was administered.

Amphetamine treatment in prior studies has produced a facilitative effect on learning and/or memory if administered within 4 hr after training [11] or 30 min before training [18]: longer intervals have not been examined. However, the most complete time-response study of amphetamine reported that posttrial facilitation was obtained at 15, but not at 30 min, while pretrial administration was effective at both those intervals. The dosages employed were optimal, as determined from dose-response curves [18]. In the research reported above, we did not administer the first injection until 24 hr after training. Although the presumptive effect of amphetamine treatment in previous studies has been to facilitate the consolidation of the memory trace, it is probable that our results were not due to consolidation effects since the posttraining interval we employed was well beyond the established range for retrograde effects on consolidation [22, 24, 25]. Thus, the temporal disparity between our research and previous investigations suggests that we are assessing effects on some aspect of long-term memory processing other than its consolidation.

Further, several of the previous studies employed only one drug injection [12, 13, 17, 19], while we gave five daily injections. If repetition of injections were producing cumulative effects, one would expect to see changes in the effects of daily drug treatment as the study progressed in those cases where multiple injections were employed. Examination of studies employing daily injections reveals contradictory evidence. On one hand, two studies gave 4 daily injections and yet reported facilitation [11,14]. One of these [11] also reported facilitation at an unusually long interval post-trial (4 hr). One study trained mice to a criterion of learning and reported facilitation [18]. Those mice given amphetamine received, on the average, about 5 daily injections. This figure was calculated from the mean Trials to Criterion for each drug group. Control mice, however, received about 15 daily injections.

On the other hand, one study employed an unusual training procedure wherein rats were food-deprived and trained in a Lashley III maze on alternate days. Saline, or

1.0 or 5.0 mg/kg d-amphetamine was given immediately after training. Both dosages facilitated retention for three injections; thereafter, no significant differences were observed until the completion of training (18 trials). Two months later all rats were retested, and the 5.0 mg/kg d-amphetamine group showed significantly impaired retention [5]. These results suggest that consolidation and long-term retention may respond differentially to the same pharmacologic manipulation, with the development of the disruptive effect of amphetamine on the long-term store requiring more time to develop than the facilitative effect on consolidation.

Thus, previous studies differ in most cases from our study, both with respect to the time at which drug treatment was initiated and, to a lesser extent, in the number of drug administrations. The difference in results may be because our treatment regimen was affecting a different process (i.e., long-term memory storage) than that affected by treatments in earlier studies (i.e., consolidation).

Learning and memory have traditionally been viewed as continuous, or even unitary processes. The technique of posttraining drug administration enables the conceptual separation of memory consolidation and acquisition processes [21]. When a particular drug is considered, however, the retrograde effects of that drug on memory consolidation do not generally differ from the effects seen when that agent is administered before training (for reviews, see [9, 15, 16, 22–25, 27]). For example, a given dosage-range of d-amphetamine will enhance acquisition and enhance memory consolidation, or disrupt them both, but it will not differentially affect them [5, 11–14, 17–19]. Thus, the pharmacologic differentiation of learning and memory consolidation has not been achieved. Although it is premature to assert on the basis of these results that the long-term store of memory and learning are supported by two distinct neurophysiological substrates, the fact that a single dosage-schedule of d-amphetamine produced opposite effects on retention and acquisition suggests that this might be the case. A subsequent study has reported an analogous differentiation of the effects of caffeine and nicotine on the long-term store and learning, supporting the general validity of the effects reported here [30].

What, then, are the possible bases for the effects of d-amphetamine on memory and upon acquisition? The disruption of the long-term store of memory by d-amphetamine could have resulted from acceleration of memory decay processes (forgetting), blockade of retrieval of an otherwise adequate engram, or interference with the neurochemical development of the engram. Analysis of the temporal relations of this effect could help to identify the correct alternative. Blockade of retrieval processes would be expected to be independent of the length of delay between initial training and initiation of the injection series. If acceleration of forgetting were the mechanism responsible for this disruption, injections soon after initial training could be expected to have a lesser effect than injections given when the decay process was measurably advanced. Finally, if specific disruption of the biochemical substrate of the formative engram were responsible, delay of injections beyond the point at which memory strength had reached its maximum could be expected to be ineffective.

Unpublished work in our laboratory indicates that the strength of long-term memory for partial training on this maze problem increases for a few days, reaching a peak at about 3 days after initial training, and that it decays thereafter monotonically. Deutsch has reported a similar non-monotonic gradient in the development of long-term memory for a different task in rats [10]. Therefore, the injections in the study reported here were probably administered during the waxing and/or peak periods of memory strength. For the reasons outlined above, this makes acceleration of decay processes seem the least likely of the 3 alternatives, but it cannot distinguish between the other 2 hypotheses. Systematic investigation of the interaction of amphetamine disruption and time after initial training that the injections are administered will be necessary to elucidate further the responsible mechanism.

At the same broad level of analysis, the proactive facilitation of learning reported here in naive mice might be the result of increased attention, vigilance, sensitization to the brightness cues in the maze, increased levels of motivation, or a specific enhancement of the neuropharmacologic substrates of the learning process. As amphetamine is a known adipsic agent, enhancement of motivation seems an unlikely alternative [6]. Attention, vigilance, and sensitization hypotheses are logically unlikely, since these results could reasonably be expected to obtain more strongly in pretrained animals than in naive animals: however, d-amphetamine treatment disrupted the performance of trained animals. Further, the neurobiological processes underlying learning are demonstrably subject to pharmacologic manipulation by repeated treatment with an excitant compound, strychnine. Several investigators have reported that repeated administration of low dosages of strychnine sulphate to rats during post-weaning development can result in enhancement (or disruption) of learning ability assessed during adulthood [20, 28, 29]. Therefore, specific enhancement of the neurochemical processes underlying acquisition seems the most likely mechanism for the mediation of the proactive facilitation.

In summary, while the conceptual differentiation of acquisition from the consolidation of memory has resulted in theoretical advances, these processes are undifferentiated in response to pharmacologic intervention. The results reported here suggest that acquisition and long-term retention may be pharmacologically differentiated. This implies a difference between consolidation processes, which apparently are labile for a few hours, and long-term memory storage processes, which are labile for more than 24 hours. Future research is needed to determine with more certainty the interrelationships among the acquisition, consolidation, and long-term memory storage processes.

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