Pharmacology Biochemistry & Behavior, Vol. 33, pp. 51–54. © Maxwell Pergamon Macmillan plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Persistence of Retrieval Enhancement by Amphetamine Following Scopolamine-Induced Amnesia

DAVID QUARTERMAIN[1] AND HERMAN JUNG

Division of Behavioral Neurology, Department of Neurology, New York University School of Medicine

Received 13 July 1988

QUARTERMAIN, D. AND H. JUNG. *Persistence of retrieval enhancement by amphetamine following scopolamine-induced amnesia.* PHARMACOL BIOCHEM BEHAV **33**(1) 51–54, 1989.—Little information is available on the permanence of pharmacologically-induced retrieval enhancement following amnesia. This was studied by comparing the rate of forgetting of a memory reactivated by d-amphetamine after amnesia with spontaneous forgetting of undisturbed fear conditioning. Mice were treated with either saline or scopolamine before conditioning and retention was tested three days later. Scopolamine-treated mice received either saline or amphetamine before testing while the saline controls received a second saline injection. The scopolamine-saline group exhibited robust amnesia, whereas both saline-saline and scopolamine-amphetamine groups showed good retention. To test the persistence of these effects mice in the three groups were subdivided and given a second retention test either 1 day, 1 week or 1 month after the first test. Amphetamine was not administered before the second test. The scopolamine-saline mice continued to exhibit amnesia for up to 1 month while the scopolamine-amphetamine and saline-saline groups continued to show strong memory with only a modest decrement in performance by 1 month after the first test. These results show that amphetamine results in a permanent recovery from scopolamine amnesia.

Scopolamine amnesia Memory retrieval Memory enhancement Forgetting

THERE is good evidence that some experimentally-induced amnesias in animals can be alleviated by pretesting administration of pharmacological agents which activate central and peripheral catecholamine (CA) systems (16, 17, 19, 21). The improved performance which follows administration of pretesting CA agents is not merely the result of increased activity (15). For example, in one study (19), forgetting of Pavlovian fear conditioning after a one-month retention interval was demonstrated using both passive and active tests. Groups treated with d-amphetamine prior to testing exhibited decreased latencies (relative to saline control groups) if an active test was used and increased latencies if retention was measured by a passive avoidance test. In addition, it has been shown that animals treated with d-amphetamine or clonidine after experimentally-induced amnesia show better retention of discrimination learning than amnestic controls if memory is indexed by relearning, but poorer retention if reversal learning is used as the test (16,18). Such findings provide strong support for the view that improved performance after pretesting administration of CA drugs results from facilitated retrieval of specific memories. In this respect, drugs appear to function like conventional reminder treatments which have been shown to improve retention by reactivating previously stored training memories (4, 13, 23).

An aspect of pharmacologically-induced retrieval enhancement which has received little attention is the persistence of memory restoration after amnesia. Presentation of conventional reminder cues such as the CS and the UCS appear to produce relatively permanent restoration of memory following amnesia (12). This may be the result of recoding during which the cueing context becomes incorporated into the training memory (4). It is not known whether pharmacological agents can produce similar long-term benefits for remembering. It has been suggested that amphetamine may improve remembering by reestablishing in the retrieval environment certain internal contextual stimuli similar to those which were present during encoding (17). It is possible that such stimuli could become incorporated into the training memory during testing and thereby increase later accessibility. On the other hand, drug treatments may provide temporary access to a weakened memory so that retention is enhanced only during the time that the drug is pharmacologically active. Some support for this conjecture is provided by the demonstration that ACTH-induced retrieval enhancement is relatively transient; remembering is improved if retention is tested shortly (1–2 hr) after treatment, but a relapse into amnesia is observed if retention is tested after longer intervals (8–24 hr) (11,20).

The intention of the present study was to determine the durability of retention enhancement induced by pretesting admin-

[1]Requests for reprints should be addressed to David Quartermain, Department of Neurology, New York University Medical Center, 550 First Avenue, New York, NY 10010.

istration of d-amphetamine by retesting groups of animals at intervals from 1 day to one month after the initial drug-induced retrieval enhancement.

METHOD

Behavioral Task

Avoidance of the stimuli on one side of a mouse shuttle box was established by the use of Pavlovian fear conditioning. Retention was tested by measuring latencies to escape from the shock compartment under extinction conditions. Previous studies have shown that substantial forgetting occurs in this task after one month (19,23) and that this forgetting can be alleviated by pharmacological (19) and conventional (24) reactivation treatments.

Animals

Male Swiss Webster mice (Taconic, Germantown, NY) approximately 12 weeks old and between 35 and 40 grams body weight were used as subjects. Animals were housed 4 per cage with food and water available ad lib.

Apparatus

Fear conditioning was carried out in one side of a two compartment mouse shuttle box (LVE MSC-002). The shock side, which was 9 cm wide, 11 cm high and 23 cm long, was enclosed by black Plexiglas walls and a black lid. The floor was made of stainless steel rods (0.3 cm dia.; 7 cm between rods) through which a scrambled shock (0.2 mA) could be delivered from a Coulbourn constant current shock source. The conditioned stimulus (CS) was a 24-V DC light that flashed on for 0.5 sec at a frequency of 1 Hz. The CS was positioned on the lid above the shock compartment. The nonshock (safe) side of the apparatus which was the same size as the shock compartment, was a white Plexiglas chamber. The two compartments were separated by a guillotine door.

Procedure

Amnestic treatment. Twenty min prior to training each mouse received an injection of either saline or 0.5 mg/kg scopolamine hydrochloride. Injections were administered subcutaneously in a volume of 0.01 ml per gram body weight.

Retrieval enhancing treatment. Thirty min before the first retention test, mice received an injection of 1.0 mg/kg d-amphetamine sulphate.

Training. Mice were first placed in the safe side of the apparatus where they remained for 90 sec. No shock was administered. The door was then opened and the animal gently pushed with a clear Plexiglas panel into the black side of the apparatus where 4 fear conditioning trails were administered. Each trial consisted of a 13-sec presentation of the CS accompanied for the last 3 sec by the UCS. Time between trials was determined by a variable interval 30-sec schedule. Following the fourth shock the door was raised and the mice were gently pushed into the safe side where they remained for 90 sec. Training consisted of a total of 12 CS-UCS pairings and exposure to the stimuli of the safe compartment for an equal amount of time.

One group of 24 mice received sham training. Each animal was treated with scopolamine and thirty min later placed in a different shock chamber in an adjacent room. The chamber was yoked to the training apparatus so that the mice received the same number and distribution of shocks as their trained counterparts. Three days after sham training mice were injected with amphetamine or saline and tested in the training apparatus. This procedure provides a means to evaluate the contribution of nonspecific (nonmemorial) effects of acute amphetamine injection on avoidance testing.

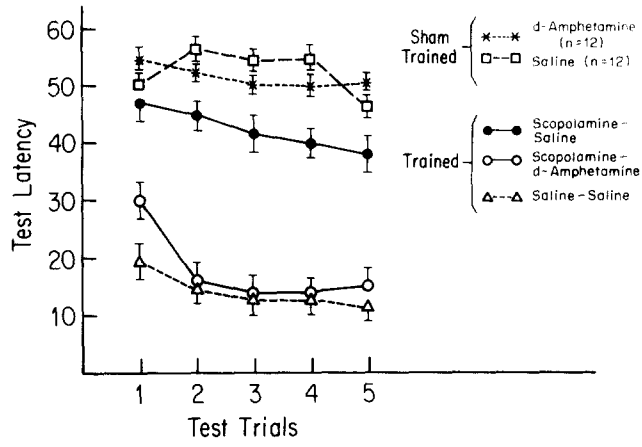

FIG. 1. Mean escape latencies in seconds (± SEM) for the five test trials on the first retention test (T1) for all animals in the three treatment groups. The scores of sham-trained mice show that amphetamine only decreases test latencies in conditioned mice.

Testing. Mice were placed in the shock compartment facing away from the door. After 5 sec the door was raised initiating the CS. When the animal crossed into the safe side the CS terminated and the latency was automatically recorded. The UCS was not present during testing. Animals failing to cross within 60 sec were given the maximum latency as a test score. The test session consisted of 5 trials with an intertrial interval of 30 sec.

Experimental design. Prior to training, 105 mice were injected with scopolamine and 45 with saline. All animals were given an initial retention test (T1) 3 days after training. Thirty min before this test 55 of the scopolamine-treated mice were treated with saline (amnestic controls) and the remaining 50 were treated with amphetamine. The saline controls were given a second injection of saline prior to the test. Following testing, animals from the scopolamine-saline, scopolamine-amphetamine and saline-saline groups were subdivided into 3 groups and given a second retention test (T2) either 1 day, 1 week or 1 month after T1. Amphetamine was not administered prior to T2. These group assignments resulted in a $3 \times 3 \times 5$ factorial design with two between subjects factors (drug group and test interval) and one within subjects factor (test trials).

RESULTS

The performance of all animals at T1 is shown in Fig. 1. The data from the trained animals was analysed by a 3×5 ANOVA. The results showed that there was a significant difference in test latency among the three drug groups, $F(2,150) = 43.88$, $p = <0.001$, and a significant effect of test trials, $F(4,150) = 13.36$, $p = <0.001$. Reference to Fig. 1 shows that animals treated with scopolamine before training and saline before testing had significantly longer latencies than the other two groups indicating a robust amnesia for fear conditioning. The scores of this group are very similar to the performance of untrained animals tested under the same conditions. The latencies of scopolamine mice treated with d-amphetamine before the test are virtually indistinguishable from those of animals with undisturbed memory. That the improved test performance following amphetamine is not merely the result of increased general activity has been shown many times in

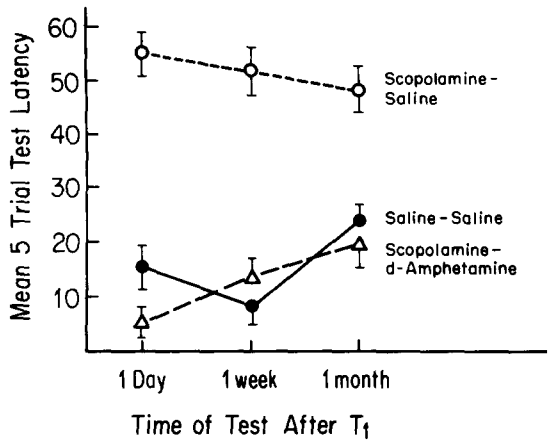

FIG. 2. Mean five trial latencies (±SEM) for mice in the three treatment groups tested either 1 day, 1 week or 1 month after T1. Amphetamine was not injected prior to the second retention test (T2).

this laboratory [e.g., (17,19)]. In the context of the present experiment this can be demonstrated by examining the performance of sham-trained mice injected with amphetamine. Their results are shown in Fig. 1 along with those of a sham-trained group which was given saline. It is apparent that amphetamine failed to decrease test latencies in animals not given formal fear conditioning.

The retest (T2) data are shown in Fig. 2. The scores of mice that failed to show improved retention following amphetamine or those that did not have a robust amnesia at T1 were not included in this analysis. Examination of the scores of the excluded subjects showed that level of retention measured at T2 remained virtually unchanged from that during T1. Mice that did not exhibit enhancement following amphetamine at T1 continued to perform like amnestic (scopolamine-saline) mice at all subsequent test intervals. Similarly, animals who were not amnestic at T1 continued to show good retention when retested. It is therefore necessary to exclude such animals in order to arrive at an accurate estimate of the durability of both the scopolamine-induced amnesia and its alleviation by amphetamine. Since we had a large pool of animals in each drug condition, we had the opportunity to make the most rigorous test of the persistence of the T1 treatment effects by examining the T2 scores of only the animals who, on the initial test, showed the strongest enhancement and the most robust amnesia. Accordingly, we discarded the scores of animals in the scopolamine-saline group if their mean 5 trial T1 latencies was less than 25.0 (N = 16 discarded) and from the scopolamine-amphetamine and the saline-saline group if their mean 5 trial T1 score was greater than 25.0 sec (N = 14 and N = 7 discarded respectively). It should be emphasised that these are rigorous selection criteria and many of the animals that were eliminated would, by statistical standards, be regarded as showing memory loss and memory facilitation. In order to simplify the statistical analysis we created equal numbers in the 9 subgroups (N = 12) by randomly eliminating 3 mice from the scopolamine-saline group and 2 from the saline-saline group. The data were analysed with a repeated measures ANOVA with two between subjects factors and one within subjects factor. Since test trials were not a significant source of variance, data are plotted as the mean of the 5 test trials. The only significant effect to emerge from the analysis was the overall difference among drug groups, $F(2,22) = 62.68$, $p < 0.001$. As shown in Fig. 2 this resulted primarily from the differences between the scopolamine-saline animals and the other two groups. Animals that showed a robust amnesia 3 days after training were still amnestic one month later. Spontaneous recovery from amnesia does not appear to occur in this experiment. Figure 2 also shows that there is no evidence for a relapse into amnesia by the scopolamine-amphetamine animals when the acute effects of amphetamine have dissipated. At the 1-day test interval the amphetamine group actually have better retention than the saline control group and they continue to exhibit good memory of the fear conditioning one month after amphetamine treatment. The performance of these animals is in all respects comparable to that of animals in which no forgetting had been induced. (It should be noted that the group differences at T2 were significant even if the scores of *all* animals tested at T1 were included in the analysis.) There was no significant effect of time of testing on retention ($F = 1.31$), neither was there a significant interaction between drug group and test interval ($F = 1.83$).

DISCUSSION

The principal finding of this study is that the facilitation of retrieval induced by pretest amphetamine administration persists after the acute effects of the drug have dissipated. This result indicates that amphetamine does not merely facilitate performance by enhancing arousal or by creating other motivational alterations which permit temporary access to a weakened trace. The memory of the fear conditioning is apparently strengthened when the avoidance response is performed under the influence of amphetamine. In this respect the effects of amphetamine on retrieval are similar to those produced by conventional reminder treatments (such as presentation of the UCS or CS) which also produce durable recovery of memory. One study employing a design similar to the present experiment has shown that the memory reactivated by a single presentation of the UCS 27 days after conditioning is forgotten more slowly than a newly acquired memory (24).

The mechanism by which amphetamine-induced reactivation strengthens the memory of fear conditioning is presently unknown. It should be emphasised that the improved retention is not the result of additional fear conditioning occurring during the retention test; the UCS was not presented during testing so that any new learning which occurred should have reduced the tendency to run from the compartment in which shock had previously been administered.

There are several mechanisms by which amphetamine could strengthen memory. One is that amphetamine treatment might induce a period of rehearsal similar to that described by Wagner, Rudy and Whitlow (26). Another is that the drug may produce internal cues similar to those which occurred following initial encoding so that animals recognise the similarity between the conditions in the retrieval environment and those that were present during conditioning. This recognition may trigger a reprocessing of the task stimuli which could result in a strengthening of the representation of the fear conditioning.

The present results provide further evidence for the similarity between memories reactivated following forgetting and those which are newly acquired. Previous studies have shown that both recently acquired and reactivated memories are susceptible to disruption by ECS and protein synthesis inhibition [e.g., (8, 9, 14)], and to facilitation by strychnine (3,6). In addition, both can act as sources of proactive interference for subsequent learning (2,7). The results of the present study indicate that memory reactivated by a pharmacological agent after experimentally-induced amnesia is forgotten at the same rate as an intact memory.

In summary, this experiment has demonstrated that amphetamine does not merely permit temporary access to a weakened

memory. After successful retrieval under amphetamine accessibility to the trance is permanently increased. This finding is consistent with recent views of retrieval which emphasize the constructive aspect of the process [e.g., (1, 10, 25)]. The act of retrieval is believed to result in the storage of a new event comprising the retrieved memory and information present in the current retrieval environment (4,5). According to this notion, a retrieved memory should have similar characteristics to one that has recently been acquired. The present findings are consistent with this idea.

ACKNOWLEDGEMENT

The research reported in this paper was supported by U.S. Public Health Service Grant MH 37326 to D.Q.

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