Pharmacology Biochemistry and Behavior, Vol. 1, pp. 295–300. ANKHO International Inc., 1973. Printed in the U.S.A.

Memory Access Pathway: Role of Adrenergic Versus Cholinergic Neurons[1]

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(Received 23 February 1973)

HAMBURG, M. D. AND R. P. COHEN. *Memory access pathway: role of adrenergic versus cholinergic neurons.* PHARMAC. BIOCHEM. BEHAV. 1(3) 295–300, 1973.–A temporary depletion of brain norepinephrine in rats produced by injection of a dopamine beta-hydroxylase inhibitor, diethyldithiocarbamate (DDC), 30 min prior to testing, prevented performance of a trained passive avoidance response 1, 3, 5 or 7 days after training. Subsequent recovery in performance indicated that the memory itself was not destroyed, but rather that the process of memory retrieval was affected. Anticholinesterase treatment produced a similar retrieval amnesia, but the effect was dependent upon the age of the memory at the time of drug injection [11]. In both cases, when the animals were presented a recall trial prior to injection, the normally observed amnesia was blocked. Animals treated with DDC up to 3 hr before training were capable of learning the passive avoidance task and of avoidance performance for a few minutes after training. However, these animals failed to produce a long-term memory of the trained response. Anticholinesterase treatment had no effect on memory formation. These results suggest different roles for adrenergic and cholinergic neurons in a pathway associated with memory storage and retrieval.

Memory Learning Retrograde amnesia Antiadrenergic Norepinephrine Dopamine beta-hydroxylase

ANTICHOLINESTERASE and anticholinergic induced retrograde amnesias have been used in a number of experiments to investigate the physiological basis of memory (see review, [4]). Either intracerebral injection of diisopropyl florophosphate (DFP) [5, 6, 17] or systemic injection of physostigmine [1, 8, 10, 11] produced a retrograde amnesia of a trained response when drug treatment occurred 5–7 days after the learning experience. No such performance decrement occurred when injection followed 1–3 days after training. The inverse temporal pattern has been observed when the anticholinergic scopolamine was administered rather than the anticholinesterase [7,18]. In all cases, subsequent recovery in performance was observed indicating that neither the memory itself nor the process of memory storage was altered, but rather that the process of memory retrieval was temporarily affected. Finally it has been reported that a recall trial conducted 30 min prior to physostigmine treatment prevented the normally observed amnesia of a 7-day old memory [11].

Our goal is to clarify the pharmacological requirements for adequate memory storage and retrieval, and for the maintenance and strengthening of stored memories. In this paper we describe the influence of a subcutaneous injection of diethyldithiocarbamate (DDC), a dopamine beta-hydroxylase inhibitor, on the learning and subsequent performance of a trained passive avoidance response. We have employed similar behavioral procedures in this study as were used in our previous investigation [11] on the effect of anticholinesterase treatment on the same passive avoidance task. This permits comparison of our current data with that of the previous study.

Dopamine beta-hydroxylase is a copper enzyme that undergoes cyclic reduction and oxidation during the conversion of dopamine to norepinephrine and is consequently inhibited by the copper chelation properties of DDC. In addition, injection of DDC has been shown to deplete brain norepinephrine in rats for a period of hours without a simultaneous decrease in the level of dopamine [9]. DDC treatment in mice has been shown to produce an early enhancement and a later impairment in performance of a step-through passive avoidance task [15].

METHOD

Animals

One hundred eighty-four male albino rats (Sprague-Dawley, Hotzman strain) (250–350 g) were used. All animals were 2–3 months old upon arrival in the laboratory and were trained 7–21 days later. Rats were housed in community cages and had access to ample food and water at all times except while they were in the experimental room.

[1] This research was supported in part by a grant from the Pharmaceutical Manufacturers Association Foundation.

Training and Testing Procedure

The task chosen was a step-down passive avoidance task [2, 3, 12, 13] in which the animals were taught to remain on a small raised platform in order to avoid foot shock. Length of time on the platform (step-down latency) served as a measure of task retention. The apparatus and procedure have been described elsewhere in detail [11]. Briefly, training consisted of 5 trials. On each trial the rat was placed on the platform and the step-down latency recorded. Animals were placed in a holding cage adjoining the experimental box for a 1 min rest period between each trial. On Trials 1–3 no foot shock was applied. On Trial 4, foot shock (approximately 0.4 mA) was administered for 15 sec. Trial 5 was conducted in a similar manner to Trial 4. On this trial, if the rat did not step down within 30 sec it was removed to the home cage and a step-down latency of 30 was recorded. On Trials 1–4 all animals stepped down within 30 sec. (Mdn step-down latency for Trial 4 was 2.31 sec.)

On the testing day, each animal was returned to the experimental room and placed on the platform for 1 trial. Upon step down or after 30 sec on the platform the experiment was terminated.

Drug Injection

Each animal received either a subcutaneous injection of DDC (250 mg/kg; 2 ml/kg injected volume) or physiological saline (2 ml/kg). This dose of DDC in mice has been shown to produce a greater than 65 percent decrease in endogenous brain norepinephrine within 30 min after injection [15]. Drug injection occurred at different times relative to training and testing as indicated in the group procedures below.

EXPERIMENT 1

The purpose of our first experiment was to determine if DDC induced norepinephrine depletion would produce a retrograde amnesia of the passive avoidance response similar in temporal aspects to the anticholinesterase induced amnesia that we have observed with the same task [11]. Thirty-nine animals were trained and assigned at random to 1 of 5 groups. Animals in Group 1 were tested 6 hr after training. For Groups 2–5 testing occurred either 1, 3, 5 or 7 days after training respectively. All animals received an injection of DDC 30 min before testing. Therefore, the difference between groups was the age of the memory (training-injection interval) at the time of drug treatment. The injection-testing interval was held constant for all groups.

Results

The mean and median step-down latency for Trials 4 and 5 of training and the testing trial was calculated for all groups. The median step-down latency for Trial 4 of training for all animals in all experiments was 2.31 sec (Mean = 2.98). The median step-down latency for Trial 5 was 30 sec (Mean = 29.03). The median testing score for each group is reported in the individual experiments. When the mean step-down latency differed from the median by more than 5 sec, the mean appears as well. The scores of each group were compared with all other groups in the experiment by Mann-Whitney U test to determine if the differences were significant. Two-tailed p values were calculated by Siegel's method for direct critical values for Mann-Whitney U test [16]. In the case of multiple comparison, the p value reported is the least significant value of the tests conducted.

Little forgetting of the passive avoidance response appeared when DDC injection and testing occurred 6 hr after training (Mdn = 30). In contrast, a significant ($p<0.05$) performance decrement resulted with treatment and testing 1, 3, 5 or 7 days after training (Mdn = 5.33, 6.61, 10.66 and 8.82; Mean score for the 1-Day Group was 11.70). The interval between injection and testing was the same for all groups, only the age of the memory at the time of injection was different. It was therefore unlikely that the performance decrement seen in Groups 2–5 was due to a toxic influence of the drug. Randt *et al.*, working with mice, have reported a similar amnesia of a step-through passive avoidance task when DDC injection and testing occurred 1 day after training [15].

Figure 1 compares the influence of DDC, physostigmine, DFP and scopolamine on the performance of a trained task as a function of the training-injection interval. Injection of either anticholinesterase (physostigmine or DFP) produced the same memory age dependent amnesia of the escape, avoidance of appetitive task. The anticholinergic scopolamine produced the inverse temporal pattern. Following normal training, norepinephrine depletion produced a retrograde amnesia of long-term memory that was not dependent upon the age of the memory at the time of drug injection. Short-term memory (6 hr) was not affected by DDC treatment.

EXPERIMENT 2

Subsequent recovery of the trained response following either physostigmine or DFP treatment suggested that the effect of the drug injection was to temporarily block the retrieval process rather than to actually destroy the stored memory [5, 8, 10, 11]. In order to determine if the observed amnesia that followed DDC treatment was also one of retrieval blockage, the following experiment was conducted.

Sixteen animals were trained and randomly assigned to 1 of 2 groups. Animals in Group 1 received an injection of DDC 1 day after training and were tested 30 min later. The animals in Group 2 also received DDC treatment 1 day after training, but following injection they were returned to their home cages and testing did not occur until 3 days later.

Results

The animals of Group 1, tested during norepinephrine depletion showed the expected amnesia (Mdn = 8.44), but no such performance decrement was observed when testing was delayed until 3 days after injection (Mdn = 30; $p<0.02$). These results suggest that depletion of brain norepinephrine prevents retrieval of long-term memory but does not destroy the memory itself.

EXPERIMENT 3

Previous studies have reported that the injection of an anticholinesterase drug prior to training did not prevent memory formation nor subsequent performance of the trained response [5,11]. Experiment 3 was conducted to determine the influence of norepinephrine depletion at the

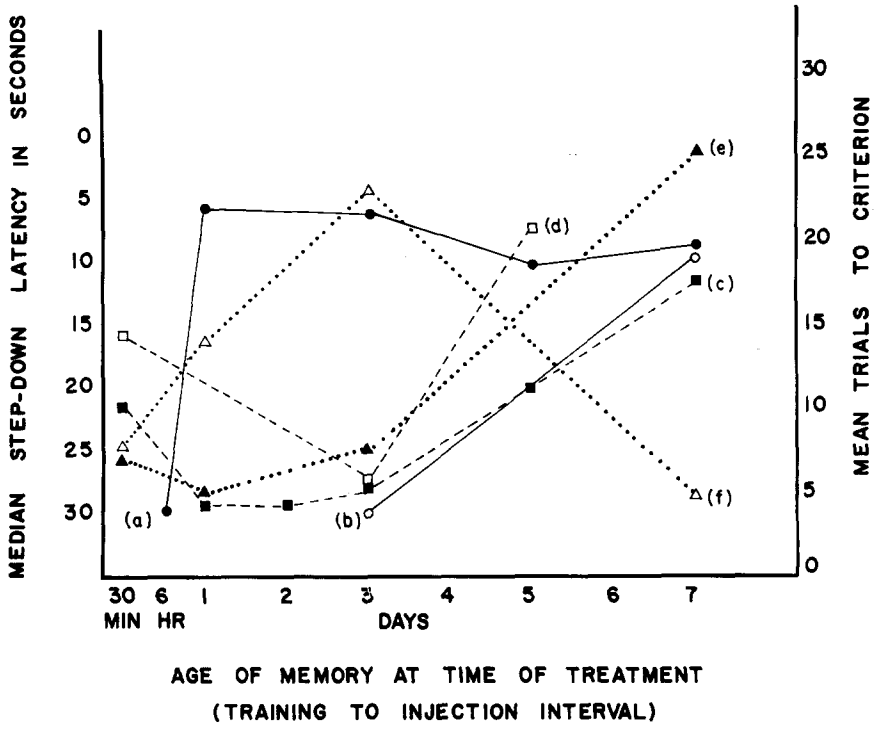

FIG. 1. The effect of a dopamine beta-hydroxylase inhibitor, an anticholinesterase and an anticholinergic on the performance of aversive and appetitive tasks as a function of the age of the memory at the time of drug treatment. (a) Subcutaneous injection of DDC 30 min prior to testing; passive avoidance task. (b) Subcutaneous injection of physostigmine 30 min prior to testing; passive avoidance task (from Hamburg and Fulton, [11]). (c) Intraperitoneal injection of physostigmine 30 min prior to testing; Y-maze escape task (from Hamburg, [10]). (d) Intracerbral injection of DFP 24 hr prior to testing; Y-maze escape task (from Deutsch, Hamburg and Dahl, [5]). (e) Intracerbral injection of DFP 24 hr prior to testing; Y-maze appetitive task (from Wiener and Deutsch, [18]). (f) Intracerebral injection of scopolamine 24 hr prior to testing; Y-maze appetitive task (from Wiener and Deutsch, [18]).

time of training on subsequent performance of the learned passive avoidance task.

Forty-six animals were injected with DDC 30 min prior to passive avoidance training and randomly assigned to 1 of 5 groups. An additional 8 animals received saline injections in place of the drug. In the 5 DDC treated groups testing occurred either 15 min, 6 hr, 1, 3 or 7 days after training. The saline control group was tested 7 days after training.

Results

Depletion of norepinephrine 30 min prior to training had no effect on the ability of rats to learn the passive avoidance task. The median score for Trials 4 and 5 of training for all animals injected with DDC 30 min prior to training was 2.58 and 30.00 sec respectively. Similar scores for noninjected and saline injected animals were 2.14 and 30.00. The difference between groups was not significant. DDC injection 30 min prior to training also did not influence subsequent performance of the trained response if testing occurred 15 min after training (see Fig. 2). However, if testing was conducted 6 hr or longer after training, a significant performance decrement was observed ($p<0.05$). Saline injected control animals performed the task 7 days after training with no difficulty (Mdn = 30) confirming previous data that indicated little natural forgetting of the passive avoidance response over this time period.

These data suggest that animals trained during a state of depleted norepinephrine learned the passive avoidance task and that short-term memory could support adequate performance for at least 30 min following training. However, these animals failed to produce a long-term memory of the trained response. Results from other studies [15] further support this interpretation. Testing scores of mice injected with DDC 30 min prior to training were normal if testing occurred within 1 hr of initial training, however, when testing was postponed until 24 hr after training, the animals showed no retention of the learned task.

EXPERIMENT 4

Two questions were asked in Experiment 4: (1) for what length of time would a DDC induced norepinephrine depletion prevent the formation of a long-term memory; and (2) could the influence of DDC treatment on memory

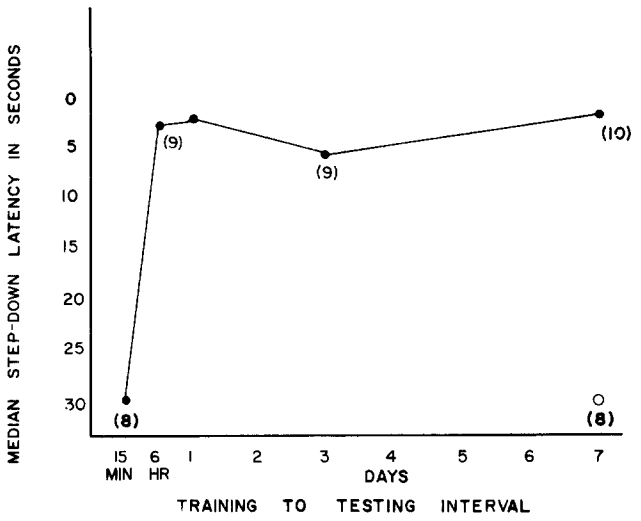

FIG. 2. The effect of depleted norepinephrine at the time of passive avoidance training on subsequent performance of the learned response. All animals received an injection of DDC 30 min prior to training. (o) Saline injected control group. Numbers in parenthesis are the N for each group.

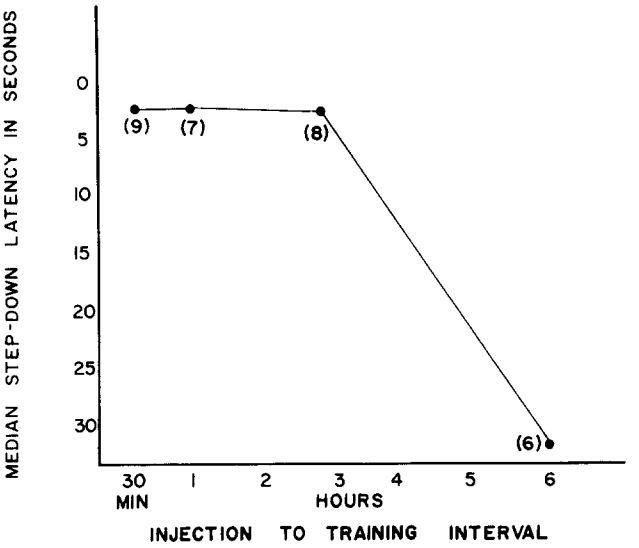

FIG. 3. The effect of DDC treatment 30 min, 1, 3 or 6 hr prior to passive avoidance training on subsequent performance of the learned response. All animals were tested 1 day after training. Numbers in parenthesis are the N for each group.

formation and retrieval be simply a state-dependency phenomenon [14]? Thirty-eight animals were injected with DDC and randomly assigned to 1 of 5 groups. Animals in Groups 1–4 were trained either 30 min, 1, 3 or 6 hr after injection and tested on the following day. Animals in Group 5 were trained 30 min after injection and then returned to their home cage. Seven days after training they received a second injection of DDC and were tested 30 min later.

Results

DDC treatment as long as 3 hr prior to training impaired memory formation and subsequent performance of the trained response (Mdn = 2.83) (see Fig. 3). However, when DDC injection occurred 6 hr prior to training, avoidance performance the following day was unaffected (Mdn = 30, Mean = 23.51). These data are in agreement with Randt's measurements on the conversion of C14 dopa to C14 norepinephrine following DDC treatment. The most effective inhibition of norepinephrine production (dopamine beta-hydroxylase activity) occurred at 90 min and 4.5 hr after administration of DDC with measurable recovery by 8.5 hr [15].

Overton [14] has reported that rats trained in a T-maze escape task following a subanesthetic dose of pentobarbital performed the task poorly when subsequently tested after the drug had worn off. The converse was also true; animals trained normally performed poorly when tested under the influence of the drug. However, animals trained under pentobarbital and tested under the drug as well performed significantly better than either of the first two groups. Good testing performance was dependent upon reinstatement of the drug condition that was present during training.

The results of Group 5 eliminated the possibility of a state-dependency explanation of our findings. These animals, both trained and tested following DDC treatment, performed no better than animals trained under DDC and tested normally, or animals trained normally and tested following drug treatment. (Group 5; Mdn = 7.25. Experiment 1, Group 5; Mdn = 8.82. Experiment 3, Group 5; Mdn = 3.43. No significant differences between groups.)

EXPERIMENT 5

A final experiment was conducted to determine the influence of a recall experience on the susceptibility of a memory to a DDC induced amnesia. Thirty-seven animals were trained and randomly assigned to 1 of 4 groups. The following day all animals were returned to the experimental room and given 1 recall trial similar to a test trial. Each rat was placed on the platform and removed after 30 sec. If the animal stepped down before 30 sec it was allowed to explore the box for the remainder of the 30 sec period without footshock. In Group 1, DDC injection occurred 30 min after the recall trial. Animals in Groups 2–4 were injected either 6 hr, 1 or 7 days after the recall trial. Testing occurred 30 min after drug injection for all groups.

Results

In Group 1, no amnesia was present at the time of testing (Mdn = 30). As with physostigmine [11], a recall trial conducted 30 min prior to DDC injection prevented the amnesia usually produced by the drug when administered 1 day after training (compared with Exp 1, Group 2; $p<0.028$).

It was possible that the recall trial served to restore memory to a short-term phase which would account for the strong passive avoidance performance observed when DDC treatment and testing occurred within 1 hour of the recall trial. Groups 2–4 served to test this hypothesis. In all cases, a strong avoidance habit was observed at the time of testing

(Mdn = 30 for all groups; Mean = 26.8, 23.73 and 23.80). A recall trial inserted at all points tested between training and injection prevented the amnesia of a long-term memory. It should be noted that animals in the first experiment which were trained 1 or 7 days prior to DDC treatment and testing showed a marked amnesia (Mdn = 5.33 and 8.82). The effect of a recall experience on the physiological basis of memory is not yet understood, but it appears to produce a dramatic resistance to DDC or physostigmine induced retrieval amnesia that is independent of the age of the memory at the time of recall or of the recall to injection interval.

DISCUSSION

In summary, our results indicate the following similarities and differences between an antiadrenergic and anticholinesterase induced amnesia.

(1) Animals preinjected with either physostigmine or DDC were capable of learning a step-down passive avoidance task, however the formation of a long-term memory was prevented during periods of norepinephrine depletion. If animals were tested within the first 3 hr after training, short-term memory stores were sufficient to produce reliable performance of the trained response, but amnesia was evident at longer testing intervals. In contrast, long-term memory storage was not affected by the injection of anticholinesterase drugs. When physostigmine preceded training, good retention was observed at all training to testing intervals.

(2) An injection of DDC 30 min prior to testing produced a retrograde amnesia, however, unlike physostigmine, the degree of amnesia produced by DDC was not dependent upon the age of the memory at the time of injection.

(3) Like physostigmine, the amnesia produced by DDC treatment prior to testing was of the retrieval process; the memory itself was unaffected as evidenced by subsequent recovery in performance.

(4) When the animal was provided a recall experience prior to either DDC or physostigmine treatment the normally observed amnesia was prevented.

The results of these experiments suggest that adrenergic fibers contribute significantly to an access pathway of long-term memory. If learning occurred during a period of depleted norepinephrine, training scores were normal and adequate performance was possible for a short period following training when, perhaps, a short-term memory storage was active. However, the formation of long-term memory was prevented and short-term stores were inadequate to support performance a few hours after training. If training occurred in a normal animal and a long-term memory was produced, then norepinephrine depletion prevented retrieval of that memory for a period of hours after injection. However, the memory remained intact and complete recovery of performance appeared.

Finally, there is suggestive evidence in the data that norepinephrine depletion also influenced the rate of memory transfer from short to long-term storage. Animals which received an injection of DDC 6 hr after training were still able to perform the task 30 min later (Mdn = 30). At this time, retrieval from long-term memory storage should be blocked, and short-term memory stores were most likely accountable for the performance of the trained response. When DDC injection occurred 6 hr before training with testing the following day, again performance was strong (Mdn = 30; Mean = 23.51). This suggested that at the time of training (or in the period following while short-term memory stores were intact), norepinephrine levels were sufficiently restored to allow formation of long-term memory. However, when DDC treatment preceded training by 30 min and testing occurred 6 hr later, a significant amnesia appeared (Mdn = 3.5; $p<0.05$). Long-term storage was prevented and under this condition short-term stores were insufficient to support adequate performance 6 hr after training. Therefore, at 6 hr post-training, a normal animal had produced a long-term memory of the trained response, but also retained sufficient short-term storage to perform despite norepinephrine depletion prior to testing. However, if norepinephrine levels were lowered earlier in this post-training period, a long-term memory was not produced, and short-term stores were dissipated faster thus preventing adequate performance 6 hours after training.

It appears likely from the converging evidence of several studies that specific neurotransmitter levels, consistant for various behaviors but different for different transmitters, are essential for proper long-term memory storage and retrieval. Of particular interest is the fact that alteration of neither the cholinergic nor adrenergic system affects memory itself, but markedly influences what appears to be the input and output pathways from long-term memory and that the role of these two neuronal systems in memory access appears to be different.

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