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Some Characteristics of Amnesia Induced by FLA-63 an Inhibitor of Dopamine Beta Hydroxylase[1]

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BOTWINICK, C. Y., D. QUARTERMAIN, L. S. FREEDMAN AND M. F. HALLOCK. *Some characteristics of amnesia induced by FLA-63, an inhibitor of dopamine beta hydroxylase.* PHARMAC. BIOCHEM. BEHAV. 6(5) 487–491, 1977. – The amnesic effects of FLA-63, a potent dopamine-beta-hydroxylase (DBH) inhibitor, were investigated in a food motivated spatial discrimination task. Groups of C57BL/6J mice were injected with either 5 mg/kg, 15 mg/kg, 25 mg/kg, 35 mg/kg or physiological saline 4 hr prior to training. Amnesia was observed 24 hr following training at all dose levels except 5 mg/kg. The performance deficit was specific to memory of the discrimination and not the result of state-dependency. Training conditions which produce an increase in habit strength prevented the amnestic effects of FLA-63. Spontaneous recovery of memory occurred 48 hr following drug administration. Recovery from amnesia was also induced by injections of a monoamine oxidase inhibitor, pargyline, administered 2 hr prior to the retention test. These data suggest that amnesia induced by norepinephrine (NE) depletion is the result of impairment of mechanisms necessary for memory retrieval.

Amnesia Dopamine-beta-hydroxylase inhibition FLA-63 Discrimination reversal learning

IT IS KNOWN that compounds which deplete catecholamines (CA) result in amnesia. For example, Seiden and Peterson [17] report a temporary failure to perform a conditioned avoidance response following injections of reserpine or alpha methyl-para-tyrosine (AMPT). Similar transient amnesias have been reported with the use of AMPT in a food-motivated discrimination reversal task [13]. Administration of a dopamine-beta-hydroxylase (DBH) inhibitor, diethyldithiocarbamate (DEDTC), which blocks the final step in the biosynthesis of norepinephrine (NE) and depletes it [8,11] produces retrograde amnesia for a food-motivated discrimination reversal [13], a one-trial passive avoidance response [3, 10, 15] and for a conditioned avoidance response [6]. We have recently demonstrated that amnesia induced by DEDTC recovers spontaneously 48 hr following training. In addition, we have shown that recovery of memory can be induced by treating amnestic animals with monoamine oxidase inhibitors prior to the retention test [3,13]. These findings suggest a role for NE in retrieval processes.

The objective of the present study was to determine whether amnesia can be induced by a different inhibitor of DBH bis-(4-methyl-1-homopiperazinyl-thiocarbonyl)-disulphide (FLA-63) [2,4]. This would indicate that the amnesic effects are not unique to a particular DBH inhibitor. A second objective was to determine the durability of the amnesia induced by FLA-63, the specificity of the effect, and whether recovery could be induced by pretest injections of the MAOI pargyline.

EXPERIMENT 1

The aim of this experiment was to study the effects of different doses of FLA-63 on retention of a food motivated spatial discrimination habit tested 24 and 48 hr following learning, and to determine whole brain NE levels at time of training and testing.

Method

Animals. Used throughout these experiments were male mice of the C57BL/6J strain, weighing approximately 25 g, obtained from the Jackson Laboratory. Each mouse was individually caged and had free access to food except during deprivation periods. Water was always available ad lib.

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Apparatus. The apparatus used throughout these experiments was a T-maze 3 in. wide and 3½ in. high. The center alley was 11¼ in. long, and each arm was 7 in. long. The initial 3½ in. of the center alley served as a start box separated from the rest of the maze by a guillotine door. Guillotine doors at the start of each arm prevented retracing. The entire maze was painted flat black and was covered with clear Plexiglas lids.

Design and procedure. Following 24 hr of food deprivation, each mouse was given a 10 min free exploration adaptation period in the maze with six 20 mg Noyes food pellets available in food cups in each arm, followed by 1.5 g of food in the home cage. On all subsequent days mice were also given 1.5 g of food when returned to home cages. On Day 1, (24 hr after adaptation) mice were given twenty reinforced trials to either the right or left arm. Reinforcement consisted of one 20 mg pellet per trial. The intertrial interval was 30 sec and a noncorrection procedure was employed. On Day 2, mice were injected with one of four doses (5, 15, 25 or 35 mg/kg) of FLA-63 (SC) or saline 4 hr before training and reversed to the side opposite that which had been correct on Day 1. Reversal training was terminated when mice had achieved a criterion of 17 correct choices. We have previously shown that this set of training conditions produces a robust and reproducible amnesia in this task following both cycloheximide and DEDTC [13,14]. Retention was tested for independent groups 24 and 48 hr. following reversal by retraining animals to the side correct on Day 2.

Biochemical methodology. Animals were sacrificed by cervical dislocation and brain regions dissected on ice. Cortex, hypothalamus, and lower brain stem (pons, midbrain and medulla) samples were homogenized in 100 volumes of 0.1 N perchloric acid (PCA) and centrifuged at 10,000 xg for 10 min. Norepinephrine in the PCA supernatant was analyzed by a modification of the radioactive enzymatic method of Coyle and Henry [5].

Results

Mean percent savings for all treatment groups are shown in Fig. 1. Percentage savings was calculated by the formula $(R-T)/(R-C) \times 100$, where R is the number of trials to reach criterion on the reversal day, T number of trials to criterion on test day, and C is criterion. Analysis of variance of these data indicate a significant drug treatment effect, $F(5,71) = 13.25$, $p = <0.025$, and a significant effect for time of testing. $F(1,71) = 27.63$, $p = <0.001$. The interaction between drug treatment and time of testing was also significant, $F(4,71) = 5.30$, $p = <0.01$. These results indicate that, with exception of 5 mg/kg, all doses of FLA-63 produced significant amnesia when retention was tested 24 hr following learning, but failed to disrupt memory when retention was tested 48 hr following learning. This indicates that memory spontaneously recovers 2 days following drug treatment. Degree of regional depletion of brain norepinephrine with 5 mg/kg and 25 mg/kg doses is shown in Table 1. A dose of 25 mg/kg was used in all subsequent experiments.

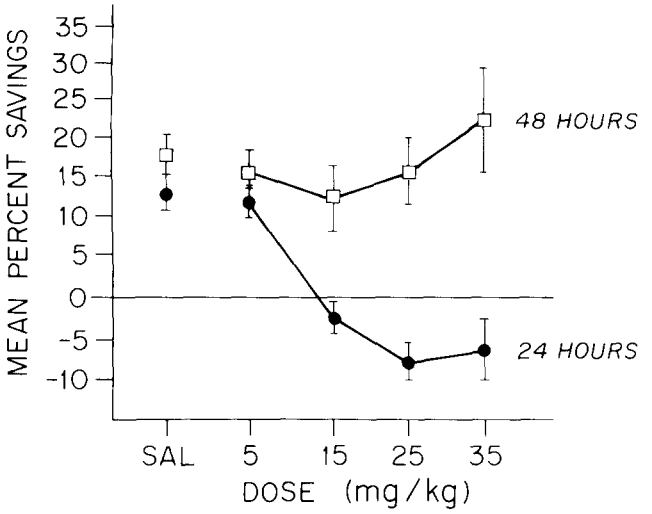

FIG. 1. Mean percent savings and ± SEM for all treatment groups tested for retention 24 hr. (filled circles) and 48 hr. (open squares) following reversal. N = 7–10 in all groups.

TABLE 1

EFFECT OF FLA-63 ON MOUSE BRAIN NOREPINEPHRINE LEVELS

	Norepinephrine % Control		
	4 hrs		24 hr
Brain Region	5 mg/kg	25 mg/kg	25 mg/kg
Cortex	103.9* ±4.60	72.4† ±2.38	99.3* ±7.82
Brain Stem	86.1* ±3.20	69.6† ±3.89	84.5* ±9.90
Hypothalamus	89.2* ±3.70	67.4† ±2.28	89.6* ±3.68

*Not significant.

†$p<0.001$ as compared to appropriate control; number of mice per group is eight.

Values expressed as mean percentage of control ± SEM; control levels (μg/g wet tissue) were: cortex 0.423, brain stem 0.816, hypothalamus 1.820.

FLA-63 was injected subcutaneously to C57BL/6J mice. Animals sacrificed at 4 or 24 hr after drug administered. Control mice injected with drug vehicle.

EXPERIMENT 2

Recent evidence from our laboratory indicates that cycloheximide (CXM) and DEDTC-induced amnesia can be prevented by training conditions which increase strength of habit [13]. The purpose of this experiment was to determine whether amnesia induced by FLA-63 is similarly sensitive to increases in training strengthen produced in the following two ways: (1) by increasing the intertrial interval (ITI) to 80 sec [13] and (2) increasing the reversal criterion from 17 to 35 correct trials.

Method

Procedure. Adaptation and Day 1 training was the same as described in Experiment 1. On Day 2, the first group of mice were reversed to a criterion of 17 correct choices with

an 80 sec ITI, 4 hr following an injection of FLA-63 (25 mg/kg, N = 10) or physiological saline (N = 10). Twenty-four hr later, all mice were tested for retention with the 80 sec ITI. For group 2 (N = 20), all mice were reversed to a criterion of 35 correct choices 4 hr following injection (FLA-63, N = 10; SAL, N = 10) on Day 2, and on Day 3 tested for retention to a criterion of 35 correct choices. The ITI for this group was 30 sec.

Results

The 25 mg/kg FLA-63 group and the saline control group from Experiment 1 were used for statistical comparison. Percentage savings scores for the two groups are shown in Table 2 along with the scores of the regularly treated groups from Experiment 1.

TABLE 2

EFFECT OF INTERTRIAL INTERVAL AND TRIALS TO CRITERION ON RETENTION IN FLA-63 AND SALINE-TREATED MICE

	Mean % Savings		
Intertrial Interval	30*	80	30
Trials to Criterion	17	17	35
FLA-63	−9.2	21.9	22.8
Saline	13.6	22.5	23.0

*Data taken from Experiment 1.

Analyses of variance carried out on the ITI data shows a significant effect for ITI, $F(1,39) = 32.55$, $p = <0.001$, and for drug treatment, $F(1,39) = 31.49$, $p = <0.001$, indicating that an 80 sec ITI produces significantly better retention than a 30 sec ITI and that SAL injected mice have overall better retention than mice treated with FLA-63. The significant drug × ITI interaction, $F(1,39) = 26.68$, $p = <0.001$, indicates that increasing the ITI to 80 sec results in a significantly greater improvement in retention in FLA-63 treated mice than it does in saline controls. Mice treated with FLA-63 are amnesic when trained and tested with a 30 sec ITI but exhibit normal retention when an 80 sec ITI is employed on training and testing.

Analysis of variance of the trials to criterion data yielded significant effects for number of trials on the reversal day, $F(1,39) = 17.48$, $p = <0.001$, and for drug treatment, $F(1,39) = 18.88$, $p = <0.001$. The interaction between these two variables was also significant, $F(1,39) = 16.93$, $p = <0.001$. These data indicate that training to a criterion of 35 correct trials prevents the expression of the amnesia which is typically present when animals are trained to a criterion of 17 correct trials. The results of this experiment show that training conditions which produce increases in habit strength can block the amnestic effects of FLA-63.

EXPERIMENT 3

The purpose of this experiment was to determine whether amnesia induced by FLA-63 is specific to the memory of the reversal, or a reflection of a nonspecific learning impairment. If the deficit is specific, FLA-63 treated mice should show significantly better learning than saline controls if the test for retention is performance on a second reversal. The following experiment tests this hypothesis.

Method

Design and procedure. The procedures for Days 1 and 2 were the same as described in Experiment 1. On Day 2, mice were injected with either FLA-63 (25 mg/kg, sec, N = 12) or physiological saline (N = 12) 4 hr prior to reversal training. On Day 3, instead of being tested to the side reinforced on Day 2, all mice were reversed to the opposite side and retrained until they had reached the criterion of 17 correct choices.

Results

The results of Experiment 3 are shown in Table 3. A within-subjects analysis of variance indicates that the groups by reversals interaction is highly significant, $F(1,47) = 35.53$, $p = <0.001$ FLA-63 treated mice take significantly fewer trials to reach criterion on the second reversal while mice treated with saline take significantly more trials. These data clearly confirm the amnesic effects of FLA-63 and rule out the possibility that the impaired performance in Experiment 1 could be attributed to toxic or other nonspecific side effects of FLA-63.

TABLE 3

MEAN TRIALS TO CRITERION

Group	Training	Reversal Test
FLA-63	26.4	24.0
Saline	26.1	28.1

EXPERIMENT 4

Recent evidence indicates that injections of FLA-63 result in state-dependent learning for a conditioned avoidance response in mice [1]. The aim of this experiment was to investigate the possibility that the amnesia observed in Experiment 1 could be a state-dependent phenomenon. This was accomplished by training animals under either FLA-63 or saline and testing half of each group under saline and the other half under FLA-63. This resulted in a 2 × 2 factorial design which is shown in Table 4.

Method

Procedure. The procedures for Days 1 and 2 were the same as described in Experiment 1. On Day 3, all mice were tested for retention to a criterion of 17 correct choices 4 hr following the second injection.

Results

Results of this experiment are shown in Table 4. Analysis of variance of these data indicate a significant drug effect for treatment on the training day, $F(1,39) = 43.75$, $p = <0.001$ and no effect of drug treatment on day of testing. The interaction between these two variables was not significant. These results indicate that FLA-63 induced amnesia is not a state-dependent phenomenon.

EXPERIMENT 5

We have previously demonstrated that DEDTC-induced amnesia for a one-trial passive-avoidance response and for a

TABLE 4

THE DESIGN OF THE STATE-DEPENDENCY EXPERIMENT AND MEAN PERCENT SAVINGS FOR FLA-63 AND SALINE INJECTED MICE

Training	Testing	Mean % Savings
FLA-63	FLA-63	−2.6
FLA-63	SAL	−9.0
SAL	SAL	22.2
SAL	FLA-63	26.4

N = 10 per group.

food-motivated spatial discrimination task can be reversed by pretest injection of the monoamine oxidase inhibitors pargyline and pheniprazine [3,13]. The aim of the present experiment was to attempt to reverse the FLA-63 induced amnesia by administering pargyline prior to the retention test.

Method

Design and procedure. The procedures for Days 1 and 2 were the same as described in Experiment 2. On Day 2, 10 mice were injected with FLA-63 (25 mg/kg, SC) and 20 mice with saline. Twenty-four hr later, the mice injected with FLA-63 on Day 2, and half of those injected with saline, were injected with pargyline (75 mg/kg, IP) 2 hr prior to the 24 hr retention test. This dose level and injection time was selected so that greater than 95% inhibition of MAO was achieved at time of testing [9,16]. The remaining 10 saline mice were injected with saline 2 hr prior to testing.

Results

Results of this experiment are shown in Table 5. Analysis of the data indicates that the FLA-63 pargyline group showed significantly greater percentage savings than the FLA-63 group (−9.0% savings) from Experiment 2 (t = 5.22, df = 18, p = <0.001). A comparison of the difference between the savings scores of the FLA-63-pargyline group and the saline-saline group was not significant. There was also no significant difference between the saline-saline group and the saline-pargyline group.

TABLE 5

EFFECT OF PARGYLINE ON RETENTION IN FLA-63 AND SALINE TREATED MICE

Drug Before Training	Drug Before Testing	Mean Percent Savings
FLA-63	Pargyline	13.3
Saline	Saline	13.9
Saline	Pargyline	13.1

EXPERIMENT 6

In our previous studies with cycloheximide and DEDTC [13], we showed that the magnitude of amnesia for a spatial habit in a T-maze was greatest when mice were first trained to one side and the following day injected with the amnestic agent and reversed to the opposite side; retention of the reversal being tested 24 hr later. The aim of the present experiment was to determine whether amnesia can be induced by FLA-63 for a spatial discrimination when a more conventional training criterion is employed and when the reversal procedure is omitted.

Method

Thirty mice were adapted as previously described. On Day 1 mice were injected with either FLA-63 (25 mg/kg, N = 20) or saline (N = 10) 4 hr prior to training. A criterion of 7 out of 8 correct choices (noncorrection) was chosen on the basis of pilot studies which indicated that mice could achieve this criterion in a single training session (range 27–40 trials) and would show approximately 60% savings 24 hr later. Animals were tested for retention 24 hr following training. Mice treated with FLA-63 were divided into two groups: Group I (N = 10) were injected with the MAOI pheniprazine (10 mg/kg, IP) one hour prior to testing and the Group II (N = 10) was injected with saline. The former group was included to determine whether any amnesia induced under the new training conditions could be attenuated by monoamine oxidase inhibition.

Results

Mean percentage savings for the saline control mice was 66.54 and −11.45 for the FLA-63 group. This difference was statistically significant (t = 9.16, p = <0.001). The savings scores of the FLA-63 group treated with pheniprazine was 57.15, which was significantly different (t = 7.17, p = <0.001) from the FLA-63 saline group, and not significantly different from the saline treated control group (mean percent savings 66.54). These results show that the use of a reversal procedure is not a necessary condition for either the production of amnesia by FLA-63 or its pharmacologically induced recovery.

DISCUSSION

The results of the experiments reported in this study indicate that the behavioral deficit induced by the administration of FLA-63 is a temporary loss of the capacity to retrieve information from storage. This deficit is specific to retrieval processes and cannot be attributed to drug induced illness, motor incapacity, disorientation or state dependent learning.

Data presented in this study indicates that the retrieval deficit induced by the inhibition of norepinephrine biosynthesis can be blocked by increases in training strength. This result confirms earlier findings with DEDTC [12,13] and indicates that norepinephrine depletion like protein synthesis inhibition [13] will not induce an amnesia if the habit is well learned. Recently Flood *et al.* [7] have shown that increasing the duration of protein synthesis inhibition by repeated doses of anisomycin can induce amnesia for relatively strong habits. This suggests the possibility that increases in duration of DBH inhibition may also abolish the protective effects of increased training strength.

It is clear from the results of this study that consolidation is not blocked by the inhibition of norepinephrine biosynthesis. At all effective doses of FLA-63, the amnesia presents 24 hr following learning spontaneously recovers 24 hr later. These data are consistent with results of

previous studies which have shown that amnesia induced by inhibition of DBH with DEDTC show spontaneous recovery [12]. The results of Experiment 5 add further support for the hypothesis that norepinephrine inhibition is impairing retrieval mechanisms by demonstrating that memory recovery can be induced by administration of a monoamine-oxidase inhibitor before the retention test.

The mechanism by which this recovery occurs is not clear at this time. Previous studies have shown that MAOI induced recovery from cycloheximide amnesia is not the result of nonspecific enhancement of relearning or facilitation of a poorly learned habit [13,14]. These data tend to rule out general enhancement of learning as an explanation of the improved retention performance in the present experiments. It is possible that the increased intraneuronal concentrations of CA's which result from MAO inhibition induced a reactivation of specific noradrenergic mechanisms which have been disrupted by FLA-63 induced inhibition of DBH activity and the resultant depletion of NE stores.

A persistent question remains concerning the fact that although norepinephrine synthesis is inhibited at time of training resulting in a depletion of endogenous NE levels, retrieval deficits are demonstrable 24 hr later at time of testing when steady state levels of NE are back to normal. The most parsimonious hypothesis concerning the neurochemical basis of this retrieval deficit is that there remains some persistent defect in the dynamic aspects of the catecholamine system that is present at 24 but not 48 hr. It should be noted that brain NE concentrations probably represent the poorest index of functional status of central noradrenergic systems. Our current research efforts are directed at assessing turnover rates, release and receptor mechanisms at the time when amnesia is present and at the time when memory has spontaneously recovered.

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