*Pharmacology Biochemistry & Behavior*, Vol 29, pp 635–639 © Pergamon Press plc, 1988 Printed in the U S A 0091-3057/88 $3 00 + 00

# Memory Enhancement in Mice: Role of Drug Dose and Training-Testing Interval

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Received 19 July 1984

FLOOD, J F , G E SMITH AND A CHERKIN *Memory enhancement in mice Role of drug dose and training-testing interval* PHARMACOL BIOCHEM BEHAV 29(3) 635–639, 1988 —Pharmacologic probes are useful for studying memory mechanisms For eight drug treatments affecting a variety of transmitter systems [arecoline, piribedil, clonidine, fluoxetine, naloxone, ACTH (4–10)], we determined how long memory retention would remain improved with a dose sufficient to improve 3-hour retention While all 6 treatments enhanced 3-hour retention test performance at $p<0$ 05, only 5 treatments significantly enhanced retention 24 hour after training and none of the treatments significantly affected retention at 168 hours A detailed analysis of the dose and retention interval interaction for arecoline indicated that at low doses retention decreased as the retention interval increased while higher doses improved retention up to 3 hours and only the highest dose tested enhanced retention at 3 and 24 hours Drug doses that enhance short-term retention (3 hours) were not adequate to enhance long term retention (168 hours) The 6 drug treatments had no significant or systematic effect on activity or on acquisition We conclude that short-term retention performance was better because of enhanced memory processing or recall and not because of performance effects per se

Long-term retention Memory Mice Short-term retention

ARTICLES summarizing drug effects on memory, frequently report inconsistent results with some studies showing enhancement of retention, others no effect and still others impairment A particularly good example is the effect of cholinergic drugs on retention [6,12] A high degree of inconsistency leads to doubt that a particular drug or class of drugs actually alters memory processes Differences in experimental parameters, i e., species, type of task, route of injection, dosage or other experimental parameters may account for the observed differences in ability to affect memory retention When species, task, method of drug administration and retention test interval were held constant, we reported that 8 compounds that decrease acetylcholine receptor activity impaired retention while 7 drugs that enhanced the receptor activity facilitated retention [6] Two parameters that are usually consistent within a laboratory's research but differ across laboratories are the time of drug administration relative to training (pre or post) and the duration of the retention test period Pharmacological studies of memory enhancement are often evaluated at a single retention test interval. The purposes of this study were to determine if short-term retention was enhanced by the same drug treatments as long-term retention and whether short- and long-term retention showed differential sensitivity to enhancement of retention test performance.

The principle findings were that drugs affecting the cholinergic, dopaminergic, serotonergic, noradrenergic transmitter systems, as well as an opioid receptor blocker and a hormonal peptide fragment enhanced short-term retention as they were reported to enhance long-term retention [8] However five out of six compounds tested enhanced short-term retention at lower doses than required to enhance long-term retention. This enhancement of short-term retention was obtained without facilitation of acquisition

## METHOD

### *Subjects*

After 1 week in the laboratory, CD-1 male mice obtained at 6 weeks of age from Charles River Breeding Laboratories, Wilmington, MA, were individually caged 24–48 hr prior to training and remained singly housed until retention was tested one week later. The median body weight was 35 g, with a range of 33 to 38 g Animal rooms are maintained on a

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TABLE 1
EFFECT OF DOSE AND RETENTION INTERVAL ON TEST PERFORMANCE

| | Arecoline (μg/mouse, SC) | | | | | | |
|---|---|---|---|---|---|---|---|
| Test Interval | 0 | 1 75 | 3 50 | 7 0 | 10 5 | 14 0 | 17 5 |
| 0 25 hr | | | | | | | |
| % Recall | 85 | 85 | 90 | 90 | 95 | 95 | 85 |
| Mean* | 2 4 | 2 6 | 2 5 | 2 2 | 2 2 | 2 1 | 2 5 |
| SEM | 0 22 | 0 20 | 0 19 | 0 20 | 0 19 | 0 18 | 0 23 |
| 1 hr | | | | | | | |
| % Recall | 80 | 80 | 85 | 75 | 80 | 75 | 85 |
| Mean* | 2 7 | 2 6 | 2 5 | 2 9 | 2 7 | 2 7 | 2 4 |
| SEM | 0 29 | 0 25 | 0 25 | 0 21 | 0 20 | 0 24 | 0 27 |
| 2 hr | | | | | | | |
| % Recall | 35 | 40 | 50 | 55 | 65 | 70 | 65 |
| Mean* | 4 0 | 3 5 | 3 4 | 3 6 | 3 2 | 3 1 | 3 2 |
| SEM | 0 28 | 0 27 | 0 25 | 0 16 | 0 29 | 0 31 | 0 32 |
| *p* value | | | | <0 05 | <0 05 | <0 01 | <0 05 |
| 3 hr | | | | | | | |
| % Recall | 30 | 35 | 55 | 60 | 75 | 80 | 75 |
| Mean* | 3 9 | 3 8 | 3 5 | 3 0 | 2 8 | 2 6 | 2 7 |
| SEM | 0 26 | 0 22 | 0 25 | 0 33 | 0 31 | 0 22 | 0 26 |
| *p* value | | | | <0 05 | <0 01 | <0 01 | <0 01 |
| 24 hr | | | | | | | |
| % Recall | 25 | 35 | 45 | 55 | 55 | 65 | 75 |
| Mean* | 4 6 | 4 4 | 4 0 | 3 9 | 3 6 | 3 3 | 3 1 |
| SEM | 0 33 | 0 34 | 0 30 | 0 28 | 0 31 | 0 28 | 0 22 |
| *p* value | | | | | <0 05 | <0 05 | <0 01 |

*Mean number of trials to the first avoidance response, for all mice in a group (N=20/group)

The *p* values given indicate statistical difference from respective control (0 μg) by Dunnett's *t*-test

12 hr light-dark cycle with light on at 0600 The mice were trained between 0700 and 1500 Mice were assigned randomly to groups of 15 unless otherwise indicated

*T-Maze Apparatus and Training*

The T-maze and training procedure was previously described [5] The maze consisted of a black plastic start alley with a start box at one end and two goal boxes at the other, a stainless steel rod floor ran throughout the maze The start box was separated from the start alley by a plastic guillotine door which prevented the mouse from moving down the alley until the training started The intertrial interval was 30 sec with a muffled doorbell-type buzzer as the conditioned stimulus and a nominal footshock of 0 30 mA (Coulbourn Instruments scrambled grid floor shocker model E13-08) unless otherwise indicated

A training trial started when a mouse was placed into the start box The guillotine door was raised and the buzzer sounded simultaneously, then 5 sec later footshock was applied At the end of each trial, the mouse was removed from the goal box and returned to its home cage. A new trial began by placing the mouse in the start box, sounding the buzzer and raising the guillotine door, with footshock beginning 5 sec later if the mouse did not move into its correct goal box

As training proceeded, a mouse made one of two types of responses A response latency longer than 5 sec was classed as an escape from the footshock A response latency less than or equal to 5 sec was considered an avoidance, since the mouse avoided receiving a footshock Mice with escape latencies greater than 20 sec were discarded as these mice rarely show evidence of learning within the three training trials The total exclusions were fewer than 5% The measure of learning and memory is the avoidance of footshock since the discrimination is easily learned and remembered by all mice

*Measures of Retention for T-Maze Footshock Avoidance Training*

To measure retention, the T-maze training was resumed until the mice made their first avoidance response Based on previous studies using our training technique (including Experiment 4 below) the correlation between mean trials to first avoidance and mean trial to a 5 avoidances in six trials criterion is greater than +0.90 Thus training mice to criterion does not in this situation provide a better measure of retention The overall significance of the drug treatment effect was determined by a one-way or two-way analysis of variance [10,16] Dunnett's *t*-test was used to make multiple comparisons between each drug group and the control group [16]

A non-parametric measure of retention was derived to better visualize the effects of drug treatments on retention test performance and to correspond with usual reporting practice For this, the number of trials to the first avoidance response was dichotomized to yield a percent recall score Those mice making their first avoidance in three trials or less were classed as remembering the original training This criterion was adopted because it has provided optimal separation between the retention test performance of naive mice (with no T-maze training) and well-trained mice [5]

For convenience, retention intervals of 0.25, 1, 2, and 3 hr were considered to reflect short-term memory (STM) retention, and intervals of 24 and 168 hr (1 week) will be considered to reflect long-term memory (LTM) retention

*Drugs*

Mice received a 0 35 ml subcutaneous injection of saline or drug solution within 2 min after training The dose of drug, expressed as $\mu$g/mouse, is given for each experiment All solutions were blind-coded to eliminate experimenter bias The drugs were obtained from the following sources arecoline hydrobromide was purchased from Sigma Chemical Co., St Louis, MO, Naloxone was a gift of Dupont Chemical Co , Wilmington, DE, ACTH (4–10) a gift of Organon International, Oss, The Netherlands, fluoxetine a gift of Ely Lilly and Co , Indianapolis, IN, clonidine from Boerhinger Ingleheim Inc , New York, NY, and piribedil hydrochloride from Les Laboratories Server, France Doses are expressed as $\mu$g of the salt Drug solutions were prepared fresh daily

EXPERIMENT 1

*The Effect of Arecoline on Retention Test Performance as a Function of Drug Dose and Training-Testing Interval*

The purpose of this experiment was to determine (a) if arecoline, which is reported to improve long-term retention in humans [15], monkeys [2] and mice [7], also improves short-term retention, and (b) to what extent the improvement persisted for 24 hr after training and drug administration Pilot data indicated that the doses which improve long-term retention impaired the ability of mice to perform in a STM test Thus, Experiment 1 seeks to determine what dose, if any, improves STM retention test performance The experiment used two variables (a) seven doses of ARE (0 00, 1 75, 3 50, 7 00, 10 50, 14 00 or 17 5 $\mu$g/mouse) and (b) five retention intervals (0 25, 1, 2, 3, or 24 hr) Mice were assigned randomly to one of the drug dose groups and one of the retention test intervals, 35 groups of mice were used (7×5 factorial) The N/group was 20

*Results*

A two-way ANOVA run on mean trials to the first avoidance response indicated that the main effects of dose, $F(6,695)=6\ 66$, $p<0\ 001$, and retention interval, $F(4,695)=35\ 37$, $p<0\ 001$, were significant but the interaction was not significant ($F<1$) A partitioning of the sum of squares indicated that only retention test intervals at 2, 3 and 24 hr contributed significantly to the main effect of retention interval Using Dunnett's $t$-test, a subsequent comparison between the mean of each control and those of the arecoline-treated mice showed that at the 0.25 and 1-hr retention test there were no significant differences as retention was uniformly good (75 to 95% recall score) At the 2-hr retention test, groups treated with 10 5, 14.0 and 17 5 $\mu$g of arecoline showed significantly better recall compared to the control group (Table 1) At the 3-hr and 24-hr retention tests, the groups treated with 7 0 to 17 5 $\mu$g of arecoline showed significantly better recall The 7 $\mu$g dose became significant because of the continuing decline in control retention test scores at the 3- and 24-hr retention tests

EXPERIMENT 2

*A Comparison of Drug Enhanced Retention on Short- and Long-Term Retention*

Compounds other than those affecting the cholinergic system can improve long-term retention [1, 3, 9, 11–14, 17] The purpose of the following experiment was to determine if compounds other than those affecting the cholinergic system and known to enhance long-term retention improved short-term retention In addition, we determined the effect to which doses that enhanced short-term retention enhanced long-term retention A diverse group of drugs was selected that included a hormonal peptide fragment (ACTH(4–10)), a noradrenergic agonist (clonidine), serotonergic uptake blocker (fluoxetine), a mu receptor antagonist (naloxone), and a dopamine agonist (piribedil) In preliminary studies we determined the dose of each compound that yielded recall scores of at least 80% compared to a control recall score of 20–25% Separate groups of mice were then trained as in Experiment 1 and tested at 3, 24 or 168 hr (1 week) after training giving a design with 5 compounds (including saline) by 3 retention intervals for 15 groups To complete the design, data for arecoline-treated mice from Experiment (3 and 24 hr) were used and 168-hr data were obtained

The results for arecoline were used in the statistical analysis since control groups were comparable Three one-way ANOVAs indicated that retention was significantly enhanced at 3 hr, $F(6,98)=7\ 35$, $p<0\ 01$, and at 24 hr, $F(6,98)=4\ 38$, $p<0\ 01$, but not at 168 hr ($F<1$) (Table 2)

A subsequent analysis of mean differences between the control and each compound using Dunnett's $t$-test indicated that treated groups made their first avoidance in significantly fewer test trials at the 3-hr retention test ($p<0\ 01$ in each case) At the 24-hr retention test, the same comparison yielded significantly fewer trials to first avoidance at $p<0.01$ for piribedil and clonidine, $p<0\ 05$ for arecoline, ACTH (4–10) and fluoxetine, naloxone did not significantly affect retention Dunnett's $t$-test indicated that at the 168-hr (1 week) retention test none of the groups differed significantly from their saline-control group

EXPERIMENT 3

*Effect of Drugs Enhancing 3-Hour Retention on Activity*

The enhanced retention observed across all 6 drugs treatments in Experiment 2 3 hours after drug administration might have occurred if the drugs enhanced activity To test this, activity was measured in an open-field 25×37 cm with 4 infra-red beams used to detect movement The equipment was fully automated (Coulbourn Instruments Inc ) Three hours prior to being tested in the open-field, mice were administered saline or one of the six drug treatments in Experiment 2 The counting began 5 min after the mice were placed in the open-field and continued for 20 min To show that the apparatus was sensitive to a general increase in activity, a group of mice injected with scopolamine (1 mg/kg, SC) was included This dose of scopolamine is well recognized as a psychomotor stimulant [4, 9, 13]

TABLE 2
EFFECT OF DRUGS ON RETENTION MEASURED 3, 24 AND 168 HOURS AFTER TRAINING

| Drug | Dose (μg/mouse) | % | Time of Retention Test 3 hr | % | 24 hr | % | 168 hr |
|---|---|---|---|---|---|---|---|
| Control | 0 | 20 | (4 27 ± 0 27) | 20 | (4 60 ± 0 33) | 20 | (4 20 ± 0 32) |
| Arecoline | 14 0 | 80 | (2 66 ± 0 22)* | 65 | (3 30 ± 0 28)* | 38 | (3 60 ± 0 31) |
| ACTH(4–10) | 17 5 | 87 | (2 60 ± 0 20)* | 67 | (3 00 ± 0 38)* | 47 | (3 53 ± 0 23) |
| Clonidine | 21 0 | 80 | (2 80 ± 0 21)* | 80 | (2 73 ± 0 28)* | 25 | (4 00 ± 0 28) |
| Fluoxetine | 70 0 | 80 | (2 67 ± 0 28)* | 73 | (3 07 ± 0 36)* | 40 | (3 67 ± 0 32) |
| Naloxone | 35 0 | 80 | (2 87 ± 0 24)* | 40 | (3 77 ± 0 33) | 33 | (3 93 ± 0 33) |
| Pirebidal | 35 0 | 87 | (2 33 ± 0 24)* | 80 | (2 47 ± 0 27)* | 33 | (3 87 ± 0 36) |
| | | | $F=7\ 35, p<0\ 01$ | | $F=4\ 38, p<0\ 01$ | | $F<1, p=$ns |

Recall score (%) is tabled with the mean trials to first avoidance (±sem)
*Indicates significant difference from control mean at $p<0\ 05$ by Dunnett's $t$-test

The number of infra-red beam interruptions were analyzed by a one-way ANOVA Dunnett $t$-tests between the saline control and each drug treated group indicated that only scopolamine significantly increased activity compared to the saline control ($t=6\ 80, p<0\ 01$) The other treatments were within less than 1 standard deviation of the mean of the saline control

## EXPERIMENT 4

### *A Test of Drug-Induced Improvement of T-Maze Footshock Avoidance Acquisition*

One interpretation of the results of enhanced short-term retention performance with the compounds used above is that retention test *performance* per se was enhanced but not STM *retention* To test this, mice were injected with the same dose of arecoline (14 μg), ACTH (4–10) (17 5 μg), fluoxetine (70 μg), naloxone (35 μg), piribedil (35 μg), clonidine (21 μg) or saline 3 hr prior to T-maze training The training was done as in Experiment 1 except that mice were trained until they made 5 avoidances in 6 consecutive training trials If retention test performance per se were affected by the administration of any of these compounds, we would expect acquisition would be more rapid compared with the saline control The N/group was 15

### *Results*

Acquisition was not significantly facilitated by a 3-hr pretraining injection of any of the compounds (ANOVA, $F<1$) Drug-treated mice did not differ significantly from saline-injected controls in mean trials to first avoidance or mean trials to criterion (Table 3)

TABLE 3
ACQUISITION 3 HOURS AFTER DRUG ADMINISTRATION

| Drug | Dose (μg/mouse) | Mean Trials to First Avoidance | Criterion |
|---|---|---|---|
| Control | 0 | 6 20 ± 0 31 | 10 40 ± 0 32 |
| Arecoline | 14 0 | 5 93 ± 0 37 | 10 20 ± 0 37 |
| ACTH(4–10) | 17 5 | 5 87 ± 0 24 | 10 00 ± 0 29 |
| Clonidine | 21 0 | 5 80 ± 0 21 | 10 00 ± 0 23 |
| Fluoxetine | 70 0 | 6 00 ± 0 29 | 10 13 ± 0 29 |
| Naloxone | 35 0 | 6 30 ± 0 32 | 10 50 ± 0 28 |
| Pirebidal | 35 0 | 6 40 ± 0 35 | 10 60 ± 0 35 |

## DISCUSSION

### *Performance Effects of Improved Retention*

One problem with administering a retention test 3 hr or less after training is that it can be interpreted as improved performance due to increased activity or enhanced acquisition rather than improved memory processing or recall Experiment 3 failed to detect a significant increase in activity with any of the 6 treatments found to enhance 3-hr retention test performance The open-field was sensitive enough to detect such an effect since scopolamine was found to increase activity If the drug treatments enhanced sensorimotor functions, then the drug treatments might be expected to facilitate acquisition of the T-maze avoidance habit Experiment 4 failed to find that any of the 6 treatments significantly enhanced acquisition Thus the enhanced retention observed at 3 hours in Experiments 1 and 2 would indicate that the compounds facilitated either memory processing or recall and the the effect was not due to increased activity or enhanced acquisition

### *Differential Effects on Short-Term and Long-Term Retention*

Table 2 clearly indicates that doses of six different drug treatments, covering a range of mechanisms of action, facilitated 3 and 24 hour retention test performance (except naloxone at 24 hr) but failed to enhance retention at 168 hours Higher doses of all six drugs have previously been reported to facilitate 168 hr retention [8]. The high doses ranged from two to five times higher except for piribedil which facilitates 3-, 24- and 168-hr retention at the same dose. Attempts to test these higher doses on 3-hr retention resulted in impaired performance and was discontinued.

Retention tested 3 hours after training is commonly considered to represent short-term memory in animal experi-

ments and 24 hr or longer to represent long-term memory Admittedly, these cutoff points are arbitrary, based in part on the findings that few drugs can be administered at longer intervals than 3 hr after training and still affect long-term retention The rationale is that as long as an intervening treatment can have an effect on retention tested 24 hr or longer after training, then the memory "engram" is still in a labile or short-term retention phase of memory processing It is interesting that 5 of the 6 drug treatments show facilitation of retention at 24 hours (Table 2) but none of the drug treatments, at these low doses, facilitated retention significantly at 168 hours. The dose-response characteristics of 3 and 24 hour retention were clearly different from 168 hr retention.

In the introduction, we asked whether differential dose effects at different retention test intervals could account for apparent inconsistencies of the effects of drugs on retention Inappropriate dose selection relative to the retention interval being tested could result in a failure to observe enhanced retention or result in impaired retention test performance. Also, differences in reported doses that facilitate retention may be related to differences in retention test interval even 24 hour versus longer retention test intervals

ACKNOWLEDGEMENTS

The research was supported by the Medical Research Service of the Veterans Administration and by the Sepulveda Geriatric Research, Education and Clinical Center (GRECC)

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