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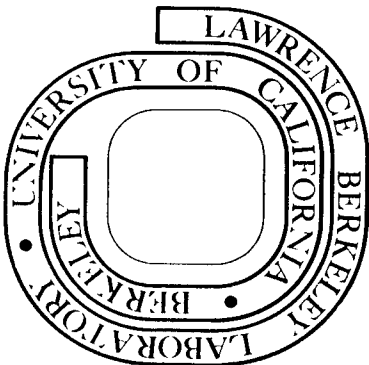
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RECOVERY AS A FUNCTION OF THE DEGREE OF AMNESIA DUE TO PROTEIN SYNTHESIS INHIBITION

Hasker P. Davis, Mark R. Rosenzweig,
Edward L. Bennett and Anne E. Orme

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Recovery as a Function of the Degree of

Amnesia Due to Protein Synthesis Inhibition[1]

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ABSTRACT

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Recovery as a function of the degree of amnesia due to protein synthesis inhibition. PHARMAC. BIOCHEM. BEHAV. Retrograde amnesia following inhibition of cerebral protein synthesis has generally been explained as either a failure of consolidation or impairment of a retrieval mechanism. Major evidence for the retrieval hypothesis is provided by studies which utilize a reminder (usually footshock) to attenuate the effect of the protein inhibitor. To examine this question, mice were injected subcutaneously with anisomycin (1 mg/animal, 7 mg/animal, or 1 mg/animal every 2 hr X 7) and given one training trial in a passive avoidance box. All subjects received a single retention test on each of four consecutive days, starting either 1, 7, or 21 days after training. One-half of the mice in each group received a foot-shock reminder 1 hr after their initial test. The footshock reminder did not attenuate the inhibitor-induced amnesia, but multiple testing did produce partial recovery in animals demonstrating some memory of training (both Saline and Anisomycin animals). The extent of amnesia and recovery were dependent upon both drug dosage and training-test interval. Implications for the consolidation and retrieval hypotheses are discussed.

Memory   Consolidation hypothesis   Retrieval hypothesis

Passive avoidance   Inhibition of cerebral protein synthesis

Anisomycin   Memory recovery   Amnesia

Running title: Amnesia, Recovery, and Protein Synthesis Inhibition

INTRODUCTION

Antibiotics, because of their inhibitory effects on protein synthesis, are frequently used in studies of memory [1,2,4,9,13,17,33,48,49,52]. Inhibition of cerebral protein synthesis that starts shortly before or shortly after training markedly impairs long-term retention in a variety of tasks and species [1,4,5,7,9,10,13,14,16,23,24,33,52]. These findings have been most frequently interpreted in terms of a consolidation deficit [1,2,5, 7,10,13,48,49,52]. That is, the blockage of protein synthesis following training prevents the permanent storage of the learning that occurred. Accordingly, an amnesic syndrome induced by protein synthesis inhibition should be of a permanent nature. However, some evidence indicates that recovery can occur in animals previously classified as amnesic [4,33-38,42, 44,45,46,47,54]. The results indicating spontaneous recovery and/or reminder-induced recovery of memory have led to questions about the adequacy of a consolidation deficit hypothesis. As an alternative, some investigators have proposed the possibility that rather than interfering with memory storage processes, protein inhibitors produce their "amnesic" effect via an impairment of the memory retrieval process(es). Thus, within the protein synthesis inhibitor literature a frequently raised theoretical question is whether retention deficits reflect a consolidation impairment in the memory storage processes or whether they represent an impairment in the retrieval process.

It has been reported that rodents injected with a protein inhibitor prior to training and classified as amnesic 1 day later demonstrate recovery of memory following a noncontingent footshock reminder given shortly after an initial retention test [37,38]. In the present experiment, we have examined the effects of a footshock "reminder" treatment on amnesia induced by protein inhibition as a function of the drug dosage and training-test

interval. In brief, the main findings were these: A large single dose or

several successive doses of anisomycin (Ani) produces a more profound

retention deficit than a small dose of Ani. Animals first tested at 21 days

after training showed a greater amnesia than animals first tested on day 1

or 7, and animals tested on day 7 show a greater amnesia than animals tested

after 1 day. All Ani animals given their initial test on day 1 showed sub-

stantial recovery on subsequent single retention tests administered on each

of the following three consecutive days. However, animals receiving a high

or multiple dosage of Ani showed little or no recovery when initial tests

were given at 7 or 21 days, whereas animals given a low dose showed signifi-

cantly improved performance on their second retention test. In contrast to

the finding that successive testing improved the retention of some groups,

a footshock reminder given 1 hr after the initial retention test was not

effective in attenuating the retention deficit. We will discuss the impli-

cations of reminder and spontaneous recovery studies for the hypotheses of

consolidation deficit $\wedge$ impairment of retrieval.
          and

Biochemical Experiments

Method

Anisomycin (2-p-methoxyphenyl-3-acetoxy-4-hydroxypyrollidine) was kindly

provided by Dr. Nathan Belcher of the Pfizer Pharmaceutical Company.

Anisomycin is now commercially available from Pfizer Diagnostics of Clifton,

New Jersey. Ani was dissolved in saline by adding an approximately equal

molar amount of 3N HCl and adjusting the pH to 6-7. Subcutaneous injections

of saline or a saline solution containing varying amounts of Ani (28 mg/ml

or 4 mg/ml) were made on the backs of male Swiss-Webster CD-1 mice 20 min

prior to training, in a volume of 0.25 ml. Animals receiving a multiple

dosage of saline or Ani (1 mg/animal/injection) were given 6 additional injections at 2 hr intervals. All pretraining injections were given under light ether anesthesia.

Evaluation of cerebral protein synthesis was achieved by subcutaneously injecting mice with radioactive (U-^{14}C)-L-valine (New England Nuclear Corp.) 20 min prior to sacrifice and then determining the ratio of (1) radioactivity resulting from incorporation of the label into trichloroacetic acid insoluble material to (2) total radioactivity in the brain sample. This provides an estimate of the protein synthesis during the 20 min period prior to sacrifice, and inhibition can be calculated by comparing Ani-treated animals to saline-injected animals. Five to 7 mice were used for each data point. Duplicate fractionations and determinations of radioactivity were made for each mouse brain. A detailed description of this procedure has been reported previously [11].

Results

A single dose of 7 mg of Ani produced a maximum inhibition of approximately 98%. This can be contrasted with an injection of 1 mg of Ani which produced a peak inhibition of approximately 92%. Seven injections of 1 mg of Ani at 2 hr intervals did not cause a detectable increase in the maximum inhibition over that obtained with a single injection of 1 mg, and only a very slight cumulative effect was observed--that is, the inhibition obtained from the seventh injection was very similar to that of the first.

Behavioral Experiments

Methods

Animals

Male Swiss-Webster CD-1 mice, 60-90 days of age, were obtained from our Lawrence Berkeley Laboratory colony. Animals were housed individually 48 hr prior to training and remained so throughout the experiments. Ad lib access to food and water was provided.

Apparatus and Procedure

Mice were given one-trial passive avoidance training in a standard step-through apparatus described previously [11]. Briefly, it consists of a black Plexiglas start box (9 cm long x 10.2 cm wide x 12.5 cm high) separated from a white Plexiglas shock compartment (35 cm long x 8.2 cm wide x 12.5 cm high) by a black panel with a 3.8 cm diameter hole at its base. Illumination of the test apparatus was provided by a 1.8 watt light bulb situated behind a white translucent Plexiglas panel at the end of the shock compartment. Entry into the shock compartment until the time of training or test was prevented by a guillotine door consisting of white translucent Plexiglas. A 0.30 mA shock was delivered through 2.4 mm diameter brass rods in the shock compartment by a constant current 18-pole shock scrambler. The apparatus was wiped clean with alcohol and allowed to dry between the testing of successive animals.

The reminder apparatus consists of a wooden trough (25.5 cm long x 3.5 cm wide at the base x 19.5 cm wide at the top x 8 cm high) with a removable door at one end. The interior sides were lined with metal plates connected to a constant current 18-pole shock scrambler.

For training, a mouse was placed into the start box for 10 sec after which the light illuminating the apparatus was turned on for 10 sec. The guillotine door blocking access to the shock compartment was removed when the animal was oriented away from the entrance. The step-through latency (STL) was measured as the time from orientation to the mouse hole entrance until the animal had all four paws on the grid of the shock compartment. Five seconds after the mouse entered the shock compartment, a continuous 0.30 mA footshock was delivered through the grid until the mouse escaped back to the start box. The guillotine door was replaced and the light turned off. After 5 sec the mouse was returned to its home cage. Animals with training STLs above 20 sec or escape latencies over 12 sec were eliminated from the experiment (total of 41 animals eliminated out of 567 trained).

All subjects received a single retention test on each of four consecutive days (designated as T_1, T_2, T_3, and T_4). The initial test (T_1) was administered either 1, 7, or 21 days after training. Testing was identical to training except that 1) no shock was delivered, and 2) animals entering the shock compartment were forced back into the start box after 5 sec by gentle touching of the hindquarters with the hand. Animals not entering the shock compartment within 600 sec were given a test score of 600. The STLs for different drug groups were compared with the Kolmogorov-Smirnov two-sample test. A within-group correlation for performance on different test days was obtained with a Pearson product-moment correlation. Within-group comparisons were made with either the Friedman two-way analysis of variance test or the Wilcoxon matched-pairs

signed-ranks test [32].

One-half of the mice in each group, selected at random, received a non-contingent footshock reminder one hr after their initial retention test. For the ∧footshock reminder treatment, an animal was placed into the dark reminder apparatus in a room separate from the training room, immediately administered a 0.30 mA footshock of 2 sec duration, and then returned to its home cage. The reminder shock strength was identical to the shock administered on training. This reminder shock procedure is similar to that employed by Quartermain et al. [37,38]. Nonreminder animals were placed in the trough in the same way, but no shock was administered.

Results

I. Training

Animals receiving subcutaneous injections of Ani (1 mg/animal or 7 mg/animal) or saline demonstrated similar STLs on training. The mean STLs were 5.7, 5.6, and 6.1 ∧sec respectively, and a one-way analysis of variance revealed no measurable effect of drug on the STLs, $F(2,525) = 1.57$, $p \geq 0.20$. There was, however, a highly significant effect on escape latencies, $F(2,421) = 7.24$, $p < 0.001$. Application of the Scheffé procedure [32] at the 0.05 level indicated this effect was primarily due to the differences between the saline and Ani (7 mg/animal) groups. The mean escape latencies for Ani (1 mg/animal; 7 mg/animal) and saline were 2.7, 3.1, and 2.3 ∧sec respectively. It has been shown previously [11] that an increase in escape latencies results in greater training strengths. Since in this experiment Ani animals show higher mean escape latencies and thus receive greater training, the amnesic effect of this agent cannot, therefore, be explained in terms of differing training strengths based on escape latencies.

II. Lack of footshock reminder effect

The median STLs of animals that did or did not receive a reminder foot-shock on retention tests at various times after training are presented in Figure 1A, B, C. To determine the effectiveness of the noncontingent foot-shock reminder, a comparison was made between reminded and nonreminded animals within an experimental treatment at each test day. No differences in STL scores were detected at any test day except for a tendency toward higher STLs on test days 3 and 4 by the saline-injected group first tested at 7 days and given a footshock reminder ($p < 0.059$ and $p < 0.055$, respectively). However, since 40 statistical comparisons were made between reminded and nonreminded animals, two results at or near the .05 level of confidence would be expected by chance. We conclude that the footshock reminder treat-ment is in and of itself an ineffective agent for attentuation of the amnesia induced by protein inhibition. This conclusion was further tested and con-firmed by performing a two-way analysis of variance with footshock reminder/ nonreminder as one factor and experimental group as the other factor. All groups were included except the saline groups first tested at 1 or 7 days, since no improvement from T_1 performance would be expected for these saline groups. The reminder shock did not significantly aid recovery even though the large N made this test as favorable as possible for detecting any difference, $F(1,286) = 1.52$, $p > 0.20$. Since none of these analyses indicated a signifi-cant effect of the reminder-shock procedure, we have therefore pooled the test scores of footshock-reminded and nonreminded animals for all other statistical tests.

III. Decline of memory with increasing training-test interval

The training-test interval (1, 7, or 21 days) exerted a significant effect upon the performance of animals on their initial retention test.

Whether animals received saline, a low dose of Ani (1 mg/animal) or a high

dosage (7 mg/animal), retention was significantly worse the longer the
                                                    seven
training-test interval (Fig. 1A, B, C). All∧differences were significant

at beyond the 0.01 level; 5 were significant beyond the 0.001 level.

IV. Amnesic effects of level and duration of protein synthesis inhibition

Animals injected with Ani, regardless of dosage, showed significantly

impaired performance as compared to saline control animals. Furthermore,

the high dose of Ani tended to produce more amnesia than the low dosage at

the two intervals where both were used (Fig. 1A, B and Table 1).

Animals receiving 7 successive injections of Ani (1 mg/animal every

2 hr) and tested on days 7-10 performed essentially like animals receiving

the equivalent dosage in a single injection (Ani 7 mg/animal). These

multiple-injected animals were significantly impaired on test days 7-10
                                    on test days 8-10 when compared with
when compared with saline controls and∧Ani 1 mg/animals (Table 1). These

results show that a more profound amnesia can be obtained by increasing the

duration or level of protein synthesis inhibition. This is in agreement

with previous studies demonstrating that duration [12] and level [52] of

protein inhibition are critical variables in determining the degree of amnesia.

V. Effects of multiple tests on retention

To determine if multiple testing affected recovery, comparisons were

made between the initial test scores and the STLs attained at each following

test day. All Ani-treated animals demonstrated recovery at the short training-

test interval (1-4 days), but at days 8-10 recovery occurred only in animals

receiving a low drug dosage (1 mg/animal) (see Table 2). When testing began

at 21 days, the saline-treated animals demonstrated a transient recovery on

day 22, whereas the Ani-injected animals (7 mg/animal) showed no improvement

of their initial poor performance. A comparison of the STLs of saline

animals first tested at 21 days with the STLs of Ani-treated animals

(1 mg/animal) first tested at 7 days showed that these groups were similar

in their initial poor retention and pattern of recovery; for all 4 test days,

Ani versus Saline, $p > 0.30$. These results indicate that recovery depends

primarily upon the degree of retention. In other words, re-exposure to the

testing situation can act as a reminder to facilitate recovery if and only

if there is a partial memory trace upon which it can exert its effect.

Although multiple testing induced recovery of memory in partially

amnesic animals, it was not capable of raising their level of performance

to that of the saline controls. An examination of Table 1 (columns T_2- T_4)

indicates that even for the drug groups that showed recovery (day 2-4:

Ani 1 mg and 7 mg; day 8-10: Ani 1 mg) there was a strong tendency to remain

impaired as compared with saline controls. These results indicate that while

animals made amnesic by a protein inhibitor may demonstrate some recovery,

they remain significantly poorer in performance than saline controls.

VI. Recovery as a function of initial retention

The conclusion of section V was based on comparisons of treatment

groups; this conclusion can be tested further by analyzing whether performance

of an animal on T_1 predicts its STLs on T_2-T_4, regardless of the treatment

group to which it belonged. To evaluate this possibility, Pearson product-

moment correlations were obtained to determine how strongly the magnitude

of the STL on a particular test was associated with the STL on the subsequent

test (Table 3). For instance, if an animal scores low on T_1, will it also

score low on T_2? Examination of the Pearson correlations indicates a highly

significant positive association between the STLs on a test and those obtained

on the following test. This relationship holds for saline-injected animals as well as anisomycin animals and across all test days. The proportion of .variance accounted for (r^2) indicates that the STL scores on a given test contribute to a considerable extent in determining the STL on the following test. The variance accounted for by initial retention ranged from 42% to 72%. While drug group and testing interval are variables that also play important roles in determining recovery, it seems clear that the degree of retention as reflected by the initial test score is the primary indicator that must be considered in determining whether or not an animal shows recovery.

The importance of initial STL scores in the determination of subsequent scores is clearly demonstrated in Figure 2 in which animals were classified solely on the basis of their STL on the initial retention test and without regard to their treatment group. It shows that animals with low initial STLs (1-7 sec) remain low on subsequent testing. Animals with intermediate STLs (8-200) show some recovery. Animals with high STLs (> 200) tend to remain high. The STL range of 1-7 was chosen for the low group because it encompassed the lower three quartiles of training STLs. The intermediate range of 8-200 was chosen because its upper value was slightly greater than the median STL of any drug-treated group. These results are in good accordance with the model to be presented in the Discussion.

VII. Controls for sickness and for effects of multiple testing

Results of control experiments suggest that the amnesic effect of Ani could not be explained by possible sickness caused by the drug. For each experimental Ani group tested at 1 or 7 days, a corresponding group was given an equivalent dosage of Ani 2 hr after training. Mice treated in this manner demonstrated retention scores on initial and subsequent tests equivalent to scores of saline controls. If the poor retention of mice injected

with Ani just before training were due to illness, then poor retention

would also have been found in groups injected 2 hr posttraining, but this

was not the case, so the hypothesis of illness is ruled out.

To evaluate the possibility that recovery was an artifact of multiple

testing, for each experimental group a corresponding group of mice was

treated and tested in an identical fashion except that they did not receive

a footshock on training; half of them did receive a "reminder" footshock

after their initial test. These controls maintained low STLs throughout

testing, and within-group comparison across test day by the Friedman two-way

analysis of variance revealed no significant differences across days for any

group. Thus, the multiple test procedure is not by itself capable of producing

the increase in STLs demonstrated by several of the experimental groups

treated with Ani. The only experimental groups showing no differences from

the nonshock controls on any tests are the animals treated with a high or

multiple dosage of Ani and first tested at day 7 or 21. Since these experi-

mental groups did not differ from naive controls, they can be considered to

be completely amnesic. The fact that some Ani-injected groups were completely

amnesic while others were only partially amnesic provides additional evidence

that both the drug dosage and training-test interval are effective methods

of manipulating the degree of amnesia.

DISCUSSION

Recovery of memory after a retrograde amnesia (RA) induced by a
disruptive agent (e.g., electroconvulsive shock, CO_2, protein synthesis
inhibition, etc.) has been demonstrated by a number of investigators
[20,22,27,28,31,33-46,50,54,55]. The resulting theoretical controversy has
centered around whether the induced retention deficit reflects a failure to
consolidate memory or whether it reflects an impairment in the retrieval
process. The arguments on each side of the issue have been basically the
same irrespective of the disruptive agent. It is not necessary to discuss
these alternative hypotheses in   great detail since excellent reviews of
the issues and evidence in support of both the consolidation hypothesis
[13,18,21,25,26] and retrieval hypothesis [4,19,29,30,33,47] have been
published.

In brief, the retrieval-impairment interpretation of RA is supported by
studies demonstrating reminder-induced or spontaneous recovery of memory.
The typical reminder study usually includes the following aspects:
1) training an animal on an avoidance or appetitive task, 2) administering
a memory disrupting treatment shortly before or after training, 3) then
providing a reminder treatment, either 3a) a physiological reminder (usually
an excitant drug) shortly after training or shortly prior to retention
testing or 3b) a behavioral reminder (usually footshock) between the initial
and a subsequent retention test. Animals receiving the reminder may show
an attentuation of their amnesia whereas animals receiving no reminder
continue to demonstrate a retention deficit. In spontaneous recovery there
is simply an attenuation of the RA with the passage of time. Thus, since
recovery from amnesia is demonstrable in animals classified as amnesic, and

because the consolidation hypothesis is interpreted as requiring an
irreversible loss of memory, these studies are frequently taken as support
for the hypothesis that the memory of the training experience is stored but
unavailable to amnesic animals prior to an effective reminder treatment
because of an impairment in the retrieval process.

I. Interpretations of RA and recovery based on the consolidation hypothesis

The response of investigators favoring an interpretation of RA as an
impairment of the storage process has been that recovery under certain
circumstances is not unexpected and thus may have little bearing upon
memory consolidation issues. Thus, Cherkin [6] pointed out that an amnestic
treatment does not necessarily have an all-or-none effect and proposed that
a reminder may raise retention above an expression threshold by summating
with a weak memory engram. Similarly, Gold and King [18] found that recovery
occurred only in animals made partially amnesic by electroconvulsive shock,
whereas animals showing a very profound amnesia were unaffected by a reminder
treatment. They argued that a footshock reminder treatment provides additional
information to an animal that is partially amnesic and that a footshock
reminder can improve the performance of normal nonamnesic controls. As
support for this contention, Gold and King cited several studies [15,20,27]
in which it was found that a reminder treatment improved the retention per-
formance of footshock control animals. Similarly, and most importantly,
they found that a noncontingent footshock reminder improved the retention
performance of poorly trained animals that received no amnestic treatment
and thus could not have had a retrieval block. A physiological reminder may
induce recovery in a similar fashion or it may improve performance by modu-
lation of arousal and/or attentional mechanisms [3,8,14]. Turning to spon-
taneous recovery, Gold and King have argued that this may be more an artifact
of the training and/or testing situation than a genuine phenomenon. Our

examination of studies reporting spontaneous recovery in animals given a protein synthesis inhibitor [36,44,45,46,50,54] showed that this phenomenon occurred only under strong training conditions or when retention was evaluated with multiple trial testing. Furthermore, one of these studies [50] that had frequently been cited as demonstrating spontaneous recovery has been reported by its authors to be unreplicable [51]. Finally, it has been pointed out that there are no reports of induced or spontaneous recovery of memory in animals that had been classified as amnesic at one week; the only reports of recovery have been following apparent amnesia one day after training [4]. Thus recovery occurs only at short training-test intervals, presumably when animals may still retain a partial memory of the training situation.

The results of our study provide further strong support for the storage impairment interpretation of RA. Mice showed different degrees of impairment as a function of the drug dosage and the training-test interval. Consequently, re-exposure to the training apparatus resulted in partial recovery of animals tested at a short training-test interval or treated with a low drug dosage. The median STL scores of groups treated in this fashion indicated a partial memory for training on the first retention trial. In contrast, the experimental groups that received a high drug dosage or tested at a long training-test interval showed a profound amnesia as indicated by their low initial median STL scores; these mice showed no significant attentuation of their amnesia after re-exposure to the training apparatus. Furthermore, when recovery from partial amnesia occurred it was not specific to animals receiving the protein inhibitor. Animals injected with saline and tested at a longer training-test interval, when they had a retention deficit similar to weakly amnesic animals, showed recovery similar to animals made partially amnesic by the protein inhibitor (see Figs. 1B and C).

The interpretation of our results as support for a consolidation hypothesis was further indicated by the analysis of performance based upon initial retention scores irrespective of treatment group. This analysis indicated that the degree of retention shown on initial testing was the strongest indicator of whether or not an animal would show partial recovery. These results are in good accord with data from other studies reporting a within-group analysis of the recovery phenomenon [6,18]. Thus, we propose that a reminder will only be effective if there is partial memory upon which it can exert its effect and, most importantly, we have demonstrated in the present study that this is true for saline-treated animals as well as for animals given an amnestic agent.

II. Consideration of studies used to support the retrieval-block hypothesis

Some investigators using antibiotics as an amnestic treatment and finding recovery have preferred to explain the amnesic effects of these drugs in terms of a retrieval block [4,36,37,38,44,45,54]. Barraco and Stettner [4, pp. 266-271] cover a number of these studies in their review of protein inhibitors and memory. Quartermain and his colleagues have been the most consistently successful in obtaining recovery of memory after treating animals with a protein inhibitor [33-38]. However, our examination of these reports leads us to conclude that an explanation in terms of a consolidation deficit is still plausible, for the following reasons: As mentioned earlier, when it is considered that an amnestic agent can have a graded effect upon memory as a function of numerous variables (e.g., shock intensity, drug-dosage level, training-test interval, task, species, etc.), then recovery is not an unexpected phenomenon when amnesia is subtotal. Furthermore, when a passive avoidance task was used, recovery following

induced RA and subsequent recovery. The basic premises of this model have been previously offered as explanations of induced or spontaneous recovery following amnestic treatment such as anesthesia or ECS [6,18,21], but the model has not been spelled out fully before. According to this model, treatment with a protein inhibitor will have a graded effect on memory as a function of various experimental variables and will result in a range of memory trace strengths (Fig. 3); memory traces, whether or not affected by drugs, will also weaken as a function of time. A partial or weak memory can be pushed above the behavioral expression criterion of an experiment by summating with a reminder treatment. The reminder may improve the performance of animals by providing additional information or via modulation of arousal and/or attentional mechanisms. Animals showing either good retention or very poor retention will show only minimal responsiveness to the reminder treatment. This lack of responsiveness could be due to one of several factors: 1) animals with good retention are already performing maximally; 2) animals with very poor retention have no memory of the training experience with which the reminder can summate; or 3) the experimental design is such that when a reminder summates with a weak memory it does not reach the expression threshold criterion (e.g., the effect of a reminder given at a 7-day interval on the lowest solid trace in Fig. 3). This model is supported by the results of this experiment and has been shown to be applicable to control animals as well as those given an amnestic treatment (Figs. 1C and 2).

Our interpretation of recovery is not meant to imply that recovery studies are unimportant. In our study the use of multiple testing to induce recovery proved to be a sensitive tool for distinguishing between degrees of memory impairment. In addition, we do not wish to give the impression that

ACKNOWLEDGMENT

Figure Captions

Fig. 1 A, B, anc C. Median step-through latencies for mice first tested at either 1, 7 or 21 days after training and then given a single retention test on each of the 3 following days. The footshock reminder treatment was administered 1 hr after the initial test. The different groups are represented as follows: Saline, footshock reminder ●——●; Saline, no footshock reminder o-----o; Ani 1 mg/animal, footshock reminder ▲——▲; Ani 1 mg/animal, no footshock reminder △-----△; Ani 7 mg/animal, footshock reminder ■——■; and Ani 7 mg/animal, no footshock reminder ▢-----▢. The N per point ranged between 19 and 29. Note that the vertical scales differ for the three panels.

Fig. 2 Median step-through latencies (STL) for mice categorized solely on the basis of their initial STL irrespective of drug or training-test interval. An explanation for the determination of STL ranges is contained in the text (p. 10). ●——● STL 1-7 sec, including the following subjects: Saline, N=27; Ani 1 mg, N=12; Ani 7 mg or Ani 1 mg x 7, N=73; Total N=112. o——o STL 8-200 sec: Saline, N=52; Ani 1 mg, N=43; Ani 7 mg or Ani 1 mg x 7, N=81; Total N=176. ▢——▢ STL 201-600 sec: Saline, N=100; Ani 1 mg, N=26; Ani 7 mg or Ani 1 mg x 7, N=10; Total N=136.

Figure Captions (contd.)

Fig. 3 An hypothesized model for explaining the effects of a reminder

and/or re-exposure treatment. The solid lines represent memory

traces of different strengths, which can be determined by such

factors as degree of training, drug treatment, and training-test

interval. Dashed lines show increases in strength of memories

caused by re-exposure treatments; the increases are small when

memory strength is either very high or very low. See text for

further explanation.
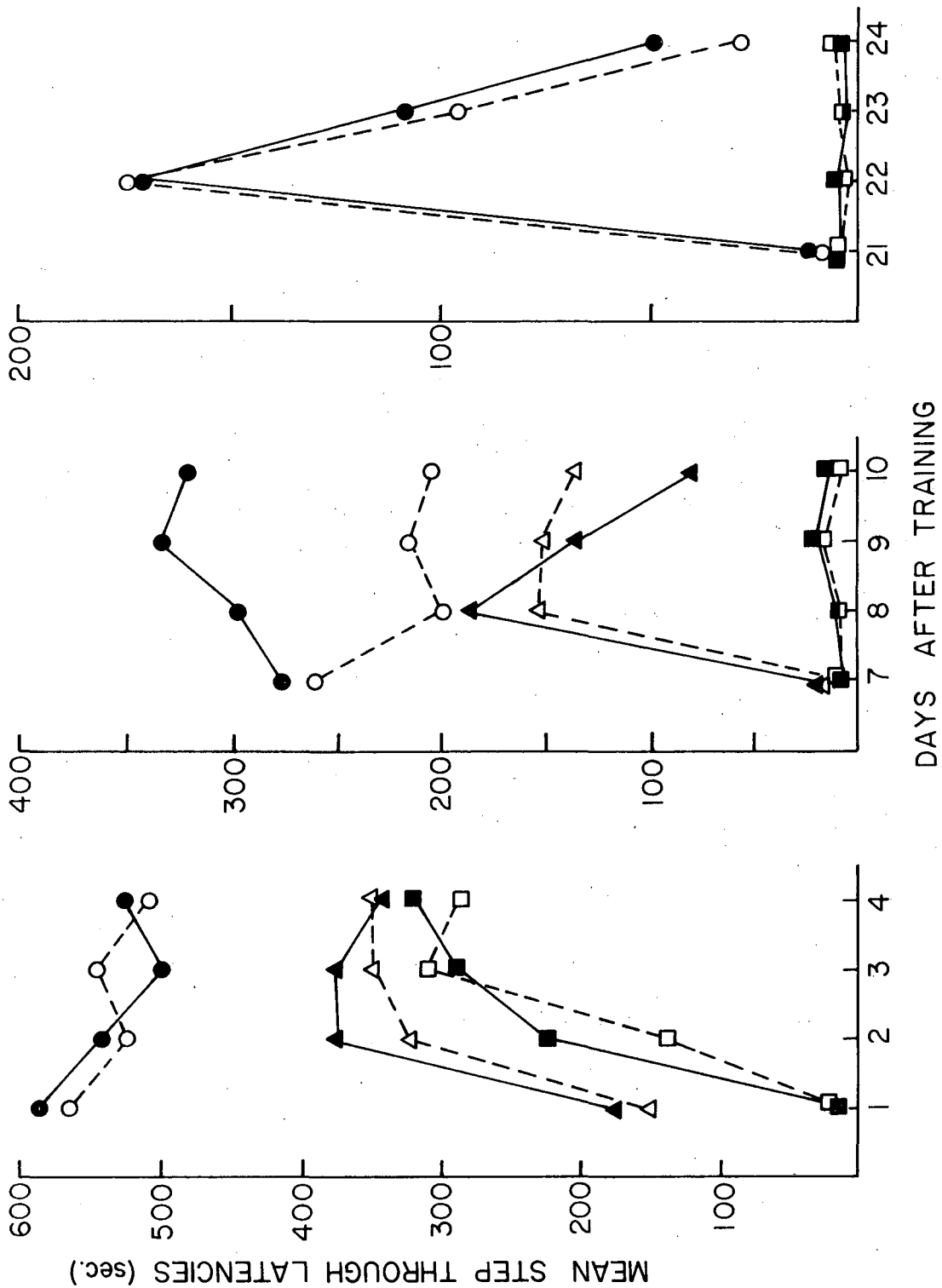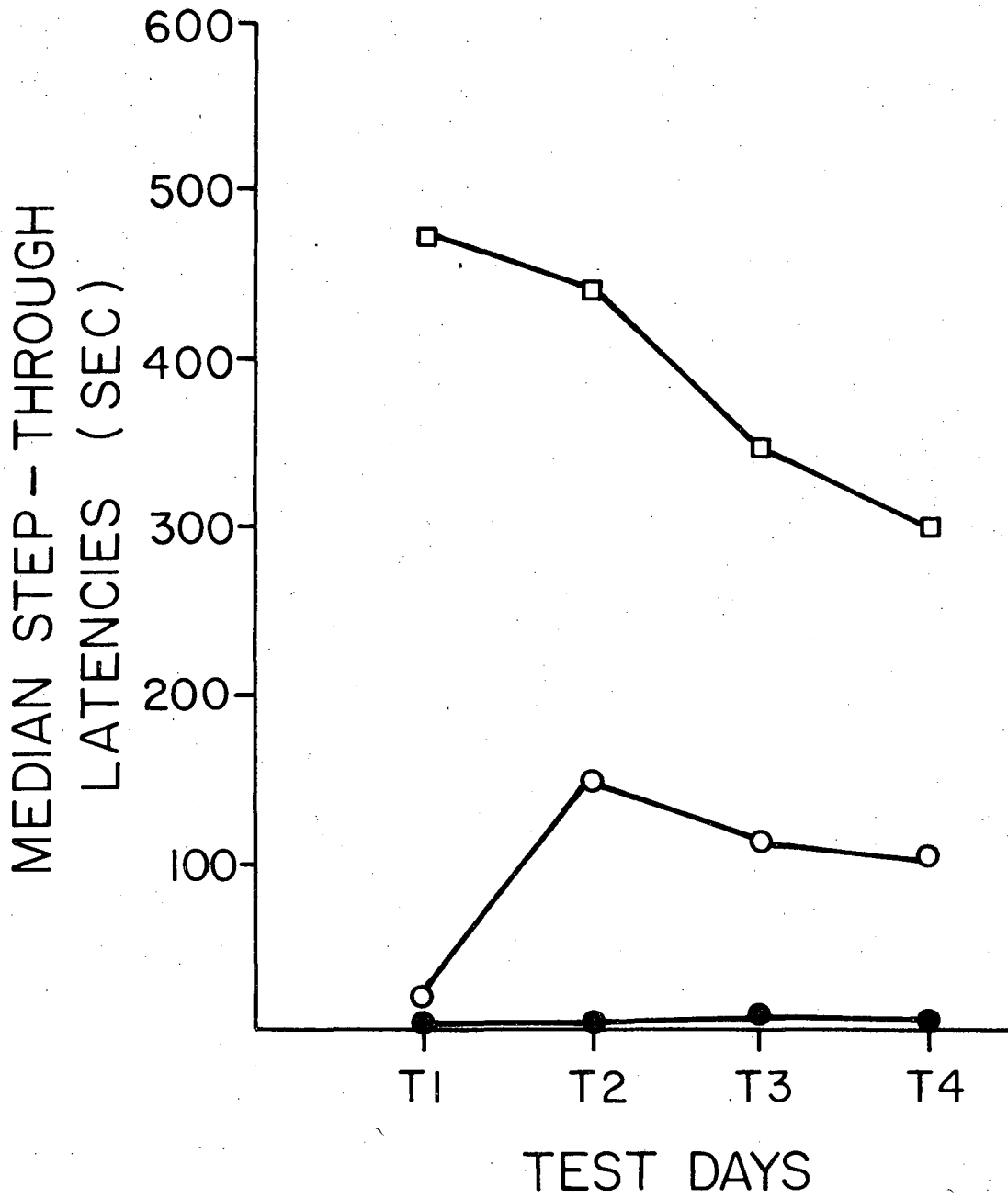
Fig. 1

XBL775-4377

Fig. 2

XBL 775 — 4397

Table 1

Effects of level and duration of inhibition of protein synthesis on memory (median step-through latencies in secs.)

		T_1	T_2	T_3	T_4
Day 1 ⟶ Day 4 [Fig. 1A]					
Ani 1mg vs. Sal	medians:	155 vs. 584	358 vs. 530	354 vs 522	343 vs. 517
	p-values:	.0001	.009	.04	.02
Ani 7mg vs. Sal	medians:	17 vs. 584	157 vs. 530	298 vs. 522	313 vs. 517
	p-values:	.0001	.0001	.07	.11
Ani 7mg vs. Ani 1mg	medians:	17 vs. 155	157 vs. 358	298 vs. 354	313 vs. 343
	p-values:	.001	.002	.79	.79
Day 7 ⟶ Day 10 [Fig. 1B]					
Ani 1mg vs. Sal	medians:	15 vs. 278	173 vs. 264	141 vs. 251	121 vs. 245
	p-values:	.004	.03	.004	.001
Ani 7mg vs. Sal	medians:	9 vs. 278	9 vs. 264	9.5 vs. 251	10 vs. 245
	p-values:	.0001	.0001	.0001	.0001
Ani 7mg vs. Ani 1mg	medians:	9 vs. 15	9 vs. 173	9.5 vs. 141	10 vs. 121
	p-values:	.07	.0001	.0002	.0001
Ani 1mg X 7 vs. Sal X 7	medians:	9.5 vs. 368	31 vs. 318	23 vs. 212	10 vs. 191
	p-values:	.0001	.0001	.005	.001
Ani 1mg X 7 vs. Ani 1mg	medians:	9.5 vs. 15	31 vs. 173	23 vs. 141	10 vs. 121
	p-values:	.62	.02	.04	.04
Ani 1mg X 7 vs. Ani 7mg	medians:	9.5 vs. 9	31 vs. 9	23 vs. 9.5	10 vs. 10
	p-values:	.79	.90	.88	.71
Day 21 ⟶ Day 24 [Fig. 1C]					
Ani 7mg vs. Sal	medians:	5 vs. 11	5 vs. 174	3.5 vs. 109	4.5 vs. 33
	p-values:	.002	.0001	.0001	.0002

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Table 2

Significance of effects of multiple tests on retention

	Trend	T_1 vs. T_2	T_1 vs. T_3	T_1 vs. T_4
Day 1 ⟶ Day 4 (Fig. 1A)				
Ani 1mg	Recovery	.01	.0001	.0001
Ani 7mg	Recovery	.0001	.0001	.0001
Sal	Decreasing Latencies	.01	.01	.01
Day 7 ⟶ Day 10 (Fig. 1B)				
Ani 1mg	Transient Recovery	.02	.01	.15
Ani 7mg	No Recovery	.08	.12	.44
Ani 1mg X 7	No Recovery	.06	.07	.87
Sal	No Recovery	.23	.58	.68
Day 21 ⟶ Day 24 (Fig. 1C)				
Ani 7mg	No Recovery	.66	.23	.51
Sal	Transient Recovery	.0001	.03	.69

Table 3

Pearson product-moment correlations for step-through latencies between test days

	T_2	T_3	T_4
T_1	.65	.54	.46
T_2		.79	.69
T_3			.85

$p \leq 0.00001$ for all correlations, $N = 424$

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REFERENCES

1. Agranoff, B. W. Effects of antibiotics on long-term memory formation in the goldfish. In: _Animal Behavior_, edited by W. K. Honig and P. H. R. James. New York: Academic Press, 1971, pp. 243-258.

2. Barondes, S. H. Protein synthesis-dependent and protein synthesis-independent memory storage processes. In: _Short Term Memory_, edited by D. Deutsch and J. A. Deutsch. New York: Academic Press, 1975, pp. 379-390.

3. Barondes, S. H. and H. D. Cohen. Arousal and the conversion of "short-term" to "long-term" memory. _Proc. Natl. Acad. Sci. USA._ 61: 923-929, 1968.

4. Barraco, R. A. and L. J. Stettner. Antibiotics and memory. _Psych. Bull._, 83: 242-302, 1976.

5. Brown, B. M. and E. P. Nobel. Cycloheximide and learning in the isolated cockroach ganglion. _Brain Res._ 6: 363-366, 1967.

6. Cherkin, A. Retrograde amnesia in the chick: Resistance to the reminder effect. _Physiol. Behav._ 8: 949-955, 1972.

7. Davis, H. P., C. W. Spanis and L. R. Squire. Inhibition of cerebral protein synthesis: Performance at different times after passive avoidance training. _Pharmac. Biochem. Behav._ 4: 13-16, 1973.

8. Dawson, R. G. and J. L. McGaugh. Drug facilitation of learning and memory. In: _The Physiological Basis of Memory_, edited by J. A. Deutsch. New York: Academic Press, 1973, pp. 77-111.

9. Flexner, L. B., J. B. Flexner and R. B. Roberts. Memory in mice analyzed with antibiotics. _Science_ 155: 1377-1383, 1967.

10. Flood, J. F., E. L. Bennett, A. E. Orme and M. R. Rosenzweig. Effects of protein synthesis inhibition on memory for active avoidance training. Physiol. Behav. 14: 177-184, 1975.

11. Flood, J. F., E. L. Bennett, M. R. Rosenzweig and A. E. Orme. Influence of training strength on amnesia induced by pretraining injections of cycloheximide. Physiol. Behav. 9: 589-600, 1972.

12. Flood, J. F., E. L. Bennett, M. R. Rosenzweig and A. E. Orme. The influence of duration of protein synthesis inhibition on memory. Physiol. Behav. 10: 555-562, 1973.

13. Flood, J. F. and M. E. Jarvik. Drug influence on learning and memory. In: Neural Mechanisms of Learning and Memory, edited by M. R. Rosenzweig and E. L. Bennett. Cambridge, MA: MIT Press, 1976, pp. 483-507.

14. Flood, J. F., M. E. Jarvik, E. L. Bennett, A. E. Orme and M. R. Rosenzweig. The effect of stimulants, depressants and protein synthesis inhibition on retention. Behav. Biol. in press.

15. Galluscio, E. H. Retrograde amnesia induced by electroconvulsive shock and carbon dioxide anesthesia in rats: An attempt to stimulate recovery. Physiol. Behav. 75: 136-140, 1971.

16. Gervai, J. and V. Csanyi. The effects of protein synthesis inhibitors on imprinting. Brain Res. 53: 151-160, 1973.

17. Gibbs, M. E. and R. F. Mark. Inhibition of Memory Formation. New York: Plenum Press, 1973.

18. Gold, P. E. and R. A. King. Retrograde amnesia: Storage failure versus retrieval failure. Psych. Rev. 81: 465-469, 1974.

19. Lewis, D. J. Sources of experimental amnesia. Psych. Rev. 76: 461-472, 1969.

20. Lewis, D. J., J. R. Misanin and R. R. Miller. Recovery of memory following amnesia. Nature 220: 704-705, 1968.

21. Mah, C. J. and D. J. Albert. Electroconvulsive shock-induced retrograde amnesia: An analysis of the variation in the length of the amnesia gradient. Behav. Biol. 9: 517-540, 1973.

22. Mah, C. J. and D. J. Albert. Reversal of ECS-induced amnesia by post-ECS injections of amphetamine. Pharmac. Biochem. Behav. 3: 1-5, 1975.

23. Mark, R. F. and M. E. Watts. Drug inhibition of memory formation in chickens: I. Long-term memory. Proc. Royal Soc. (Series B) 178: 439-454, 1971.

24. Mayor, S. J. Memory in the Japanese quail. Effects of puromycin and acetoxycycloheximide. Science 166: 1165-1167, 1969.

25. McGaugh, J. L. Time-dependent processes in memory storage. Science 153: 1351-1358, 1966.

26. McGaugh, J. L. and M. J. Herz. Contemporary Issues in Psychobiology: Memory Consolidation. San Francisco: Albion, 1972.

27. Miller, R. and A. Springer. Induced recovery of memory following electroconvulsive shock. Physiol. Behav. 8: 645-651, 1972.

28. Miller, R. and A. Springer. Recovery from amnesia following trans-corneal electroconvulsive shock. Psychonomic Soc. 28: 7-9, 1972.

29. Miller, R. R. and A. D. Springer. Amnesia, consolidation, and retrieval. Psych. Rev. 80: 69-79, 1973.

30. Miller, R. R. and A. D. Springer. Implications of recovery from experimental amnesia. Psych. Rev. 81: 470-473, 1974.

31. Nachman, M. and R. O. Meinecke. Lack of retrograde amnesia effects of repeated electroconvulsive shock and carbon dioxide treatments. J. Comp. Physiol. Psychol. 68: 631-636, 1969.

32. Nie, N. H., C. H. Hull, J. G. Jenkins, K. Steinbrenner and D. H. Bent. Statistical Package for the Social Sciences. 2nd edition. New York: McGraw Hill, 1975.

33. Quartermain, D. The influence of drugs on learning and memory. In: Neural Mechanisms of Learning and Memory, edited by M. R. Rosenzweig and E. L. Bennett. Cambridge, MA: MIT Press, 1976, pp. 508-518.

34. Quartermain, D. and C. Y. Botwinick. The role of biogenic amines in the reversal of cycloheximide-induced amnesia. J. Comp. Physiol. Psychol. 88: 286-401, 1975.

35. Quartermain, D. and C. Y. Botwinick. Effect of age of habit on susceptibility to cycloheximide-induced amnesia. J. Comp. Physiol. Psychol. 89: 803-809, 1975.

36. Quartermain, D. and B. S. McEwen. Temporal characteristics of amnesia induced by protein synthesis inhibitor: Determination by shock level. Nature 228: 677, 678, 1970.

37. Quartermain, D., B. S. McEwen and E. C. Azmitia. Amnesia produced by electroconvulsive shock or cycloheximide: Conditions for recovery. Science 169: 683-686, 1970.

38. Quartermain, D., B. S. McEwen and E. C. Azmitia. Recovery of memory following amnesia in the rat and mouse. J. Comp. Physiol. Psychol. 79: 360-370, 1972.

39. Riege, W. H. and A. Cherkin. Sensitive period for reversal of carbon-dioxide amnesia in goldfish. Behav. Biol. 12: 477-490, 1974.

40. Rigter, H. and H. van Riezen. Anti-amnesic effect of $ACTH_{4-10}$: Its
    independence of the nature of the amnesic agent and the behavioral
    test. Physiol. Behav. 14: 563-566, 1975.

41. Rigter, H., H. van Riezen and D. de Wied. The effects of ACTH- and
    vasopressin-analogues on CO_2-induced retrograde amnesia in rats.
    Physiol. Behav. 13: 381-388, 1974.

42. Roberts, R. B., J. B. Flexner and L. B. Flexner. Some evidence for the
    involvement of adrenergic sites in the memory trace. Proc. Natl. Acad.
    Sci. USA. 66: 310-313, 1970.

43. Sara, S. J. Recovery from hypoxia and ECS-induced amnesia after a
    single exposure to training environment. Physiol. Behav. 10: 85-89,
    1973.

44. Schmaltz, G. and B. Delerm. Effects of cycloheximide on acquisition
    and retention of an avoidance learning in the rat: Recovery of memory.
    Physiol. Behav. 13: 211-220, 1974.

45. Serota, R. G. Acetoxycycloheximide and transient amnesia in the rat.
    Proc. Natl. Acad. Sci. USA 68: 1249-1250, 1971.

46. Serota, R. G., R. B. Roberts and L. B. Flexner. Acetoxycycloheximide-
    induced transient amnesia: Protective effects of adrenergic stimulants.
    Proc. Natl. Acad. Sci. USA 69: 340-342, 1972.

47. Spear, N. E. Retrieval of memory in animals. Psychol. Rev. 80: 163-194,
    1973.

48. Squire, L. B. Short-term memory as a biological entity. In: Short-Term
    Memory, edited by D. Deutsch and J. A. Deutsch. New York: Academic
    Press, 1975, pp. 1-40.

49. Squire, L. R. and S. H. Barondes. Inhibition of cerebral protein or RNA synthesis and memory. In: Macromolecules and Behavior, edited by J. Gaito, second edition. New York: Appleton-Century-Crofts, 1972, pp. 61-82.

50. Squire, L. R. and S. H. Barondes. Variable decay of memory and its recovery in cycloheximide-treated mice. Proc. Natl. Acad. Sci. USA 69: 1416-1420, 1972.

51. Squire, L. R. and S. H. Barondes. Anisomycin, like other inhibitors of cerebral protein synthesis, impairs "long-term" memory of a discrimination task. Brain Res. 66: 301-308, 1974.

52. Squire, L. R. and H. P. Davis. Cerebral protein synthesis inhibition and discrimination training: Effects of extent and duration of inhibition. Behav. Biol. 13: 49-59, 1975.

53. Squire, L. R., G. A. Smith and S. H. Barondes. Cycloheximide affects memory within minutes after the onset of training. Nature 242: 201-202, 1973.

54. Van Abeelen, J., J. Daems and G. Douma. Memory storage in three inbred mouse strains after injection of cycloheximide. Physiol. Behav. 10: 751-753, 1973.

55. Zinkin, S. and A. J. Miller. Recovery of memory after amnesia induced by electroconvulsive shock. Science 155: 102-104, 1967.