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Recovery as a Function of the Degree of Amnesia Due to Protein Synthesis Inhibition[1]

HASKER P. DAVIS AND MARK R. ROSENZWEIG

Department of Psychology, University of California, Berkeley, CA 94720

EDWARD L. BENNETT AND ANN E. ORME

Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory, University of California Berkeley, CA 94720

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DAVIS, H. P., M. R. ROSENZWEIG, E. L. BENNETT AND A. E. ORME. *Recovery as a function of the degree of amnesia due to protein synthesis inhibition.* PHARMAC. BIOCHEM. BEHAV. 8(6) 701–710, 1978. – 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 × 7) and given one training trial in a passive avoidance box. All animals 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 footshock 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). Animals injected with anisomycin whose testing began 1 day after training demonstrated partial recovery irrespective of drug dosage level. 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 Amnesia Passive avoidance
Inhibition of cerebral protein synthesis Anisomycin Memory recovery

ANTIBIOTICS, because of their inhibitory effects on protein synthesis, are frequently used in studies of memory [1, 2, 4, 7]. 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, 6, 8, 18, 19]. These findings have been most frequently interpreted in terms of a consolidation deficit [1, 2, 6, 33]. 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, 28, 29, 30, 34]. Thus, it has been reported that rodents injected with a protein synthesis 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 [25,26]. 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).

In the present experiment, we have examined the effects of a footshock reminder treatment on amnesia induced by inhibition of protein synthesis as a function of the drug dosage and training-test interval. In brief, the main findings were these: Retention declined with increased drug dosage and/or greater training-test intervals. Partial recovery was demonstrated with a low drug dosage and at a short training-test interval irrespective of drug dosage. Mice showed little or no recovery at long training-test intervals when high or multiple doses of Anisomycin were given. In

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contrast to the finding that successive testing improved the retention of some groups, a footshock reminder was not effective in attenuating the retention deficit within our experimental conditions. We will discuss the implications of reminder and spontaneous recovery studies for the hypotheses of consolidation deficit and impairment of retrieval.

BIOCHEMICAL EXPERIMENTS

Method

Anisomycin (2-p-methoxyphenyl-3-acetoxy-4-hydroxypyrollidine) was kindly provided by Dr. Nathan Belcher of the Pfizer Pharmaceutical Company. Anisomycin (Ani) is now commercially available from Pfizer Diagnostics of Clifton, NJ. Ani was dissolved in saline by adding an approximately equal molar amount of 3N HCl and adjusting the pH to 6–7 with 0.1 NaOH. 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.

Evaluation of cerebral protein synthesis and its inhibition by Ani was achieved by the following procedure: Mice were injected subcutaneously with L-[U-^{14}C] valine (New England Nuclear Corp.) at various times after the administration of Ani. Twenty min after the radioactive isotope injection, mice were sacrificed by cervical dislocation; brains were quickly removed, frozen on dry ice and stored at −20°C until analyzed. At the time of analysis brains were weighed and then homogenized at a concentration of 20 mg/ml in 0.1 N NaOH. The protein in a 2 ml aliquot was precipitated by the addition of 5 ml of 14% trichloracetic acid (TCA). The supernatant after centrifugation was saved for determination of radioactivity, and the precipitate was washed twice by resuspending and recentrifugation in 5 ml of 10% TCA. These washes were discarded. One ml of Biosolv BSS-3 solubilizer was added to the TCA-precipitate (P). The precipitate was mixed and allowed to dissolve overnight, transferred to vials with four to five 3 ml rinses of toluene-Fluor II scintillation fluid and counted. One ml aliquots of the TCA-supernatant (S) were added to 10 ml of Aquasol 2 scintillation fluid and counted. The degree of incorporation was calculated by determining the ratio [P/(P+S)] of (1) radioactivity resulting from incorporation of the label into TCA insoluble material (P) to (2) total radioactivity in the brain sample (P+S). This provides an estimate of the protein synthesis during the 20 min period prior to sacrifice. The percent inhibition was calculated by comparing this ratio for Ani-treated animals to saline-treated animals. Five to seven mice were used for each data point. Duplicate fractionation and determinations of radioactivity were made for each mouse brain. Additional data concerning the amount of radioactivity in the brain and the distribution of radioactivity between supernatant and precipitate have been presented [12].

Results

Determinations of the percent inhibition of protein synthesis produced by varying dosages and by repeated injections of Ani are given in Fig. 1. 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

Method

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. Animals were given a subcutaneous injection 20 min prior to training; as in the biochemical experiment the injection consisted of either saline or a saline solution containing varying amounts of Ani (28 mg/ml or 4 mg/ml) and were made in a volume of 0.25 ml. Six additional injections of sal or Ani (1 mg/animal/injection) were given at 2 hr intervals to animals receiving the multiple dosage series. When Ani 1 mg was administered in this fashion an inhibition of protein synthesis greater than 80% was maintained for approximately 14 hr (see Fig. 1). All pretraining injections were given under light ether anesthesia.

Mice were given one-trial passive avoidance training in a standard step-through apparatus described previously [9]. Briefly, it consists of a black Plexiglas start box (9 cm long × 10.2 cm wide × 12.5 cm high) separated from a white Plexiglas shock compartment (35 cm long × 8.2 cm wide × 12.5 cm high) by a black panel with a 3.8 cm dia. hole at its base. Illumination of the test apparatus was provided by a 1.8 W 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 dia. 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 (24.5 cm long × 3.1 cm wide at the base × 19.0 cm wide at the top × 8.0 cm high) with a removable door at one end. The interior sides were lined with metal plates separated at the base by a 0.9 cm gap and 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

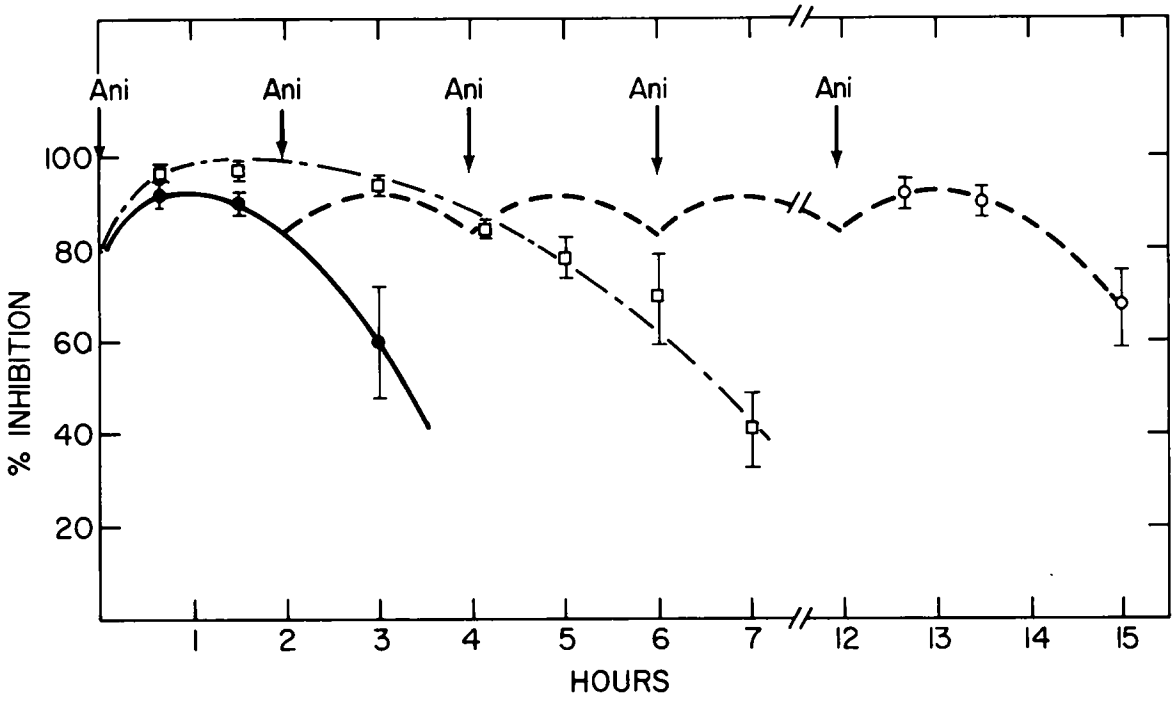

FIG. 1. Inhibition of protein synthesis by 1 mg of Ani (●———●) or after the 7th injection of Ani (○- - - -○) and by 7 mg of Ani (□- - - -□) are presented. Five to seven mice were used for each data point, and the standard deviations are shown by the vertical bars. The dashed curve (- - - -) denotes the inhibition produced by successive injections of 1 mg of Ani at 2 hr intervals (indicated by ↓) and has been derived from numerous other experiments carried out at this laboratory in which a series of injections of Ani has been used (see for example [12]).

from the experiment (total of 41 animals eliminated out of 567 trained).

All animals 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-rank test [23].

One-half of the mice in each group, selected at random, received a noncontingent footshock reminder 1 hr after the 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 footshock of 2 sec duration and approximately 0.30 mA, and then returned to its home cage. The immediate application of the footshock is very important in assuring that animals receive the footshock. It is only when animals are first placed into the reminder apparatus that one can be confident that they are bridging the two plates through which the shock is delivered. Each of the mice was observed to jump when the current was applied. This reminder shock procedure is similar to that employed by Quartermain *et al.* [25,26]. The primary distinction between our reminder procedure and Quartermain's procedure was the application of a scrambled shock to parallel plates instead of a grid. The intensity of the reminder shock in our procedure may have been slightly lower than the training shock. However, pilot work indicated that for our experimental conditions higher shock intensities did not attenuate the performance deficit of animals classified as strongly amnesic. Nonreminder animals were placed in the trough in the same way, but no shock was administered. Table 1 shows the main experimental groups and the number of animals in each group.

Control groups for sickness at testing and for effects of multiple testing were trained and tested at the same times as the experimental groups. Sickness control animals received Ani 2 hr after training. Controls for multiple testing were treated and tested in a fashion identical to the experimental animals except that they did not receive a footshock on training; half of them did receive a reminder footshock after their initial test.

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, $F(2,421) = 7.24$,

Table 1

Experimental Groups with N

Drug Cond.[a]	Day of T_1 1 day	7 day	21 day
Sal	42	41	57
Ani 1 mg	42	39	—
Ani 7 mg	42	40	40
Ani 1 mg x 7	—	42	—
Sal x 7	—	39	—

[a] Half of each group received reminder shock

$p<0.001$. Application of the Scheffé procedure [23] 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 [9] 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 footshock on retention test at various times after training are presented in Figure 2A, B, C. To determine the effectiveness of the noncontingent footshock 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 0.05 level of confidence would be expected by chance. We conclude that for the experimental conditions of this study the footshock reminder treatment is in and of itself an ineffective agent for the attentuation of the amnesia induced by protein synthesis inhibition. This conclusion was further tested and confirmed by performing a two-way analysis of variance with footshock reminder/nonreminder as the between subjects factor and test days as the within subjects factor. All groups were included except the saline groups first tested at 1 day because any improvement from T_1 performance could have been

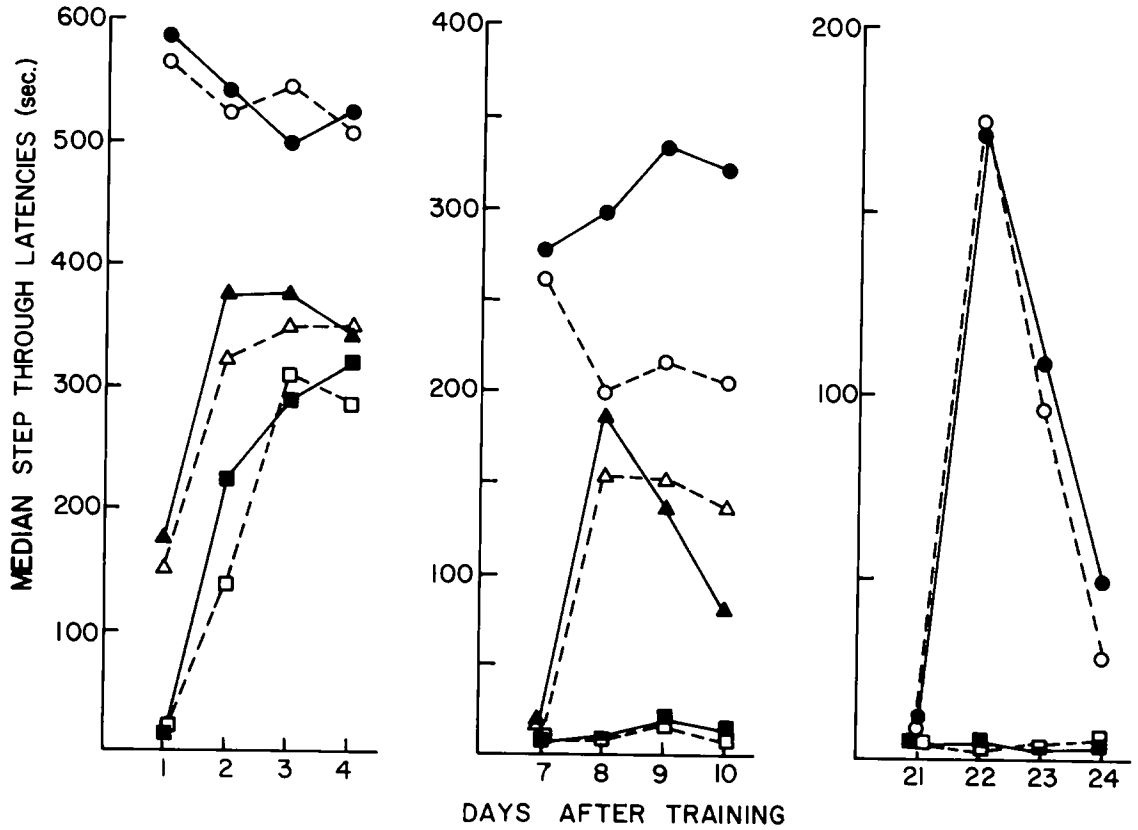

FIG. 2 A, B, and C. Median step-through latencies for mice first tested at either 1, 7 or 21 days after training and then given a single retention test on each of the 3 following days. The footshock reminder treatment was administered 1 hr after the initial test. The different groups are represented as follows: Saline, footshock reminder ●——●; Saline, no footshocks reminder ○ - - - - ○; 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.

Table 2

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:	95 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

masked by a ceiling effect 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, $F(1,380) = 1.28$, $p > 0.20$. Since none of these analyses indicated a significant 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 training-test interval (Fig. 2A, B, C). Five of these differences were significant beyond the 0.001 level (Sal 1 day vs. Sal 7 days, Sal 1 day vs. Sal 21 days, Sal 7 days vs. Sal 21 days, Ani 1 mg 1 day vs. Ani 1 mg 7 days, Ani 7 mg 1 day vs. Ani 7 mg 21 days); the remaining two were significant at beyond the 0.01 level (Ani 7 mg 1 day vs. Ani 7 mg 7 days, Ani 7 mg 7 days vs. Ani 7 mg 21 days).

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. 2A, B and Table 2).

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 when compared with saline controls and on Test Days 8–10 when compared with Ani 1 mg/animals (Table 2). 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 [10] and level [33] of protein synthesis 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 (2–4 days), but at Days 8–10 recovery occurred only in animals receiving a low drug dosage (1 mg/animal) (see Table 3). 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 acted as a reminder for both controls and drug treated animals only

Table 3

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

when their initial STLs indicated partial retention of the original training.

Although mutliple 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 2 (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 synthesis 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 4). 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 Ani 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 predicting 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 a 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 Fig. 3 in which animals were classified solely on the basis of their STL on the initial retention test and without regard to their

Table 4

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

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.

The high correlations obtained between initial test scores and retest scores (Table 4) are compatable with an interpretation of equal recovery in all animals irrespective of initial STL. However, this interpretation appears unlikely because, as demonstrated in Fig. 3, only animals showing an initial intermediate latency demonstrated recovery on a subsequent test.

VII. Controls for sickness and for effects of multiple testing. Results of control groups show that the amnesic effect of Ani could not be explained by possible prolonged sickness caused by the drug. For each experimental Ani

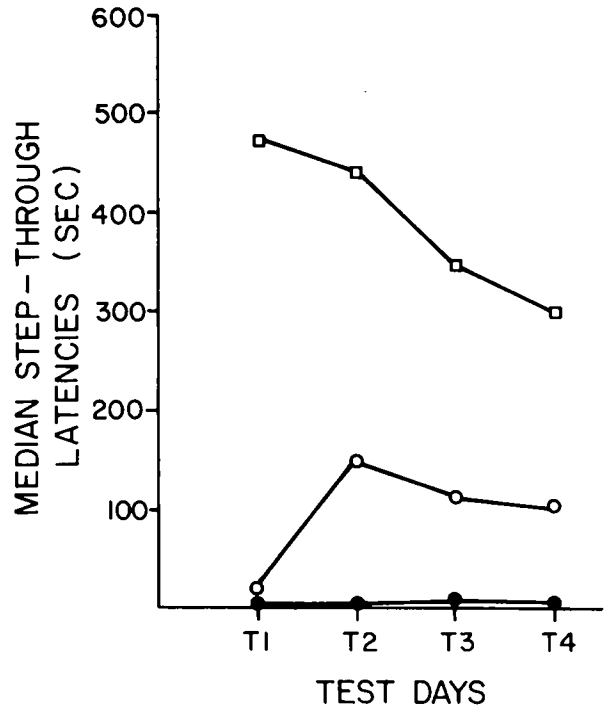

FIG. 3. Median step-through latencies (STL) for mice categorized solely on the basis of their initial STL irrespective of drug or training-test interval. An explanation for the determination of STL ranges is contained in the text. •———• STL 1–7 sec, including the following animals: Saline, N=27; Ani 1 mg, N=12; Ani 7 mg or Ani 1 mg × 7, N=73; Total N=112. o———o STL 8–200 sec: Saline, N=52; Ani mg, N=43; Ani 7 mg or Ani 1 ×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 × 7, N=10; Total N=136.

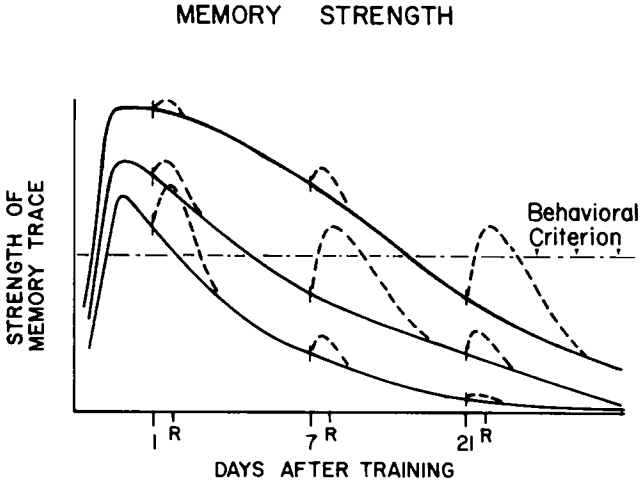

FIG. 4. An hypothesized model for explaining the effects of a reminder and/or re-exposure treatment. The solid lines represent memory traces of different strengths, which can be determined by such factors as degree of training, drug treatment, and training-test interval. Dashed lines show increases in 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.

group tested at 1 or 7 days, a corresponding group (N = 10 per 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 that did not differ significantly from scores of saline controls (Table 5). 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.

The control groups for the effects of multiple testing 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 ($p \geq 0.20$ for all comparisons except Ani 7 mg at 21 days which demonstrated $p < 0.06$ but with the difference detected being opposite to what recovery would have produced). The median STLs for these groups across all test days ranged from a low of 4 sec to a high of 14 sec. 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.

These controls that did not receive footshock on training thus afford a clear baseline against which to compare even weak memories. The only experimental groups showing no differences from the nonshock controls on one or more of the four tests were the animals treated with a high or multiple dose of Ani and first tested at Day 7 or 21: Day 7 (Ani 7 mg T_1 :$p \geq 0.74$, T_2 :$p \geq 0.10$, T_3 :$p \leq 0.05$, T_4 :$p \geq 0.10$; Ani 1 mg × 7 T_1 :$p \geq 0.15$, T_2 :$p \leq 0.05$, T_3 :$p \leq 0.05$, T_4 :$p \geq 0.15$); Day 21 (Ani 7 mg T_1 :$p \geq 0.73$, T_2 :$p \geq 0.81$, T_3 :$p \leq 0.01$ with the control group median being greater, T_4 :$p \geq 0.46$). Since these experimental groups did not differ from naive controls at several test points, they can be considered to be strongly amnesic. The fact that some Ani-injected groups demonstrated a near total amnesia while others were only partially amnesic provides additional evidence that both drug dosage and training-test interval are effective methods of manipulating the degree of amnesia.

The possibility that the behavioral deficit observed in Ani treated animals is due to a lack of acquisition rather than a lack of retention has been considered previously by one of the authors [6]. In general, a consistent finding in studies utilizing protein synthesis inhibitors has been their lack of effect upon acquisition and short-term memory, in contrast to their effects on long-term memory [4, 6, 10, 32, 33].

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 [16, 21, 25, 26, 27, 30, 34, 35]. 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 [14, 17, 20] and retrieval hypothesis [4, 15, 22] have been published.

Table 5

Results of tests for drug-induced sickness, drug animals injected 2 hr. after training*

		T_1	T_2	T_3	T_4
Day 1 ⟶ Day 4					
Ani 1mg vs. Sal	medians:	483 vs. 584	504 vs. 530	506 vs. 522	526 vs. 517
	p-values:	.20	.87	.95	.99
Ani 7mg vs. Sal	medians:	527 vs. 584	499 vs. 530	449 vs. 522	510 vs. 517
	p-values:	.61	.70	.90	.99
Day 7 ⟶ Day 10					
Ani 1 mg vs. Sal	medians:	483 vs. 278	504 vs. 264	282 vs. 251	259 vs. 245
	p-values:	.63	.91	.99	.99
Ani 7mg vs. Sal	medians:	359 vs. 278	332 vs. 264	306 vs. 251	267 vs. 245
	p-values:	.17	.85	.95	.94
Ani 1mg X 7 vs Sal X 7	medians:	400 vs. 368	346 vs. 318	365 vs. 212	389 vs. 191
	p-values:	.54	.92	.38	.12

*N=10 for all sickness control groups

In brief, the retrieval-impairment interpretation of RA is supported by studies demonstrating reminder-induced or spontaneous recovery of memory. 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. Interpretation 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 [5] 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 [14] found that recovery occurred only in animals made partially amnesic by electroconvulsive shock (ECS), 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 [13, 16, 21] in which it was found that a reminder treatment improved the retention performance of poorly trained animals that received no amnestic treatment and thus could not have had a retrieval block induced by ECS. A physiological reminder may induce recovery in a similar fashion or it may improve performance by modulation of arousal and/or attentional mechanisms [3,11]. Turning to spontaneous 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 [24, 28, 29, 30, 31, 34] showed that this phenomenon occurred only under strong training conditions or when retention was evaluated with multiple testing. Furthermore, one of these studies [31] that had frequently been cited as demonstrating spontaneous recovery has been reported by its authors to be unreproducible after changing their animal supply source [32]. 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 are consistent with 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 synthesis 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 synthesis inhibitor (see Figs. 2B and C).

The interpretation of our results as consistent with 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 a partial recovery. These results are in good accord with data from other studies reporting a within-group analysis of the recovery phenomenon [5,14].

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, 24, 25, 26, 28, 29, 34]. 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 training occurred only at a short training-test interval [24, 25, 26] and/or following strong training [24]. Thus, recovery occurred under conditions when it would be likely that animals would have partial retention of the training conditions and when a behavioral reminder [25,26] or multiple retention tests [24, 25, 26] could summate with the existing memory engram.

In presenting evidence for the retrieval hypothesis, Quartermain *et al.* have reported the degree of recovery to be nearly 100% and the durability of the recovered memory to be equivalent to the memory demonstrated by saline controls [24, 25, 26]. However, because these investigators used a relatively low ceiling for latencies in retention testing (180 sec) it is questionable whether recovery was 100% and durable. For example, if in the present study we had used a 180 sec STL score cut-off, we could likewise have reported that inhibitor-treated mice showed total recovery and a durability of memory equivalent to that of saline controls for the retention tests given on Test Days 1–4. However, by increasing the observation period to 600 sec we found that the animals treated with a protein inhibitor recovered only partially. The partial recovery shown by animals tested on Days 7–10 was attenuated on T_3 and T_4 (Fig. 2B) and was not completely durable. Thus, the recovery of amnesic animals after a footshock reminder and/or re-exposure to the training situation does not necessitate invoking the retrieval hypothesis to explain the RA induced by protein synthesis inhibition.

III. A model reconciling recovery with the consolidation hypothesis. A simple model based on the consolidation hypothesis is capable of encompassing and reconciling the data obtained to date on protein inhibitor 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 [5, 14, 17], but the model has not been spelled out fully before. According to this model, treatment with a protein synthesis 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. 4); 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. 4). 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. 2C and 3).

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 the results of this experiment refute the retrieval hypothesis. The partial recovery from amnesia following a reminder in these experiments could be accounted for by either the consolidation or retrieval hypothesis. However, the finding that retention in normal (non-drug treated) animals 21 days after training is similarly improved by a reminder treatment severely limits the interpretation that inhibitors of protein synthesis induce a blockage of retrieval. The recovery of control animals does not necessarily exclude a retrieval hypothesis nor does it directly support a consolidation hypothesis since it is possible that normal forgetting is a retrieval deficit. However, for the retrieval hypothesis to be consistent with the general finding that amnesia occurs only when inhibition of protein synthesis is achieved during or shortly after training, it is necessary to make the additional assumption that during this time a physiological process for the storage of memory is left intact while a process for retrieving the memory is impaired. Such an assumption seems unwarranted considering our limited knowledge of the physiological mechanisms subserving memory. Instead, we would propose that a reminder in some manner increases the sensitivity of the behavioral measure for both normal and experimental animals, that there is presently no justification for compartmentalizing memories into retrieval and storage components, and that recovery is not unexpected under certain conditions and thus does not necessarily lend support to a retrieval hypothesis. While a definitive distinction between the consolidation and retrieval hypotheses is not likely to be achieved until investigators have elucidated the physiological substrate of memory, we believe, based upon the behavioral data available to date, that the consolidation hypothesis offers the most parsimonious explanation for memory trace formation, interference by amnestic agents, and recovery from amnesia.

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