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Author

Flood, James F.

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James F. Flood, Murray E. Jarvik, Edward L. Bennett,
Ann E. Orme, and Mark R. Rosenzweig

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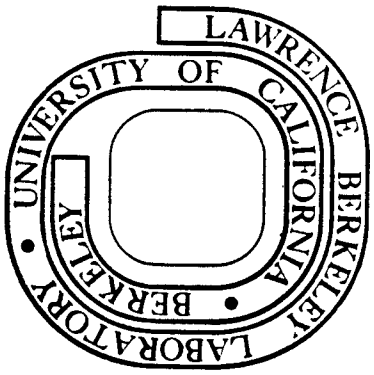

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Protein Synthesis Inhibition and Memory Modification With Stimulants and Depressants

Lawrence Berkeley Laboratory
University of California
Berkeley, CA 94720

ABSTRACT

Post-training administration of the stimulants caffeine or nicotine blocked the amnesia for a passive avoidance task produced by pretraining administration of the protein synthesis inhibitor anisomycin. Using more vigorous training conditions in which anisomycin did not produce amnesia by itself, post-training administration of the depressants chloral hydrate or sodium phenobarbital caused amnesia. Stimulants and depressants did not have an appreciable influence on the degree of inhibition produced by anisomycin. The results supported the hypothesis that post-training arousal is an important factor in the conversion of short-term to long-term memory.

To test the hypothesis that arousal facilitates memory consolidation, we have determined whether excitant drugs could counteract the amnesic effects caused by inhibition of cerebral protein synthesis and whether depressant drugs would enhance the amnesia. Some evidence has already been presented that amphetamine administered after training can block the amnesia induced when either cycloheximide or acetoxycycloheximide inhibit protein synthesis. (1). We have tested the generality of these findings by using the stimulants nicotine and caffeine and by using anisomycin (Ani) to inhibit protein synthesis. We have also extended the scope of such experiments by using depressants (chloral hydrate and sodium phenobarbital) as well as stimulants. Since the drug producing arousal and depression were found to produce significant effects on retention seven days after training and drug administration, we have tested whether the excitant or depressant drugs affected the extent and/or duration of protein synthesis inhibition caused by Ani.

The subjects were CD-1 male albino mice, 60-80 days of age and were obtained from Charles Rivers Breeding Laboratories. The mice were trained on a one-trial, step-through passive avoidance task which has been described elsewhere (2). In brief, the passive avoidance apparatus consists of a black start compartment joined to a white shock compartment by a partition containing a mouse-hole. Subjects were permitted to enter the white compartment through the mouse-hole where they received footshock (0.32 ma) until they returned to the black start compartment. On the retention test given one week after training, the mice were placed into the black compartment and the time required to enter the white compartment was taken as a measure of retention. A latency-to-enter the white shock compartment on the

test day of 20 sec or less was defined as amnesia, since this represents the longest entry time for naive mice. Most trained non-amnesic mice did not enter the white compartment within three minutes. The percentage of mice not entering within 20 sec is defined as "% amnesia".

Ani was dissolved in an approximately equal molar amount of HCl and the pH was finally adjusted to 6-7. The final solution was 2.0 mg/ml in 0.9% saline. Mice received the first subcutaneous Ani injection (20 mg/kg) 15 min prior to training, the second 1-3/4 hrs after training. When a third injection was used it was given 3-3/4 hrs after training. Saline was administered subcutaneously to other groups as a control for the stress of the Ani injections. Animals that received stimulants were administered low doses of caffeine citrate (20 mg/kg) or nicotine hydrochloride (0.5 mg/kg) intraperitoneally (IP), 30 min after training. Saline was administered, IP, 30 min after training to control for the arousal and stress of the injection procedure. The 8 groups in the experiment are shown in Table 1.

Two or three successive injections of Ani (group 3 and 4) caused significant amnesia (74%, 70% respectively), whereas two or three successive injections of saline (groups 1 and 2) caused little amnesia (17%, 20%). The amnesia caused by two successive injections of Ani was blocked by the post-training administration of either caffeine (group 5, 23% amnesia) or nicotine (group 7, 21% amnesia). Extending the duration of protein synthesis inhibition by a third Ani injection blocked the antiamnesic effect of the stimulants and re-established high levels of forgetting (group 6, caffeine and three Ani injections

67%; group 8, nicotine and three Ani injections, 72% amnesia). We have in addition unpublished data that indicate that similar effects can be obtained with amphetamine, strychnine, and picrotoxin. Each stimulant acts as an antiamnesic, but its effect can be blocked by giving additional injections of Ani.

If stimulants act as antiamnesics then depressants might be expected to potentiate the effect of protein systhesis inhibition on memory. To test this, chloral hydrate and sodium phenobarbital was administered to mice/two successive injections of Ani, given It was necessary to increase the footstock intensity to 0.36 ma so that the two Ani injections would not cause amnesia. The apparatus, subjects and other conditions were as described above. The three Ani injection times were #1, 15 min prior to training, #2, 1-3/4 hrs after training, and #3 (if given), 3-3/4 hrs after training. Sodium phenobarbital (Pheno) 125 mg/kg or chloral hydrate (CH) 300 mg/kg were administered IP, 30 min after training. Saline was administered IP to control for the stress of the depressant injections. The groups are shown in Figure 1.

Under the higher footshock conditions, two successive injections of Ani did not cause amnesia. This replicates previous findings that as footshock intensity increases a given number of Ani injections has a decreasing amnesic effect (3). Three successive injections of Ani caused significantly greater amnesia (75%) than was present in the saline control group (0%) or in the mice receiving two successive injections of Ani (10% amnesia). (Note that with the stronger training, the saline-only control group showed less amnesia than the saline-only groups 1 and 2 in Experiment 1.) Both chloral hydrate and sodium phenobarbital potentiated the effect of two successive injections of

Ani, so that Ani(CH)Ani yielded 80% anmesia and Ani(Pheno)Ani caused 70% amnesia; these values did not differ significantly from those obtained after three successive injections of Ani [Ani(Sal)Ani + Ani] (75% amnesia) (Fig. 1). Pilot work done prior to the study showed that neither depressant given alone 30 min after training had an effect upon retention when the 0.36 ma footshock was used for training. Depressants thus have the opposite effect of the stimulants in that they potentiate the amnesia caused by Ani.

The results of these experiments support the hypothesis that post-training arousal is an important factor in the conversion from short-term to long-term memory known as consolidation. It is very important for the interpretation of these results to know whether the stimulants and depressants affected protein synthesis inhibition. One possible interpretation of the results above is that the stimulants blocked or reduced the inhibition of protein synthesis, while the depressants increased the extent and/or duration of protein synthesis inhibition. To test this possibility, we administered the drugs and determined the ratio of radioactivity resulting from incorporation of subcutaneously administered [^{14}C(U)]-L-valine into the trichloracetic acid insoluble protein fraction to the total activity of the brain sample by techniques previously described (2). From previously published work (4), it was determined that a single injection of Ani inhibits protein synthesis at 80% or more for 2 hrs and each successive injection of Ani administered every two hours extends the duration of inhibition by 2 hrs. The duration of inhibition by anisomycin was not affected either by the stimulants or by the depressants; nor did they have an appreciable effect on the degree of inhibition. We also studied the effect of the stimulants and

depressants in mice not given Ani and found that only very low levels of inhibition for short durations resulted after the administration of either stimulants or depressants. Thus the mechanisms of action whereby the stimulants and depressants are able to affect long-term memory loss do not involve direct modification of protein synthesis.

Another interpretation to be considered is that the stimulants and depressants had proactive effects on the retention test performance: that is, that the drugs did not affect consolidation of memory but affected performance during recall. Such an interpretation is more plausible when the retention test occurs only minutes following administration of ampehtamine (5) than in our experiments where the retention test follows one week after training and drug treatment. In addition, we have found that other stimulants (amphetamine, strychnine and picrotoxin) show time-dependent antiamnesic effects such that they are most likely to block Ani-induced amnesia the closer to training they are administered (and thus, the farther from testing). We have not found any stimulant to block Ani-induced amnesia when administered 240 min after training.

Another possible interpretation is that the stimulants and depressants alter the life of the short-term memory trace. If stimulants enable the short-term trace to persist beyond the period of protein inhibition, then the conversion from short-term to long-term can proceed when protein synthesis has recovered. This could account for the low level of amnesia in groups 5 and 7 of Experiment 1. But the effects of the stimulants could then be overcome by extending the duration of inhibition beyond the life of the short-term trace, and amnesia would again result. This was found to occur in groups 6 and 8 of Experiment 1.

If arousal (pharmacologically induced) extends the life of the short-term trace, then depressants should reduce the life of the short-term memory trace. Thus amnesia would occur even with relatively short periods of protein synthesis inhibition. This was found to occur in Experiment 2. Two successive injections of Ani did not cause amnesia but Ani(CH)Ani yielded 80% amnesia which did not differ from the effect of three successive Ani injections (Ani(Sal)Ani+Ani = 75% amnesia).

The level of posttraining arousal can also be varied according to whether or not footshock is used to motivate the animal. We have shown elsewhere that the number of seconds during which shock is given to mice throughout training is directly related to the duration of inhibition of protein synthesis required to cause amnesia for the training (4). Thus the level of arousal, whether manipulated by drugs or by training procedures, appears to affect the life of the short-term memory trace; this in turn controls the time period over which conversion from short-term memory can occur. The longer the period available for this conversion, the stronger is the memory trace or the less susceptible is the process of consolidation of long-term memory to disruption by inhibition of protein synthesis.

James F. Flood

Murray E. Jarvik

Department of Psychiatry
University of California
Los Angeles, 90024

Edward L. Bennett

Ann E. Orme

Lawrence Berkeley Laboratory
University of California
Berkeley, 94720

Mark R. Rosenzweig

Department of Psychology
University of California
Berkeley, 94720

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Figure 1 Effect of chloral hydrate and phenobarbital on anisomycin-induced amnesia for passive avoidance training. The depressants increased amnesia, and the resulting amnesia was equivalent to that obtained with three injections of Ani. The N for each group was not less than 20 mice with the largest group having 31 mice.

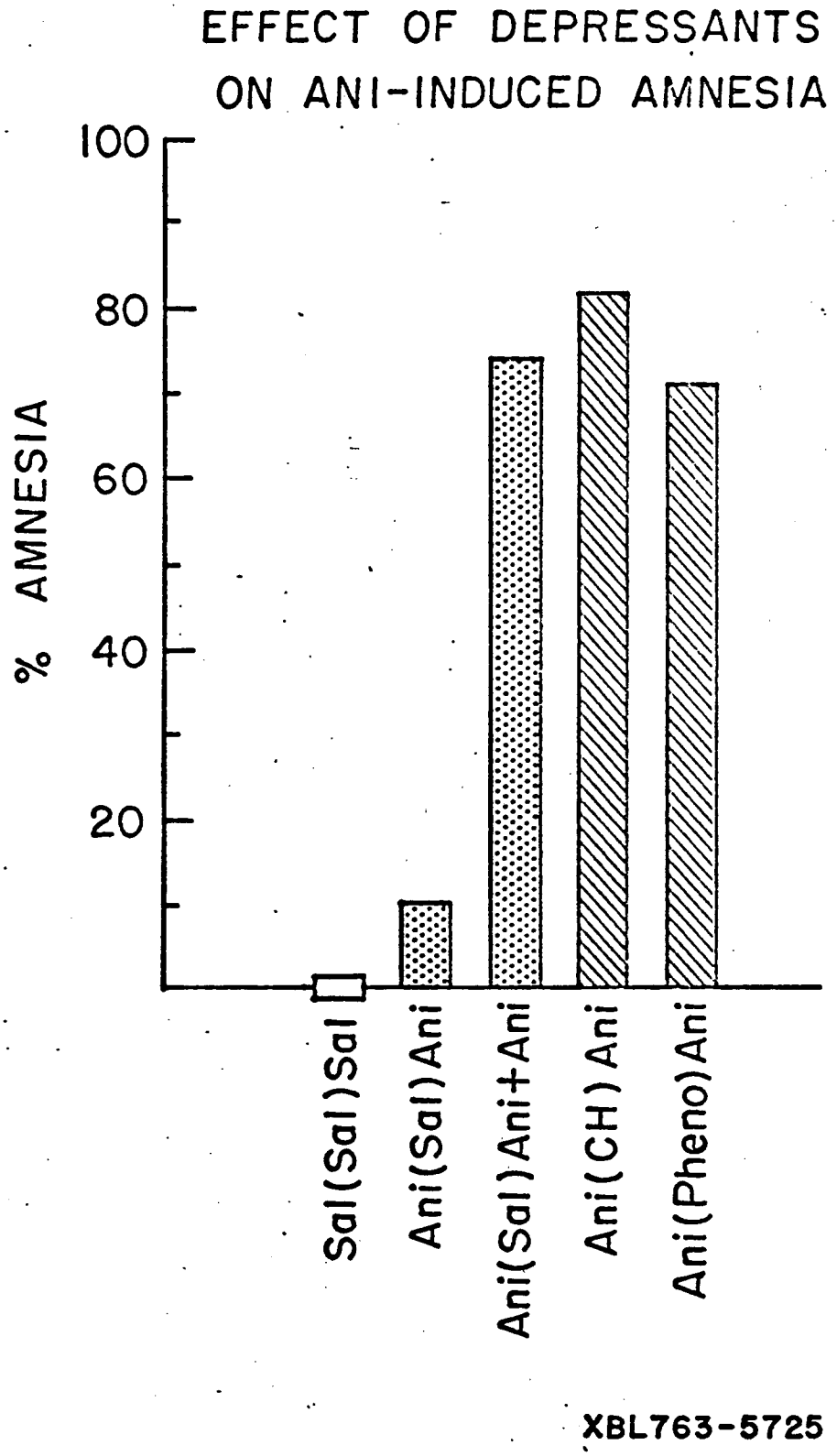

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Fig. 1

Table 1. Effect of caffeine or nicotine on Anisomycin induced amnesia for a passive avoidance test using CD-1 male albino mice. An additional injection of Ani overcame the anmesia-blocking effects of the stimulants.

Group	Injection: First (Subcut) -1/4 h	Second (IP) 1/2 h	Third (Subcut) 1-3/4 h	Fourth (Subcut) 3-3/4 h	N	% Amnesic Mice*
			Time from Training			
1	Sal	Saline	Sal	-	23	17
2	Sal	Saline	Sal	Sal	25	20
3	Ani	Saline	Ani	-	23	74
4	Ani	Saline	Ani	Ani	20	70
5	Ani	Caffeine	Ani	-	21	23
6	Ani	Caffeine	Ani	Ani	21	67
7	Ani	Nicotine	Ani	-	19	21
8	Ani	Nicotine	Ani	Ani	25	72

* Group 5 and 7 each differ from Group 3 beyond the .001 level; Group 8 differs from Group 7 beyond the .001 level, and Group 6 differs from Group 5 beyond the .005 level.

TECHNICAL INFORMATION DIVISION
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720