moved only modulating influences, we should see behavior typical of an earlier rather than of a later developmental stage.

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4. The characteristic response during sleep and before 2 months of age is solely or primarily accelerative, as are the "defensive" and startle reflexes elicited by intense and sudden stimulation, respectively. In contrast, the cardiac orienting response to novel or informative stimuli shows monophasic deceleration beginning within the first or second cardiac cycle and lasting for several seconds, generally peaking between 3 and 7 seconds. The shift from an accelerative to a decelerative response during early development is not an all-or-none matter but a change in the probabilities that specific stimuli will elicit slowing. Under optimal conditions of state and stimulus characteristics, deceleration has been obtained even in the newborn. For reviews, see F. K. Graham and J. C. Jackson (*5*) and R. K. Clifton [in *Cardiovascular Psychophysiology*, P. Obrist, A. H. Black, J. Brener, L. DiCara, Eds. (Aldine, Chicago, 1974), p. 479]. See also W. K. Berg [*J. Exp. Child Psychol.* **17**, 303 (1974)], L. A. Leavitt *et al.* (*6*), and J. W. Brown, L. A. Leavitt, F. K. Graham [*Devel. Psychobiol.* **10**, 255 (1977)].
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14. The number and kind of stimulations given at each intensity.

db	Sine wave	FM triangular wave	Speech syllable	White noise
75	12	8	10	0
81	49	44	96	0
95	27	42	0	0
109	20	0	0	22

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Memory: Modification of Anisomycin-Induced Amnesia by Stimulants and Depressants

Abstract. *Mice were trained in a passive (foot shock) avoidance task. When administered after training, the stimulants caffeine or nicotine blocked amnesia for the task that had been produced by injections of the protein synthesis inhibitor anisomycin given prior to training. With foot shock at a higher intensity, anisomycin did not produce amnesia by itself, but the administration of the depressants chloral hydrate or sodium phenobarbital after training did cause amnesia. Stimulants and depressants did not have an appreciable influence on the overall degree of protein synthesis inhibition produced by anisomycin. The results support the hypothesis that arousal after training is an important factor in the conversion of short-term to long-term memory.*

To test the hypothesis that arousal facilitates memory consolidation, we determined whether excitant drugs could counteract the amnesic effects caused by inhibition of cerebral protein synthesis and whether depressant drugs could enhance the amnesia. Amphetamine administered after training can block the amnesia induced when cycloheximide or acetoxycycloheximide inhibit protein synthesis (*1*). We tested the generality of these findings by using the stimulants nicotine and caffeine and by using anisomycin to inhibit protein synthesis. We also extended the scope of such experiments by using depressants (chloral hydrate and sodium phenobarbital). Since the drugs producing arousal and depression produced significant effects on retention 7 days after training and drug administration, we tested whether the drugs affected the extent and duration of protein synthesis inhibition caused by anisomycin.

The subjects, CD-1 male albino mice 60 to 80 days of age (Charles River), were trained on a one-trial, step-through passive avoidance task (*2*). In brief, the avoidance apparatus consisted of a black compartment (start) joined to a white compartment (where shock was administered) by a partition containing a mousehole. Subjects were permitted to enter the white compartment through the mousehole where they received foot shock (0.32 ma) until they returned to the black compartment. The subjects were trained in a black-to-white situation because we found that this produced a considerably smaller variation in the time (latency) taken by the mice to enter the shock compartment than when subjects were trained in the opposite direction; typically, 80 percent of the mice enter in 2 seconds. To reduce and control the variability of the latencies to enter and escape the shock compartment, which control the degree of learning (*2*), only subjects with latencies of 1 to 3 seconds in entering and 1 to 4 seconds in escaping the shock compartment were used. On the retention test given 1 week after training, the mice were again placed into the black compartment and the time each mouse 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 seconds or less was defined as amnesia, since this represented the longest entry time for naive mice. Most trained nonamnesic mice did not enter the white compartment within 3 minutes. The percentage of mice entering within 20 seconds was defined as "percentage amnesia."

Anisomycin was dissolved in an approximately equal molar amount of 3*N* HCl, and the *p*H was finally adjusted to 6 to 7 with dilute HCl or NaOH as required. The final solution was 2.0 mg/ml in 0.9 percent saline. Mice received the first subcutaneous anisomycin injection (20 mg/kg) 15 minutes prior to training, the second 1.75 hours after training. When a third injection was used it was given 3.75 hours after training. Saline was administered subcutaneously to other groups as a control for the stress of the

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anisomycin injections. Injections prior to training were made under light ether anesthesia. Animals that received stimulants were administered low doses of caffeine citrate (20 mg/kg) or nicotine hydrochloride (0.5 mg/kg) intraperitoneally 30 minutes after training. Saline was administered intraperitoneally 30 minutes after training to control for the arousal and stress of the injection procedure. The ten groups in the experiment are shown in Table 1.

We have additional data (*3*) that indicate that effects similar to those shown in Table 1 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 anisomycin. Not unexpectedly, caffeine and nicotine produced lower amnesic scores when administered to saline-injected subjects than was found in the saline controls. However, the difference was not statistically significant (groups 9 and 10 versus group 1).

If stimulants act as antiamnesics, then depressants might be expected to potentiate the effect of protein synthesis inhibition on memory. To test this, chloral hydrate or sodium phenobarbital was administered to mice given two successive injections of anisomycin. It was necessary to increase the foot-shock intensity to 0.36 ma so that the two injections of anisomycin would not cause amnesia. In addition, to further control the degree and the variability of learning, subjects had to enter the shock compartment in 2 to 4 seconds and escape in 1 to 4 seconds (the mean latencies for entering were between 2.2 and 2.4 seconds across groups; the mean latencies for escaping were between 2.4 and 2.6 seconds across groups; the standard deviations were of similar magnitude with the largest being ± 0.93). The apparatus, subjects, and other conditions were as described above. Three injections of anisomycin were given: first, 0.25 hour prior to training; second, 1.75 hours after training; and third (if given), 3.75 hours after training. Sodium phenobarbital (125 mg/kg) or chloral hydrate (300 mg/kg) were administered intraperitoneally 30 minutes after training. Saline was administered intraperitoneally to control for the stress of the depressant injections. The groups are shown in Fig. 1.

Under the higher foot-shock and latency conditions used, two successive injections of anisomycin did not cause amnesia. This replicates previous findings that as training strength increases, a given number of anisomycin injections has a decreasing amnesic effect (*4*). With the higher intensity of foot shock, the saline-only control group showed less amnesia than the groups given saline only (Table 1, groups 1 and 2). In a study run prior to that shown in Fig. 1, the groups Sal(Sal)Sal, Sal(Pheno)Sal, and Sal(CH)Sal yielded 0, 0, and 10 percent amnesic scores, respectively (N = 10 in each group). The conditions of training, testing, drug administration, and dosage were the same as above. Thus, under these conditions of training, the depressants alone do not account for the high percentage of subjects having amnesia when anisomycin and a depressant are used together. Depressants thus had the opposite effect of the stimulants in that they potentiated the amnesia caused by anisomycin.

Table 1. Effect in CD-1 male albino mice of caffeine or nicotine on anisomycin-induced amnesia for a passive avoidance test. An additional injection of anisomycin overcame the amnesia-blocking effects of the stimulants. The second injection was given intraperitoneally; all others were given subcutaneously. The first injection was given prior to training; the second, third, and fourth injections were given after training.

Group	Injection and time from training (hours)				N	Amnesic mice* (%)
	First 0.25	Second 0.50	Third 1.75	Fourth 3.75		
1	Saline	Saline	Saline		23	17
2	Saline	Saline	Saline	Saline	25	20
3	Anisomycin	Saline	Anisomycin		23	74
4	Anisomycin	Saline	Anisomycin	Anisomycin	20	70
5	Anisomycin	Caffeine	Anisomycin		21	23
6	Anisomycin	Caffeine	Anisomycin	Anisomycin	21	67
7	Anisomycin	Nicotine	Anisomycin		19	21
8	Anisomycin	Nicotine	Anisomycin	Anisomycin	25	72
9	Saline	Caffeine	Saline		20	5
10	Saline	Nicotine	Saline		20	10

*Groups 5 and 7 each differ from group 3 at $P < .001$; group 8 differs from group 7 at $P < .001$; and group 6 differs from group 5 at $P < .005$ (chi-square test).

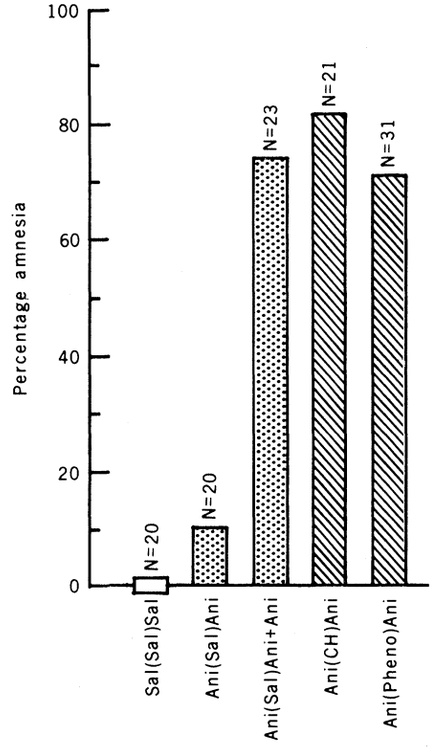

Fig. 1. Effect of chloral hydrate (CH) and phenobarbital (Pheno) on anisomycin(Ani)-induced amnesia for passive avoidance training. The depressants increased amnesia significantly [Ani(Sal)Ani versus Ani(CH)Ani, $P < .001$, $\chi^2 = 27.86$; Ani(Pheno)Ani, $P < .001$, $\chi^2 = 18.97$]. The depressants increased the level of amnesia to that comparable with three injections of anisomycin.

The results of these experiments support the hypothesis that arousal after training 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 is that the stimulants blocked or reduced the inhibition of protein synthesis, while the depressants increased the extent 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 [U-^{14}C]-L-valine into the trichloroacetic acid insoluble protein fraction to the total activity of the brain sample by techniques previously described (*2*). A single injection of anisomycin inhibits protein synthesis 80 percent or more for 2 hours, and each successive injection of anisomycin administered every 2 hours extends the duration of inhibition by 2 hours (Fig. 2A). Chloral hydrate or phenobarbital caused a maximum inhibition of protein synthesis of approximately 35 percent at 1.5 hours after administration. In addition, the duration of inhibition of protein synthesis was not affected by either depres-

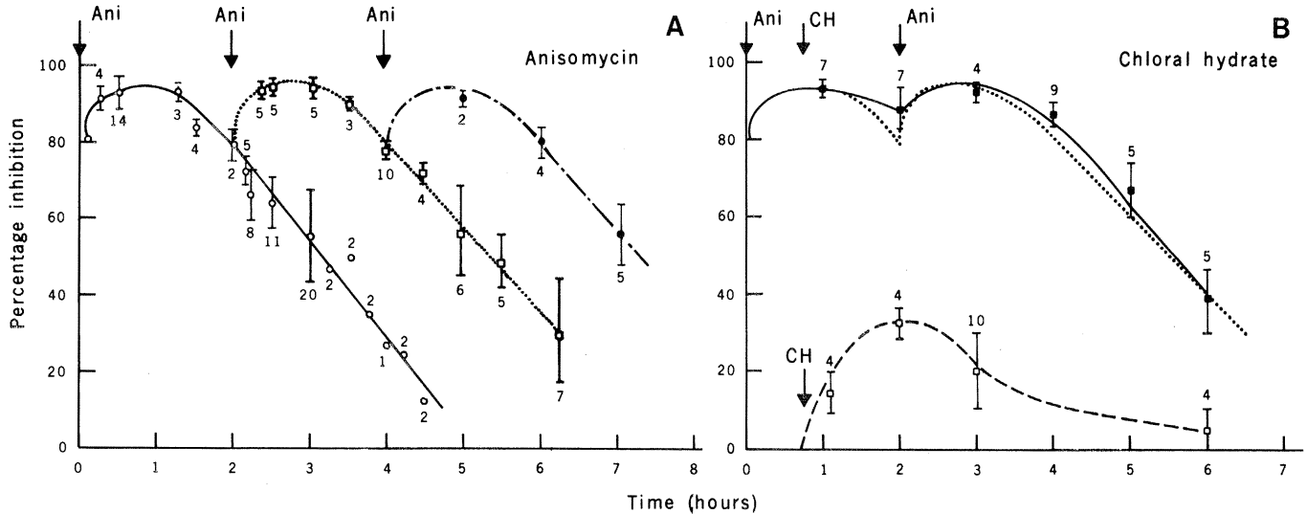

Fig. 2. (A) Inhibition of the rate of protein synthesis by successive subcutaneous administration at 2-hour intervals of one, two, or three doses of anisomycin (20 mg/kg) to Swiss mice. The successive curves show the inhibition produced by a single injection of anisomycin (———), by a second injection of anisomycin (·····), and by a third injection (-·-·-·). (B) The influence of chloral hydrate (300 mg/kg) alone (------), and in combination with anisomycin (———). The inhibition by two successive doses of anisomycin is also shown (·····). The results obtained with phenobarbital are similar to those obtained with chloral hydrate. The number (N) per group is shown at each data point.

sant, nor did the depressants have an appreciable effect on the degree of inhibition (Fig. 2B). Caffeine or nicotine did not decrease the extent or duration of anisomycin-induced inhibition. In the absence of anisomycin, a small (<10 percent) apparent inhibition rather than stimulation of protein synthesis was observed 1.5 hours after administration of the stimulants. Thus the mechanisms of action whereby the stimulants and depressants are able to affect long-term memory loss do not involve direct modification of the rate of protein synthesis.

Another interpretation 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 plausible when the retention test occurs only minutes after the administration of amphetamine (5), but not in our experiments where the retention test follows 1 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 anisomycin-induced amnesia the closer to training they are administered (and thus, the farther from testing). We have not found any stimulant to block anisomycin-induced amnesia when administered 4 hours 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 (Table 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 (Table 1).

If pharmacologically induced arousal 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 occurred in the experiment in Fig. 1. Two successive injections of anisomycin did not cause amnesia, but the sequence Ani(CH)Ani (Fig. 1) yielded 80 percent amnesia which did not differ from the effect of three successive anisomycin injections [Ani(Sal)Ani + Ani, 75 percent amnesia].

The level of arousal after training can also be varied according to whether or not foot shock 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 (5). 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 consolidation can occur. The longer the period available for consolidation, the stronger is the memory trace or the less susceptible is the process of consolidation to disruption by inhibition of protein synthesis.

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