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Publication Date

1979-06-01



Lawrence Berkeley Laboratory

UNIVERSITY OF CALIFORNIA

CHEMICAL BIODYNAMICS DIVISION

Submitted to Behavioral and Neurobiology

LAWRENCE
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JUL 9 1979

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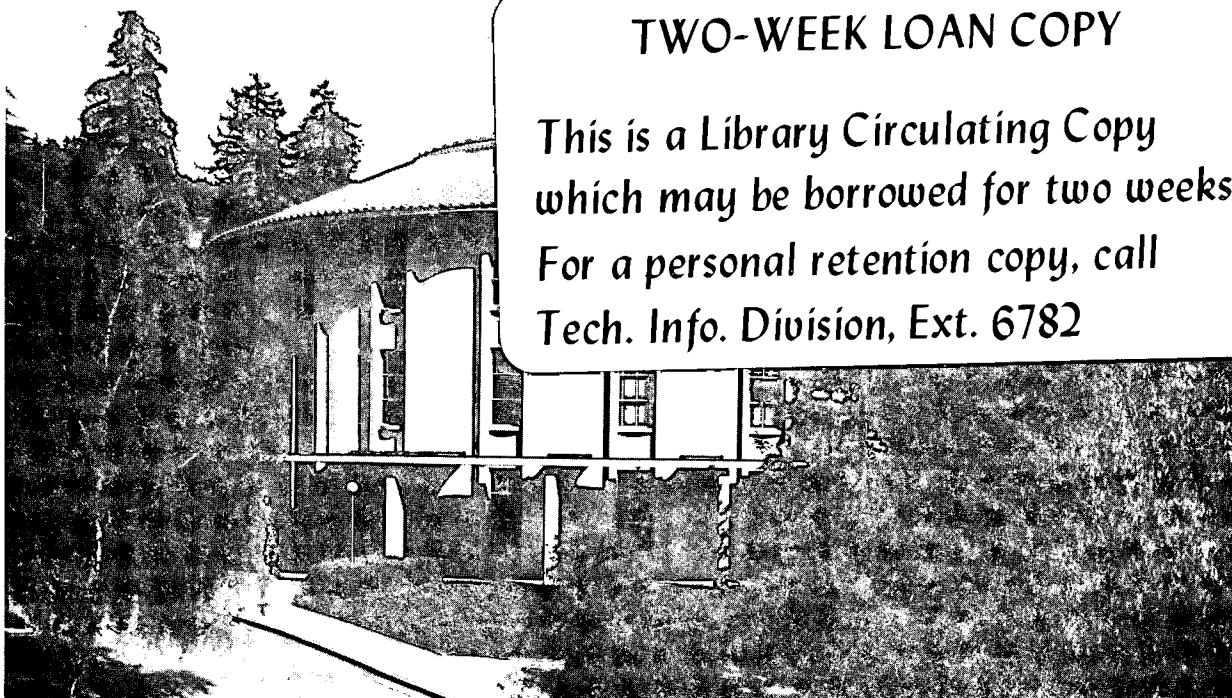
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June 1979

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LBL-9338 C. 2

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INHIBITION OF CEREBRAL PROTEIN SYNTHESIS: DISSOCIATION
OF NONSPECIFIC EFFECTS AND AMNESIC EFFECTS.

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Abstract

Injection of 210 mg/kg of anisomycin 5 hr prior to training produced more nonspecific behavioral side effects at the time of training than did a low dosage (30 mg/kg) given 20 min prior to training. Yet the low dosage 20 min pre-training produced greater protein synthesis inhibition at training and greater impairment of retention of passive avoidance training than did the high dosage 5 hr pre-training. These results demonstrate that the level of protein synthesis inhibition at or near the time of training is the critical factor for inducing amnesia, and not nonspecific side effects following treatment with a protein synthesis inhibiting drug. Conditions required for inducing amnesia according to various alternative hypotheses are also satisfied better by the high dosage of anisomycin given 5 hr prior to training than by the amnesic low dose given 20 min prior to training. Thus, these results provide further support for the hypothesis that brain protein synthesis is required for long-term memory formation.

When antibiotic drugs that inhibit cerebral protein synthesis are administered shortly before or shortly after training, they markedly impair long-term retention in a variety of species and for a variety of tasks (Flood & Jarvik, 1976). The usual interpretation of these findings is that cerebral protein synthesis is not required for acquisition or short-term memory but is required for the formation of long-term memory. It is possible, however, that these drugs act by producing some nonspecific effect on acquisition or retention rather than by specifically inhibiting the synthesis of cerebral proteins required for memory formation. One possible way this might occur is that inhibiting protein synthesis causes sickness.

The possible role of sickness is typically evaluated by administering the protein synthesis inhibitor several hours after training and then testing for retention after the drug has been metabolized. If the poor retention of animals treated prior to training were due to illness, then animals injected several hours after training should have as poor retention as animals injected before training. Since animals injected hours after training have normal retention, illness has usually been considered not to contribute to poor retention. A difficulty with this type of control group is that it rules out only those nonspecific effects that might occur after the time of injection. Nonspecific effects that could operate closer to the time of training have been evaluated by comparing the amnesic effect of a dosage of protein synthesis inhibitor given minutes before training with the effect of the same dosage given hours before training

(Barondes & Cohen, 1967; Squire & Barondes, 1976). Although no independent measure of nonspecific side effects was obtained in these studies, injections given hours before training did not effect retention, provided capacity for cerebral protein synthesis had sufficiently recovered by the time of training. For a more vigorous test of the nonspecific side effects of the protein synthesis inhibitors, the present study treated animals with different drug dosages at different times prior to training and made assessments of the resultant nonspecific side effects at the time of training.

Specifically, we injected mice with a high dose (210 mg/kg) of anisomycin (ANI) 5 hr prior to training or a low dose (ANI 30 mg/kg) 20 min prior to training. Nonspecific effects were assessed by observing locomotor activity and asking raters to make blind judgments for symptoms of overt sickness. We found that sickness at the time of training was greater following the high dose of ANI. However, because ANI-induced inhibition of synthesis begins declining after approximately 2 hr (Davis, Rosenzweig, Bennett, & Orme, 1978), the level of inhibition of brain protein synthesis was marginal for the induction of amnesia. In the case of the low dose of ANI, inhibition of cerebral protein synthesis was high at the time of training, but sickness was relatively absent. The rationale for this experiment is illustrated in Table 1. If the illness hypothesis is correct, then the large dose of ANI (210 mg/kg) would produce the greater impairment of performance at test (B' in Table 1), but if the level of protein synthesis inhibition is critical, then the

low dose of ANI (30 mg/kg) should produce the greater impairment of retention (A' in Table 1).

Male Swiss-Webster CD-1 mice were used for both the biochemical and behavioral parts of this experiment. The method for evaluating protein synthesis inhibition has been described in detail previously (Davis et al., 1978). In brief, mice were injected subcutaneously with L-[U-¹⁴C] valine at various times after the injection of ANI (210 mg/kg or 30 mg/kg) or saline, and then sacrificed 20 min later. An estimate of protein synthesis during the 20 min prior to sacrifice was calculated by 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. The percent inhibition was calculated by comparing this ratio for ANI-treated animals to saline-treated animals.

Determinations of the percent inhibition of protein synthesis produced by the different doses of ANI and their relation to the training time for the behavioral experiments are given in Fig. 1. A large dosage of ANI (210 mg/kg) inhibits protein synthesis to a greater extent and duration than does a low dose of ANI (30 mg/kg). However, at the time when animals are trained, the high dose of ANI (210 mg/kg) given 5 hr previously is inhibiting protein synthesis at a level of about 80%, whereas the level of protein synthesis inhibition is high (90%) for the 30 mg/kg dose of ANI given only 20 min previously.

Evaluation of nonspecific behavioral effects was achieved by automatically measuring locomotor activity in an activity box

(30.5 cm square x 15.5 cm high) painted flat black. The box was divided into four quadrants by photocells, and measurement of the number of crossings was made for the different drug dosages at a time that would correspond with the time of training. Additionally, at a time corresponding to training time 15 triplet sets of mice that had received either ANI 210 mg/kg, ANI 30 mg/kg, or saline were assessed for overt signs of illness by 3 individuals experienced in handling laboratory animals. Sickness assessment, as well as behavioral training and testing, was done without knowledge of the drug condition of the animal.

The results clearly indicate that animals that received a high dose of ANI 5 hr prior to evaluation demonstrated greater nonspecific behavioral effects than did animals that received a low dose of ANI 20 min prior to evaluation. The mean number of quadrant crossings during a 10 min period for ANI 210 mg/kg, ANI 30 mg/kg, and saline were 120, 180, and 218, respectively. The ANI 210 mg/kg mice were significantly different from the ANI 30 mg/kg and saline mice when evaluated by the Duncan test at the 0.05 level. The saline and ANI 30 mg/kg mice were not significantly different. On ratings for overt sickness, the mice receiving the high dose of ANI were rated as most sick, compared to the other two groups, on 14 out of 15 comparisons ($p < 0.01$ for ANI 210 mg/kg vs ANI 30 mg/kg or saline). By contrast, the mice receiving a low dose of ANI were considered sicker than saline mice on 9 out of 15 comparisons ($p > 0.30$). Thus, both measures consistently indicated differential degrees of nonspecific side effects for the two dosages of ANI.

Retention was evaluated 1 or 7 days after one-trial passive avoidance training in a standard step-through apparatus described previously (Davis et al., 1978). 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 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. A 0.30 mA shock was delivered through 2.4 mm diameter brass rods by 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 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 entrance until the animal had all four paws on the shock grid. Five seconds after the mouse entered, a footshock was delivered until the mouse escaped back to the start box. The guillotine door was replaced, the light turned off, and after approximately 5 sec the mouse was returned to its home cage. Mice were treated in an identical fashion at test, except that no footshock was delivered.

The retention performance of mice injected with either ANI 210 mg/kg, ANI 30 mg/kg, or saline 5 hr or 20 min prior to

training is shown in Table II. These results demonstrate that the important factor for inducing amnesia is the level of protein synthesis inhibition at or shortly after the time of training and not some nonspecific side effect of the protein synthesis inhibitors observable during this time. As the level of protein synthesis inhibition increased, the retention of animals decreased, as indicated by percent amnesia. In contrast, the mice that showed the greatest nonspecific effects of protein synthesis inhibition at the time of training (ANI 210 mg/kg 5 hr pre-training) did not show the greatest impairment of retention.

There are two possible objections that might be raised to our interpretation of the results from this experiment. First, the normal passive avoidance retention of mice treated with ANI (210 mg/kg) 5 hr prior to training and tested at 1 day might be interpreted as an artifact of low locomotor activity due to some lingering side effect of the high drug dosage. This possibility is ruled out, however, by the significant impairment of retention at 1 day when the same drug dosage was given 20 min prior to training. A second possible objection comes from the finding that mice treated with ANI (210 mg/kg) 5 hr prior to training demonstrated impaired retention at 7 days. This finding does not, however, provide support for the nonspecific illness hypothesis for the following reasons: 1) This group is less impaired than the group tested at 7 days that received ANI 30 mg/kg 20 min prior to training, and which demonstrated no nonspecific side effects at the time of training. 2) The level of protein synthesis inhibition achieved in this group is sufficient to cause some degree of

amnesia, particularly at a long training-test interval, since memory strength declines over time (Davis, et al., 1978).

The results of this experiment, in conjunction with experiments using post-training injected sickness controls (Davis et al., 1978), rule out nonspecific illness prior to, at, or after training as an explanation for amnesia following protein synthesis inhibition. In addition, we believe that the present study has implications for most, if not all, of the alternative hypotheses that have been offered to explain amnesia following inhibition; specific studies have in the past dealt directly with alternative hypotheses for the amnesic effects of protein synthesis inhibition. These include altered locomotor activity (Squire & Barondes, 1974), conditioned aversion (Squire, Emanuel, Davis, & Deutsch, 1975), altered cerebral electrical activity (Cohen, Ervin, & Barondes, 1966) inhibition of tyrosine hydroxylase activity (Squire, Kuczynski, & Barondes, 1974), induction of an abnormal brain state by accumulation of a metabolite or depletion of a short half-life protein (Squire & Barondes, 1976), accumulation of brain tyrosine (Spanis & Squire, 1978), and inhibition of adrenal steroidogenesis (Squire, St. John, & Davis, 1976). The conditions required for inducing amnesia according to these alternative hypotheses would be satisfied by the high dose of ANI 5 hr prior to training, at least as well as the low dose of ANI 20 min prior to training, but the low dose of ANI produced a significantly greater impairment of retention than did the high dose. Thus, the results of the present and previous studies are consistent with the hypothesis that the protein synthesis

inhibitors induce amnesia by blocking the synthesis of brain protein specifically required for the formation of long-term memory.

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Acknowledgements

This research was supported by ADAMHA Grant R01MH26704 and from the Division of Biomedical and Environmental Research of the U.S. Department of Energy, Contract number W-7405-ENG-48. L.R.S. was supported by the Medical Research Service of the Veterans Administration and by a grant from the Spencer Foundation. We thank Ann E. Orme for biochemical assistance.

Table I
 RATIONALE FOR THE EXPERIMENT

	Conditions		Amnesia Hypotheses	
	A	B	A'	B'
	Protein Synthesis Inhibition at Training	Sickness at Training	Protein Synthesis Inhibition	Illness
ANI 210 mg/kg	Lower	Higher	Lower	Higher
ANI 30 mg/kg	Higher	Lower	Higher	Lower

Table II

Results: Percent amnesia at recall and nonspecific effects at training.

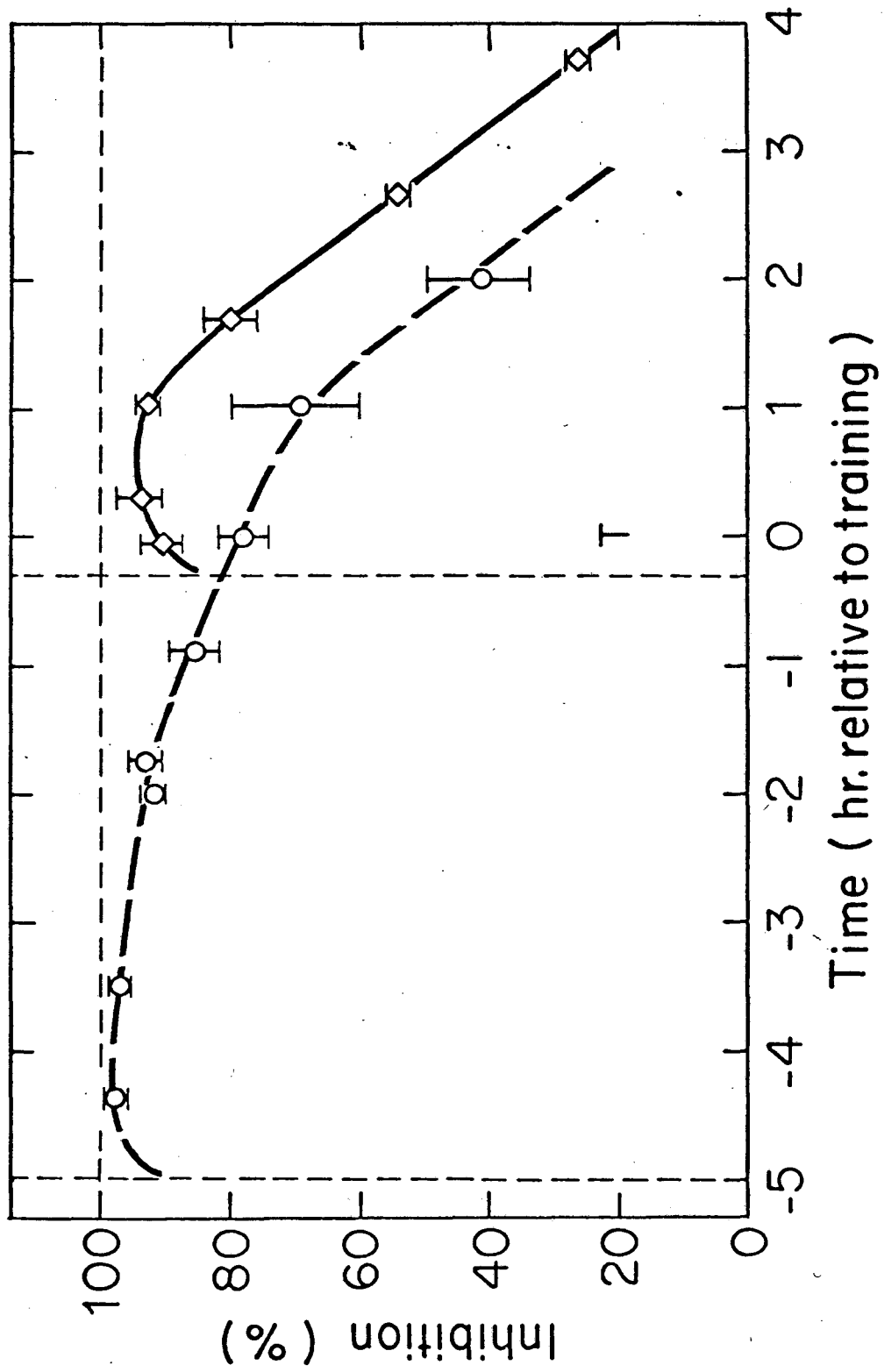
	Time of pretraining injection	N	% amnesic	% inhibition at training	Sickness at training
1 Day Test					
	Saline	20	15	0	1
	ANI 30 mg/kg	20	5 ⁺⁺	5	3 [▲]
	ANI 210 mg/kg	20	10 ⁺	80	5
	ANI 30 mg/kg	20	50 [*]	90	2
	ANI 210 mg/kg	20	75 ^{***}	95	4 [▲]
7 Day Test					
	Saline	25	8		
	ANI 30 mg/kg	20	15 ⁺⁺		
	ANI 210 mg/kg	24	46 ^{**}		
	ANI 30 mg/kg	21	62 ^{***}		
	ANI 210 mg/kg	20	90 ^{***}		

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ as compared to saline. ⁺ $p < 0.05$ and ⁺⁺ $p < 0.01$ as compared to ANI 30 mg/kg 20 min pretraining. [▲] rank order determined on basis of activity scores only.

Figure Caption

Figure 1. Percent inhibition of protein synthesis by ANI 210 mg/kg (O-----O) and ANI 30 mg/kg (◇-----◇) are presented in relation to training time (T).

Five mice were used for each data point, and the standard deviations are shown by the vertical bars. These inhibition curves have been derived, in part, from numerous other experiments carried out in this laboratory.



XBL 795-4768

This report was done with support from the Department of Energy. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the Department of Energy.

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