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Publication Date
1977

Submitted to Behavioral Biology

LBL-5302 Rev.
Preprint c.1

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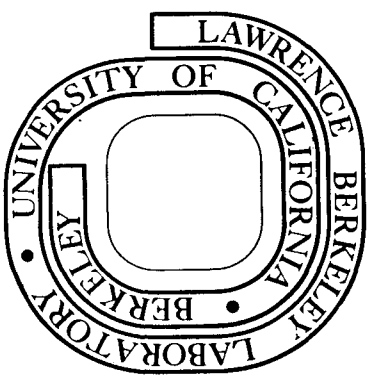
January 1977

Prepared for the U. S. Energy Research and
Development Administration under Contract W-7405-ENG-48



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DISCLAIMER

THE EFFECT OF STIMULANTS, DEPRESSANTS AND PROTEIN SYNTHESIS INHIBITION ON RETENTION

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Running title: Arousal, protein synthesis, and amnesia

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ABSTRACT

This study tested the hypothesis that the level of arousal is an important determinant of memory formation. The experiments measured the amnesia caused in mice by inhibition of cerebral protein synthesis using anisomycin. The level of arousal was modified by the use of excitant and depressant drugs. Post training administration of stimulant--d-amphetamine, strychnine, or picrotoxin,--counteracts the amnesic effects of protein synthesis inhibition, so that amnesia does not occur unless the duration of inhibition is lengthened. Stimulants show a time dependency, since they are less effective when administered at longer intervals after training. Depressants enhance the amnesia resulting from protein synthesis inhibition. Biochemical experiments showed that depressants alone had only slight effects on the rate of protein synthesis. In combination with anisomycin, the depressants did not markedly prolong the duration or increase the degree of inhibition. Stimulants, either by themselves or in combination with the inhibitors, had little or no effect on protein synthesis. Other alternative hypotheses are considered, but the results are all consistent with the hypothesis that the level of arousal following acquisition plays an important role in determining the length of time over which the biosynthetic phase of memory formation will last.

KEY WORDS

Anisomycin	Amnesia	Valine-^{14}C
Depressants	Arousal	d-Amphetamine
Memory	Chloral Hydrate	Brain
Passive Avoidance Test	Excitants	Inhibition
Protein Synthesis Inhibition	Picrotoxin	Protein Synthesis
Strychnine	Sodium Phenobarbital	Stimulants

In general, stimulants facilitate and depressants impair retention (McGaugh, 1973; Dawson and McGaugh, 1973; Jarvik, 1964). Some evidence suggests that stimulants prolong the labile or short-term memory trace (Gibbs, 1976); it should also follow that depressants reduce the life of the short-term memory trace. The relation of this type of memory modification to the blocking of long-term memory storage by inhibitors of protein synthesis is not clear. If we make the assumptions that (a) protein synthesis is necessary for long-term memory formation and (b) the short-term memory trace must be present at the time of memory-related protein synthesis for long-term memory storage to occur, then stimulants should antagonize amnesia by extending the life of the short-term memory trace beyond the duration of protein synthesis inhibition. On the other hand, depressants by reducing the life of the short-term memory trace should enhance the amnesic effect obtained with inhibitors of protein synthesis.

Some evidence has already been obtained which shows that stimulants can block the amnesia induced by inhibitors of protein synthesis. Amphetamine administered <u>after training</u> can block the amnesia induced by acetoxycycloheximide or cyloheximide (Serota, Roberts and Flexner, 1972; Barondes and Cohen, 1968). Hall, Schlesinger and Stamm (1976) found that puromycin-induced amnesia could be prevented by post training injections of amphetamine, strychnine or pentylenetetrazol. They concluded that their results showed "that the amnesic effects of puromycin can be counteracted by a state of heightened nervous system excitation." Gibbs (1976) blocked cycloheximide-induced amnesia with a post training injection of amphetamine. She interpreted her results as indicating that amphetamine extended the life of the labile or short-term memory

trace and thus allowed "consolidation into permanent memory at a time later than normal."

In this article we confirm the anti-amnesic effects of stimulants by demonstrating that amphetamine, strychnine and picrotoxin block anisomycin-induced amnesia; we further show that two depressants--chloral hydrate and sodium phenobarbital--have an opposite effect from the stimulants; the depressants enhance anisomycin-induced amnesia.

PROCEDURE

GENERAL DESCRIPTION - BEHAVIORAL

Animals

The animals were Swiss Webster (CD-1) male, albino mice, 60-80 days of age at the time of training. The mice were obtained from Charles River Breeding Laboratories at 6 weeks of age. They were housed singly 24 hr prior to training and remained so housed until tested for retention 1 week after training.

Apparatus and Training Procedures

The apparatus and training procedures for our step-through passive avoidance task have been described in detail previously (Flood, Bennett, Rosenzweig and Orme, 1972, 1974). In brief, the one trial, step-through passive avoidance apparatus consists of a black start compartment joined to a white shock compartment by a partition containing a mousehole. Mice were permitted to enter the white compartment through a mousehole where they received footshock until they returned to the black compartment. To control the strength of learning, only subjects entering in 2 seconds and escaping in 2 seconds were used. On the retention test given one week after training, the mice were placed into the black compartment and the time required for the subjects to enter the white compartment

was taken as a measure of retention. An entry time into the white shock compartment on the test day of 20 sec or less was defined as amnesia. Percentage amnesia is defined as the percentage of mice having an entry time less than 20 sec. Most trained non-amnesic mice did not enter the white compartment within three minutes. Throughout, training and testing were done between the hours of 7:30 AM and 2:00 PM.

Drugs

Anisomycin (Ani) was a gift from Pfizer Pharmaceutical Co., Groton, Conn., through the generosity of Dr. N. Belcher or was obtained from Pfizer Diagnostics, Clifton, N.J. In order to dissolve Ani, an approximately equal molar amount of dilute HCl was added, and the pH was finally adjusted to 6-7. The final solution was 2.0 mg/ml in 0.9% saline and was injected at a dosage of 20 mg/kg subcutaneously in the back. When the saline or Ani was administered prior to training the subject was lightly anesthesized with ether. The other drugs were obtained from commercial sources and were administered intraperitoneally (IP) at the following doses: sodium phenobarbital (Pheno), 125 mg/kg; chloral hydrate (CH), 300 mg/kg; d-amphetamine (Amph), 2 mg/kg; strychnine (Stry), 0.1 mg/kg; and picrotoxin (Pic), 1.0 mg/kg). The concentrations of the depressants and stimulants were such that the desired dose could be obtained by the administration of 0.25 ml/25 g mouse.

Experiment 1

The purpose of this experiment was to test if depressants would enhance the amnesic effect of Ani. The mice received two successive subcutaneous injections of either Ani or saline. The first injection was given 15 min prior to training and the second 1-3/4 hr after training. The footshock was set at the fairly high level of 0.36 mA so that the two injections of Ani alone would not cause a high level of amnesia. Chloral hydrate or sodium phenobarbital was administered (IP) 30 min

after training to Ani-injected mice. A saline injection served as a control injection for the depressant in Ani-injected mice. One group of mice received three successive injections of Ani (15 min before training and 1-3/4 and 3-3/4 hr after training). The groups used were these: Sal(Sal)Sal, Ani(Sal) Ani, Ani(Sal)Ani+Ani, Ani(CH)Ani and Ani(Pheno)Ani. (The parentheses indicate IP injections given 30 min after training.)

Results

Under these conditions of training, three successive injections of Ani caused amnesia, while two successive injections of Ani had no greater effect on retention test performance than saline (Table 1). The groups given the depressants and two injections of Ani differed significantly from those receiving only the two injections of Ani and a control injection of saline 30 min after training (Table 1). Chloral hydrate and phenobarbital increased the amnesia by 60 to 70 percent, and the resulting amnesia was equivalent to that obtained with three successive injections of Ani. Thus the effects of these depressants was to enhance the amnesia induced by anisomycin. It should be noted that the doses of depressants used were such that when administered alone they did not have a significant effect on retention. Thus the results demonstrated an interaction between the depressant and the inhibitor of protein synthesis.

Experiment 2

The purpose of this experiment was to test whether stimulants would block Ani-induced amnesia. The following schedule of three successive injections was used: Ani or Sal, 15 min prior to training; Sal or one of the stimulants, (d-amphetamine, strychnine, or picrotoxin)

30 min after training; and Ani or Sal, 1-3/4 hr after training. To control for non-specific effects of the injections or material injected, 8 mice in each of the 5 conditions received pseudo-training in which they were injected and allowed to step into the white box, but were not shocked. Footshock was set at the relatively lower intensity of 0.32 mA for subjects being trained so that two successive injections of Ani would cause amnesia. The groups used were these; Sal(Sal)Sal (N=51), Ani(Sal)Ani (N=44), Ani(Amph)Ani (N=30), Ani(Stry)Ani (N=38), and Ani(Pic)Ani (N=47).

Results

Two successive injections of Ani with an IP injection of saline-Ani(Sal)Ani--caused significant amnesia compared to the saline control--Sal(Sal)Sal (73% versus 8% amnesia; $P < .001$, χ^2 Test). Any of the stimulants administered 30 min after training significantly decreased the percentage of amnesia in Ani-injected mice: Ani(Sal)Ani = 73% amnesia; Ani(Amph)Ani = 7% amnesia; Ani(Stry)Ani = 18% amnesia and Ani (Pic) Ani = 17% amnesia. The effect of saline versus any of the three stimulants differed at $P < .001$, χ^2 Test.

In addition, the groups injected and given psuedo-training showed 100% amnesia; that is, 100% stepped into the white compartment on the retention test within 20 sec. The non-specific effects of the injection procedure or the material injected *per se* did not influence the latency-to-enter the shock compartment at the time of the retention test.

Experiment 3

The purpose of this experiment was to test the time-dependency of the effect observed in Experiment 2 by varying the time when the IP injections of d-amphetamine, strychnine, or picrotoxin were given

after passive avoidance training. The stimulants or a contol saline injection were at 30, 90, 150, and 210 min after training. The subcutaneous injections of Ani or saline were given as before at 15 min prior to training and then again at 1-3/4 hr after training. Other conditions were as in Experiment 2. The N was 20 per group.

Results

The longer after training each of the stimulants was injected, the less effectively they reduced Ani-induced amnesia (Fig. 1). None of the stimulants significantly reduced amnesia when given at 210 min after training. The clearest example of a time-dependent effect was obtained with d-amphetamine. When d-amphetamine was administered 30 min after training to an Ani-injected subject [Ani(Amph 30)Ani], 20% amnesia occurred; when Amph was injected at 90 min, 15% amnesia occurred; at 150 min, 50% amnesia; and at 210 min, 80% amnesia (Fig. 1A). The time-dependent effect had shorter gradients with strychnine and picrotoxin in that injections given 150 and 90 min respectively after training failed to reduce the amnesia caused by two successive injections of Ani (Fig. 1B and 1C).

Experiment 4

The results of Experiment 2 suggested that pharmacologically induced arousal can reduce the effectiveness of Ani as an amnestic agent. This experiment tested whether the anti-amnesic effect of stimulant drugs could be counteracted by prolonging the duration of the inhibition of protein synthesis caused by Ani.

The subjects, training apparatus, and conditions were as for Experiments 2 and 3. The IP injection of saline or one of the stimulants was administered at 30 min after training. The number of subcutaneous Ani or

saline injections was verified as follows: Ani was administered 2,3, or 4 times. The first injection was 15 min prior to training, the 2nd injection 1-3/4 hr after training, the 3rd injection, if given, 3-3/4 hr after training and the 4th injection, if given, at 5-3/4 hr after training.

Thus there were 9 experimental groups: the three stimulant drugs (d-amphetamine, strychnine, picrotoxin) by three durations of inhibition (produced by either 2, 3, or 4 successive injections of Ani giving durations of 4, 6, or 8 hr inhibition). In addition, the possible extent of Ani-induced amnesia without the stimulants was measured in two groups: Ani(Sal)Ani, and Ani(Sal)Ani+Ani+Ani. Saline controls were run only for the extreme numbers of injections: Sal(Sal)Sal and Sal(Sal)Sal+Sal+Sal.

Results

The results showed that, as the number of Ani injections increased, the effectiveness of the stimulants in preventing amnesia decreased. As had been found in Experiments 2 and 3, all three stimulants blocked the amnesia that occurred as the result of giving two successive injections of Ani. But as the number of Ani injections increased, the stimulants lost their ability to block amnesia. d-Amphetamine, probably due to its relatively long period of action, required four injections of Ani to block the anti-amnesic effect (Fig. 2A). The anti-amnesic effect of strychnine and picrotoxin were blocked by three successive injections of Ani (Figs. 2B and 2C).

BIOCHEMICAL EXPERIMENTS

In order to interpret the behavioral results, it is essential to determine what effect(s) the stimulants and depressants had on protein synthesis and on protein synthesis inhibition induced by Ani. If the

al., 1972). Duplicate fractionations and determinations of radioactivity were made for each mouse brain. The stimulants, depressants, and Ani were obtained from the same sources and used in the same manner as described in the Behavioral Procedures. The [U-^{14}C]-L-valine was obtained from New England Nuclear Corp.

EFFECTS OF DEPRESSANTS ON PROTEIN SYNTHESIS

A large number of experiments were carried out to test the effects of Ani, depressants, and Ani plus depressants on inhibition of protein synthesis. In these experiments, we determined (a) the inhibition due to a single injection of Ani at several intervals during the time period 1/2 hr to 4-1/2 hr following the injection, (b) the inhibition produced by the series of injections Ani(Sal)Ani up to 6-1/2 hr following the initial injection, (c) the inhibition caused by the depressant alone from 20 min to approximately 7 hr after administration, (d) the inhibition caused by Ani plus the depressant 1 and 2 hr after administration of Ani, and (e) the inhibition produced by the series of injections Ani(Sal)Ani and Ani(Depressant)Ani over the time interval 3 hr to 6 hr after the initial injection of Ani.

Results

After a single injection of Ani, the inhibition of protein synthesis rose rapidly to 90% and fell to 80% after 2 hr. A second injection of Ani resulted in an inhibition curve similar to the first one and maintained inhibition at 80% or more for a total of 4 hr (Fig. 3A). It should be emphasized that training of mice occurred in the behavioral experiments 15 min after the first injection of Ani or saline.

Neither of the depressants, chloral hydrate or phenobarbital, exerted a large effect on protein synthesis, either alone or in combina-

tion with Ani. The maximum inhibition caused by either depressant alone was 30% to 35%; this occurred 1-1/4 hr after administration of the depressant (Fig. 3B and 3C). (It should be noted that no effects on memory have ever been reported unless protein inhibition was 80% or more.) Protein synthesis inhibition by either depressant persisted no more than 3 hr after its initial administration. Depressant in combination with Ani increased the protein synthesis inhibition at 2 hr from 80 to approximately 90%. No significant increase in inhibition was found at 4 hr from Ani(Depressant)Ani when compared to Ani(Sal)Ani. In addition, inhibition above 80% obtained with Ani(Depressant)Ani was not extended beyond that obtained with Ani(Sal)Ani alone. Thus neither the extent or duration of inhibition of protein synthesis was significantly altered by administering a combination of Ani and depressant.

EFFECTS OF STIMULANTS ON PROTEIN SYNTHESIS

The procedures were the same as for the biochemical studies with depressants. The effects of the stimulants amphetamine, strychnine and picrotoxin on protein synthesis, both in the presence and absence of Ani, were investigated. The effects on protein synthesis were determined 1-1/4, and 2 hr after administration of Ani, and 1/2 and 1-1/4 hr after administration of the stimulants.

Results

The stimulants produced either a slight inhibition of or no effect on protein synthesis. That is, although they reduced amnesia, whatever effect they had on protein synthesis was in the same direction as Ani. Stimulants did not modify the inhibition produced by Ani. The results are summarized in Table 2.

DISCUSSION

The behavioral and biochemical experiments reported here demonstrated that post training injections of stimulant or depressant drugs modify significantly the amnesia caused by inhibition of protein synthesis but the stimulants or depressants do not produce these effects by altering the inhibition of protein synthesis. Let us consider whether the mechanism of action of these drugs on formation of long-term memory may be either (a) some common pharmacological effect on a particular neurotransmitter or (b) the ultimate effect of these drugs on arousal (i.e., level of brain excitability) where the mechanism of action is likely to involve more than one neurotransmitter.

Neuropharmacology of Stimulants and Depressants

The excitants and depressants used in this study and in our previous paper (Flood et al., 1977) were chosen to act on a variety of neurotransmitter systems in order to determine whether the effects on memory were specific to certain neurotransmitter systems or are general to excitation or depression. The drugs used exert their influence in the CNS by a variety of actions which are briefly reviewed below.

The primary effect of d-amphetamine appears to be that of increasing the release and blocking the reuptake of catecholamines (Besson et al., 1971; Glowinski and Axelrod, 1966; Thornburg and Moore, 1973).
The primary action of strychnine appears to be as an antagonist to glycine, thereby affecting the postsynaptic glycine receptor and selectivity blocking inhibition (Curtis, Duggan, and Johnston, 1971; Curtis, 1969; Franz, 1975; and Dreifuss and Andrews, 1972). However, the action of strychnine is not entirely specific (Krnjevic, 1974, p.459; Phillis, 1970). Picrotoxin, by interaction with the GABA receptor, blocks presynaptic inhibition and affects all portions of the CNS (Snyder, 1975;

Krnjević, 1974). Nicotine acts on the nicotinic acetylcholine receptors in brain. Cells with nicotinic cholinergic receptors are predominantly excitatory when activated (Krnjević, 1974), and the presence of such receptors in the CNS has recently been demonstrated (Moore and Loy, 1972; Salvaterra, Mahler, and Moore, 1975; Eterović and Bennett, 1974).

Surprisingly little information appears to exist on the mode of action of the depressants. Sodium phenobarbital, in common with other barbiturates, appears to cause a decreased turnover of dopamine, serotonin and noradrenaline in the CNS (Harvey, 1975; Corrodi, Fuxe, and Hökfelt, 1966, 1967, Corrodi et al., 1971, Lidbrink et al., 1972). The CNS depression produced by chloral hydrate does not appear to have been linked to any particular neurotransmitter system. Thus based on the combined results of the passive and active avoidance tests (Flood et al., 1977), it appears that formation of memory can be modulated by any of the following transmitter systems--cholinergic, adrenergic, serotonergic, and GABA-glycine. Since no particular neurotransmitter system seems to be able to account for the mode of action of all these drugs, we hypothesize that it is the net increase or decrease in arousal which primarily effects memory formation modulated by changes in the degree of the transmitter-receptor interaction.

Related Behavioral Studies

The importance of arousal during acquisition of a habit has been recognized for some time. More recently it was suggested that arousal that follows the acquisition of a habit plays an important role in memory formation. The physiological mechanisms that mediate post-training arousal may involve norepinephrine and other biogenic amines (Kety, 1976; Stein, Belluzzi, and Wise, 1975), hormones such as ACTH, corticosteroids

and vasopressin (Rigter, Van Riezen and de Wied, 1974; Flood and Jarvik, 1976; Flood, et al., 1976), and adrenergic and cholinergic neurotransmitters (McGaugh, 1973; Deutsch, 1973). The results of Experiment 2 showed that pharmacologically induced arousal can reduce the effectiveness of Ani as an amnestic agent. These results mimicked our previous finding that greater training strength (i.e., more or stronger footshock, more training trials) which probably involved greater arousal, can decrease the amnestic effectiveness of a given number of Ani injections (Flood et al., 1973, 1974, 1975a).

The present results agree with previous reports that administration of stimulants and depressants shortly after training can influence retention test performance. They also confirm reports by Serota, Roberts and Flexner (1972) Barondes and Cohen (1968), Hall, Schlesinger and Stamm (1976) and Gibbs (1976) that stimulants have an anti-amnesic effect against inhibitors of protein synthesis. An anti-amnesic effect has been demonstrated with d-amphetamine, strychnine, pentylenetrazol, picrotoxin, caffeine and nicotine (submitted). In addition, we have shown that depressants enhanced amnesia induced by protein synthesis inhibition.

Barondes and Cohen (1968) found that d-amphetamine blocked amnesia "when cerebral protein synthesis inhibition is slight and when 'short-term' memory remains." In their experiment, amnesia was prevented from occurring by the administration of amphetamine 3 hr or more after training when protein synthesis was recovering from cycloheximide-induced inhibition. But if inhibition of protein synthesis was maintained by administering acetoxycycloheximide prior to the amphetamine, then amnesia resulted. This agrees with our findings (Experiment 4) that extending the duration of inhibition of protein synthesis reduced or blocked the anti-amnesic

effect of the stimulant (Flood et al., 1977). In the Barondes and Cohen study, the recovery of protein synthesis and the presence of a short-term memory occurred together. Thus it is not possible to determine whether the anti-amnesic effect was dependent on the presence of protein synthesis or short-term memory or both. Experiments 2 through 4 above and those of Gibbs (1976) and Flood et al., (1977) show that amnesia can be blocked with stimulants when the inhibition of protein synthesis caused by either Ani or cycloheximide is high (at least 85% inhibition). Our results (Experiment 3) and those of Gibbs demonstrate a clear time-dependent anti-amnesic effect of administering stimulants. With the possible exception of amphetamine, the stimulants are very rapidly metabolized (Franz, 1975; Innes and Nickerson, 1975). Therefore, most of the stimulants were probably no longer active when protein synthesis recovered 3 to 5 hr after 30 min post-training administration. Thus it does not appear to be necessary for stimulants to be administered or to be active when protein synthesis recovers in order for them to block amnesia.

The anti-amnesic effect is dependent on the integrity of the short-term memory trace since both our Experiment 3 and those of Gibbs (1976) demonstrate clear time dependency, with the clearest anti-amnesic effect resulting from injections given close to the end of training. We would also point out that stimulants administered 150 or 210 min after training would very likely be active when protein synthesis resumed, but this did not prevent amnesia.

Related Biochemical Studies

One possible alternative interpretation could be that stimulants and depressants directly counteract or augment protein synthesis inhibition.

None of the behavioral studies cited above determined if inhibition of protein synthesis was altered by the stimulants employed. A paucity of literature exists on direct effects of acute administration of either excitants or depressants on protein synthesis. Jaboubek and Semiginovsky (1970) reviewed the literature and concluded that it is likely that there is a correlation between protein and nucleic acid synthesis and increased functional activity (produced by any of a number of stimuli such as motor activity, electrical stimulation and excitants). Satake (1972) similarly concluded that trans-synaptic stimulation seems to activate protein metabolism in the neuron. McMahon and Blaschko (1971) have found that chloral hydrate inhibits protein synthesis in Chlamydomonas reinhardii. However, the concentrations were considerably higher than used in our studies. In fact this high concentration (0.01 M) eventually blocked cell division. Edström and Larsson (1974) showed that high concentrations of barbiturates were relatively ineffective in inhibiting protein synthesis in vitro in the sciatic nerve of the frog.

In our experiments, each of these depressants caused a modest inhibition of protein synthesis which is of relatively brief duration. The stimulants did not cause any marked increase in protein synthesis; on the contrary, in several cases slight inhibition was noted. Recently, several investigators have reported that d-amphetamine administered in vivo interfered with subsequent protein synthesis measured in vitro. Generally the effective dose ranges have been high or even lethal (Loh, Hitzemann and Stolman, 1973). It should be noted that high doses of stimulants cause convulsions and impair rather than facilitate retention. Disaggregation of brain polysomes has been shown (Moskowitz, Weiss, Lytle, Munro, and Wurtman, 1975; Widelitz, Coryell, Widelitz, and Avadhani, 1975), and

it now appears that amphetamine interferes with initiation of protein synthesis through a step related to formation of the RNA-ribosome complex.

Neither the depressants nor the stimulants markedly alter the inhibition of protein synthesis produced by Ani. Thus it would appear that we can rule out any direct action of the drugs on protein synthesis which would have altered the inhibition caused by Ani.

Another possibility to be considered is that Ani had direct effects on neurotransmitter synthesis and that the stimulants and depressants either antagonize or potentiate the effect. This is of particular concern since Flexner and Goodman, (1975) have suggested that an important side effect of all protein synthesis inhibitors is that they depress the rate of accumulation of norepinephrine, dopamine and total catecholamines and at the same time markedly elevate levels of tyrosine. Unfortunately, in the case of anisomycin, Flexner and Goodman present data for only one dosage and one time point after administration (2 hr); until more complete data are available, it is difficult to evaluate the significance of these results for the interpretation of our behavioral experiments. Even if it occurs, the inhibition of catecholamine synthesis may be a side effect that has no appreciable contribution to the amnesia caused by protein synthesis inhibitors. Squire, Kuczenski and Barondes (1974) have studied the inhibition of brain tyrosine hydroxylase activity by cycloheximide and Ani and by doses of alpha-methyl-para-tyrosine (AMPT) that depress tyrosine hydroxylase activity as much as or more than either cycloheximide or Ani. They showed that AMPT did not cause amnesia under the same training condition for which cycloheximide and Ani caused amnesia. They concluded that the effect of protein synthesis inhibition

on brain tyrosine hydroxylase activity was not sufficient to explain the amnesic effect of protein synthesis inhibitors. Unpublished experiments (Flood et al.) that have used drugs that specifically modify the levels of catecholamines have shown that these agents can cause amnesia but only when training is very weak. The range of training strengths for which Ani will cause amnesia is far greater. It is our general conclusion at this time that interference with synthesis of catecholamines, serotonin or acetylcholine is not sufficient by itself to account for the amnesia induced by Ani. Thus the present evidence suggests that effects of a protein synthesis inhibitor on neurotransmitter synthesis is not the primary mechanism by which amnesia is caused; however, the results are consistent with the hypothesis that protein synthesis is involved in memory formation.

We believe that our data and those of others are consistent with the hypothesis that the level of arousal following acquisition plays an important role in determining the length of time over which the biosynthetic phase of memory formation will last.

TABLE 1

EFFECT OF DEPRESSANTS ON ANI-INDUCED AMNESIA

Group	N	% Amnesic Mice
Sal(Sal)Sal	20	0
Ani(Sal)Ani	20	10
Ani(Sal)Ani+Ani	23	74*
Ani(CH)Ani	31	84*
Ani(Pheno)Ani	21	71*

* % amnesic mice differed from either of the control groups at <.001 level.

TABLE 2

EFFECTS OF STIMULANTS ON CEREBRAL PROTEIN SYNTHESIS

	Saline Injected Mice		Anisomycin Injected Mice	
	Time Administered Prior to Sacrifice (min)			
Saline or Anisomycin	75	120	75	120
Stimulant	30	75	30	75
Stimulant	% Inhibition of [^{14}C]-Valine Incorporation			
None	-	-	93 ± 2	77 ± 5
d-Amphetamine 2 mg/kg	9 ± 12	26 ± 10	92 ± 1	88 ± 2
Strychnine sulfate 0.1 mg/kg	20 ± 8	2 ± 5	91 ± 2	76 ± 3
Picrotoxin 1 mg/kg	5 ± 4	5 ± 6	92 ± 3	76 ± 4

* [^{14}C]- Valine was administered 20 min prior to sacrifice; 3 mice were used for each data point.

FIGURE CAPTIONS

Figure 1. Time-dependent effects of stimulants on anisomycin-induced amnesia (Exp. 3: N = 20/group). A. d-Amphetamine blocked amnesia caused by anisomycin when given 30 or 90 min after passive avoidance training. d-Amphetamine failed to block amnesia when given 210 min after training. Thus proactive effects of d-amphetamine cannot explain the effect obtained with a 30 min post-training injection of d-amphetamine. B. Amnesia was blocked with a 30 min post-training injection of strychnine, and a slight effect was present with a 90 min post-training injection. Strychnine did not block anisomycin induced amnesia when given 150 or 210 min after training. C. Picrotoxin only blocked amnesia when administered 30 min after training.

Figure 2. Effect of the number of anisomycin (A) injections (duration of inhibition of protein synthesis) on amnesia blocked by stimulants (Expt. 4). A. Four successive injections of anisomycin were required to regain the high percent amnesia lost by injecting d-amphetamine 30 min after training. Thus the capacity for memory related protein synthesis extends 3-4 hr longer in A(Amph-30)A than in A(Sal)A mice (N/group = 15). B. Three successive injections of anisomycin were required to regain the high percent amnesia lost by injecting strychnine 30 min after training. Thus the capacity for memory related protein synthesis extend 1-2 hr longer in A(Stry)A than in A(Sal)A mice. (N/group = 20).

C. Three successive injections of anisomycin were required to regain the high percent of amnesia lost by injecting picrotoxin 30 min after training. The capacity for memory related protein synthesis extends 1-2 hr longer in A(Pic)A than in A(Sal)A mice (N/group = 15 except for the A(Sal)A and A(Sal)A+A+A where N = 10).

Figure 3. A. Inhibition of cerebral protein synthesis in Swiss Webster male mice obtained by subcutaneous injections of Ani, and Ani+Ani. B. The inhibition of protein synthesis by chloral hydrate alone (□ --- □) or Ani(CH)Ani (■ --- ■). The inhibition by anisomycin without chloral hydrate (.....) is redrawn in 3B. C. The inhibition of protein synthesis by phenobarbital alone (□ --- □) or Ani(Pheno)Ani (■ --- ■). The inhibition by anisomycin without phenobarbital (.....) is redrawn in 3C.

The doses of drugs and the injection schedule were the same as used for the behavioral experiments. The number of mice and standard deviation are shown for each data point where more than two mice were used.

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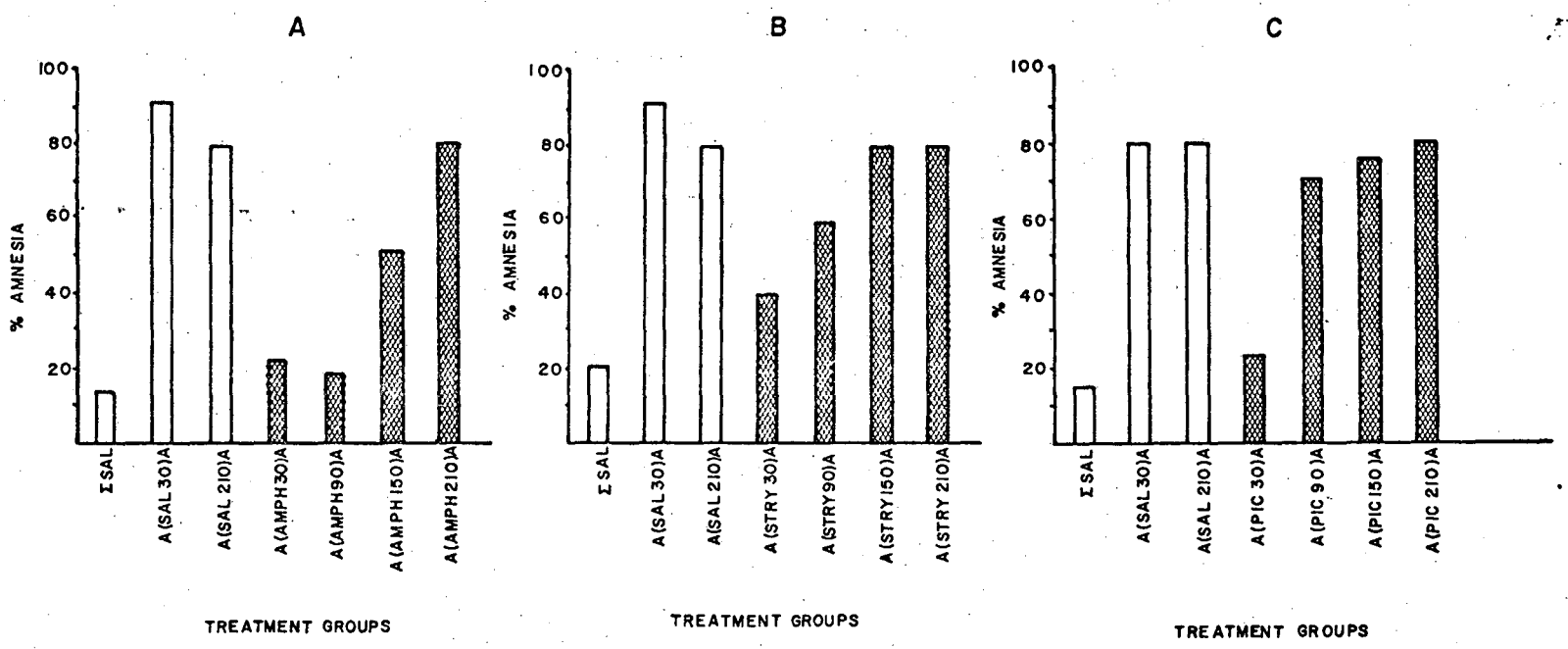
ACKNOWLEDGEMENTS

We wish to express our appreciation to N. Belcher of Pfizer Pharmaceuticals for the generous gift of anisomycin and to Sergio A. Vasquez and Rosalie Chang for skilled assistance for the behavioral experiments. The behavioral research was supported by NIMH grant NH 26608-02 to M. E. Jarvik, M.D., and the biochemical research by the U. S. Energy Research and Development Administration through the Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory.



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Flood, et al.

Fig. 1

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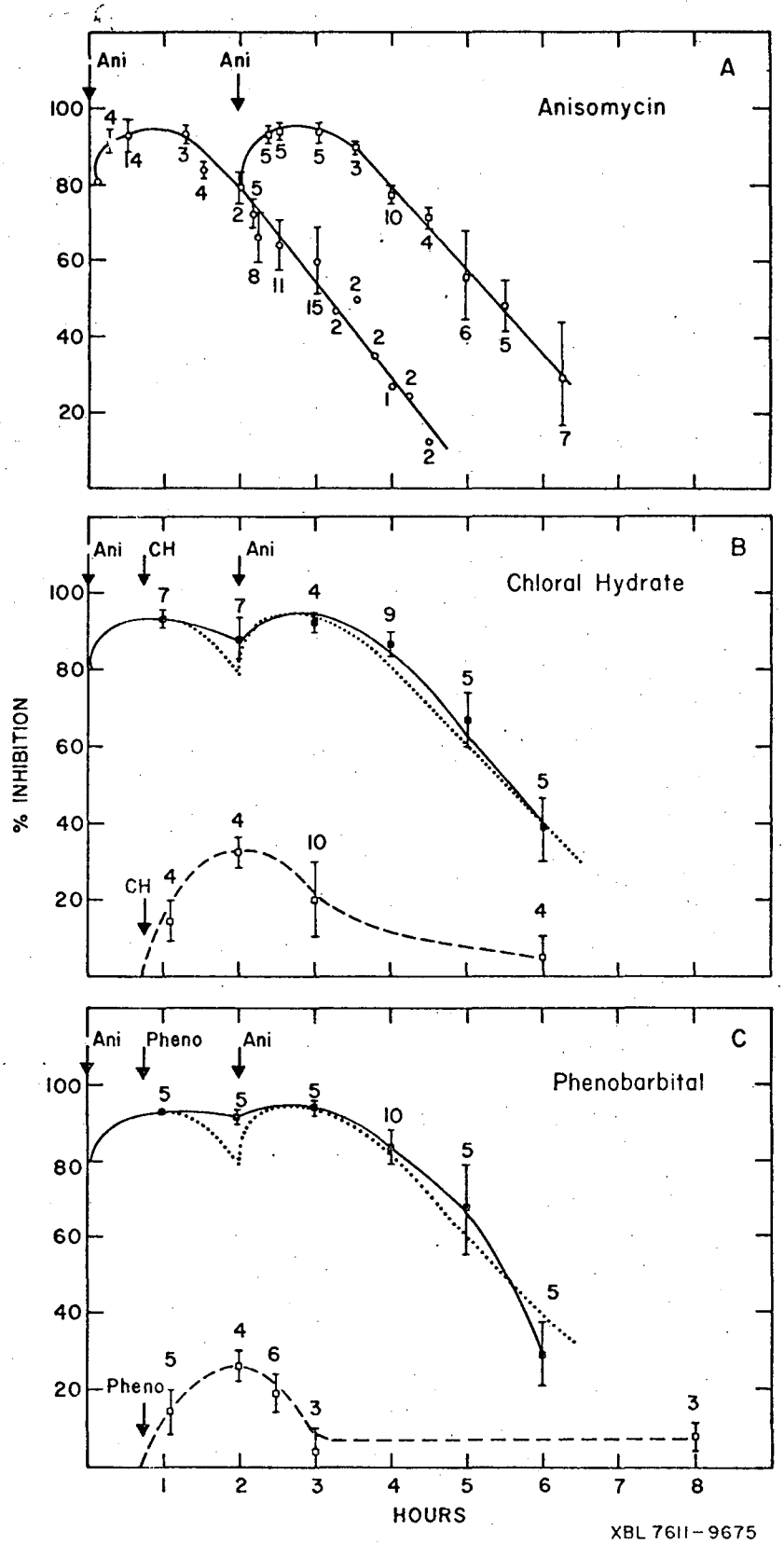

Flood et al.

Fig. 3

This report was done with support from the United States Energy Research and Development Administration. 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 United States Energy Research and Development Administration.

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