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DISCLAIMER

EFFECTS OF CHANGES IN AROUSAL ON AMNESIA INDUCED BY INHIBITION OF BRAIN PROTEIN SYNTHESIS

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KEY WORDS

Active Avoidance Test

Amnesia

d-Amphetamine

Anisomycin

Arousal

Brain

Caffeine

Chloral Hydrate

Cycloheximide

Depressants

Excitants

Inhibition

Memory

Meprobamate

Nicotine

Passive Avoidance Test

Picrotoxin

Protein Synthesis

Protein Synthesis Inhibition

Sodium Phenobarbital

Stimulants

Strychnine

Valine-^{14}C

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 and cycloheximide. The level of arousal was modified either by varying the difficulty of the training task or by the use of excitant and depressant drugs. The pole-jump active avoidance task is intermediate in difficulty between the passive avoidance task and the T-maze. The duration of inhibition of cerebral protein synthesis required after pole-jump training to obtain amnesia is intermediate to that found for these other two tests. Post-training administration of stimulants--d-amphetamine, strychnine, picrotoxin, caffeine, or nicotine--counteracts the amnestic 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 and cycloheximide, 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.

Inhibition of protein synthesis during and after training has been found in many cases to lead to a permanent amnesia (Barondes and Cohen, 1968; Cohen and Barondes, 1968; Roberts and Flexner, 1969; Quartermain et al., 1970; Geller et al., 1970; Randt et al., 1971; Squire and Barondes, 1972a,b; Andry and Luttges, 1972; Flood et al., 1972, 1973, 1974, 1975a,b; Agranoff, 1972; Ungerer, 1973; and Mayor, 1973). Control over the parameters of acquisition is needed since it was shown that failure to do so can reduce or obliterate the amnesic effect (Flood et al., 1972, 1974, Quartermain and Botwinick, 1975). Recently it was reported that as the duration of inhibition of brain protein synthesis increased after passive avoidance training, the percentage of subjects classed as amnesic increased (Flood et al., 1973, 1974). This was also reported for T-maze footshock avoidance training (Flood et al., 1975a), but the authors found that the parameters controlling acquisition of T-maze avoidance conditioning were too numerous and the duration of inhibition required for strong amnesic affects too long (14 h) for this task to be used regularly in studies of memory formation.

In attempting to account for the differences in time of inhibition required to produce permanent amnesia in the passive avoidance task and the T-maze, Flood and Jarvik (1976) have hypothesized that the greater the number of training trials and the resulting stress, the longer the duration of protein synthesis inhibition required to obtain amnesia. In the present series of experiments, we have used the jump pole active avoidance training task which is intermediate in difficulty between the passive avoidance task and the T-maze task. It was hypothesized that the duration of inhibition required to obtain amnesia for the jump pole task would be intermediate between that required for the passive avoidance task and the T-maze avoidance training.

To test further the hypothesis that the number of training trials and the difficulty of the training task are related to the level and duration

of arousal and stress that accompany and follow training, we have modified arousal by means of pharmacological agents. Decreased arousal, which is associated with training on easier tasks, might be mimicked by administering depressants after training on more difficult tasks. For a given duration of post-training inhibition of protein synthesis, amnesia should be greater with either an easier task or a pharmacologically induced decrease in post-training arousal. Sodium phenobarbital, chloral hydrate and meprobamate were used to test the hypothesis that decreasing post-training arousal will decrease the duration of inhibition of protein synthesis needed to cause amnesia.

Further, it was hypothesized that increased arousal, which is associated with training on more difficult tasks, might be mimicked by administering a stimulant after training on a relatively easy task such as the one-trial, step-through passive avoidance task. For a given duration of post-training inhibition, amnesia should be less with either a more difficult task or a pharmacologically induced increase in post-training arousal.

Evidence that stimulants administered during or shortly after training can facilitate memory has been provided by a number of investigations. Picrotoxin enhances retention for maze and shock avoidance tasks (McGaugh and Petrinovich, 1965; Petrinovich, 1967; Breen and McGaugh, 1961). Strychnine in low doses has been reported to facilitate habituation (Andry and Luttges, 1971) and food motivated visual discrimination (McGaugh and Krivanek, 1970) and to improve passive avoidance (Duncan and Hunt, 1972; Gordon and Spear, 1973). Amphetamine administered after training facilitates a food-rewarded visual discrimination (Krivanek and McGaugh, 1969), active avoidance (Del Rio, 1971; Evangelista et al., 1970, 1971) and Y-maze water escape task (Castellano, 1974). Post training injections of nicotine have been reported to facilitate retention of maze learning (Garg and Holland, 1969) and of active avoidance training (Oliverio, 1968; Erickson, 1971). Caffeine has not been studied much but Pare

(1961) reported facilitation of retention. A recent and noteable exception to these studies by Stripling and Alpern (1974) and Crabbe and Alpern (1973) reported disruptive affects of caffeine and nicotine. A possible reason for this discrepancy may be that appetitive conditioning was used and the subjects were given several daily injections between training and testing (injections started 24 hrs after training and terminated 48 hrs prior to testing). For more extensive reviews of the stimulant and depressant literature, see McGaugh (1973), Dawson and McGaugh (1973) and Jarvik (1964). Although positive effects have not been reported uniformly, the majority of reports support the hypothesis that stimulants improve memory consolidation and depressants impair it.

Some evidence has already been obtained which shows that amphetamine administered after training can block the amnesia induced by inhibition of protein synthesis caused by cycloheximide or acetoxycycloheximide (Serota et al., 1972; Barondes and Cohen, 1968). The authors suggested that the mechanism responsible for preventing amnesia was arousal. We report here the effects of five stimulants--d-amphetamine, strychnine, picrotoxin, nicotine and caffeine--, and the effects of three depressants--meprobamate, chloral hydrate, and phenobarbital--on amnesia induced by an inhibitor of brain protein synthesis, anisomycin. Anisomycin is far less toxic than cycloheximide and acetoxycycloheximide and has the important advantage in that it may be injected several times without causing death or severe illness. An injection of anisomycin causes inhibition at 80% or more for 2 hr, so by employing a schedule of injections spaced 2 hours apart, it has been possible to control the duration of inhibition of protein synthesis (Flood et al., 1973) and thus show that amnesia increased as the duration of inhibition increased (Flood et al., 1973, 1974, 1975a,b).

PROCEDURES

GENERAL DESCRIPTION - BEHAVIORAL

Animals

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

Apparatus and Training Procedures

Pole jump task: The training apparatus for the pole jump task consisted of an alley 30 cm long, 11.5 cm wide and 18 cm high divided into 2 compartments by a guillotine door. A brass grid floor was used to deliver footshock (0.35 ma) in both compartments. The smaller compartment (9 cm long) was a start box. The other compartment (21 cm long) contained a vertical plastic pole in the center. The pole (2.5 cm diameter) was covered with 1/2 inch wire mesh which started just above the shock grid. The pole could be removed easily with the mouse on it. The apparatus was built of black plastic except for the pole, which was white. A loud door bell buzzer was used as the CS. The training room was dark except for a bright Tensor lamp illuminating the apparatus.

The training procedure consisted of placing the mouse in the small compartment and after approximately 15 sec lifting the guillotine door to give access to the pole compartment. Simultaneously with removing the guillotine door, the buzzer began to sound, and 5 sec later footshock was administered if the mouse had not climbed onto the pole. The buzzer and shock were manually terminated as soon as the mouse climbed onto the pole. An avoidance response was scored if the mouse climbed onto the pole within the 5 sec safe period.

After each trial the mouse was returned to its home cage from the pole

compartment by carefully removing the pole (with the mouse on it) and placing the pole in the home cage. Most mice quickly climbed off the pole, but occasionally a light touch to the hind quarters was used to encourage the mouse to dismount. Subsequent trials (training or testing) were run in the same manner. The intertrial interval was about 45 sec. Subjects received only 2 training trials.

The retention test followed 1 week after training, and consisted of retraining a mouse until it made one avoidance response. The number of trials prior to making the first avoidance response was taken as a measure of retention. In this experiment, amnesia is defined as taking 3 or more test trials to make an avoidance response. This criterion was chosen because it classified 79% of naive mice as amnesic, and 92% of previously trained NaCl-injected control mice as non-amnesic (Fig. 1, panels A and B). Training and testing were always done between 8 a.m. and 2 p.m.

Ten of the saline-injected subjects were given 10 test trials each in order to test whether a subject continues to avoid after making its first avoidance response. The mean percent avoidance responses after each mouse made its first avoidance response was 97.5% across the 10 subjects. Only 2 mice received additional shock--one a shock on the 6th trial and the other on the 7th trial. Thus retraining the mice to a 9 out of 10 criterion on the retention test would have provided little additional information. Also, more retention trials can confuse the distinction between retention of a habit vs maintenance of a habit.

Step-through passive avoidance task: The procedure for training and testing mice for the one-trial step-through passive avoidance task has been described in detail previously (Flood et al., 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. Subjects were permitted to enter the white compartment through

the mousehole where they immediately received footshock (0.30 mA, except as noted) until they returned to the black compartment. 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. A latency-to-enter the white shock compartment on the test day of 20 sec or less was defined as amnesia. Most trained non-amnesic animals did not enter the white compartment within three minutes. Throughout, training and testing were done between the hours of 7:30 AM and 2 PM.

Drugs

Anisomycin (Ani) was a gift from Charles Pfizer Co., Groton, Conn., through the generosity of Dr. N. Belcher. 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; 0.25 ml was injected subcutaneously in the back. Cycloheximide (Cyclo), the depressants (sodium phenobarbital, chloral hydrate and meprobamate) and the stimulants (d-amphetamine hydrochloride, strychnine sulphate, picrotoxin, nicotine hydrochloride, and caffeine citrate) were obtained from commercial suppliers. Meprobamate was obtained as a suspension (Equinil) from Wyeth Labs. The concentrations of the depressants and stimulants were such that the desired dose could be obtained by the intraperitoneal injection of 0.25 ml/25 g mouse.

BEHAVIORAL EXPERIMENTS

Jump Pole Task

Experiment 1

Design: The purpose of this experiment was to study the effect of duration of inhibition of protein synthesis on retention for jump pole

training. Two training trials were used because pilot work showed that saline-injected controls performed equally well on the retention test whether given 2, 4 or 6 training trials. Four drug groups were used. These were Ani (single pretraining injection 15 min prior to training), and Ani^2 + Cyclo (a single pretraining injection of Ani followed 1-3/4 hrs after training by another injection of Ani and then an injection of Cyclo 3-3/4 hrs after training). Another drug group, Ani^3 + Cyclo, received one injection prior to training, 2 Ani injections after training at 1-3/4 and at 3-3/4 hr, and Cyclo at 5-3/4 hrs after training. The last drug group received 2.5 mg of Ani 15 minutes prior to training. Three saline control groups were run; they received saline injections at the time the comparable drug groups received their injections. An eighth group was used to establish the performance of naive subjects. This naive baseline group was isolated at the time when the other groups were being trained and received no injections. The naive group was first trained when the other groups were being tested for retention. Ani was administered subcutaneously (s.c.) at a dose of 0.5 mg/mouse/injection (0.25 ml); Cyclo was administered s.c. at a dose of 2.5 mg/mouse (0.25 ml). The last drug group was given 2.5 mg of Ani (10 mg/ml) in a single injection (5 Ani). The N's are given in Fig. 1.

Results: The saline control animals combined (Fig. 1B) and the group given a single 0.5 mg Ani injection (Fig. 1C) showed good retention. Only 8% and 9% of these groups, respectively, were classed as amnesic. Both groups differ clearly from the naive baseline group (Fig. 1A) in which 79% of the subjects were scored as amnesic. Because some of the naive mice learned the task in 1 or 2 training trials, the percent amnesia was not 100%. The Ani^2 + Cyclo group (Fig. 1D), which had 6 hrs of protein synthesis inhibition at 80% or greater, yielded 38% amnesic animals; this was significantly different from both the saline controls and the groups that received only a single pretraining injection of Ani ($P < .001$, χ^2 test. Sixty-six percent of the Ani^3 + Cyclo

group (Fig. 1E), which had 8 hrs of inhibition of protein synthesis, were amnesic; this percentage of amnesic mice differed significantly from that of the Ani^2 + Cyclo group ($P < .025$) but did not differ significantlly from the naive baseline group ($P < .25$). The 5 Ani group (Fig. 1F) yielded 81% amnesia, which differed significantly from the Ani ($P < .001$) and Ani^2 + Cyclo groups ($P < .001$) but did not differ significantly from Ani^3 + Cyclo ($P < .5$, χ^2 test). Thus, increased durations of inhibition of protein synthesis (6 or 8 hr) led to increased amnesia, and so did a large initial dose of Ani.

Experiment 2

Design: The purpose of this experiment was to determine if depressants (sodium phenobarbital, chloral hydrate, meprobamate) administered after training on the pole jump task would influence the amnesia caused by protein synthesis inhibition. The experiment tested the hypothesis that a decrease in post-training arousal will reduce the duration of inhibition of protein synthesis required to produce a high degree of amnesia.

The mice, training, and testing on the pole jump task were as in Experiment 1. The doses of depressants were these: sodium phenobarbital (Pheno), 125 mg/kg; chloral hydrate (CH), 300 mg/kg; meprobamate (M), 150 mg/kg as Equanil suspension. For the control group that received saline instead of a depressant drug, Ani was injected 15 min prior to training, saline was injected IP 30 min after training, Ani was again injected 1-3/4 hrs after training and Cyclo 3-3/4 hrs after training; this group is therefore designated as Ani(Sal) Ani+Cyclo. For the experimental groups -- Ani(Pheno)Ani+Cyclo, Ani(CH)Ani+ Cyclo, and Ani(M)Ani+Cyclo -- the Ani and Cyclo injections were given subcutaneously at the same times as for Ani(Sal)Ani+Cyclo, and ip injections of depressants were given 30 min after training. The doses of Ani (0.5 mg/mouse) and Cyclo (2.5 mg/mouse) were as in Experiment 1. Amnesia was again defined as requiring 3 or more trials to make an avoidance response. N per group was 20.

Results: Ani^2+Cyclo of Experiment 1 and Ani(Sal)Ani+Cyclo of Experiment 2 caused amnesia in about the same percentage of animals (38% and 30% respectively). All three of the groups given depressants yielded a significantly higher percent amnesia than the Ani(Sal)Ani+Cyclo control group (Fig. 2). Ani(Pheno)Ani+Cyclo yielded 65% amnesia ($P < .05$); Ani(CH)Ani+Cyclo, 80% amnesia ($P < .005$), and Ani(M)Ani+Cyclo, 75% amnesia ($P < .005$, χ^2 Test). The depressant groups showed about the same level of amnesia as Ani^3+Cyclo or the naive group of Exp. 1. The results are consistent with the hypothesis that lower arousal after training impairs memory formation.

Step-Through Passive Avoidance Task

Experiment 3

In Experiment 2, depressants were shown to increase the amnesia caused by a given duration of inhibition of protein synthesis in an active avoidance task (Pole Jump). Depressants were used on the active avoidance task because this type of training has always been more resistant to amnesic treatments than passive avoidance. In this experiment, the effects of two of the depressants were studied in passive avoidance. Chloral hydrate and sodium phenobarbital were administered 30 min after training at the doses given in Experiment 2. The footshock intensity was 0.32 ma. The injections of Ani or saline were administered 15 min prior to training, and/1-3/4 hrs after training; when a third injection was used, it was given 3-3/4 hrs after training.

Results: Under these conditions of training, three successive injections of Ani caused greater amnesia than two successive injections (74% vs 10% amnesia). 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 (Fig. 3). 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

depressants on retention reported in Experiment 2 were not unique to active avoidance.

Design: Experiments 4 through 7 use the step-through passive-avoidance task to determine if the stimulants d-amphetamine (2 mg/kg), strychnine (0.1 mg/kg) or picrotoxin (1.0 mg/kg) would block amnesia for a one-trial step-through passive avoidance task induced by injection of anisomycin (0.5 mg/mouse/injection). The hypothesis tested is that increased arousal lengthens the period of time over which the capacity for memory related protein synthesis in the CNS can occur; that is, arousal extends the length of time that inhibition of protein synthesis is required in order to obtain a high degree of amnesia. Therefore, stimulants will reduce amnesia.

Experiment 4

The following injection schedule was used for Experiment 4: Ani or Sal, 15 min prior to training; Sal or one of the stimulants, 30 min after training; and Ani or Sal 1-3/4 hrs after training. Ani was administered subcutaneously, while the stimulants were administered intraperitoneally. To control for the stress of the injections, Sal was administered as appropriate in place of Ani or any of the stimulants. 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.

Results: Two successive injections of Ani with an ip injection of saline--Ani(Sal)Ani (N=44) caused significant amnesia compared to the saline-injected group (N=51) (73% versus 8%; $P < .001$, χ^2 Test). Any of the stimulants administered 30 min after training significantly decreased the percentage of amnesia of Ani-injected subjects. d-Amphetamine caused the biggest decrease in amnesia: Ani(Sal)Ani = 73% amnesia (N=44); Ani(Amph)Ani = 7% amnesia (N=30), $P < .001$, χ^2 Test. Strychnine [Ani(Stry)Ani] (N=38) and picrotoxin [Ani(Pic)Ani]

(N=47) reduced amnesia to 18% and 17% respectively and differed significantly from Ani(Sal)Ani, $P < .001$.

In addition, the groups injected and given pseudo-training showed 100% amnesia; that is, 100% stepped into the white box on the retention test within 20 sec. Thus 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 5

Design: The purpose of this experiment was to test the time-dependency of the effect observed in Experiment 4 by varying the time when the ip injections of d-amphetamine, strychnine, or picrotoxin were given after passive avoidance training. The stimulants or a control saline ip injection were given at 30, 90, 150, or 210 min after training. The subcutaneous injections of Ani or saline were given as before at 15 min prior to training and then again 1-3/4 hrs after training. Other conditions are as in Experiment 4. The N was 20 per group.

Results: The longer after training each of the stimulants was injected, the less effectively they reduced amnesia (Fig. 4). None of the stimulants significantly reduced amnesia when given at 210 min after training. The clearest example of time-dependent effect was obtained with d-amphetamine. When d-amphetamine was injected 30 min after training to Ani-injected subjects [Ani(Amph 30)Ani] 20% amnesia occurred, at 90 min 15% amnesia, at 150 min 50% amnesia, and at 210 min 80% amnesia (Fig. 4A). The time-dependent effect had a shorter gradient with strychnine and picrotoxin in that injections given 150 and 90 min respectively after training failed to reduce the amnesia caused by the two successive Ani injections (Fig. 4B and C).

Experiment 6

Design: This experiment tried to establish a relation between the effect of behavioral and pharmacologically induced arousal on amnesia. The results of Experiment 5 suggested that pharmacologically induced arousal can reduce the effectiveness of Ani as an amnestic agent. These results mimicked the 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). However, increasing the number of Ani injections and thus the duration of inhibition can reestablish a high level of amnesia in spite of increased training strength (Flood et al., 1973). The purpose of Experiment 6 was to see if longer durations of inhibition of protein synthesis (accomplished by giving more Ani injections) would block the effect of the stimulants that were reported in Experiments 4 and 5.

The subjects, training conditions and apparatus were as for Experiments 4 and 5. 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 varied 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 hrs after training, the 3rd injection, if given, 3-3/4 hrs after training, and the 4th injection, if given, at 5-3/4 hrs after training.

Thus there were 9 experimental groups: the 3 stimulant drugs (d-amphetamine, strychnine, picrotoxin) by 3 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: A(Sal)A, and A(Sal)A+A+A. 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 4 and 5, d-amphetamine was the most effective of the three stimulants in overcoming the amnesia produced by Ani since inhibition of protein synthesis had to be maintained for 8 hrs by 4 injections of Ani to obtain a high degree of amnesia (Fig. 5A). Strychnine was the next most effective, and a clear gradient of increasing amnesia with increased duration of inhibition of protein synthesis was obtained (Fig. 5B). Picrotoxin had the shortest post-training gradient, and its effect was blocked by a third injection of Ani (Fig. 5C).

Experiment 7

A further test of the effects of stimulants on anisomycin-induced amnesia was carried out using low doses of caffeine citrate (20 mg/kg) and nicotine hydrochloride (0.5 mg/kg) administered 30 min after passive avoidance training. The experimental design combines those of Experiments 4 and 6 in that the effect of caffeine and nicotine on retention were assessed in subjects given two versus three successive injections of Ani. The first Ani injection was given 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. The footshock was 0.35 ma.

Results: Nicotine and caffeine, when administered 30 min after training to mice given two successive injections of Ani, produced significantly less amnesia than was observed in the comparable Ani(Sal)Ani group (Fig. 6). Ani(Caf)Ani vs Ani(Sal)Ani yielded 22% vs 74% amnesia ($P < .001$, χ^2 Test); Ani(Nic)Ani vs Ani(Sal)Ani, 21% vs 74% ($P < .001$, χ^2 Test). Giving an additional Ani injection to these groups blocked the anti-amnesic effect of caffeine and nicotine. Ani(Caf)Ani differed from Ani(Caf)Ani+Ani at $P < .005$, 22% vs 67%, Ani(Nic)Ani differed from Ani(Nic)Ani+Ani at $P < .001$, 21% vs 76%. Thus, these two additional stimulants demonstrate an ability to block amnesia induced by

protein synthesis inhibition. But the amnesic effect was regained by giving only one additional injection of Ani which extended the duration of inhibition by two additional hours; whereas with d-amphetamine and strychnine, two additional injections of Ani extending the inhibition four hours were required to block the effects of the stimulants.

BIOCHEMICAL EXPERIMENTS

The purpose of the biochemical experiments was to determine to what extent the behavioral results described above might be explained by modification of cerebral protein synthesis from depressants or stimulants alone or in combination with the protein synthesis inhibitors, anisomycin and cycloheximide. The first series of experiments tested the effects of depressants; the second series tested stimulants.

PROCEDURES

GENERAL DESCRIPTION - BIOCHEMICAL

Animals

The mice used for the biochemical experiments were male Swiss Webster mice; they were first or second generation of a stock obtained from the Charles Rivers Breeding Laboratories and raised at the Lawrence Berkeley Laboratory; recent behavioral comparisons of our own bred Swiss Websters and those obtained directly from Charles Rivers showed no significant differences. At testing the mice were 60-80 days of age and weighed 30-35 g.

Drugs

The inhibitors, stimulants, and depressants were obtained from the same sources and used in the same manner as described in Behavioral Procedures. [^{14}C(U)]-L-Valine was obtained from New England Nuclear Corp.

Determination of Protein Synthesis

Protein synthesis was determined by the ratio of[a] radioactivity resulting from incorporation of subcutaneously administered [^{14}C(U)]-L-valine into the trichloracetic acid insoluble fraction to[b] the total activity in the brain sample. The radioactive amino acid was injected 20 min prior to sacrifice. The percent

inhibition or stimulation was determined by a comparison of this ratio in the control and experimental mice. The experimental procedures have been described in detail (Flood et al., 1972). Duplicate fractionations and determinations of radioactivity were made for each mouse brain.

Experimental Series 1: Effects of Depressants

Design: A large number of experiments were carried out to demonstrate the effects of inhibitors, depressants, and inhibitors 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 caused by the depressant alone from 1/2 to 9 hr after administration, and (c) the inhibition caused by Ani plus the depressant over the same time period. In addition, the inhibition produced by the series of injections Ani(Sal)Ani, Ani(Sal)Ani+Cyclo, Ani(depressant)Ani, and Ani(depressant)Ani+Cyclo was determined over the time interval 4 hr to 9 hr after the initial injection of Ani.

Results: The experimental results for this series of experiments, which used over 500 mice, are given for Ani (Fig. 7), meprobamate (Fig. 8), chloral hydrate (Fig. 9), and sodium phenobarbitol (Fig. 10). After an injection of Ani, the inhibition of protein synthesis rises rapidly to 90%, and falls to 80% after 2 hr. A subsequent injection of Ani results in an inhibition curve similar to the first one. The inhibition obtained by an injection of Cyclo falls to 80% more quickly than does the inhibition obtained with Ani, but the subsequent decay is less rapid. The curve for Ani+Ani is a composite curve incorporating data from both C_{57}Bl/Jf and Swiss male mice. We have found that C_{57}Bl/Jf and Swiss mice have essentially identical inhibition resulting from a single dose of Ani and similar degrees of inhibition at the 4 hr and 5 hr Ani+Ani data points shown in Figure 7. The inhibition curves for Ani and Ani(Sal)Ani+Cyclo are repeated on each figure.

The number of mice used to obtain each data point is indicated on the figures.

It should be noted that training of mice occurred in the behavioral experiments 15 min after the first injection of Ani or saline.

No depressant exerted a large effect on protein synthesis, either by itself or in combination with the protein synthesis inhibitors. The maximum inhibition caused by a depressant alone was 30% found with the meprobamate; this occurred approximately 2-1/4 hr after administration of meprobamate (Fig. 8). Protein synthesis inhibition by meprobamate persisted no more than 5 hr after its initial administration. Meprobamate in combination with Ani increased the protein synthesis inhibition at 2 hr from 80 to 90%. No significant increase in inhibition was found at 4 hr from Ani(Mep)Ani when compared with Ani(Sal)Ani. In addition, the duration of inhibition above 80% obtained with Ani(Mep)Ani+Cyclo was not extended beyond the 6 hr above 80% obtained with Ani(Sal)Ani+Cyclo.

Chloral hydrate (Fig. 9) produced a maximum of 30% inhibition of protein synthesis 1-1/4 hr after its injection. The inhibition persisted for no more than 3 hr after administration. In combination with Ani an increase of 12% in inhibition was noted 2 hr after administration of Ani (1-1/4 hr after administration of chloral hydrate), but not at the later time points.

Phenobarbitol (Fig. 10) gave results very similar to chloral hydrate and to meprobamate in that only slight and brief inhibition was found when it was administered by itself, and an increase in inhibition 2 hr after Ani injection (1-1/2 hr after phenobarbitol administration), but no significant increase in inhibition at the later time intervals.

Experimental Series 2: Effects of Stimulants

Design: The effects of the stimulants amphetamine, strychnine, picrotoxin, caffeine, and nicotine on protein synthesis, both in the presence and absence of anisomycin was investigated. The effects on protein synthesis were determined 1-1/4 hr and 2 hr after administration of Ani, and 1/2 hr and 2-1/4 hr after the administration of the stimulant. Three mice were used for each data point. [^{14}C(U)]-L-Valine was administered 20 min prior to sacrifice.

Results: The results are summarized in Table 1. Somewhat surprisingly, the stimulants produced either a slight inhibition of protein synthesis 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 anisomycin. Stimulants did not modify the inhibition produced by anisomycin.

DISCUSSION

The importance of arousal during acquisition of a habit has been recognized for some time. More recently it has been suggested that arousal that follows the acquisition of a habit plays an important role in memory formation. The physiological mechanisms which mediate post-training arousal may involve norepinephrine and other biogenic amines (Kety, 1976; Stein, 1975), hormones such as ACTH and vasopressin (Rigter, Van Riezen and deWied, 1974), and adrenergic and cholinergic neurotransmitters (McGaugh, 1973). We hypothesize that the more difficult a task, the longer the period of arousal that persists after the training. In the case of many standard laboratory tasks, greater difficulty is associated with more training trials and with a greater total exposure to shock. In order to prevent the formation of memory, protein synthesis must be prevented until such time as the neurophysiological effects of the arousal have ceased or considerably diminished. The neurophysiological effects (e.g., hormone release, increase in neurotransmitter release, increased activation of RNA, prolonged periods of memory-related protein synthesis) will diminish as the time from training increases. Let us note how the present experiments relate to this hypothesis and point out some problems of interpretation and some areas requiring further research.

Primary Tests of the Arousal Hypothesis

The present experiments give considerable support to the hypothesis that arousal following training affects the formation of long-term memory. Experiment 2 demonstrated that administering depressants (sodium phenobarbitol, chloral hydrate, or meprobamate) after training on the pole jump task significantly increased the amnesia caused by inhibition of protein synthesis that resulted from administration of Ani. Experiment 7 showed that the depressant effect was not task dependent in that similar results were obtained with passive avoidance. Experiments 4 through 7 showed that administering stimulants (d-amphetamine, strychnine, picrotoxin, nicotine, caffeine) after passive avoidance training significantly reduced the amnesia caused by inhibition of protein synthesis. Experiment 5 showed that the effect of administering stimulants was greater the closer the stimulants followed upon training; therefore, this was not a proactive effect on retrieval during the retention test. Experiments 6 and 7 showed that the effect of post-training stimulants was not absolute--it could be blocked by further increases in the duration of inhibition of protein synthesis (i.e., additional Ani injections). All of these results are consistent with the arousal hypothesis and suggest that arousal promotes memory formation.

Some Alternative Interpretations

In order to properly interpret the behavioral results and to evaluate some alternative explanations, a brief review of the modes of action of the drugs used in these experiments is desirable.

It is generally accepted that the excitants and depressants act by modifying the action of the various neurotransmitter systems in the central and peripheral nervous system. The actions of the neurotransmitters are complex, and in many cases they are localized in discrete anatomical areas. Nevertheless, for

the present purposes it suffices to indicate that the transmitter functions are frequently grouped by such classifications as cholinergic, adrenergic, excitatory, and inhibitory. An excellent review of the actions of neurotransmitters has recently appeared (Krnjevic, 1974) and hence no attempt will be made here to summarize their actions.

The excitants and depressants used in this study were chosen to act on a variety of neurotransmitter systems in order to assess whether the effects on memory are specific to certain neurotransmitter systems or are general to excitation or depression. These agents exert their influence in the CNS by a variety of actions. The principal modes of action of the stimulants appear to be better defined than those of the depressants. At the risk of oversimplification, their actions are summarized 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; Von Voigtlander and Moore, 1973). The actions of d-amphetamine are on the dopaminergic (Chiueh and Moore, 1973; Thornburg and Moore, 1973; Costa, Groppetti and Naimzada, 1972) and perhaps the noradrenergic systems (Snyder et al., 1970). Leonard (1972) suggested that the predominant effects of d-amphetamine are on the adrenergic system.

As its primary action, strychnine appears to act as an antagonist to glycine, thereby affecting the postsynaptic glycine receptor and selectively blocking inhibition (Curtis, Duggan, and Johnston, 1971; Curtis, 1969; Franz, 1975; Dreifuss and Andrews, 1972). However, the action of strychnine is not entirely specific (see Krnjevic, 1974, p. 459; Phillis, 1970).

Picrotoxin, by interaction with the GABA receptor, blocks presynaptic inhibition and affects all portions of the CNS. It is of interest that Snyder has estimated that as many as 30% of the brain synapses are GABAnergic (1975). Since the main action of GABA is to increase the membrane permeability to small

anions, especially chloride, picrotoxin ultimately will modify membrane permeability (Krnjevic, 1974, p. 448 et seq.).

Nicotine acts on the nicotinic acetylcholine receptors in brain. These nicotinic cholinergic cells are predominantly excitatory when activated (Krnjevic, 1974; p. 435 et seq.). The CNS effects of nicotine appear to result from the activation of these cholinergic receptors and appear to be partially dependent on endogenous catecholamine interactions. The presence of nicotinic acetylcholine receptors in brain has recently been demonstrated (Moore and Loy, 1972; Salvaterra, Mahler, and Moore, 1975; Eterovic and Bennett, 1974). The CNS effects of nicotine appear to be partially dependent upon endogenous catecholamine interactions (Sabelli and Giardine, 1972).

Caffeine excites the CNS at all levels, acting first on the cortex and then on the medulla (Ritchie, 1975). The mode of action of caffeine appears to be by inhibition of cyclic nucleotide phosphodiesterase (Fuxe and Ungerstedt, 1974). The inhibition of the phosphodiesterase leads to an increase in the cyclic AMP concentration of the brain. Waldeck (1973) has suggested that cyclic AMP is involved in central catecholamine receptor mechanisms. Greengaard (1976) has suggested multiple roles for cyclic nucleotides and protein phosphorylation in neuronal function. Caffeine has also been reported to raise the brainstem level of serotonin by 40 - 100%, either by preventing its release or by increasing the rate of serotonin synthesis (Berkowitz and Spector, 1973). It has also been shown to increase the conversion rate of tyrosine to noradrenaline and dopamine (Waldeck, 1971; Waldeck, 1972). A marked increase in the accumulation of labeled catecholamine from DOPA was also stimulated by caffeine.

Surprisingly little information appears to exist on the mode of action of the depressants. Sodium phenobarbital, in common with other barbiturates, appears particularly to affect synaptic transmission across neuronal and neuro-effector junctions (Harvey, 1975). It has been shown to decrease the turnover

of brain dopamine and serotonin (Corrodi, Fuxe, and Hohfelt, 1966, 1967) and lead to a reduction in the turnover of noradrenaline in cortical noradrenergic nerve terminals (Corrodi et al., 1971; Lidbrink et al., 1972). A number of rather non-specific actions are attributed to sodium phenobarbital such as a general reduction in the energy yielding and synthetic reaction of the brain (Harvey, 1975). While the locus and mode of action of meprobamate are not established, the pharmacological effects of meprobamate appear to be very similar to those of the barbiturates (Byck, 1975). Corrodi, Fuxe, and Hohfelt (1966) have reported that meprobamate reduces the utilization of forebrain dopamine but that it does not decrease the turnover of noradrenaline in the cortical noradrenergic nerve terminals. The CNS depression produced by chloral hydrate is believed to be caused by its reduction product trichloroethanol (Harvey, 1975). This reduction in brain is carried out by aldehyde reductase. Tabakoff et al. (1974) have suggested that one of the effects of chloral hydrate may be an increased steady state of the biogenic amine metabolism.

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 increased functional activity produced by a number of stimuli such as motor activity, electrical stimulation, narcotics and excitants, and protein and nucleic synthesis. Satake (1972) similarly concluded that trans-synaptic stimulation seems to activate protein metabolism in the neuron. With respect to specific experiments utilizing the drugs discussed above, Von Voigtlander (1974) has shown in the frog that d-amphetamine reduces the rate of transport in the nigrostriatal pathway, but no evidence was presented that it reduced the rate of protein synthesis. He ascribed the reduced rate of transport to the reduced rate of firing of neurons. McMahon and Blaschko (1971) have found that chloral hydrate inhibits protein synthesis in Chlamydomonas reinhardii. However,

it should be noted that high concentrations (0.01 M) were required to achieve greater than 90% inhibition. With longer exposure, cell division is inhibited, but this observation would not appear to be relevant for understanding the mechanism of action for the present studies. Edstrom and Larsson (1974) showed that high concentrations of barbiturates were relatively ineffective in inhibiting protein synthesis in vitro in the sciatic system of the frog.

In our experiment, we have found that each of the depressants caused a modest inhibition of protein synthesis which was 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. Neither the depressants nor the stimulants markedly altered the inhibition of protein synthesis produced by anisomycin.

Few studies have been made of the side effects of anisomycin on brain neurochemistry, especially on the neurotransmitter systems. Flexner and Goodman (1975) have raised questions about the interpretation of experiments using inhibitors of protein synthesis. They pointed out that important side effects on the central adrenergic system appear to be common to all inhibitors of protein synthesis and that these side effects may contribute to the amnesia. Indeed, they conclude that the behavioral manifestations may not be attritutable solely, or at all, to inhibition of protein synthesis. They showed that protein synthesis inhibitors--cycloheximide, puromycin, anisomycin, and acetoxycycloheximide--had the common property of depressing the rate of accumulation of norepinephrine, dopamine, and total catecholamines and at the same time markedly elevating the levels of tyrosine. However, in the case of anisomycin, Flexner and Goodman presented data for only one dosage and one time point after administration (2 h), and until more complete data are available, it is difficult to evaluate the significance of these results for the interpretation of our behavioral experiments. Squire, Kuczenski, and Barondes (1974) have studied the inhibition

of brain tyrosine hydroxylase activity by cycloheximide and anisomycin, and by doses of α-methyl-p-tyrosine which depressed tyrosine hydroxylase activity as much as or more than either cycloheximide or anisomycin. They concluded that the effect of protein synthesis inhibition on brain tyrosine hydroxylase activity is not sufficient to explain the amnesic effect. Unpublished experiments (Flood et al.) which have used drugs which specifically modify the levels of catecholamines have shown that these agents are much less effective than anisomycin as amnesic agents. Other experiments (Flood et al., in preparation) have demonstrated that anisomycin is effective as an amnestic agent within minutes after its administration. No rapid effects of anisomycin on neurotransmitter systems have yet been reported; however, we are now investigating this possibility.

All five stimulants enhanced memory formation in that they blocked or reduced the amnesia induced by the anisomycin injections. The results of the biochemical experiments summarized in Table 1 showed that the stimulants did not significantly affect protein synthesis nor did they decrease significantly the inhibition produced by Ani.

The extensive biochemical tests reported in this paper showed that none of the depressants affected protein synthesis to any important extent. Since all three depressants had similar effects in enhancing amnesia, it appears that the effect of these drugs on memory is due to the general pharmacological actions of these agents as depressants rather than to their more specific effects.

Therefore, at the present time, we prefer to believe that the neurological actions commonly referred to as "arousal" provide a mechanism by which protein synthesis is rapidly modified in "activated pathways" leading eventually to long-lasting anatomical--synaptic and/or dendritic--changes. Many factors, including shock intensity and duration, excitant and depressant and drugs, may serve to modify the degree of arousal of the animal and thus the magnitude of the protein synthesis evoked and eventually the strength of the memory formation.

A question frequently asked is whether the injection of Ani 15 min before training was sufficient to disrupt learning or to impair memory formation. Figure 1C demonstrated that the group given only a pretrial injection of Ani showed good retention as did saline-injected controls (Fig. 1B). Thus, unless the inhibition is maintained by subsequent post-training injections of Ani, memory formation is not impaired (Flood et al., 1973, 1975a, b).

Type of Learning Task and Amnesia

Different types of training seem to require shorter or longer periods of inhibition of protein synthesis to induce amnesia. We believe that these observations can be related to the arousal hypothesis.

Passive avoidance can be learned in a single trial, and 75-85% amnesia can be induced by one or two injections of Ani (2-4 hrs of inhibition) depending on the degree of training (Flood et al., 1973, 1974). The jump pole task is learned well in two trials, and it required 8 hr of inhibition to yield 66% amnesia. We have previously shown that T-maze avoidance conditioning can be acquired in 5 trials, but production of an amnesic effect required 14 hrs of inhibition of protein synthesis. Using a still more difficult rod-discrimination task involving 20 training trials, Squire and Davis (1975) failed to produce substantial amnesia with about 10-11 hrs of inhibition. These tasks can also be compared in terms of the duration of footshock that the subjects received during training: passive avoidance, 1-4 sec; jump pole, 12-20 sec; T-maze, 20-45 sec; and the rod-discrimination task, at least 60 sec. Thus among these training procedures employing shock, the length of inhibition required to produce amnesia is related to both the number of trials and the duration of shock received. Consistent with this is the fact that within a given task, greater shock strength reduces the likelihood of amnesia (Flood et al., 1972, 1973, 1974).

Further evidence on this question comes from the studies in which shock was not employed for training and Ani was used to induce amnesia. Squire and Becker (1975) found that a single, immediate low dose injection of Ani impaired retention for habituation to a novel sound; the measure was the degree of drink suppression when the sound was subsequently presented. Also we have recently found that retention for learned extinction of an active avoidance response was very poor in Ani-injected animals. Although the initial training in this case involved shock, no shock was employed during the extinction trials. number of trials to reach extinction criterion was relatively large (8-10), (2-4 hrs) only relatively short duration of inhibition was needed to disrupt retention for the learned extinction that did not involve shock. Thus it seems that retention for non-shock motivated learning tasks is retention for may be more susceptible to Ani interference than shock-motivated learning tasks. Considering both shock-motivated and non-shock-motivated tests, it appears that the differing degrees of arousal may be the important factor in determining how susceptible the formation of memory is to inhibition of protein synthesis.

Anomalous Effects with Large Doses

A large dose of Ani when given prior to training, but not when given after training, results in far greater amnesia than one would expect on the basis of the duration of inhibition (Flood et al., 1973, Exp. 7; 1975a). In the present Exp. 1, 5 Ani (5 times the amount normally given) results in about 3 hrs of inhibition at 80% or greater yet caused significantly greater amnesia than a single low dose of Ani. However, we previously found that the same large dose given after training (e.g. Ani+5Ani) did not cause any greater amnesia than would be expected on the basis of the resulting duration of inhibition of protein synthesis (Flood et al., 1975a). Squire and Davis (1975) have also observed that a large pretraining dose of Ani (equivalent to 9 Ani) produced greater amnesia than a low dose of Ani on the rod-discrimination task.

They suggested that the slightly higher inhibition of protein synthesis at the time of training--and therefore less "leakage" of continuing synthesis--might account for the greater effectiveness of large doses of Ani. However, if "leakage" of protein synthesis occurred throughout the multiple-injection Ani experiments, then the later injections of Ani should not have caused amnesia since the protein would already have been synthesized by this time. Another possibility may be that such large doses not only affect protein synthesis but also produce substantial side effects which are insignificant when smaller doses of Ani are given. It may well be that there are such side effects on acquisition but not on memory formation / since large doses administered before training increased amnesia whereas large doses given posttrial did not cause any greater amnesia than would be expected on the basis of the resulting duration of inhibition of protein synthesis.

Proposed Relation between Stimulants, Depressants and Memory-Related Protein Synthesis

Since our results show clear effects of depressants and excitants on establishment of long-term memory, we should inquire about the mechanisms or processes whereby these drugs affect memory. Several alternative routes might be possible, and our experiments allow us to exclude some of these. First, the drugs might affect protein synthesis directly or might modulate the inhibition caused by Ani. Our biochemical experiments rule out this route, since the drugs did not affect inhibition of protein synthesis markedly. Secondly, the depressants and excitants could act by altering the initial acquisition. In the present experiments, however, the drugs were given after training, so that they could not have affected learning. Some of the excitants promoted

memory formation even when administered 90 min after training. Third, the drugs might operate by modifying the level of arousal during the post-training period and thus altering the excitability of tissues involved in memory formation; this may change the capacity for the CNS to direct memory-related protein synthesis. More specifically, we suggest that the mechanism by which stimulants improve retention is by prolonging the period of time over which the CNS has the capacity to synthesize memory-related protein(s). This is inferred from the results of Exp. 6 and 7 which showed that additional hrs of protein synthesis inhibition were required to yield amnesia when the excitants were used. Furthermore, the excitants differed in effectiveness, since 4 hrs of additional inhibition was required after d-amphetamine, whereas an additional 2 hrs of inhibition sufficed with the other stimulants tested.

The depressants on the other hand reduce the time during which the CNS retains the capacity for such protein synthesis. This is inferred from the observation that injecting a depressant in these experiments made it possible to produce the same degree of amnesia with 2 hrs less inhibition of protein synthesis than would otherwise have been necessary.

Another relevant finding previously reported is that permitting a brief pulse of protein synthesis produced greater retention the closer to training it occurred (Flood et al., 1975b). From this and other results it was inferred that the rate of memory-related protein synthesis decreased as the time since training increased. The modulation of protein synthesis resulting from training must wane during a period of hours; stimulants may maintain such modulation for a longer period of time, while depressants accelerate the decay.

Our 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.

Table 1

EFFECT OF STIMULANTS ON CEREBRAL PROTEIN SYNTHESIS

	Saline Injected Mice			Anisomycin Injected Mice		
	Time Administered Prior to Sacrifice (min)					
Saline or Anisomycin	75	120	180	75	120	180
Stimulant	30	75	135	30	75	135
	% Inhibition of [^{14}C]-Valine Incorporation					
Stimulant						
None	-	-		93 ± 2	77 ± 5	56 ± 8
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	
Caffeine citrate 20 mg/kg			5 ± 7			45 ± 8
Nicotine hydrochloride 0.5 mg/kg			6 ± 5			50 ± 8

* [^{14}C]-Valine was administered 20 min prior to sacrifice.

REFERENCES

Agranoff, B. W. (1972). Effects of antibiotics in long-term memory formation in the goldfish. In W. K. Honig and P. H. James (Eds.), "Animal Memory", p. 243-258. New York: Academic Press.

Andry, D. K., and Luttges, M. W. (1971). Facilitated habituation: Strychnine dose response effects on neural and behavioral habituation. Agents and Actions 2, 103-117.

Andry, D. K., and Luttges, M. W. (1972). Memory traces: Experimental separation by cycloheximide and electroconvulsive shock. Science 178, 518-520.

Barondes, S. H., and Cohen, H. D. (1968). Arousal and the conversion of "short-term" to "long-term" memory. Proc. Natl. Acad. Sci. USA 61, 923-929.

Berkowitz, B., and Spector, S. (1973). Role of brain serotonin in the pharmacological effects of the methyl xanthines. In J. Barchas and E. Usdin (Eds.), "Symposium on Behavioral Effects of Changes in Brain Serotonin, Stanford University, 1972", p. 137-147. New York: Academic Press.

Besson, M. J., Cheramy, A., Feltz, P., and Glowinski, J. (1971). Dopamine: spontaneous and drug-induced release from the caudate nucleus in the cat. Brain Res. 32, 407-424.

Breen, R. A., and McGaugh, J. L. (1961). Facilitation of maze learning with posttrial injections of picrotoxin. J. Comp. Physiol. Psychol. 54, 498-501.

Byck, R. (1975). Drugs and the treatment of psychiatric disorders. In L. S. Goodman and A. Gilman (Eds.), "The Pharmacological Basis of Therapeutics", p. 152-200. New York: MacMillan.

Castellano, C. (1974). Cocaine, pemoline, and amphetamine on learning and retention of a discrimination test in mice. Psychopharmacologia 36, 67-76.

Chiueh, C. C., and Moore, K. E. (1973). Release of endogenously synthesized

catechols from the caudate nucleus by stimulation of the nigro-striatal pathway and by the administration of d-amphetamine. Brain Res. 50, 221-225.

Cohen, H. D., and Barondes, S. H. (1968). Effects of acetoxycycloheximide on learning and memory of a light-dark discrimination. Nature (London) 218, 271-273.

Corrodi, H., Fuxe, K., and Hökfelt, T. (1966). The effect of barbiturates on the activity of the catecholamine neurons in the rat brain. J. Pharm. Pharmacol. 18, 556-558.

Corrodi, H., Fuxe, K., and Hökfelt, T. (1967). The effect of some psychoactive drugs on central monamine neurons. Europ. J. Pharmacol. 1, 363-368.

Corrodi, H., Fuxe, K., Lidbring, P., and Olson, L. (1971). Minor tranquilizers, stress and central catecholamine neurons. Brain Res. 29, 1-16.

Costa, E., Gropetti, A., and Naimzada, M. K. (1972). Effects of amphetamine on the turnover rate of brain catecholamines and motor activity. Brit. J. Pharmacol. 44, 742-751.

Crabbe, J. C., and Alpern, H. P. (1973). Facilitation and disruption of the long-term store of memory with neural excitants. Pharmacol. Biochem. Behav. 1, 197-202.

Curtis, D. R. (1969). The pharmacology of postsynaptic inhibition. Prog. Brain Res. 31, 171-189.

Curtis, D. R., Duggan, A. W., and Johnston, G. A. R. (1971). The specificity of strychnine as a glycine antagonist in the mammalian spinal cord. Exp. Brain Res. 12, 547-565.

Dawson, R. G., and McGaugh, J. L. (1973). Drug facilitation of learning memory. In J. A. Deutsch (Ed.), "The Physiological Basis of Memory", p. 77-111. New York: Academic Press.

Del Rio, J. (1971). Facilitation of some chlorpromazine-D-amphetamine mixtures on avoidance learning. Psychopharmacologia 21, 39-48.

Dreifuss, J. J., and Andrews, E. K. (1972). Antagonism between strychnine and glycine, and bicuculline and GABA in the ventromedial hypothalamus. Brain Res. 45, 599-603.

Duncan, N., and Hunt, E. Reduction of ECS produced retrograde amnesia by post-trial introduction of strychnine. Physiol. Behav. 9, 295-300.

Edström, A., and Larsson, H. (1974). Effects of barbiturates on synthesis and rapid axonal transport of protein in vitro in the sciatic system of the frog. Acta Physiol. Scand. 91, 433-440.

Erickson, C. K. (1971). Studies on the mechanism of avoidance facilitation by nicotine. Psychopharmacologia 22, 357-368.

Eterovic, V. A., and Bennett, E. L. (1974). Nicotinic cholinergic receptor in brain detected by binding of α-[^{3}H]bungarotoxin. Biochim. Biophys. Acta. 362, 346-355.

Evangelista, A. M., Gattoni, R. C., and Isquierdo, I. (1970). The effect of amphetamine, nicotine and heximenthonium on performance of a conditioned response during acquisition and retention trials. Pharmacol. 3, 91-96.

Evangalista, A. M., and Isquierdo, I. (1971). The effect of pre- and post-trial amphetamine injection on avoidance response of rats. Psychopharmacologia 20, 42-47.

Flexner, L. B., and Goodman, R. H. (1975). Studies on memory: Inhibitors of protein synthesis also inhibit catecholamine synthesis. Proc. Nat. Acad. Sci. USA 72, 4660-4663.

Flood, J. F., Bennett, E. L., Rosenzweig, M. R., and Orme, A. E. (1972). Influence of training strength on amnesia induced by pretraining injections of cycloheximide. Physiol. Behav. 9, 589-600.

Flood, J. F., Bennett, E. L., Rosenzweig, M. R., and Orme, A. E. (1973). The influence of duration of protein synthesis inhibition on memory. Physiol. Behav. 10, 555-562.

Flood, J. F., Bennett, E. L., Rosenzweig, M. R., and Orme, A. E. (1974). Comparison of the effects of anisomycin on memory across six strains of mice. Behavior. Biol. 10, 147-160.

Floow, J. F., Bennett, E. L., Orme, A. E., and Rosenzweig, M. (1975a). Effects of protein synthesis inhibition on memory for active avoidance training. Physiol. Behav. 14, 177-184.

Flood, J. F., Bennett, E. L., Orme, A. E., and Rosenzweig, M. R. (1975b). Relation of memory formation to controlled amounts of brain protein synthesis. Physiol. Behav. 15, 97-102.

Flood, J. F., and Jarvik, M. E. (1976). Drug influence on learning and memory. In M. R. Rosenzweig and E. L. Bennett (Eds.), "Neural Mechanisms of Learning and Memory", p. 483-507. Cambridge, Mass.: MIT Press.

Franz, D. N. (1975). Central nervous system stimulants. Strychnine, picrotoxin, pentylenetetrazol, and miscellaneous agents (doxapram, ethamivan, nikethamide, flurothyl, methylphenidate). In L. S. Goodman and A. Gilman (Eds.), "The Pharmacological Basis of Therapeutics", p. 359-366. New York: MacMillan Publ. Co.

Fuxe, K., and Ungerstedt, U. (1974). Action of caffeine and theophyllamine on supersensitive domamine receptors. Considerable enhancement of receptor response to treatment with dopa and dopamine receptor agonists. Med. Biol. 52, 48-54.

Garg, M., and Holland, H. C. (1969). Consolidation and maze learning: A study of some strain/drug interactions. Psychopharamacologia 14, 426-431.

Geller, A., Robustelli, F., and Jarvik, M. E. (1970). A parallel study of the amnesic effects of cycloheximide and ECS under different strengths of conditioning. Psychopharmacologia 16, 281-289.

Glowinski, J., and Axelrod, J. (1966). G. effects of drugs on the disposition of H^3-norepinephrine in the rat brain. Pharmacol. Rev. 18, 775-785.

Gordon, W. C., and Spear, N. E. (1973). Effects of strychnine on recently acquired and reactivated passive avoidance memories. Physiol. Behav. 10, 1071-1075.

Greengard, P. (1976). Possible role for cyclic nucleotides and phosphorylated membrane proteins in postsynaptic actions of neurotransmitters. Nature 260, 101-108.

Harvey, S. C. (1975). Hypnotics and sedatives: The barbiturates. In L. S. Goodman and A. Gilman (Eds.), "The Pharmacological Basis of Therapeutics", p. 102-123. New York: MacMillan.

Jakoubek, B., and Semiginovsky, B. (1970). The effect of increased functional activity on the protein metabolism of the nervous system. Int. Rev. Neurobiol. 13, 255-288.

Jarvik, M. E. (1964). The influence of drugs upon memory. In H. Steinbery, A. V. S. de Reuck, and J. Knight (Eds.), "Animal Behavior and Drug Action", p. 44-64. Boston: Little, Brown and Co.

Kety, S. (1976). Biological concomitants of affective states and their possible roles in memory processes. In M. R. Rosenzweig and E. L. Bennett (Eds.), "Neural Mechanisms of Learning and Memory", p. 321-326. Cambridge, Mass.: MIT Press.

Krivanek, J. A., and McGaugh, J. L. (1969). Facilitating effects of pre- and posttrial amphetamine administration on discrimination learning in mice. Agents and Actions 1, 36-42.

Krnjevic, K. (1974). Chemical nature of synaptic transmission in vertebrates. Physiol. Rev. 54, 418-540.

Leonard, B. E. (1972). Effect of four amphetamines on brain biogenic amines and their metabolites. Biochem. Pharmacol. 21, 1289-1297.

Lidbrink, P., Corrodi, H., Fuxe, K., and Olson, L. (1972). Barbiturates and meprobamate: decreases in catecholamine turnover of central dopamine and

noradrenaline neuronal systems and the influence of immobilization stress. Brain Res. 45, 507-524.

McGaugh, J. L. (1973). Drug facilitation of learning and memory. Annu. Rev. Pharmacol. 13, 229-241.

McGaugh, J. L., and Krivanek, J. A. (1970). Strychnine effects on discrimination learning in mice: Effects of dose and time of administration. Physiol. Behav. 5, 1437-1442.

McGaugh, J. L., and Petrinovich, L. (1965). Effects of drugs on learning and memory. Int. Rev. Neurobiol. 8, 139-196.

McMahon, D., and Blaschko, W. (1971). Chloral hydrate inhibits protein synthesis in vivo. Biochim. Biophys. Acta 238, 338-342.

Mayor, S. J. (1973). Puromycin's effects on long-term memory and the acquisition of two successive visual discriminiation tasks in Japanese quail. Physiol. Psychol. 1, 33-36.

Moore, W. J., and Loy, N. J. (1972). Irreversible binding of a Krait neurotoxin to membrane proteins from eel electroplax and hog brain. Biochem. Biophys. Res. Commun. 46, 2093-2099.

Oliverio, A. (1968). Effects of nicotine and strychnine on transfer of avoidance learning in the mouse. Life Sci. 7, 1163-1167.

Pare, W. (1961). The effect of caffeine and seconal on a visual discrimination task. J. Comp. Physiol. Psychol. 54, 506-509.

Petrinovich, L. F. (1967). Drug facilitation of learning: Strain differences. Psychopharmologia 10, 375-378.

Phillis, J. W. (1970). "The Pharmacology of Synapses", New York: Pergamon.

Quartermain , D., and Botwinick, C. Y. (1975). The role of biogenic amines in the reversal of cycloheximide-induced amnesia. J. Comp. Physiol. Psychol. 88, 386-401.

Quartermain, D., McEwen, B. S., and Azmitia, E. C. Jr. (1970). Amnesia produced by electroconvulsive shock or cycloheximide: conditions for recovery. Science 169, 683-686.

Randt, C. T., Barnett, B. M., McEwen, B. S., and Quartermain, D. (1971). Amnestic effects of cycloheximide on two strains of mice with different memory characteristics. Exp. Neurol. 30, 467-474.

Rigter, H., van Riezen, H., and deWied, D. (1974). The effects of ACTH- and vasopressin-analogues on CO_2-induced retrograde amnesia in rats. Physiol. Behav. 13, 381-388.

Ritchie, J. M. (1975). Central nervous system stimulants. The xanthines. In L. S. Goodman and A. Gilman (Eds.), "The Pharmacological Basis of Therapeutics", p. 367-378. New York: MacMillan.

Roberta, R. B., and Flexner, L. B. (1969). The biochemical basis of long-term memory. Quart. Rev. Biophys. 2, 135-173.

Sabelli, H. C., and Giardina, W. J. (1972). Tranquilizing and electrophysiological effects of nicotine. Biol. Psychiat. 4, 105-130.

Salvaterra, P. M., Mahler, H. R., and Moore, W. J. (1975). Subcellular and regional distribution of ^{125}I-labeled α-bungarotoxin binding in rat brain and its relationship to acetylcholinesterase and choline acetyltransferase. J. Biol. Chem. 250, 6469-6475.

Satake, M. (1972). Some aspects of protein metabolism in the neuron. Int. Rev. Neurobiol. 15, 189-213.

Serota, G. R., Roberts, R. B., and Flexner, L. B. (1972). Acetyoxycycloheximide-induced amnesia: Protective effects of adrenergic stimulants. Proc. Natl. Acad. Sci. USA 69, 340-342.

Snyder, S. H. (1975). Neurotransmitter and drug receptors in the brain. Biochem. Pharmacol. 24, 1371-1374.

Snyder, S. H., Taylor, K. M., Coyle, J. T., and Meyerhoff, J. L. (1970). The role

of brain dopamine in behavioral regulation and the actions of psychotropic drugs. Am. J. Psychiatry 127, 199-207.

Squire, L. R., and Barondes, S. H. (1972a). Inhibition of cerebral protein or RNA synthesis and memory. In J. Gaito (Ed.), "Macromolecules and Behavior", (2nd ed.), p. 61-82. New York: Appleton-Century-Crofts.

Squire, L. R., and Barondes, S. H. (1972b). Variable decay of memory and its recovery in cycloheximide-treated mice. Proc. Natl. Acad. Sci. USA 69, 1416-1420.

Squire, L. R., and Becker, C. K. (1975). Inhibition of cerebral protein synthesis impairs long-term habituation.

Squire, L. R., and Davis, H. P. (1975). Cerebral protein synthesis inhibition and discrimination training: Effects of extent and duration of inhibition. Behav. Biol. 13, 49-57.

Squire, L. R., Kuczenski, R., and Barondes, S. H. (1974). Tyrosine hydroxylase inhibition by cycloheximide and anisomycin is not responsible for their amnesic effect. Brain Res. 82, 241-248.

Stein, L., Belluzzi, J. D., and Wise, C. D. (1975). Memory enhancement by central administration of norepinephrine. Brain Res. 84, 329-335.

Stripling, J. S., and Alpern, H. P. (1974). Nicotine and caffeine: Disruption of the long-term store of memory and proactive facilitation of learning in mice. Psychopharmacologia 38, 187-200.

Tabakoff, B., Vugrincic, C. Anderson, R., and Alivisatos, S. G. A. (1974). Reduction of chloral hydrate to trichloroethanol in brain extracts. Biochem. Pharmacol. 23, 455-460.

Thornburg, J. E., and Moore, K. E. (1973). The relative importance of dopaminergic and noradrenergic neuronal systems for the stimulation of locomotor activity induced by amphetamine and other drugs. Neuropharmacol. 12, 853-866.

Ungerer, A. (1973). Nature et ampleur des effets de l'acetoxycycloheximide sur

la retention d'un apprentissage instrumental chez la souris. Physiol. Behav. 11, 323-327.

Von Voigtlander, P. F. (1974). Axonal transport: Reciprocal effects of neuroleptics and D-amphetamine. Brain Res. 72, 370-374.

Waldeck, B. (1971). Effects of caffeine and aminophylline on the turnover of catecholamines in the brain. J. Pharm. Pharmacol. 23, 824-830.

Waldeck, B. (1972). Increased accumulation of [^{3}H]catacholamines formed from [^{3}H]dopa after treatment with caffeine and aminophylline. J. Pharm. Pharmacol. 24, 654-655.

Waldeck, B. (1973). Sensitization by caffeine of central catechol amine receptors. J. Neural Transm. 34, 61-72.

FIGURE CAPTIONS

Figure 1. Effects of duration of inhibition of protein synthesis on retention for jump pole training (Exp. 1). As the number of injections of inhibitor increased from one to three (Ani, Ani^2, Ani^3), increasing the duration of inhibition, the percentage of mice showing amnesia increased. The amnesia resulting from a high dose (5 ANI) is unexpectedly large, since the duration of inhibition resulting from this dose is not much longer than that from a single dose.

Figure 2. The effects of depressants (phenobarbital, chloral hydrate, meprobamate) on the level of anisomycin-induced amnesia for the pole jump task (Exp. 2; N/group = 20). Each depressant significantly enhanced the amnesic effects of the inhibition of protein synthesis.

Figure 3. Effect of chloral hydrate and phenobarbital on anisomycin-induced amnesia for passive avoidance training [Exp. 3; N's: Sal(Sal)Sal, 20; Ani(Sal)Ani, 20; Ani(Sal)Ani+Ani, 23; Ani(CH)Ani, 31; and Ani(Pheno)-Ani, 21]. The depressants increased amnesia, and the resulting amnesia was equivalent to that obtained with three injections of Ani.

Figure 4. Time-dependent effects of stimulants on anisomycin-induced amnesia (Exp. 5; 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 5. Effect of the number of anisomycin injections (duration of inhibition of protein synthesis) on amnesia blocked by stimulants (Exp. 6). 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 hrs 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 extends 1-2 hrs 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 hrs 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 6. Effect of caffeine and nicotine on Ani-induced amnesia (Exp. 7). An additional injection of Ani was required to overcome the amnesia-blocking effects of the stimulants.

Figure 7. Inhibition of cerebral protein synthesis in Swiss Webster male mice obtained by subcutaneous injections of Ani, Ani+Ani, and Ani+Ani+Cyclo. The number of mice and the standard deviation are shown for each data point where more than two mice were used. The doses (0.5 mg Ani, 2.5 mg cycloheximide) and the injection schedule were the same as used for the behavorial experiments. Two major series of experiments done two years apart are represented by O and □.

Figure 8. The inhibition of protein synthesis by meprobamate alone (□ ---- □) or meprobamate and Ani+Ani+Cyclo (■ ---- ■). The inhibition by protein synthesis inhibitors without meprobamate (— · — · —) is redrawn from Fig. 7. The number of mice and standard deviation are shown for each data point. Administering meprobamate with the inhibitors neither increased the maximum inhibition nor prolonged its duration.

Figure 9. The inhibition of protein synthesis by chloral hydrate alone (□--□) or phenobarbital and Ani+Ani+Cyclo (■ ---- ■). The inhibition by protein synthesis inhibitors without chloral hydrate (— · — · —) is redrawn in Fig. 7. The number of mice and standard deviation are shown for each data point.

Figure 10. The inhibition of protein synthesis by phenobarbital alone (□--□) or phenobarbital and Ani+Ani+Cyclo (■---- ■). The inhibition by protein synthesis inhibitors without phenobarbital (— · — · —) is redrawn from Fig. 7. The number of mice and standard deviation are shown for each data point.

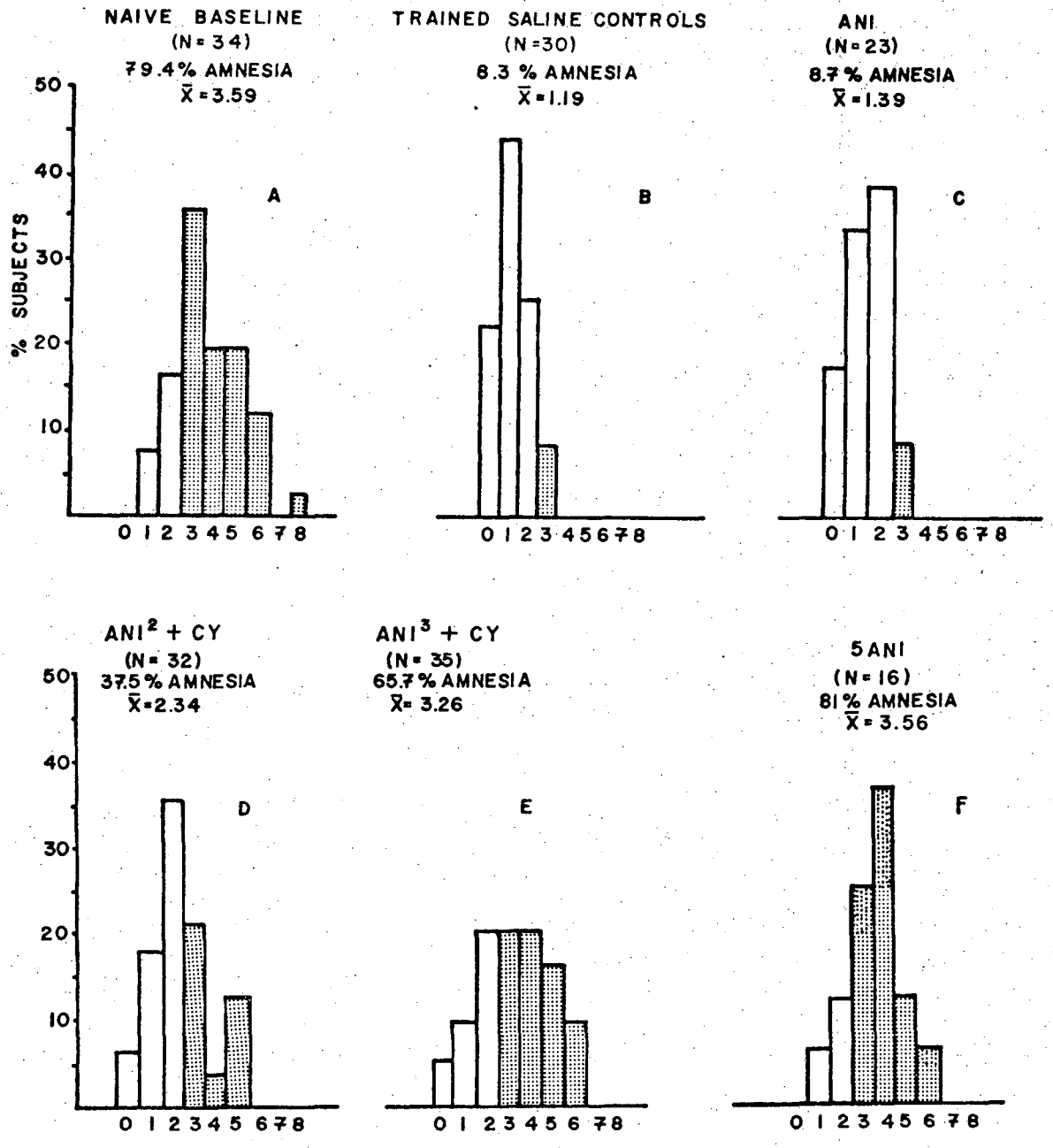

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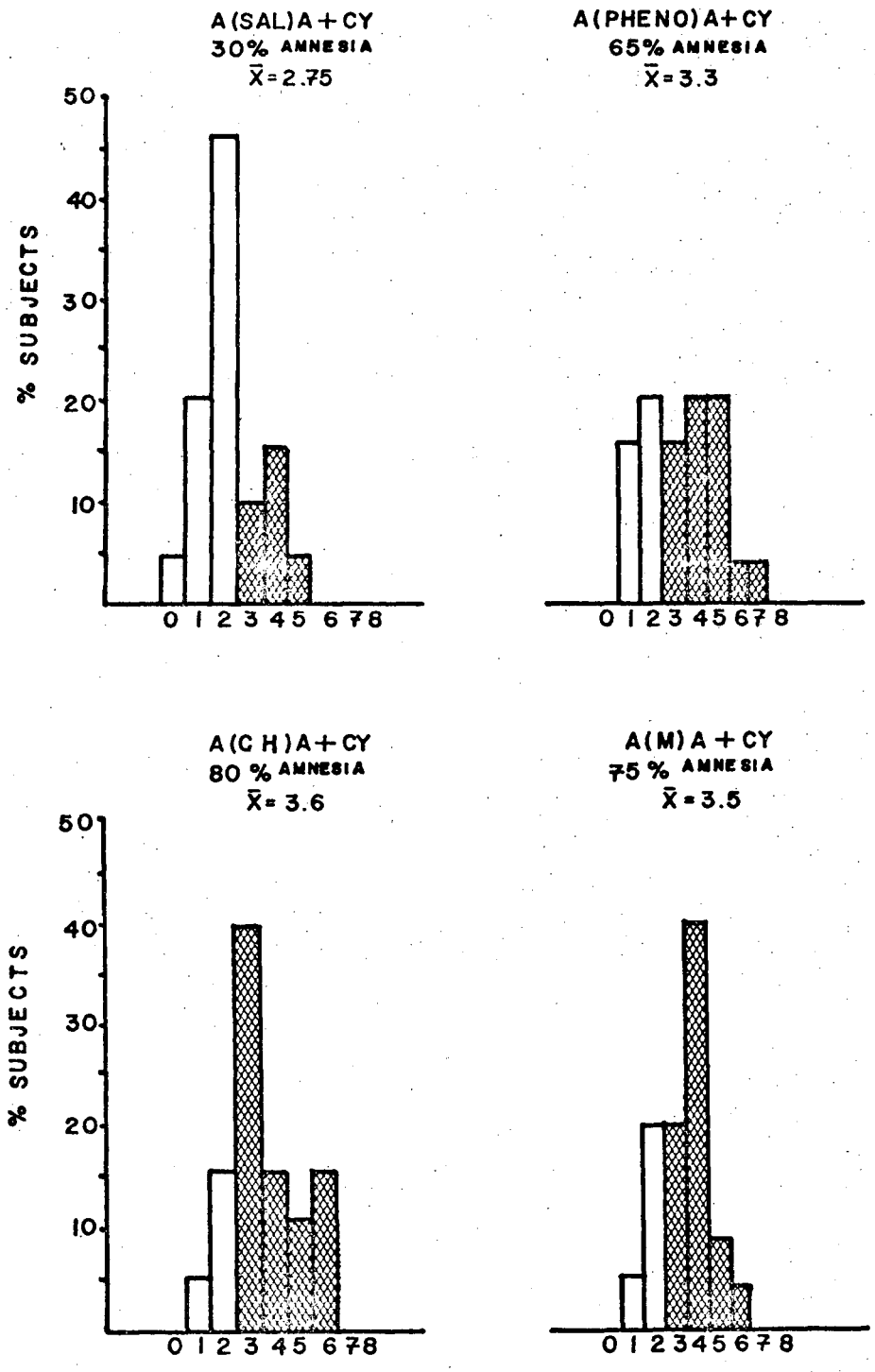
Fig. 1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A(PHENO)A+CY
65% AMNESIA
X̄=3.3
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80 % AMNESIA
X̄=3.6
A(M)A+CY
75 % AMNESIA
X̄=3.5
% SUBJECTS
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40
30
20
10
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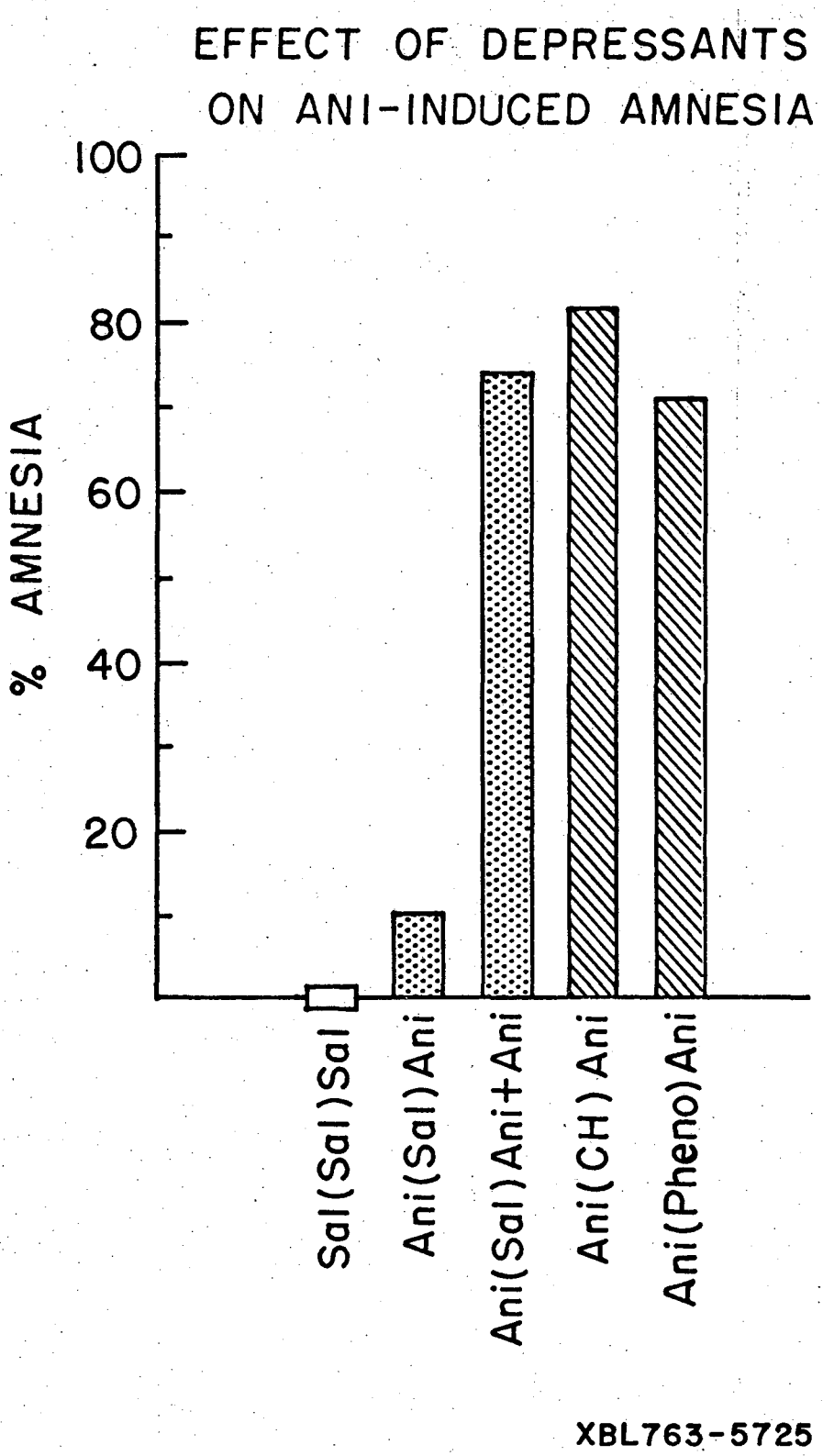
TRIAL OF FIRST AVOIDANCE RESPONSE
SV

Fig. 2



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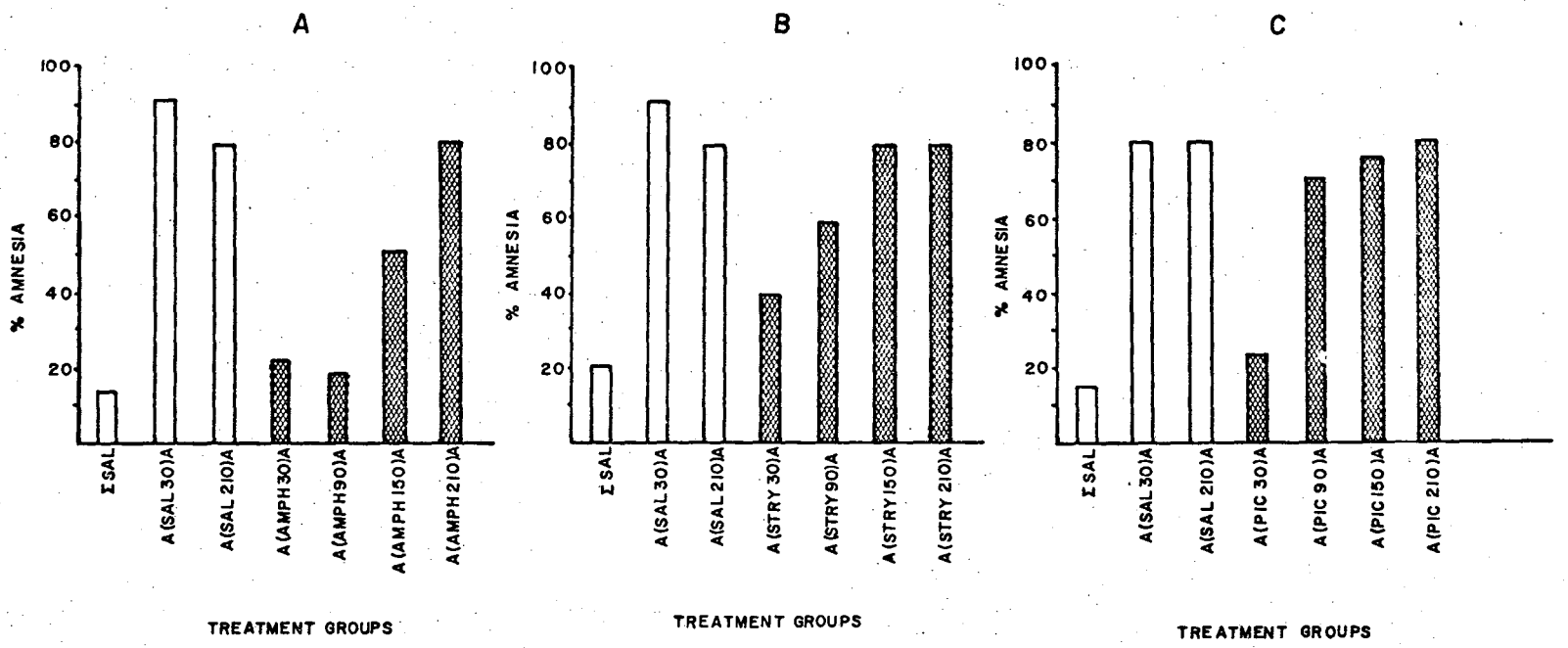

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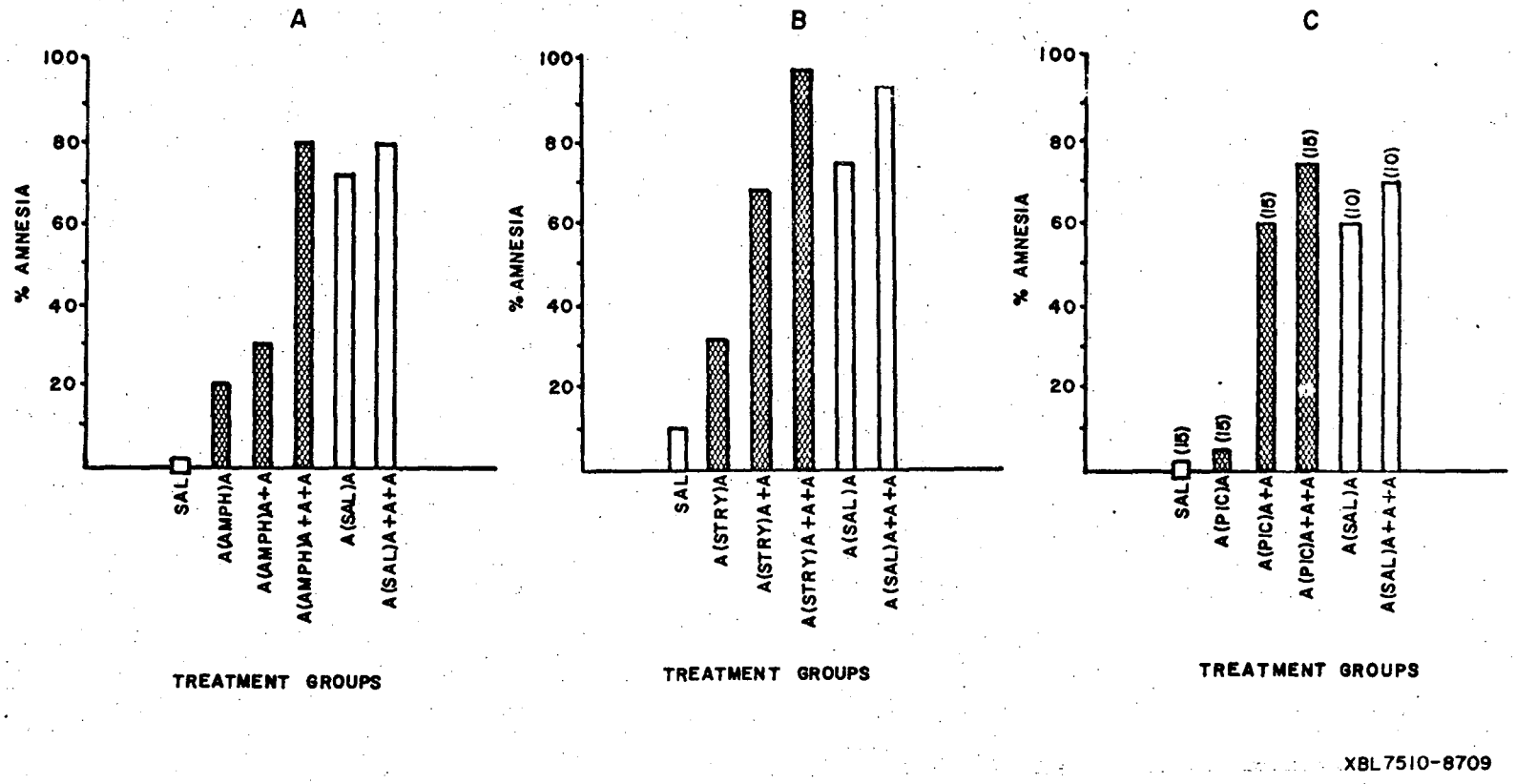
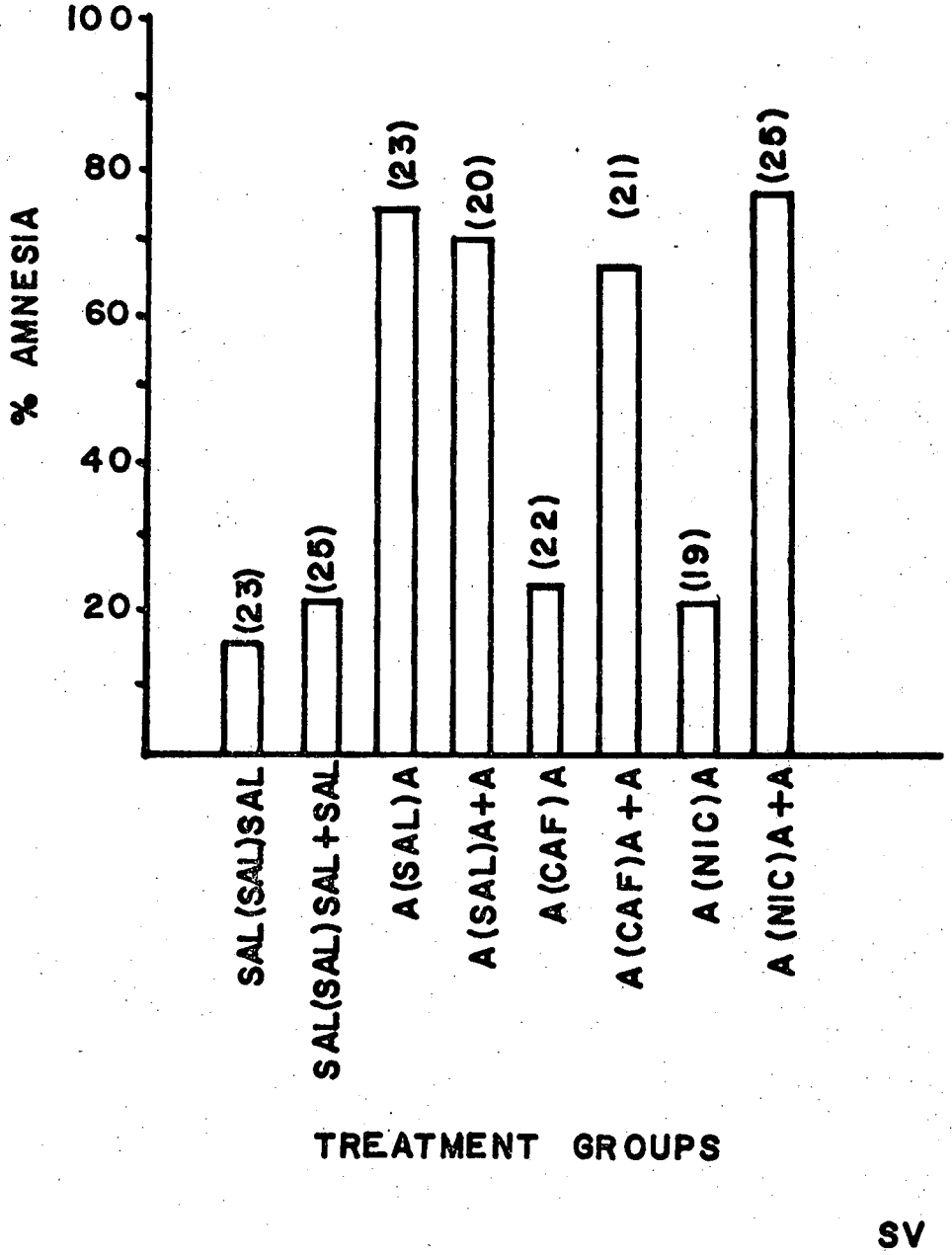


Fig. 5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SAL(SAL)SAL
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A(SAL)A
A(SAL)A+A
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TREATMENT GROUPS
SV

Fig. 6

Fig. 6

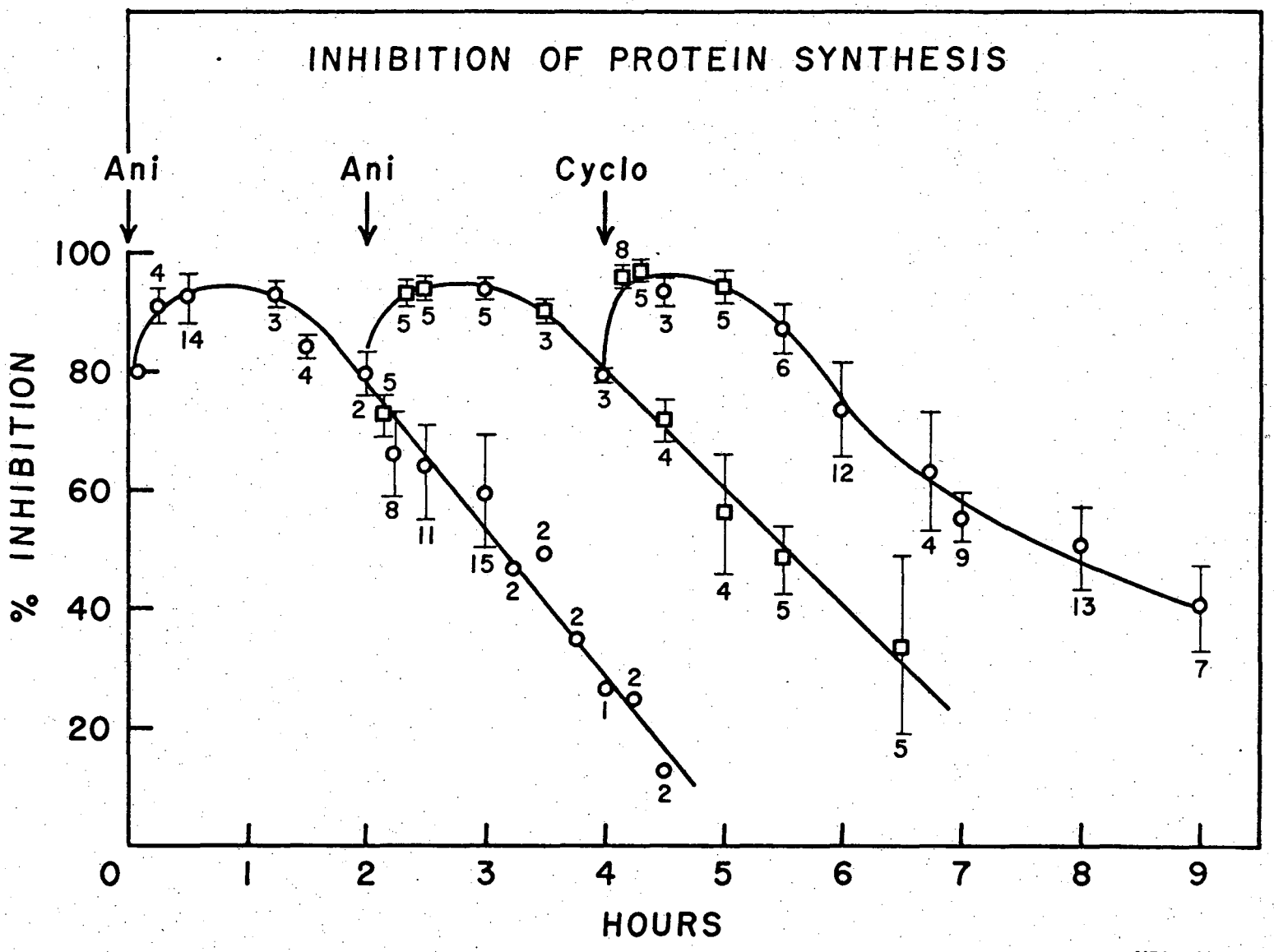

Fig. 7

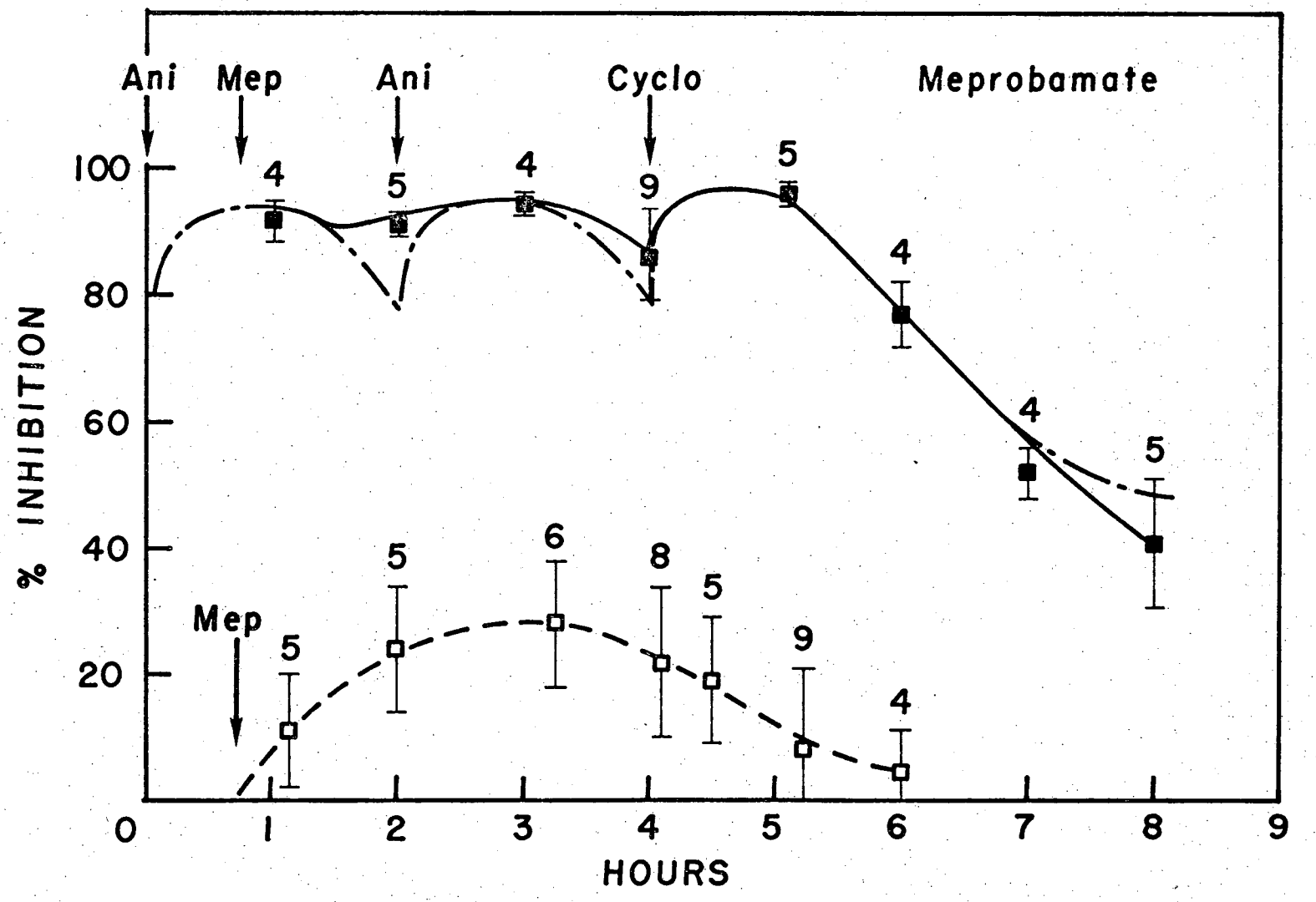

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Fig. 8

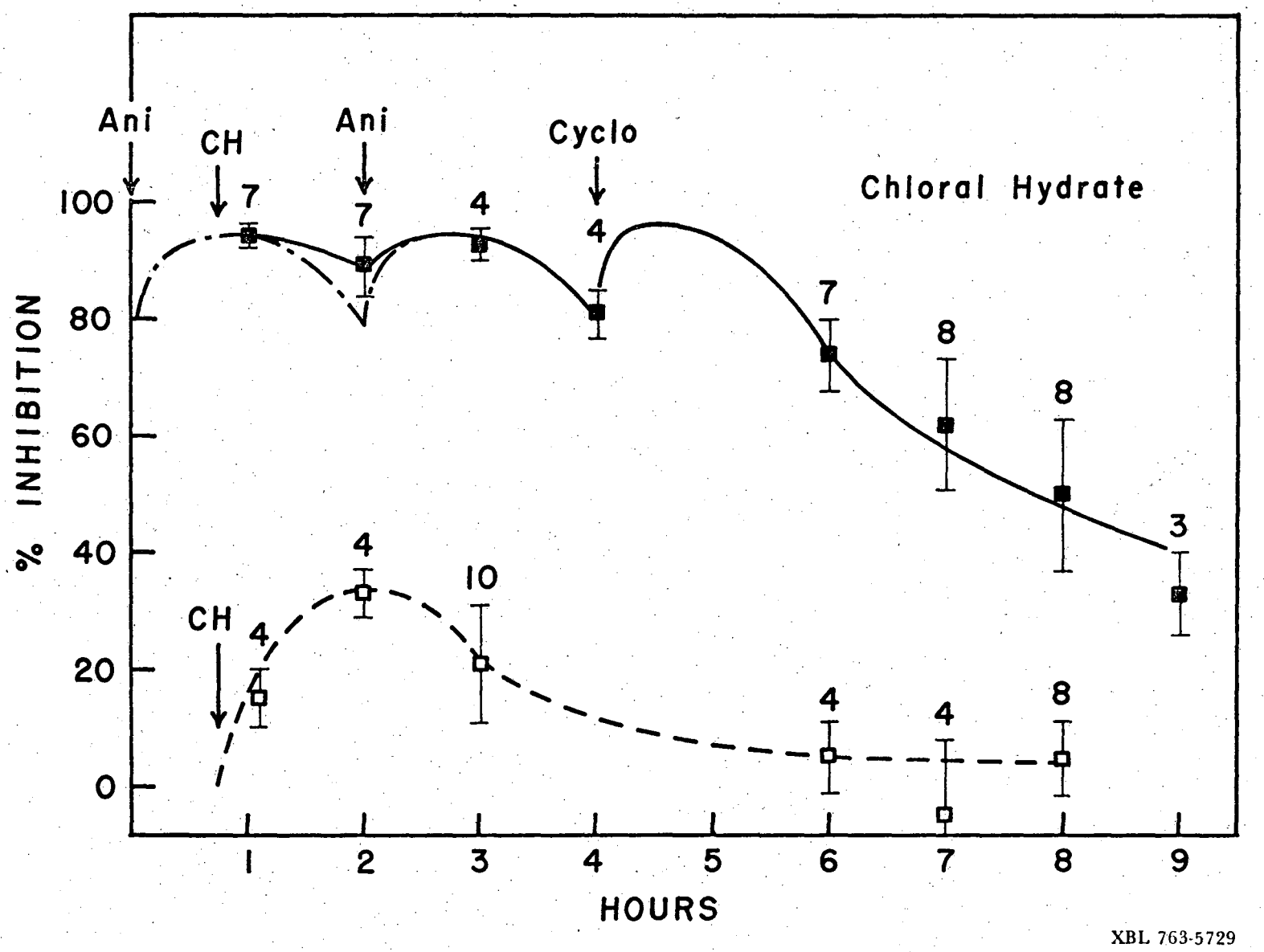

Fig. 9

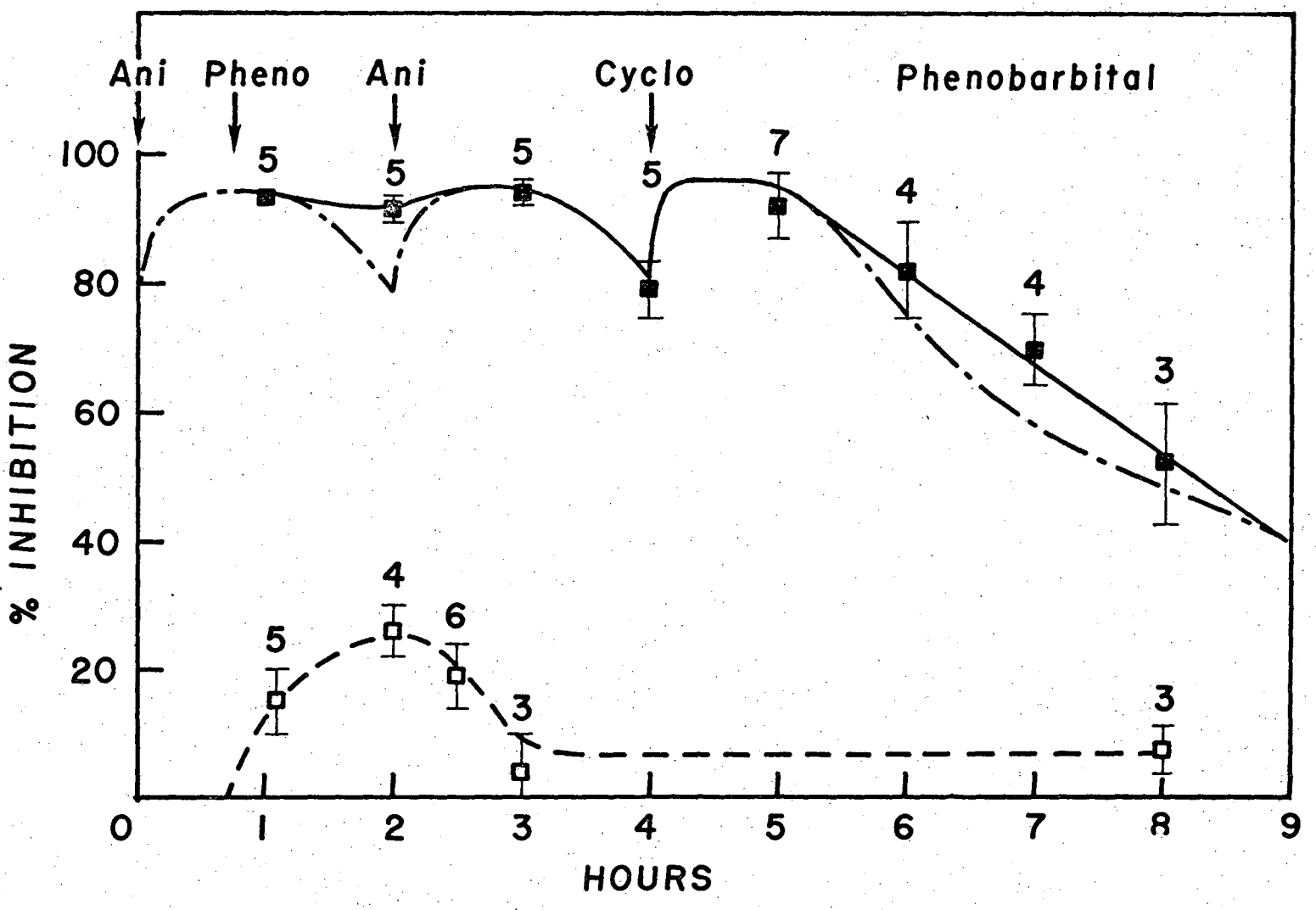

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Fig. 10

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