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EFFECTS OF ACTH PEPTIDE FRAGMENTS ON MEMORY FORMATION

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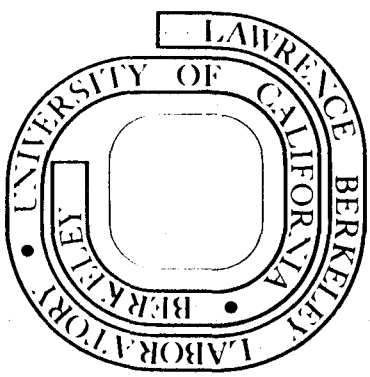

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DISCLAIMER

EFFECTS OF ACTH PEPTIDE FRAGMENTS ON MEMORY FORMATION

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Running Title: Effects of ACTH Peptides on Memory Formation

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ABSTRACT

The effects of peptides derived from ACTH on the formation of long-term memory have been investigated in male mice. Post-training administration of $ACTH_{4-10}$-L-Phe_7 (ACTH-L) improved retention for both passive and active avoidance tasks. Administration of $ACTH_{4-10}$-D-Phe_7 (ACTH-D) impaired retention for both tasks. The optimum dose for ACTH-L was about 0.3 mg/kg; the optimum dose for ACTH-D was in the range of 1.0 to 3.0 mg-kg. Using the passive avoidance task, it was shown that either drug had to be administered within 60 min of training to be highly effective.

Amnesia produced by anisomycin (Ani), an inhibitor of protein synthesis, was lessened by ACTH-L and increased by ACTH-D. ACTH-D opposed the memory-facilitating effects of ACTH-L.

Using intact mice, ACTH-L or ACTH-D did not significantly change the incorporation of valine into protein, nor did these peptides influence the inhibition of protein synthesis caused by anisomycin.

The results show that ACTH may play a major role in memory processing, perhaps by facilitating essential protein synthesis at sites specific for the memory being established.

Active avoidance
Anisomycin
$ACTH_{4-10}$-L-Phe_7
Passive avoidance
Protein synthesis
$ACTH_{4-10}$-DPhe$_7$
Arousal
Inhibition of protein synthesis
Peptides

EFFECTS OF ACTH PEPTIDE FRAGMENTS ON MEMORY FORMATION

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Since the early 1960's a great deal of research has been done on the role of the pituitary-adrenocortical axis in animal learning where footshock was the main motivation (for a review, see [3]). More recently attention has turned to the effects of ACTH and related compounds on retrieval of stored information through studies of the effect of ACTH peptides on extinction of a habit.

De Wied and his coworkers have suggested that $ACTH_{4-10}$-L-Phe_7 (ACTH-L) is the portion of ACTH which affects memory and that this portion has no adrenocorticotropic activity. In addition $ACTH_{4-10}$-D-Phe_7 (ACTH-D) opposes the activity of ACTH-L. Bohus and De Wied [1] reported that the decapeptide, $ACTH_{1-10}$-D-Phe_7, increased the rate of extinction for shuttlebox training. Greven and De Wied [16] studied different peptide fragments of ACTH to determine the shortest possible sequence of amino acids that would have the same effect on extinction as the full sequence of ACTH. ACTH-L was reported to be as effective as the naturally occurring ACTH in delaying extinction of shuttlebox performance, jump-pole response, and passive avoidance. ACTH-D facilitated shuttlebox and jump-pole extinction but delayed extinction for passive avoidance. The $ACTH_{4-10}$ peptides possess almost none of the adrenocorticotropic effects of the parent molecule ACTH [3]. Consistent with the above findings is a report by Rigter, Van Riezen and de Wied [21] that ACTH-L administered prior to the retention test could alleviate the amnesia caused by CO_2 or electroconvulsive shock.

Greven and de Wied [16] suggested that the ACTH peptide acts on membranes of specific target cells and by inducing conformational changes, stimulates

cyclic AMP production. This in turn stimulates protein synthesis and leads to the establishment of new synaptic junctions. Gispen et al. and Walter have outlined at this Symposium rather similar postulated mechanisms for actions of ACTH peptides on receptors leading to membrane changes through the cyclic nucleotide system. Greengaard [15] has recently discussed the multiple roles that cyclic nucleotides may play in synaptic function.

The research that we have undertaken focuses on the effects of the ACTH peptides on memory formation as opposed to the experimental designs used to study its effects on learning and retrieval. For this reason, we have investigated the effects of $ACTH_{4-10}$-L-Phe_7 and $ACTH_{4-10}$-D-Phe_7 either alone or in combination with anisomycin, a protein synthesis inhibtor, on retention and protein synthesis. A number of studies have now shown that anisomycin is an effective inhibitor of memory formation [8, 9, 11-13, 24, 25]. In all of the experiments in this report, the ACTH peptide fragments were injected within 4 h after training and thus 1 week prior to the retention test. This makes it unlikely that the effects of the peptides on retention are due to influences on the acquisition of the habit or on retrieval. Both active and passive avoidance tasks were used.

The effects of ACTH and derivative peptides have been studied in many laboratories under a variety of conditions. Macromolecular effects of ACTH and its analogues in the brain including effects on protein synthesis have recently been reviewed or reported de Wied [4], Dunn [7], Rees et al. [18], Dunn et al. (submitted to Neurochemical Research), and at this Symposium by Dunn, Rees, and Iuvone. Most of the studies cited investigated the effects of either ACTH or its analogues using either hypophysectomized or adrenalectomized mice or rats, or have used <u>in vitro</u> systems. However, no study was noted in which the compounds, mode of injection, and time parameters appeared

to be relevant for our behavioral studies. The main objectives of our biochemical experiments were twofold: (1) to determine any gross effects of $ACTH_{4-10}$ peptides on cerebral protein synthesis within hours after a single subcutaneous administration, and (2) to determine if some unanticipated interaction occurred between anisomycin and either peptide to greatly modify the inhibition of protein synthesis.

MATERIALS AND PROCEDURES

Behavioral Experiments

For the behavioral experiments, CD-1 male mice from Charles Rivers Breeding Laboratories, Wilmington, MA were obtained at 6 weeks of age. After 1 week in the laboratory, the mice were individually housed in small cages 24 h prior to training. After training the mice were returned to individual cages until the retention test was given one week later. The mice were trained on a one-trial step-through passive avoidance or on a T-maze active avoidance task. Mice were tested and trained between 0800 and 1400 h.

Passive Avoidance

The passive avoidance training and apparatus have been described previously [10]. In brief, the apparatus consists of a 44 cm long alley divided into a small, black start box and a longer white shock compartment. The two compartments are separated by a panel which contains a mouse hole. Entry into the white compartment was prevented until the appropriate time by a translucent guillotine door. The shock was administered by a high voltage, constant current 18 pole shock scrambler through a brass floor grid in the white box. The footshock intensity is given in each experimental description.

The training trial consisted of placing a mouse in the black start box for 20 sec, then illuminating the white shock box and the mouse hole for an additional 20 sec. Next, the guillotine door was removed while the mouse was facing away from it. The latency-to-enter was determined from the time the mouse oriented toward the mouse hole until it had entered completely the white compartment. The shock was turned on when the mouse was half-way down the alley (about 5 sec after entry), and was left on until the mouse was removed from the training box and was returned to its own cage.

The retention test followed the same procedure as for training except that no footshock was given. Mice not entering into the white compartment within 180 sec were removed and given a score of 180 sec. Amnesia was defined as entering into the white shock compartment in 20 sec or less. Retention was defined as not entering the shock compartment within 20 sec.

T-Maze Active Avoidance

The T-maze training and apparatus have been previously described [8]. The training apparatus consisted of a black Plexiglas start alley with a start box at one end and two goal boxes at the other. A brass floor grid ran throughout the entire maze. Each goal box was fitted with a clear Plexiglas liner, the bottom of which went below the shock grid. This liner was used to remove the animal from the goal box. The start box was separated from the rest of the start alley by a black Plexiglas guillotine door which prevented the animal from moving down the start alley until the trial started. Animals were not permitted to explore the maze prior to training. The conditioned stimulus (CS) was a door bell type buzzer. The intertrial interval was about 40 sec; 0.37 ma shock was used in all active avoidance tasks.

A training trial consisted of placing the mouse in the start box, then raising the guillotine door and simultaneously sounding the buzzer. Mice not moving to the correct goal box within 5 sec were shocked until they did so. The side preference was determined on the first training trial by forcing all mice to go to the side opposite to their first response. On subsequent trials, the correct goal box was the non-preferred side for each mouse. At the end of each trial, the mouse was removed to its home cage by carefully removing the liner and placing it into the mouse cage. As training proceeded a mouse could

make one of two types of responses: (a) a response latency longer than 5 sec was an escape, (b) a response latency less than or equal to 5 sec was an avoidance. The retention test given 1 week after training consisted of retraining to a criterion of 1 avoidance response. Mice requiring more than 3 trials to make the first avoidance response were scored as amnesic.

Injections

When pretraining injections of saline or anisomycin (2-p-methoxyphenyl-3-acetoxy-4-hydroxy-pyrrolidine) (20 mg/kg) were employed, they were administered under very light ether anesthesia. All ACTH peptide or control injections were given without ether. All injections were given subcutaneously in a volume of about 0.35 ml. Solutions of the ACTH fragments used for the behavioral studies were prepared in saline after first dissolving in a small quantity of 0.01 N HCl.

Biochemical Experiments

The effects of the ACTH peptides, either alone or in combination with anisomycin, on protein synthesis was determined by measuring the incorporation of $[^{14}C(U)]$-L-valine into the trichloracetic acid fraction of either whole brain in the first experiment, or into brain stem and rest of brain separately in the second. For the first experiment, mice were sacrificed 75, 100, and

135 min after subcutaneous administration of 0.5 mg of anisomycin. ACTH-D, ACTH-L, or saline was administered 45 min after the anisomycin as had been done in the behavioral experiments. [^{14}C]-L- valine was administered 15 min prior to sacrifice. The design of Experiment 2 was similar except mice were sacrificed 135 or 180 min after anisomycin injection. In the biochemical experiments (but not in the behavioral experiments), mannitol had been used to stabilize the peptide solutions. Therefore, in addition to the usual saline controls, some mice were administered mannitol. Experimental procedures for determining protein synthesis have been described in further detail [10]. Duplicate fractionations and determinations of radioactivity were determined for each brain sample. Four intact male Swiss mice, which were offspring of mice obtained from Charles River Breeding Laboratories, were used for each data point in the first experiment and 2 mice per data point in the second experiment.

BEHAVIORAL EXPERIMENTS AND RESULTS

Effects of $ACTH_{4-10}$-L-Phe_7 on Retention

Experiment 1 - 3

Experiment 1: Dose Response

The purpose of the experiment was to determine the dose range that might facilitate retention. $ACTH_{4-10}$-L-Phe_7 (ACTH-L) was administered 30 min after passive avoidance training. A low shock level of 0.30 ma was chosen so that controls would show poor performance and thus improvement of retention could be measured. As a further means of setting the proper level of training, we retained only those mice with latencies-to-enter by latencies-to-escape of 2 sec x 1 sec, 2x2, 2x3, 3x1, or 3x2 (values rounded to the nearest second). These latency values are very important since they determine the degree of learning. ACTH-L was administered at 0, 0.1, 0.3, 1.0 and 3.0 mg/kg. The 0.1 and 0.3 mg/kg doses are values similar to those used by de Wied and his associates.

Results

The results summarized in Fig. 1 show that for this passive avoidance task, the 0.1, 0.3 and 1.0 mg/kg doses of ACTH-L facilitated retention significantly and equally, that is, these groups had all showed about 80% retention, whereas the group receiving no ACTH-L showed only 27% (Fig. 1). There is some indication that too much of the peptide produces less than optimal facilitation of memory formation since the 3.0 mg/kg showed 50% retention.

Experiment 2: Interaction of Dose and Time of Administration

The purpose of this experiment was to determine if (a) ACTH-L could produce a better dose response curve with a decrease in training strength from that used in Experiment 1, and (b) if the effect of ACTH-L was dependent on the time of drug administration after training. The mice were trained (shock intensity 0.30 ma) and injected in the same manner as in Exp. 1 except that the latency-to-enter had to be 2 sec and the latencies-to-escape 1 or 2 sec. This shifted the distribution of training values toward those producing somewhat poorer retention; that is the controls were amnesic. Thus the test should be more sensitive to the effects of ACTH-L. The N per group was 20.

Results

The dose response portion of the ACTH-L curve over which retention improved was between 0.03 and 0.1 mg/kg. The dose of 0.3 mg/kg seemed to produce the optimal level of improvement in retention (Table 1). However, the effective dose range was still 0.1 to 1.0 mg/kg. The effect of ACTH-L on retention was dependent on the time of its administration. Administration of the peptide at 30 or 60 min after training had similar effects on retention. But ACTH-L was less effective in improving retention when administered 90 min after training and an additional loss of effectiveness was observed when administration occurred 240 min after training (Table 1). The decrease in

effectiveness of the higher ACTH-L doses was found again. In addition, this modified training procedure was an improvement over the procedure previously used since it made it possible to demonstrate that a dose of 0.3 mg/kg was optimal.

Experiment 3: ACTH-L on Retention using T-Maze Active Avoidance

The purpose of this experiment was to test if ACTH-L would have the same effect on memory formation for an active avoidance task as it did in the passive avoidance task. The mice were given 4 training trials on the T-maze using a muffled door bell buzzer as CS. ACTH-L was administered within 1 min after training at doses of 0.3 or 3.0 mg/kg. A saline-injected group served as control. The N per group was 10.

Results

Administration of ACTH-L immediately after active avoidance training improved retention scores both as measured by the percentage of mice requiring more than 3 trials to make the first avoidance response i.e., amnesic mice (Fig. 2B), and by the number of trials for the first avoidance (Fig. 2A). Again as in the passive avoidance task, the higher dose was less effective than the lower dose of ACTH-L.

Effects of $ACTH_{4-10}$-D-Phe_7 on Retention

Experiments 4 - 5

Experiment 4: Interaction of Dose and Time of Administration of ACTH-D

The purpose of the experiment was twofold: (1) to determine what doses of $ACTH_{4-10}$-D-Phe_7 (ACTH-D) would affect retention test scores and (2) to determine the influence of time of administration on the effect. The mice were trained on one-trial passive avoidance. In Experiment 2 it was necessary that the control animals would be only minimally trained so that facilitation of memory could be demonstrated. In this experiment control animals need to be well trained so

that impairment of memory formation by ACTH-D could be shown. One way of increasing training strength is to raise the level of shock intensity. For this reason shock intensity was .33 ma rather than .30 ma as in Experiment 2. ACTH-D was administered 30, 60, 90, or 240 min after training at doses of 0.3, 1.0 or 3.0 mg/kg. Saline was administered as a control for the stress of the injection. We retained only subjects with latencies-to-enter of 2 sec and latencies-to-escape of 2 sec (when rounded off to the nearest second). The N per group was 20.

Results

All three doses of ACTH-D were effective at reducing retention scores and thus increasing amnesia when administered 30 or 60 min after training. There is a trend under these conditions of training for the high dose of ACTH-D to be more effective than the low dose (Table 2). As was found with ACTH-L in Experiment 2, ACTH-D exerted a greater effect when injected 30 or 60 min than when injected 90 or 240 min after training.

Experiment 5: ACTH-D effect on T-Maze Active Avoidance

The purpose of the experiment was to determine if ACTH-D would have the same effect on retention for an active avoidance task as it did in a passive avoidance task. In Experiment 3 we examined the facilitating effect of ACTH-L on an active avoidance task, therefore it was necessary for controls to be minimally trained so only 4 training trials were given. In this experiment we are looking for impairment of memory, therefore well-trained controls were needed. For this reason the mice were given 5 training trials with a loud door bell buzzer as CS. In order to control the degree of learning a training performance criterion was imposed. Subjects were not included if they made an avoidance response during original training or if they failed to make one correct escape response. ACTH-D was administered within 1 min after training

at doses of 0.0, 0.3, 1.0 or 3.0 mg/kg. The retention test, as in all experiments of this series, was one week after training. The N was 20 per group.

Results

ACTH-D at 1.0 or 3.0 mg/kg significantly impaired retention; that is the percentage of amnesic mice increased (Table 3). As with Experiment 4, a significant difference was not found between 1.0 and 3.0 mg/kg doses although there is indication of greater effect with the larger dose (compare Tables 2 and 3).

The subjects which made avoidance responses during the original training were analyzed separately to see if ACTH-D would still have an effect (Table 4). Interestingly, this seems to have brought out the differences in dose response. ACTH-D at 3.0 mg/kg caused more amnesia. Owing to the small N's, no differences were significant but the group given 3.0 mg/kg differed from the control group at approximately $P < .06$.

Effects of ACTH on Amnesia produced by Anisomycin

Experiments 6 - 9

Experiment 6: Effects of ACTH-L

Protein synthesis inhibition by anisomycin (Ani) was used to cause amnesia in this experiment. In some of the mice ACTH-L was administered shortly after training (30 min) to determine if the ACTH-L would block the amnesia. Other groups received more injections of Ani to test if longer durations of inhibition of protein synthesis would block the anti-amnesic effect of ACTH-L.

Mice were trained on passive avoidance at a footshock of 0.36 ma. This is a relatively high footshock and produces strong training. Ani was administered 15 min prior to training and again at 1-3/4 h after training. Some groups received a third injection of Ani 3-3/4 h after training. Saline-injected

mice served as controls. Saline, 0.3 or 3.0 mg/kg of ACTH-L was administered 30 min after training. N per group was 20.

Results

As expected, two or three successive injections of Ani caused significantly greater amnesia than was found in the saline control (80 and 85% vs 15%). An injection of ACTH-L at the dose of 3.0 mg/kg significantly reduced this amnesia in mice given 2 successive injections of Ani but not in those given 3 successive injections of Ani (Fig. 3). The effect of 0.3 mg/kg ACTH-L, while significant, did not cause such a large drop in the percent amnesia. ACTH-L did block the amnestic effect of two successive injections of Ani but increasing the number of injections (and thus the duration of inhibition) blocked the anti-amnestic effects of the ACTH-L.

The high dose of ACTH was more effective at reducing the level of amnesia than the lower dose. However, an additional injection of Ani which prolongs inhibition of protein synthesis by 2 h at 80% or more blocked the anti-amnestic effect of the ACTH-L.

Experiment 7: Effects of Number of Injections of ACTH-L

The purpose of this experiment was to determine if additional injections of ACTH-L would block the effect of additional injections of Ani as observed in Experiment 6. The conditions of training were the same as in Experiment 6. The injection groups are given in Fig. 4. Ani or saline were administered 15 min prior to training and 1-3/4 h after training. Some groups received a 3rd injection 3-3/4 h after training. ACTH-L or its control injection (saline) was administered 1/2 h or 1/2 h and 2-1/2 h after training. ACTH-L was administered at a dose of 3.0 mg/kg. N per group was 10.

Results

As in experiment 6, two injections of Ani caused significantly more amnesia than did injections of saline. ACTH-L blocked the amnesia in groups receiving only two successive injections of Ani. Neither a single or double injection of ACTH-L blocked the amnesia induced by three successive injections of Ani (Fig. 4). These data, like the time dependent results of Experiment 2, suggest that ACTH-L alters memory formation only when administered within the first hour after training.

Experiment 8: Effects of ACTH-D

Under appropriately high conditions of training the amnestic effect of high levels of protein synthesis inhibition by anisomycin can be reduced so that one can test if another substance will increase amnesia.

Subjects were trained on the passive avoidance task. They received either two injections of Ani or saline 15 min prior to training and 1-3/4 h after training. ACTH-D was administered 30 min after training at 0.0, 0.3, 0.1 or 3.0 mg/kg. The latency-to-enter and escape was 2 sec by 2 sec. The footshock intensity was .34 ma. The N per group was 20.

Results

Two successive injections of Ani alone did not cause significant amnesia. The groups receiving ACTH-D all differ significantly from the group receiving only [A(ACTH-D 0)A] (Fig. 5). The 3.0 and 1.0 mg/kg doses differ at $P < .001$. The groups receiving ACTH-D did not differ significantly from each other but a strong indication of the weaker effect of the low ACTH-D dose versus the high is present. The doses 1.0 and 3.0 mg/kg in combination with Ani induced the greatest retention deficit or amnesia.

Opposing Effects of $ACTH_{4-10}$ L- and D-Phe_7 on Retention

Experiment 9A

The results of Experiments 1 through 8 indicated that ACTH-L facilitated memory formation while ACTH-D impaired memory formation. As a further test of the opposing effects of these peptide fragments, we administered ACTH-L at its optimal dose (0.3 mg/kg) and combined this with various doses of ACTH-D (0.3 to 6.0 mg/kg) to determine how much ACTH-D was required to block the facilitating effects of ACTH-L.

The mice were trained on passive avoidance at a footshock intensity of 0.30 ma. Subjects had to have latencies-to-enter of 2 sec and latencies-to-escape of 2 sec to be retained. The ACTH-L, ACTH-L + ACTH-D (combined in one injection), or saline were administered 18 sec after training. The doses of the peptides and the N's per group are given in Fig. 6A.

Results

ACTH-L given alone significantly enhanced retention when compared with the saline controls (Fig. 6A). A dose of ACTH-D at 3.0 or 6.0 mg/kg was sufficient to block the effect of ACTH-L. It appeared that 6.0 mg/kg of ACTH-D completely blocked the effect of ACTH-L (0.3 mg/kg).

Experiment 9B

As a further check on whether such a high dose of ACTH-D was required to block the ACTH-L effect on retention, Experiment 9A was repeated except that the footshock was reduced slightly to 0.28 ma. This was done to increase the percentage of amnesic mice in the control group. The groups used are shown in Fig. 6B. Other conditions were as in experiment 9A.

Results

ACTH-L again enhanced retention. In fact the percent difference was similar under the two training conditions (a difference of 45% for Experiment

9A, 58% for Experiment 9B; ACTH-L versus Saline). Under less vigorous training only the 6.0 mg/kg dose of ACTH-D completely blocked the effect of ACTH-L (Fig. 6B).

BIOCHEMICAL RESULTS

The results of Biochemical Experiments 1 and 2 indicate that ACTH-L or ACTH-D, when administered subcutaneously to intact mice, did not alter cerebral protein synthesis by more than 10% within 2-1/4 hrs (Tables 5 and 6). Furthermore, these peptides did not significantly modify the inhibition caused by anisomycin. Mannitol, which had been used to solubilize the ACTH peptides for the biochemical experiments, by itself caused a slight increase of valine incorporation. It should be emphasized that mannitol was not used in the behavioral experiments since it introduced side effects there also.

DISCUSSION

ACTH effects on memory formation.

The finding of primary importance in these experiments was that ACTH-L, a peptide fragment of ACTH, administered shortly after training can influence subsequent retention test performance and thus aid memory formation (Experiment 1-3). ACTH-D, containing the unnatural isomer of phenylalanine, impairs memory formation (Experiments 4-5). These effects do not appear to be proactive since it was found that the effect of the ACTH peptides was time dependent. Such peptides can have an effect on retention test performance when administered shortly prior to testing [2, 5, 6, 16, 27] and for the most part these effects are consistent with those found in our studies.

The results confirm previous reports that $ACTH_{4-10}$-L-Phe_7 and $ACTH_{4-10}$-D-Phe_7 have opposite effects on retention for an active avoidance task [1, 16, 27] and for an appetitive task [14]. Gray and Garrud (this Symposium) have reported that ACTH-L and ACTH-D given during acquisition had opposite effects during the non-rewarded extinction test; the L-peptide blocked the effect of the non-reward, the D-peptide accelerated the extinction. ACTH-L consistently enhanced retention in active and passive avoidance tasks (Experiments 1, 2, 3). ACTH-D impaired retention in active and passive avoidance (Experiments 4, 5). However, Greven and de Wied [16] reported that $ACTH_{1-10}$-L-Phe_7 did not show the anticipated reversal of the effects of $ACTH_{1-10}$-L-Phe_7 for a passive avoidance task. Consistent with the opposing actions of the two peptides was the finding that ACTH-L acted as an anti-amnestic agent (Experiment 6) and ACTH-D potentiated amnesia caused by brain protein synthesis inhibition (Experiment 8).

Effective dose range of the ACTH peptides.

The effective dose range of the peptides was similar to that reported for

extinction test studies [16, 21, 27]. ACTH-L was effective over a dose range of 0.1 to 1.0 mg/kg or 3 to 30 µg per 35 gm mouse. The optimal dose was 0.3 mg/kg or 10 µg per mouse. The ACTH-D effective dose range was somewhat higher than reported for extinction studies. Doses from 0.3 to 3.0 mg/kg were effective and 1.0 mg/kg µg/mouse seemed to be the most effective dose. Extinction studies have shown that the effective dose range of the L- and D-ACTH peptide fragments is about the same. However, when administered different ratios of the peptides after training, ACTH-D seemed to have significantly less affinity for the receptor site than the L form, since it took a ratio of ACTH-D to ACTH-L of at least 10 to 1 to block ACTH-L facilitation (Experiment 9A and 9B). It is also possible different receptors are involved with the ACTH-D receptor being less active. A third possibility would be differences in effective concentration of the two drugs at the active sites. De Wied has presented evidence that different receptor sites exist for the two forms of the peptide [6].

Anti-amnestic studies.

We have shown that anisomysin administered at 2 hr intervals will maintain a high level of inhibition of protein synthesis [11]. There is an interaction between the strength of training as determined by several factors including the shock intensity, duration of shock, etc., and the duration of inhibition required to produce amnesia [10-12]. We have recently shown that a number of stimulants and depressants, which act by a variety of mechanisms, can modify the amnesia produced by anisomysin (Flood et al., submitted to Behavioral Biology). In the present series of experiments, it was found that ACTH-L and ACTH-D can also modify anisomycin-induced amnesia. $ACTH_{4-10}$-L-Phe_7 was found to block anisomycin-induced amnesia when the peptide was injected 30 min after training. However, extending the duration of inhibition by an additional 2 hr by a 3rd injection of Ani re-established the amnesia (Experiment 6). An

additional injection of the peptide was not able to block amnesia when three successive anisomycin injections were used (Experiment 7).

ACTH-D increased a partial amnesia caused by anisomycin after strong training. We would like to caution against an interpretation that the anti-amnestic effect of ACTH-L suggests that anisomycin causes amnesia by interfering with ACTH function or vice versa. In this series of experiments, we were unable to show that ACTH-L or ACTH-D had more than a slight generalized effect on protein synthesis as measured in large brain areas. In addition, neither ACTH-L nor ACTH-D appeared to change significantly the overall inhibition of protein synthesis produced by anisomycin. In interpreting the effects of ACTH peptides on memory other results that we have obtained should also be considered:

(a) ACTH-L improves retention in poorly trained mice,

(b) stimulants including d-amphetamine, strychnine, picrotoxin, caffeine, nicotine, and bicuculine all blocked amnesia induced by anisomycin and the amnesia could be regained by extending the duration of inhibition of protein synthesis (Flood et al., ibid, and unpublished observations),

(c) dexamethasone and hydrocortisone could block amnesia induced by inhibition of protein synthesis,

(d) depressants, including sodium phenobarbital, chloral hydrate, chloropromazine, meprobamate, potentiated anisomycin-induced amnesia (Flood et al., ibid).

It seems unlikely that pituitary function or dysfunction can explain all the drug effects. A common factor in all cases may be the net effect of hormones or drug on arousal. Increases in arousal improved retention and counteracted amnestic treatments, while decreases in arousal impaired retention and potentiated the effects of amnestic treatments. We have discussed recently the role of arousal in memory formation (Flood et al., ibid).

The ACTH peptides did not have any effect on open-field type activity which is normally associated with arousal or stimulants. One might think of arousal as a group of changes that serves several functions (i.e., prepares an animal to defend itself, flee, look for food, improve memory storage). The ACTH peptides would seem to be very specific in that they only affect memory storage and retrieval and have little or no effect on gross behavior as does amphetamine. Direct administration of the peptides to various areas of the brain suggests arousal may be involved since the active sites are primarily located in or near the reticular formation and its projections [27]. Consistent with ACTH affecting arousal levels is a report that ACTH-L shifted hippocampal theta activity [26].

Previous work with the peptides and the question of permanence of amnesia has employed pretesting injections of ACTH-L to alleviate amnesia [20, 21] have attempted to obtain similar effects using anisomycin to impair retention, but as yet we have not yet been successful in overcoming amnesia. A possible reason for the difference in results is that many studies compare trained treated and untreated subjects. This comparison, while showing a loss of retention due to some amnestic treatment, does not necessarily imply that the loss of memory was complete or even substantial. In our studies, treated and untreated mice were compared against a performance criterion that represents the performance of naive mice. Treatments that can alleviate amnesia using pretesting injections may only be successful if the degree of memory loss is substantially less than complete. We recently reported that d-amphetamine reversed amnesia in anisomycin-injected mice which had long amnesic latencies in a passive avoidance task but had no effect on mice that originally had very short test latencies [13]. Presumably the amnesia was more severe in the case of those mice with very short test latencies and increased arousal was not

to aid recall. In addition, amphetamine had the same effect of improving recall in poorly trained saline-injected mice. Thus anti-amnestic effects are not necessarily restricted to animals receiving amnestic treatments. It might be said that anti-amnestic treatments have the ability to improve the recall of stored memories that are poorly stored either because of drug interference with consolidation or because of weak training as in the case of poorly trained undrugged subjects.

Biochemical Effects of ACTH Peptides

While it is generally stated that ACTH and selected analogues stimulate protein synthesis, the clearest evidence for this stimulation comes from studies of hypophysectomized animals. In this system, either single or chronic injections have been shown to produce large increases in protein synthesis [17, 22, 23]. It should be noted that protein synthesis is depressed in these animals, and the effect of the peptides has been to restore protein synthesis to a more normal value. Another commonly used system to investigate the effects of ACTH analogues has been tissue slices of brain derived from both normal and operated animals. Here also stimulation of protein synthesis to a normal value was observed in the operated animal by *in vitro* addition of ACTH [19]. A surprising paucity of data appears to exist on the acute effects of ACTH and its analogues on cerebral protein synthesis. Rees, Dunn and Iuvone [18] have recently shown that ACTH peptides administered intracerebrally causes a slight stimulation of cerebral protein synthesis within 90 min after administration. Dunn, Rees, and Iuvone (submitted, Neurochem. Res., and this Symposium) have reported that a small but significant increased incorporation of lysine into brain protein within 15-25 min after administration of ACTH-L. Our studies are in general agreement with those of Rees, Dunn and Iuvone in that any effects we found were small. It is clear that further research needs to be done to

determine the effects of ACTH derivatives on protein synthesis at specific sites (micro effects).

In our experiments ACTH peptides had the ability to modify memory processing. ACTH-L facilitated memory processing and ACTH-D impaired processing. The effects were time-dependent and were observed in both passive and active avoidance tests. However, until more is known of the mechanics of action of these ACTH peptides, their role in memory processing will be uncertain.

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TABLE 1

Amnesia as a Function of Time and Dose of ACTH-L

Passive Avoidance Task

Time of Injection after training (min)	Dosage in mg/kg					
	0.0	0.03	0.1	0.3	1.0	3.0
			% Amnesic Mice *			
30	80%	60%	30%	15%	35%	55%
60	80%	60%	25%	20%	50%	60%
90	85%	75%	50%	40%	50%	75%
240	80%	80%	75%	65%	75%	75%

*N=20/group

TABLE 2

Amnesia as a Function of Time and Dose of ACTH-D

Passive Avoidance Task

Time of Injection after training (min)	Dosage in mg/kg			
	0.0	0.3	1.0	3.0
		% Amnesic Mice *		
30	20	55	65	75
60	15	55	70	70
90	15	30	35	40
240	20	10	30	45

*N=20/group

TABLE 3

Amnesia as a Function of Dose of ACTH-D

Active Avoidance Task

Dose in mg/kg	0.0	0.3	1.0	3.0
% amnesic mice*	20%	40%	60%	65%
P value from 0.0		NS	.05	.02

*N=20/group. Mice not making the first avoidance response in 3 trials or less were scored as amnesic.

TABLE 4

Amnesia as a Function of Dose of ACTH-D

Selected Subjects* - Active Avoidance Task

Dose in mg/kg	0.0	0.3	1.0	3.0
N	10	8	7	8
% Amnesia	10%	12%	14%	50%

*Only those subjects which made an avoidance during the original training were used. Amnesic mice defined as in Table 3.

TABLE 5

Effect of ACTH-L, ACTH-D, and Anisomycin

on [^{14}C]-Valine Incorporation in Whole Brain

% Inhibition of Valine Incorporation

Sal or Ani time (min.)	75		100		135	
Total ACTH (min.)	30		55		90	
Valine Incorporation (min.)	15		15		15	
	Sal	Ani	Sal	Ani	Sal	Ani
ACTH none	0	90	0	85	0	73
ACTH-L 2.0 mg/kg	5	90	13	85	10	76
ACTH-D 0.5 mg/kg	7	90	6	88	-3	69

Table 6

Effect of ACTH-L, ACTH-D and Anisomycin on [^{14}C]-Valine Incorporation

% Inhibition of Valine Incorporation

	Rest of Brain				Brain Stem			
Sal or Ani time (min.)	135		180		135		180	
Total ACTH time (min.)	90		135		90		135	
Valine Incorporation (min.)	15		15		15		15	
	Sal	Ani	Sal	Ani	Sal	Ani	Sal	Ani
ACTH added								
none	0	68	0	53	0	53	0	38
ACTH-L 0.5mg/kg	13	71	-13	61	2	64	-1	43
2.0mg/kg	-1	68			-10	58		
ACTH-D 0.5mg/kg	-11	76	4	63	-20	61	-8	44
Mannitol 333.3mg/kg	-11		-12	63	5			49

(-) denotes stimulation

Figure Captions

Fig. 1 The effects of dose of ACTH-L on retention for passive avoidance training. The ACTH was administered 30 min after training. Retention was tested one week later. N/group was between 23 and 28.

Fig. 2 Effect of dose of ACTH-L on active avoidance training. ACTH-L was administered immediately after training. A, (left) Mean number of trials to first avoidance; B, (right) Percentage of mice requiring more than 3 trials to make first active avoidance response.

Fig. 3 Effects of Anisomysin (A) and Dose of ACTH-L on retention for a passive avoidance task. A was administered 15 min prior to training and subsequently at 2 h intervals as indicated. ACTH or saline (S) was administered 30 min after training. ACTH-L overcame the amnestic effects of 2 doses of A, but not of 3 doses. The 3.0 mg/kg dose of ACTH was more effective than 0.3 mg/kg.

Fig. 4 Effect of number of injections of ACTH-L on amnesia produced by anisomycin: Anisomycin (A) was administered 15 min prior to training on a passive avoidance task and subsequently at 2 h intervals. ACTH-L or saline (S) was administered 30 min after training and subsequently at 2 h intervals as shown. ACTH-L did not overcome the amnesia produced by 6 h of high inhibition of protein synthesis from 3 injections of anisomycin.

Fig. 5 Effect of ACTH-D and anisomycin on amnesia. With strong training, for a passive avoidance task, anisomycin administered 15 min prior and 1 3/4h after training caused only a low percentage of amnesic mice. ACTH-D increased the amnesia; high doses were more effective than low doses.

Fig. 6 Interraction of ACTH-D and ACTH-L on retention for a passive avoidance task. Under either condition of strong training (high foot-shock)(left)or weak training (right), ACTH-D blocked the enhanced retention produced by ACTH-L. Drugs were administered 18 sec. after training. N's/group and significance of difference from the control groups are shown above the data bars.

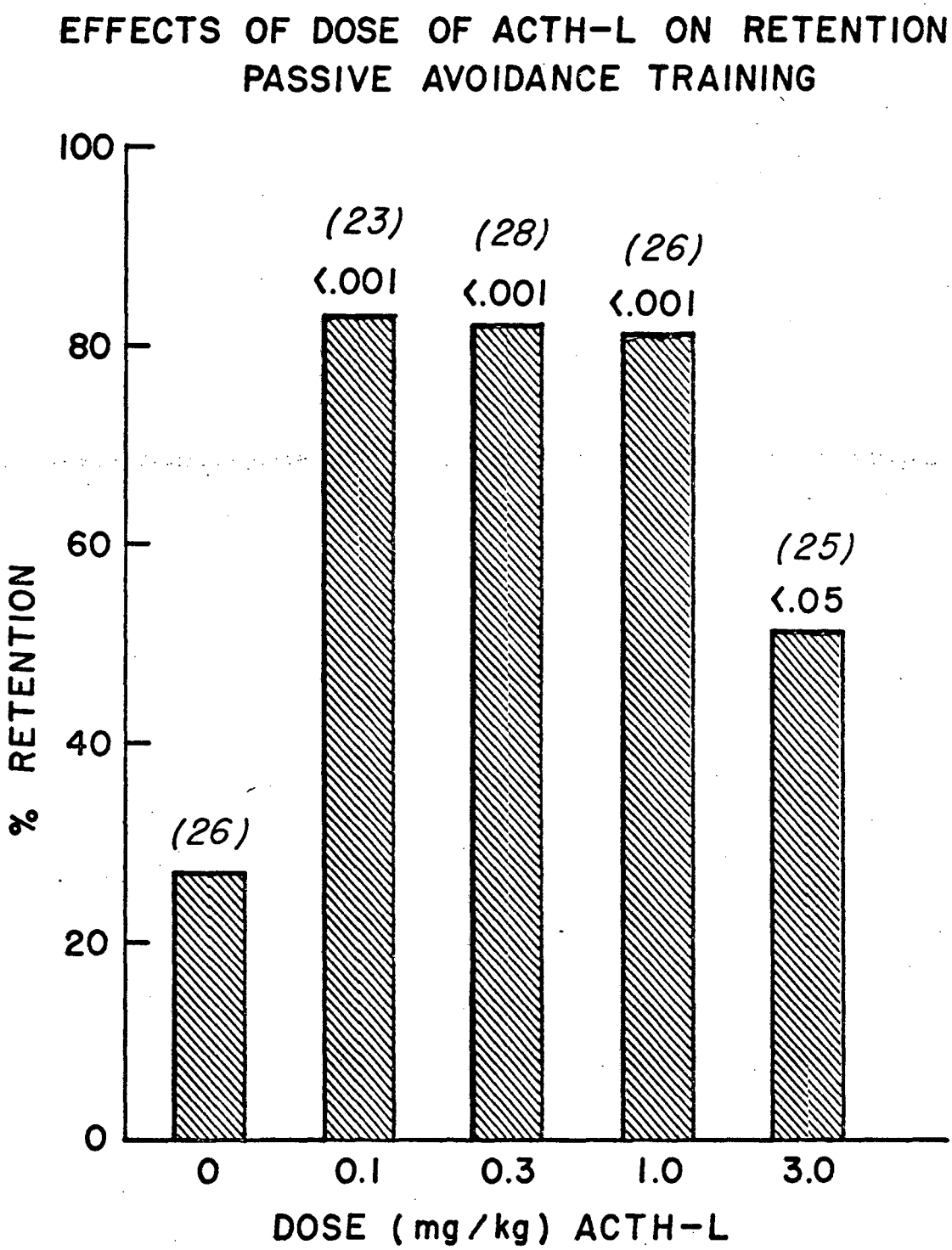

Fig. 1

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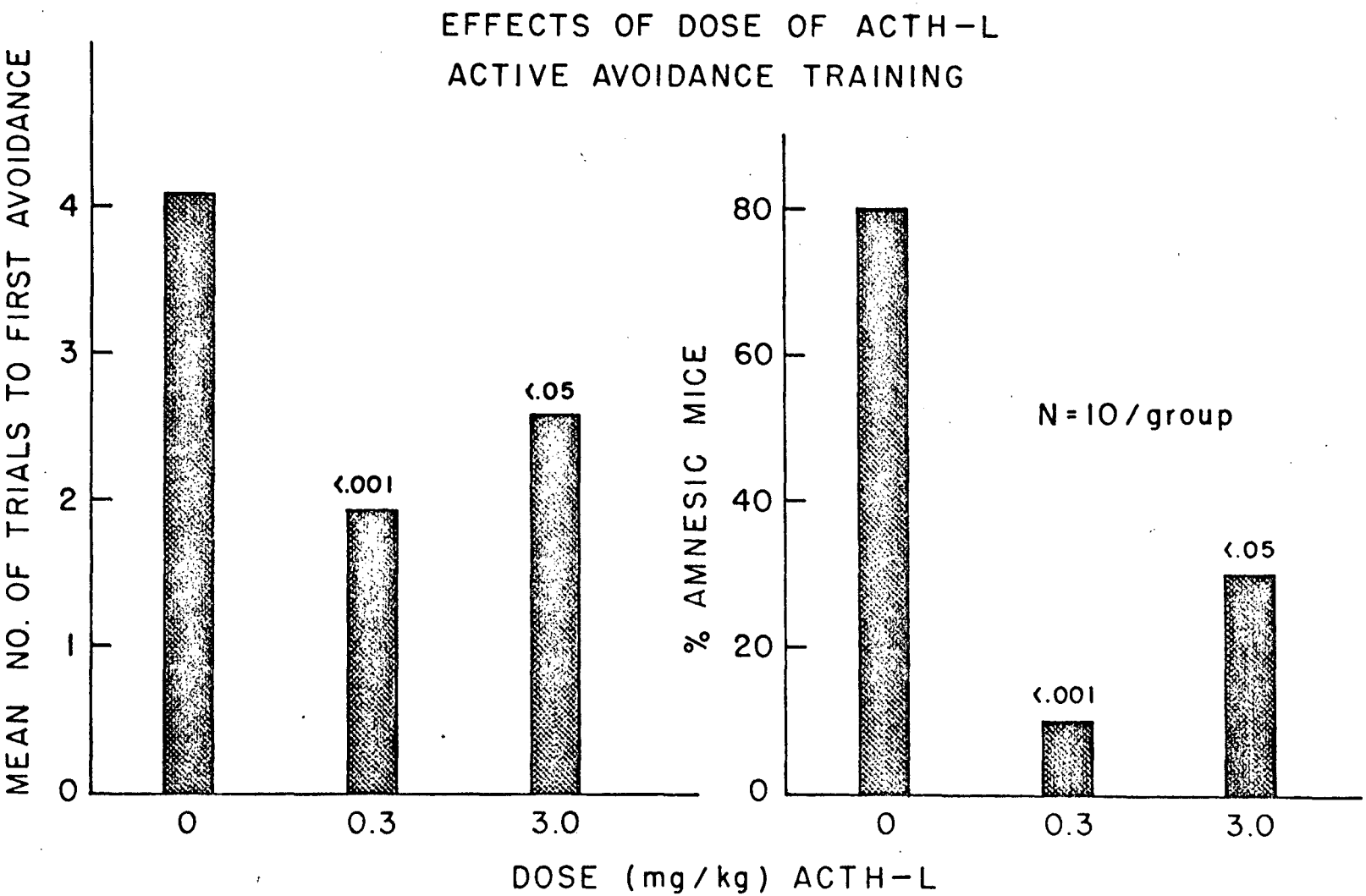

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Figure 2

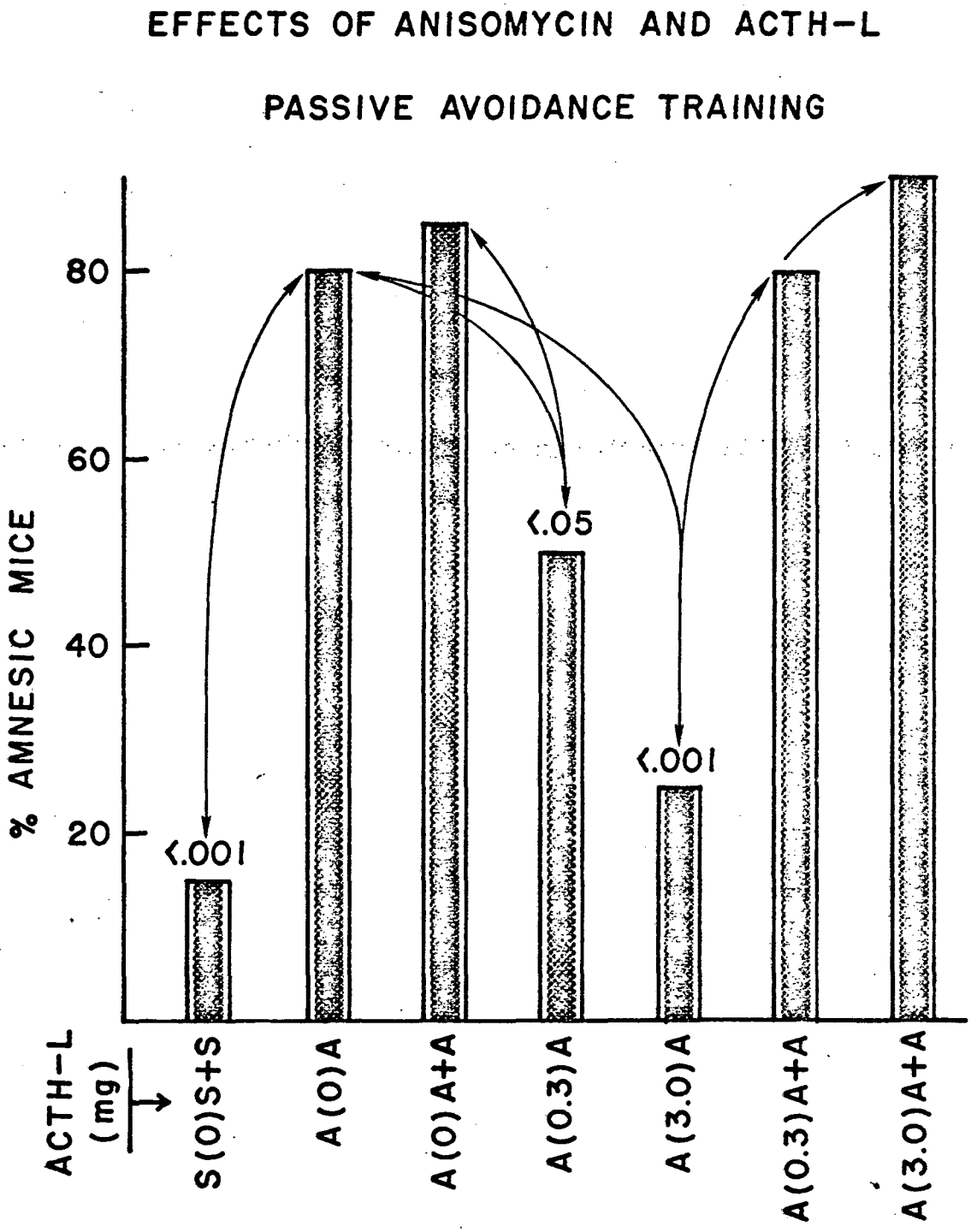

Fig. 3

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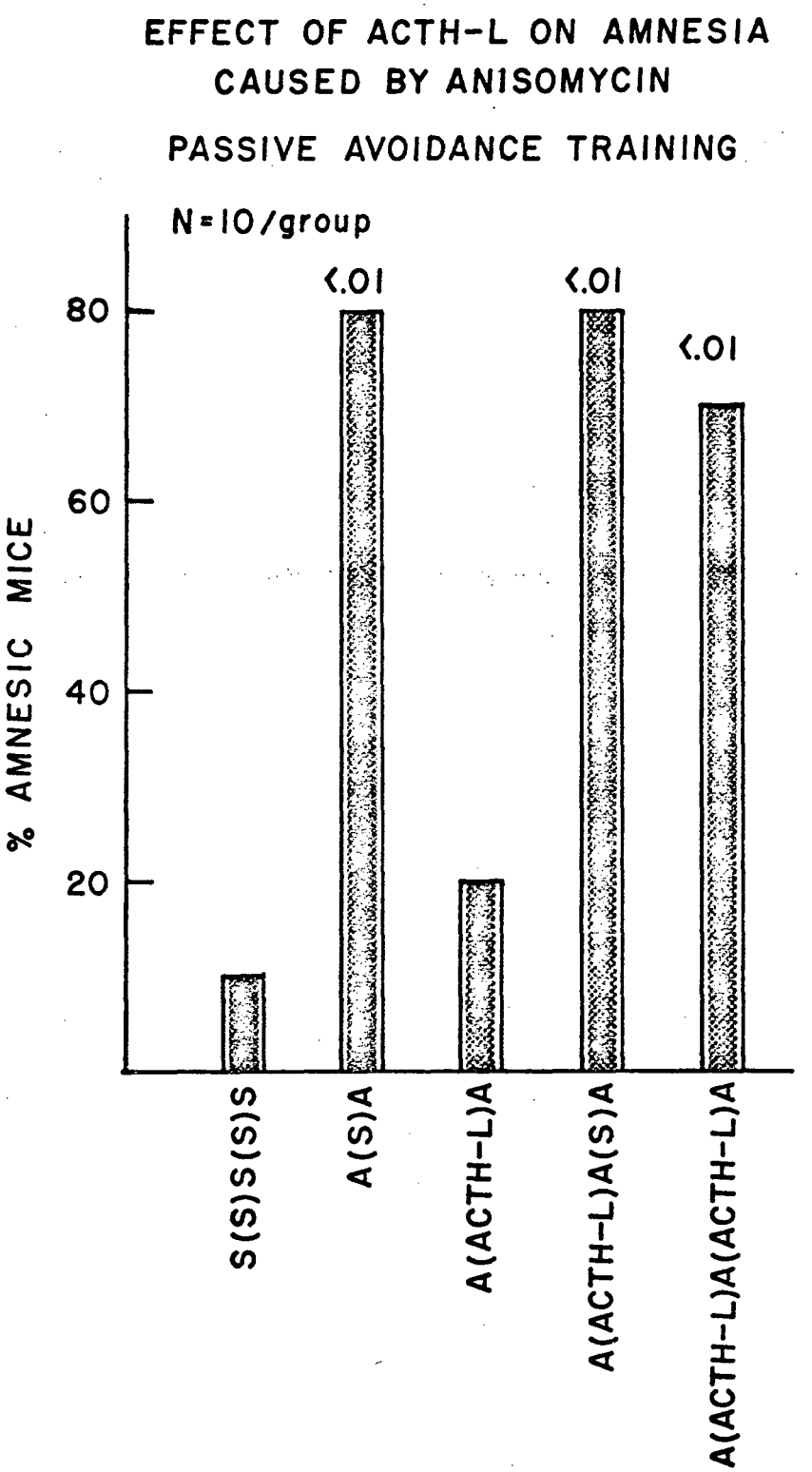

Fig. 4

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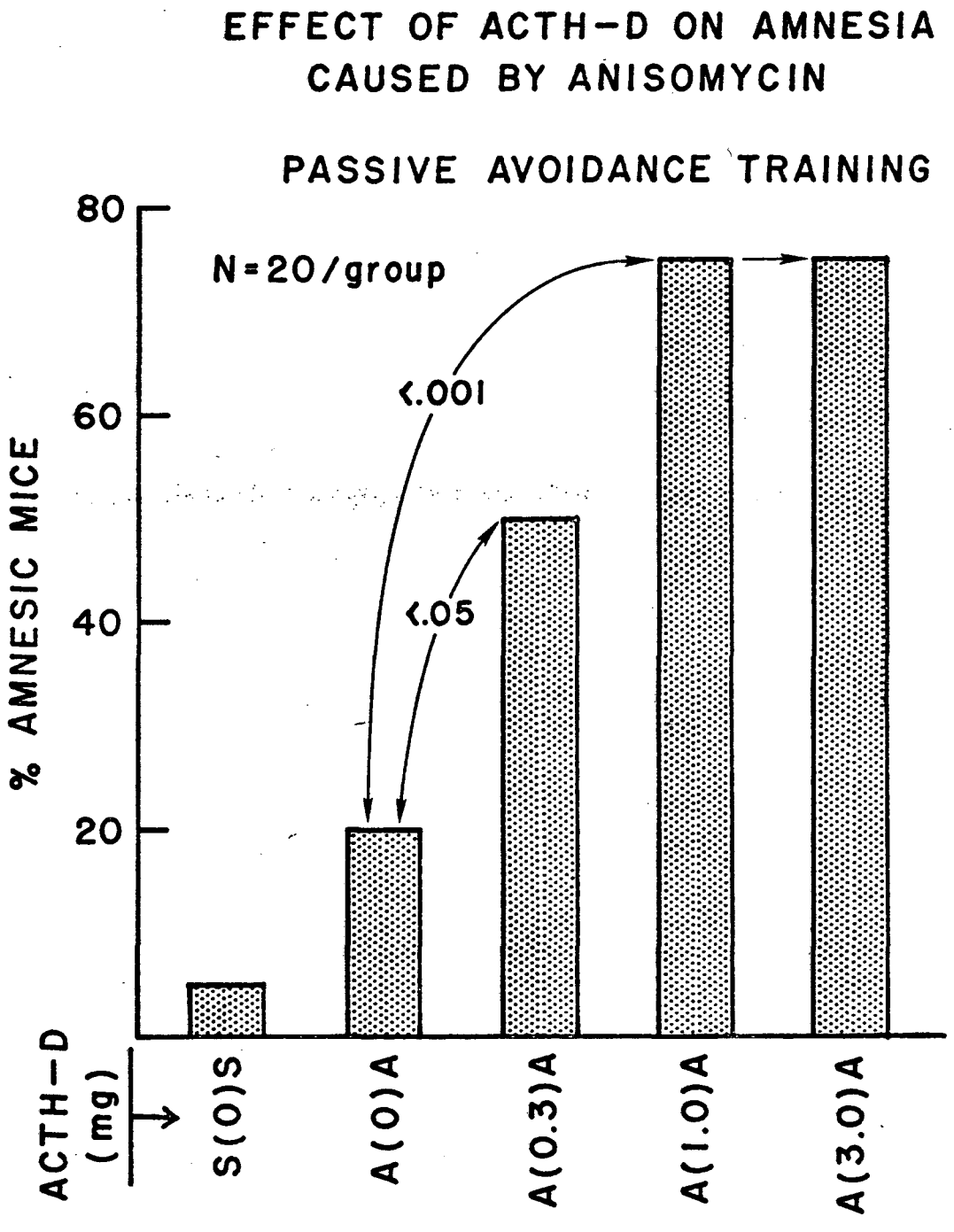

Fig. 5

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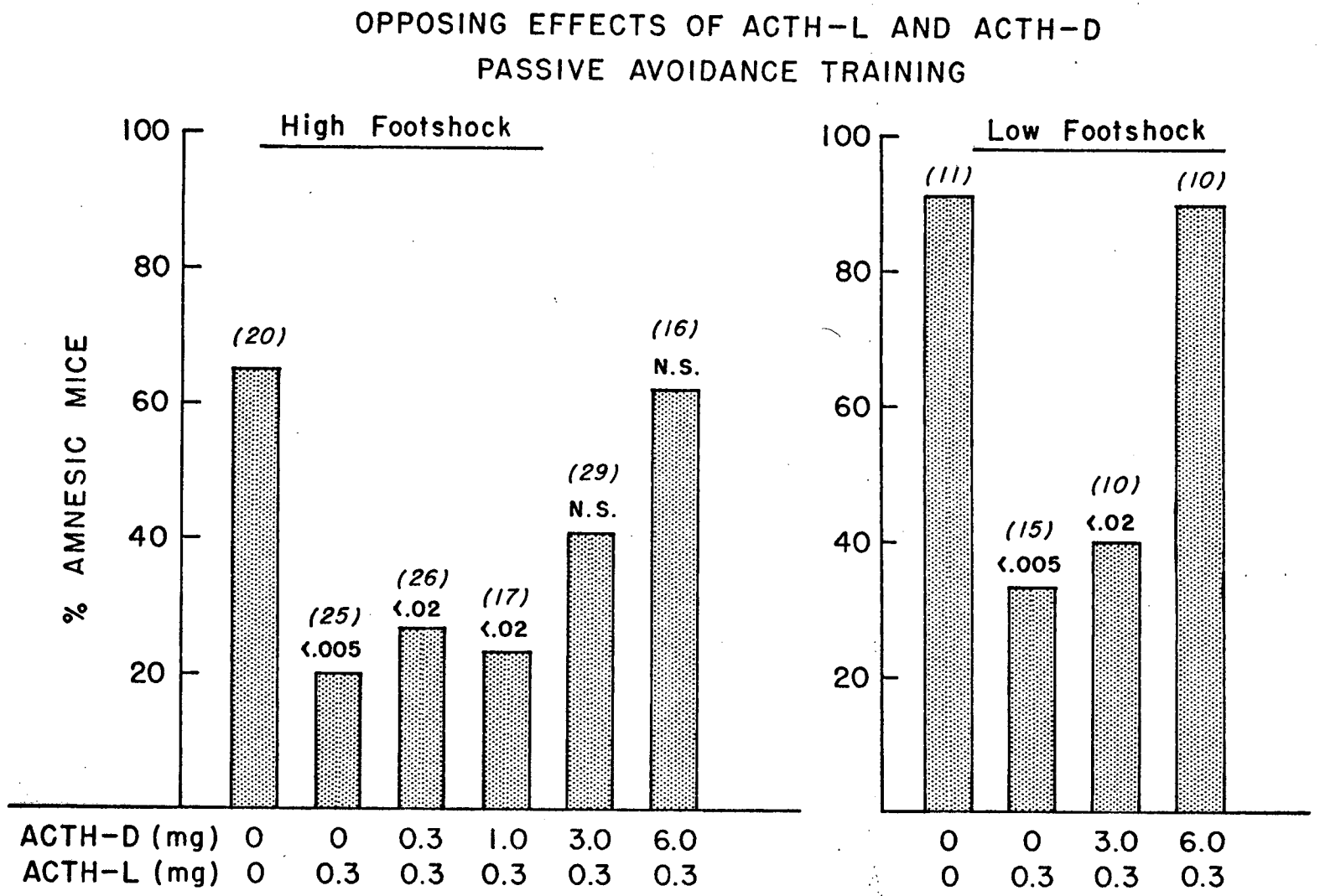

Fig. 6

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