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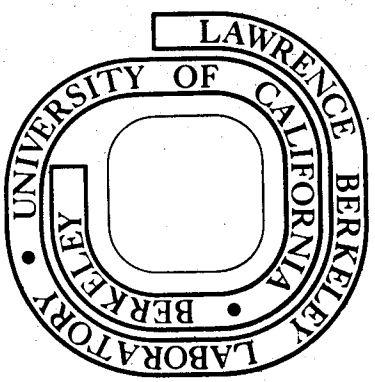

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PROTEIN SYNTHESIS DEPENDENT GRADIENT OF ECS RETROGRADE AMNESIA

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ABSTRACT

The interacting amnesic effect of a protein synthesis inhibitor, anisomycin, and ECS were studied in active and passive avoidance tasks. By giving one to three injections of anisomycin, the duration of inhibition of protein synthesis was inhibited from 2 to 6 hr at 80% or greater. ECS was administered at various times after training (1 min to 9 hr) to both inhibited and uninhibited mice. The ECS gradient in uninhibited mice was never greater than 30 min. The ECS gradient in anisomycin-treated mice ranged from 3 hr to 8 hr depending on the training strength and the number of anisomycin injections. The ECS gradient of retrograde amnesia consistently developed at about 1 hr after the recovery of protein synthesis began and this displaced the ECS amnesic gradients by as much as 8-9 hr. The study also determined that ECS caused only a transient, low percent inhibition of protein synthesis in uninhibited mice. The ECS given to anisomycin-treated mice had only a very slight effect on inhibition of protein synthesis and did not seem to increase the inhibition enough to account for the amnesia observed. The results are discussed in terms of the ECS amnesic gradient being dependent on memory-related protein synthesis that preceeds ECS administration.

Most theories about memory consolidation have two components: a short-term electrical phase and a long-term macromolecular synthesis phase (Agranoff, 1971; Andry and Luttges, 1973; Davis and Klinger, 1969; John, 1967; Landauer, 1964; McGaugh, 1967; Schneider and Sherman, 1968; Watts and Mark, 1971). The short-term phase is usually believed to control or lead to the long-term phase of memory consolidation. In spite of this suggested relationship between short- and long-term phases of memory trace formation, only a few studies have attempted to test for an interaction between these two phases. In many studies, electroconvulsive shock (ECS) has been employed to disrupt short-term memory mechanisms. Long-term memory disruption studies, on the other hand, often employed the use of antibiotics to inhibit brain protein synthesis.

A test of the interaction between ECS and inhibition of protein synthesis was reported by Andry and Luttges (1973). In a one-trial passive avoidance training task, ECS and inhibition of protein synthesis (using cycloheximide) caused a more rapid onset of amnesia compared to mice receiving only cycloheximide. The mice receiving both ECS and cycloheximide had a considerably longer post-training amnesic gradient than mice given only ECS. In fact, the ECS gradient was extended from less than 30 min in mice receiving only ECS to at least 3 hr in mice treated with cycloheximide and ECS. However, when an active avoidance task was employed, the results showed that neither an ECS given immediately after training nor ECS and cycloheximide yielded amnesia when tested one week after training. A possible reason for failing to find an effect with active avoidance may have been the large number of training trials used (50 in 30 min). Others have reported that extended training reduced or blocked the amnesic effect of ECS (Geller et al, 1970; Keyes, 1973; Quartermain et al, 1970)

or of protein synthesis inhibition (Barondes, 1970; Flood et al, 1972, 1973; Schmaltz and Delerm, 1974).

The purpose of this series of experiments was to replicate the extended ECS gradient reported by Andry and Luttges using passive and active avoidance tasks. With a step-through, passive avoidance task, the duration of protein synthesis inhibition was controlled by using anisomycin, an inhibitor of protein synthesis which can be administered repeatedly at 2 hr intervals to maintain high levels of inhibition for various lengths of time. An interaction betwen the number of anisomycin injections and the shape of the ECS retrograde amnesic gradient was studied. A similar study using the pole jump apparatus, an active avoidance task, which could be learned in 3-4 trials was conducted to test the generality of Andry and Luttges findings.

GENERAL DESCRIPTION -- BEHAVIORAL EXPERIMENTS

Materials and Procedures

In the behavioral experiments, CD-1 male, albino mice from Charles River Breeding Laboratories, Wilmington, Mass. were obtained at 6 weeks of age. After 1 week in the laboratory, the mice were individually housed in small cages 24 hr prior to training. After training, the mice were returned to individual cages until the retention test one week later. The mice were trained on one-trial, step-through passive avoidance or on a pole jump shock avoidance task.

Anisomycin (Ani) was obtained through the generosity of N. Belcher as a gift from the Pfizer Pharmaceutical Co., Groton, Connecticut. 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. The injections were given subcutaneously over the back of the mice under light ether anesthesia.

Passive Avoidance

The passive avoidance training and apparatus have been described previously (Flood et al, 1972). In brief, the apparatus consists of a long alley divided into a small black start box and a long white shock compartment. The two compartments were separated by a panel which contained a mouse hole. The entry into the white compartment was prevented until the appropriate time by a translucent guillotine door. The white shock compartment was illuminated by a dim lamp situated at the end of the alley. Shock was delivered by a high voltage, constant current 18 pole shockscrambler. The shock was administered through a brass floor grid in the white box. The footshock intensity was varied according to each experiment.

A training trial consisted of placing a mouse into the black start box for 20 sec. Next, the guillotine door was removed while the mouse was facing away from the hole giving the mouse access to the white box. The latency-to-enter the white compartment was determined from the time the mouse oriented toward the mouse hole until it had completely entered the white compartment. The shock was turned on when the mouse was halfway down the alley (approximately 5 sec after entering) and was left on until the mouse escaped back into the black box. This ended the training and the mouse was returned to its own cage until the retention test was given 1 week later. The retention test followed the same procedure as for training except that no footshock was given if the animal entered the white compartment. Amnesia was defined as entering the white shock compartment in 20 sec or less. Mice not entering the white shock compartment within 180 sec were removed and given a score of 180 sec. Training and testing were done between 8:00 AM and 2:00 PM.

Pole Jump

The pole jump apparatus consisted of an 11.5 cm wide and 18 cm high alley divided into two compartments by a guillotine door; one small compartment (9 cm long) was a start box, the other larger compartment (21 cm long) contained the pole in the center. A brass grid floor which was used to deliver the footshock (0.35 ma) ran through both compartments. The pole (1" diameter, hollow, rigid, plastic tubing covered with 1/2" wire mesh) did not deliver any shock. The pole could be easily removed with the mouse on it. The apparatus was built of black Plexiglas except for the pole which was white and the lid which was clear. A loud doorbell type buzzer was used as the conditioned stimulus. The training room was dark except for a bright Tensor lamp illuminating the apparatus.

The training procedure consisted of placing the mouse into the small compartment and after approximately 15 sec lifting the guillotine door, thus giving the mouse access to the pole compartment. The buzzer was sounded at the same time the guillotine door was removed. This was followed 5 sec later by the administration of footshock if the mouse had not climbed onto the pole. The buzzer and/or shock were terminated as soon as the mouse climbed onto the pole. An escape response was scored if the mouse climbed onto the pole after 5 sec; an avoidance response was scored if the mouse climbed onto the pole within 5 sec. The mouse was returned to its home cage from the pole compartment by carefully removing the pole (with the mouse on it) and placing it in the home cage. Most mice quickly climbed off the pole. Occasionally, however, a light touch to the hind quarter was needed to encourage the mouse to dismount. Twenty seconds after being returned to its home cage the mouse was returned to the black start box and the next trial was given; each mouse received three training trials. The retention test was given one week after training and consisted of retraining the mice until one avoidance response was made.

The retention measure was the number of trials required to make the first avoidance response. Amnesia was defined as taking 4 or more trials to make an avoidance response.

Inhibition of Protein Synthesis and Administration of ECS

Various durations of inhibition of protein synthesis were possible by giving one or more subcutaneous injections of anisomycin (Ani) at 2 hr intervals at a dose of 0.5 mg/mouse/injection. The first injection of Ani was given 15 min prior to passive or active avoidance training (no drug was used in Exp.1). The first injection of either Ani or saline was administered under very light ether anesthesia. When additional injections of Ani were used they were administered 1-3/4 or 1-3/4 and 3-3/4 hrs after traininig.

Electroconvulsive shock (ECS) was administered transcorneally at 8 ma for 0.2 seconds at 60 Hz. Mice not showing both tonic and clonic convulsions were discarded. Mice given pseudo-electroconvulsive shock (P-ECS) were handled in the same manner as the ECS mice except that no current was delivered.

Experiment 1

The purpose of this experiment was to determine what influence footshock intensity in passive avoidance had on the shape of the post-training gradient for ECS-induced amnesia. The mice were trained on the passive avoidance task as described above. The mice were divided into three groups by footshock intensity: 0.34, 0.38 or 0.42 ma. Only mice that had a latency-to-enter the white shock compartment between 1.5 and 2.4 sec inclusive, and at the same time had a latency-to-escape between 1.0 and 3.4 sec were used in the study; all others were discarded. The three footshock intensity groups were further divided into animals receiving ECS or P-ECS at 1, 30 or 60 min after training. Therefore, the experiment included a total of 18

groups with 20 subjects in each group. The retention test was given 1 week after training.

Results

When ECS was administered within 1 min after training the percentage of amnesic mice was high at all footshock intensities. But when ECS was administered 30 or 60 min after training, the effectiveness of ECS as an amnestic treatment decreased as the footshock intensity increased. This was particularly noticeable when ECS was administered 30 min after training. Furthermore, the longer ECS administration was delayed beyond training for all footshock intensity conditions, the less was the amnesia that occurred (Table 1). The P-ECS controls showed 10 to 35% forgetting depending on the footshock intensity. The 0.34 ma footshock intensity was marginal training in that the P-ECS groups showed 25 to 35% forgetting.

Table 1 about here

Experiment 2

The purpose of this experiment was to determine if the ECS amnesic gradient was dependent on the duration of memory-related protein synthesis occurring during and after training but prior to the administration of ECS. To test this, the protein synthesis inhibitor Ani was administered prior to training (training conditions being those for which one injection of Ani did not cause amnesia) and ECS was administered at times during the inhibition and as protein synthesis was recovering. The mice were trained on passive avoidance at a footshock of 0.38 ma. Four basic groups were used: Saline+ ECS, Saline+P-ECS, Ani+ECS and Ani+P-ECS. Ani or saline was administered

15 min prior to training. ECS or P-ECS was administered 1, 30, 60, 120, 180, 210 or 240 min after training. To control the degree of learning, only mice with the following combinations of latencies were used: 1X3, 1X4, 2X2, 2X3, 2X4, 3X1, 3X2 (latency-to-enter by latency-to-escape to the nearest second). Other conditions and procedures were as described above.

Results

The results indicate that Ani delayed the onset of the ECS amnesic gradient by 150 min (Table 2). Ani+ECS differed significantly from Saline+ECS for ECS treatment groups 30 to 180 min (at 30 min $P < .01$, for 60 to 180 min $P < .001$, χ^2Test). The Saline+ECS gradient was much shorter since Saline+ECS and Saline+P-ECS only differed at the 1 min treatment time ($P < .001$, $\chi^2 = 32.4$, df=1). At 30 min after training, Saline+ECS did not differ significantly from Saline+P-ECS ($\chi^2 = 3.53$; however, $P < .05$ is equal to $\chi^2 = 3.84$). As is clear from Table 2, Ani+P-ECS did not cause amnesia.

Table 2 about here

Experiment 2A

Additional groups were tested to determine if extensive inhibition of brain protein synthesis at 180 min after training would cause amnesia when an additional injection of Ani was given. The training conditions were the same as in Experiment 2. The Ani+Ani$_{180}$ group received 1 injection of Ani 15 min prior to training and another injection at 180 min after training when ECS would have been administered. As previously reported (Flood et al, 1975b), this injection schedule creates about a 60 min gap in inhibition of protein synthesis during which time substantial recovery of protein synthesis occurs.

The second group received two injections 2 hr apart (no gap in inhibition) resulting in 4 continuous hours of inhibition at 80% or more. Ani+Ani$_{180}$ yielded only 15% amnesia which was within the range of amnesia for either Saline or Ani groups given P-ECS, and differed from Ani+ECS$_{180}$ (75% amnesia). The Ani+Ani group yielded 75% amnesia; thus, 2 injections of Ani given 2 hr apart were required to cause as high a percentage of amnesic mice as in the Ani+ECS$_{180}$ group.

Experiment 3

The purpose of this experiment was to test the generality of the results of Experiment 2 over a longer time period. The training strength was increased so that three successive injections of Ani would not cause amnesia for passive avoidance training. This was done by increasing the footshock intensity to 0.42 ma and by lengthening the shock escape latencies. Only the following combinations of latencies-to-enter by latencies-to-escape (in seconds) were used: 2X3, 2X4, 3X2, 3X4. The same four basic groups were used as in Experiment 2. ECS or P-ECS was administered at 1 min, 30 min, 1, 3, 5, 6, 7, 8, or 9 hr after training. Ani was administered 15 min prior to training and at 1-3/4 hr and at 3-3/4 hr after training. Two additional groups were run to determine how many Ani injections were required to obtain amnesia under these conditions of training. These groups received a total of 4 or 5 successive injections of Ani each 2 hr apart beginning 15 min prior to training.

Results

The results of the retention test given 1 week after training showed that Ani had shifted the ECS amnesic gradient 7 hours. This is based on a comparison between Ani^3+ECS and Saline^3+ECS where the percent amnesia differed signifi-

cantly for ECS treatment groups 30 min to 7 hr, but did not differ significantly for ECS treatment groups 8 or 9 hr after training (Table 3). The three injections of Ani were not solely responsible for the amnesia as Ani^3+P-ECS and $Saine^3$+P-ECS did not differ. $Saline^3$+ECS resulted in an amnesic gradient of less than 30 min as $Saline^3$+ECS and $Saline^3$+P-ECS only differed significantly at the 1 min treatment time (90% vs 10% amnesia, $P<.001$, $\chi^2=18.11$). A series of four or five Ani injections were required to yield high levels of amnesia (65% and 95% amnesia respectively). These results also confirm previous reports that amnesia will occur in well-trained mice if an appropriate number of Ani injections are given (Flood et al, 1973, 1975a).

Experiment 4

In this experiment, the dependency of the ECS amnesic gradient on the number of Ani injections was tested in an active avoidance task. Mice were trained and tested on the pole jump task as described above. The retention test was given 1 week after training, drug and ECS treatment. The same four basic groups were used as in Experiments 2 and 3. Ani-injected mice received two successive injections, the first 15 min prior to training and the second 1-3/4 hr after training. ECS or P-ECS was administered at 1 min, 30 min or 5, 6, or 7 hr after training. These times were chosen because in Experiments 2 and 3 the Saline-ECS amnesic gradient appeared only over the first 30 min after training and the Ani+ECS amnesic gradient appeared only as recovery of protein synthesis was occurring.

Results

In an active avoidance situation, Ani delayed the gradient of ECS induced amnesia. The percent amnesia for Ani^2+ECS and for $Saline^2$+ECS differed significantly for treatment groups 30 min, 5 and 6 hr but not for 1 min or 7 hr

(Table 4). The ECS gradient was displaced by 5 to 6 hours. The Saline^2+ECS and Saline^2+P-ECS differed only when ECS had been administered within 1 min of training. Since Ani^2+P-ECS and Saline^2+P-ECS did not differ, it was clear that Ani alone was not responsible for the amnesia.

Table 4 about here

BIOCHEMICAL EXPERIMENTS

The purpose of this experiment was to evaluate the effect of ECS on protein synthesis in mice given either saline or Ani. The same four basic groups of mice were used: Ani+ECS, Saline+ECS, Ani+P-ECS and Saline+P-ECS plus two groups receiving either saline or Ani alone.

GENERAL DESCRIPTION - BIOCHEMICAL

Materials and Procedures

The CD-1 male mice were obtained and housed as described in the behavioral section. Anisomycin was used in the same manner as in the behavioral experiments. [^{14}C (U)]-L-Valine was obtained from New England Nuclear Corp. and diluted with 0.9% saline to contain 50 µc/ml.

Mice received a subcutaneous injection of Ani or saline at "zero time" and ECS or P-ECS at 180, 210 or 240 min later. ECS or P-ECS was administered as described for the behavioral experiments. Subsequently, each mouse received 5 µc of [^{14}C]-valine subcutaneously administered either 5 or 25 min after ECS or P-ECS. After a ten minute incorporation period, the animals were decapitated, the brains excised and frozen. There were 3 or 4 subjects per data point.

Protein synthesis was determined by the ratio of (a) radioactivity resulting from incorporation of [^{14}C (U)]-L-valine into the trichloracetic acid insoluble fraction to (b) the total radioactivity in the brain sample. The percent inhibition or stimulation was determined by a comparison of this ratio in the control (saline injected mice) and experimental mice. The experimental procedures have been described in detail (Flood et al, 1972). Duplicate fractions and determinations of radioactivity were made for each mouse brain.

Results

Dunn et al (1971), Cotman et al (1971) and Kelly and Luttges (1976) have reported that ECS caused an immediate but transient inhibition of protein synthesis up to 50%. For this reason we measured incorporation of L-valine from 5 to 15 min as well as from 25 to 35 min after ECS or P-ECS. In our studies, we found that ECS resulted in a maximum of 28% inhibition of protein synthesis in saline-injected mice over the time period 5 to 15 min after ECS. This inhibition was within the anticipated range when the intensity and duration of the ECS and time parameters are considered. As anticipated, Table 5a shows that the trend was similar whether ECS was given 180 or 210 min after saline. P-ECS caused a transient stimulation of protein synthesis of 19% over a time period of 5 to 15 min after ECS compared to saline-injected control mice. The stimulation of protein synthesis declined to 8% when measured 25 to 35 min after ECS.

Ani and ECS resulted in somewhat higher inhibition of brain protein synthesis than Ani alone. At 180 min after an Ani injection, protein synthesis was 48% inhibited, while at the same time Ani+ECS showed 53% inhibition. The additional inhibition due to ECS became more apparent as the level of inhibition due to Ani alone decreased. At 240 min after the administration of Ani alone,

only 19% of the protein synthesis was inhibited compared to the Ani+ECS group which showed 32% inhibition. P-ECS reduced slightly the level of inhibition due to Ani; thus it stimulated protein synthesis as it did in the saline-injected mice (Table 5b).

Table 5a and 5b about here

DISCUSSION

Dependence of ECS amnesic gradient on protein synthesis

The primary importance of these experiments was the finding that Ani delayed the onset of the gradient of ECS retrograde amnesia. These results are interpreted on the basis of the inhibition of brain protein synthesis which resulted from one or more injections of Ani. In Experiments 2, 3, and 4, therefore, the ECS amnesic gradient appeared 3 to 5 hr after the last injection of Ani or 1 to 3 hr after the initial recovery of protein synthesis. For example in Experiment 2, a single injection of Ani inhibited protein synthesis at 80% or more for about 2 hr. At 3 hr after the Ani injection, the inhibition had dropped from above 80% to about 50%. By the 4th hour, the inhibition was about 20% (Table 5b). The ECS gradient appeared between 3 and 4 hr after the training when protein synthesis recovered from 50% to 80%. The relationship between the decreasing effectiveness of ECS as an amnestic treatment and the recovery of protein synthesis for Experiments 2, 3, and 4 is shown in Figure 1a, b, and c.

Figure 1a, b, and c about here

Figure 1b shows recovery of protein synthesis 4-7 hr after the first Ani injection. The percent amnesia for Ani^2+ECS mice was still high at 5 hr but declined rapidly when ECS was administered 6 to 7 hr after training. Similarly, Figure 1c shows recovery of protein synthesis 6 to 9 hr after the first Ani injection and the gradient for ECS amnesia appeared 8 to 9 hr after training. The figure also shows that the ECS alone was most effective as an amnesic treatment when administered within 1 min after training. The delay of the gradient of ECS retrograde amnesia showed the same pattern for passive avoidance (Figure 1a,c) and active avoidance (Figure 1b).

Possible Interpretations

An Inhibition Interpretation

One possible interpretation of these results is that ECS and Ani together increased protein synthesis inhibition, thus preventing the formation of the memory trace. However, three points indicate that this is an unlikely explanation: (a) ECS caused only a small transient increase in protein synthesis inhibition compared to Ani given alone, (b) amnesia resulted when ECS was administered during maximal periods of Ani-induced inhibition of protein synthesis - a period when sizeable further increases in inhibition were not possible and (c) no amnesia occurred when Ani was administered in place of ECS (Exp. 2A).

The inhibition of protein synthesis increased slightly when ECS and Ani were combined. The inhibition achieved with Ani+ECS (during the recovery phase of protein synthesis) was 53% at 180 min after a single injection of Ani (Table 5b). This level of inhibition was not significantly greater than that resulting from Ani given alone (48% inhibition). At 210 min after train-

ing, Ani+ECS yielded 35% amnesia as compared with 75% amnesia for mice in the 180 min Ani+ECS group. The inhibition levels for Ani+ECS was 53% at 180 min and 50% at 210 min. Thus a decrease in amnesia occurred even though the inhibition level remained nearly constant. ECS administered alone caused a maximum of 28% inhibition which decreased over the next 20 min. This maximal level of ECS-induced inhibition of protein synthesis is too low and lasts for too short a time to account for the amnesia in the Saline+ECS groups.

The level of inhibition due to ECS alone is consistent with estimates obtained by Dunn et al (1971), Cotman et al (1971) and Kelly and Luttges (1976). Kelly and Luttges (1976) found that the combination of ECS and cycloheximide did not produce any greater protein synthesis inhibition than cycloheximide alone. In fact, during the recovery phase of protein synthesis inhibition, ECS appeared to reduce the protein synthesis inhibition slightly. MacInnes and Luttges (1972) have also noted that combined ECS and cycloheximide treatment produced no greater brain polysome disaggregation than cycloheximide alone. We would agree with the conclusion of Kelly and Luttges (1976) that ECS and cycloheximide or Ani produced additive memory deficits through different effects on the underlying memory storage mechanisms.

When ECS was administered during peak levels of Ani-induced protein synthesis inhibition, any further increase in inhibition would necessarily have to be very small. Yet Ani+ECS caused significantly more amnesia than Ani+P-ECS or Saline+ECS. This is clearly demonstrated in Experiment 3 in which inhibition due to Ani was maintained at well above 80% for 6 hr. Ani+P-ECS did not cause amnesia when P-ECS was administered during this time period. Ani+ECS however resulted in 85 to 100% amnesia for those groups receiving ECS 1 min to 6 hr after training. Since protein synthesis inhibition

was near maximum for both Ani+P-ECS and Ani+ECS, it seems unlikely that the additional small increase in inhibition could account for nearly 70% greater amnesia in the Ani+ECS groups.

In Experiment 2, Ani+Ani$_{180}$ group received one injection of Ani 15 min prior to training and a second injection 180 min after training. Since Ani would normally have been administered 105 min after training this 75 min delay permitted some recovery of protein synthesis prior to the second Ani injection (Flood et al, 1975). If the high percent and long duration of inhibition of protein synthesis caused by the second injection of Ani at 180 min after training did not result in amnesia (15% amnesia), then it is unlikely that the low percent and short duration of protein synthesis inhibition due to Ani+ECS when ECS was given at 180 min after training could be the cause of the significant difference in amnesia (15% vs 75% amnesia).

A Protein Synthesis Dependent Interpretation

A second interpretation based on a protein synthesis hypothesis can better explain the data. The degree to which ECS can induce amnesia is dependent on how much memory-related protein synthesis occurs prior to ECS treatment. The mechanism of action of ECS may be related to its ability to cause conformational changes in the synaptic membrane similar to those that store short-term memory and thus disrupt the pattern of synaptic changes that occurred due to training.

In order to claim that the ECS amnesic gradient is controlled by the amount of memory-related protein synthesis that occurs prior to ECS treatment, it must first be shown that protein synthesis which is necessary for long-term memory can and must occur over the time period in question. In Experiment 2, a single injection of Ani resulted in a mean of 12.1% amnesia while two successive injections of Ani yielded 75% amnesia. The additional 2 hr of inhibition of

protein synthesis from a second injection of Ani was necessary to block long-term memory trace formation. The second injection of Ani extended inhibition at 80% or greater from 1-3/4 hr to 3-3/4 hrs. The ECS amnesic gradient in this experiment appeared over the time period 3 to 4 hr after training (Figure 1a). Thus for retention, protein synthesis was required over the time period that the ECS amnesic gradient appeared. Similarly in Experiment 3, three successive injections of Ani did not cause amnesia because the mice were trained with a higher footshock intensity; a fourth or a fifth injection of Ani extending inhibition at 80% or greater from 5-3/4 to 9-3/4 hr after training was necessary to cause amnesia. The ECS amnesic gradient for Ani^3+ECS groups appeared between 7 and 9 hr after training (Figure 1c). In both of these experiments, the ECS amnesic gradient appeared during a time period for which one or more injections of Ani were required to obtain a high percent amnesia - presumably because memory-related protein(s) continued to be synthesized beyond the duration of inhibition used in each experiment.

We suggest that ECS disrupts the pattern of synaptic changes at the synaptic membrane which are the short-term trace and the sites at which the conversion to long-term memory storage occurs. ECS would presumably result in a release of neurotransmitter from most synapses on a neuron thus disrupting the pattern of selective changes induced by training. Protein(s) synthesized prior to ECS would migrate and bind to short-term storage sites, but once the ECS has been administered such migration would no longer be selective as most synapses would at this time have been activated by the ECS current or resulting convulsions. Thus little protein would reach the synapses activated during training.

TABLE 1

Interaction of Footshock Intensity on Post Training Gradient of ECS Induced Amnesia

Footshock Intensity (ma)	Treatment	Percent Amnesia Time of Treatment (min)		
		1	30	60
0.34	ECS	90	70	40
	P-ECS	25	30	35
0.38	ECS	80	40	30
	P-ECS	15	10	15
0.42	ECS	95	25	10
	P-ECS	10	15	15

Amnesia is defined as a step-through latency of 20 sec or less on a retention test for passive avoidance training. The retention test was given 1 week after training and electroconvulsive shock (ECS) or pseudo-electroconvulsive shock (P-ECS).

TABLE 2

Effect of a Protein Synthesis Inhibitor, Anisomycin, on the Post Training Gradient of ECS Induced Amnesia

Drug	ECS	Percent Amnesia Time of ECS Treatment after Training (min)						
		1	30	60	120	180	210	240
Ani	ECS	90	85	85	80	75	35	20
Ani	P-ECS	5	10	15	5	20	20	10
Saline	ECS	95	40	10	10	10	15	15
Saline	P-ECS	5	15	10	10	15	10	5

$Ani+Ani_{180}$ = 15% amnesia

Ani+Ani = 75% amnesia

Ani without ECS did not result in amnesia for passive avoidance training. When Ani and ECS treatments were combined the ECS gradient of amnesia was displaced from 30 min or less to about 3 hrs. N per cell equals 20.

TABLE 3

Effect of ECS and Anisomycin on Retention for Passive Avoidance

Drug	ECS	Percent Amnesia Time of ECS Treatment after Training								
		1 min	30 min	1 hr	3 hr	5 hr	6 hr	7 hr	8 hr	9 hr
Ani^3	ECS	90	100	90	100	90	85	90	40	15
Ani^3	P-ECS	10	10	0	0	10	0	20	10	0
$Saline^3$	ECS	90	20	0	10	10	0	10	10	0
$Saline^3$	P-ECS	10	10	0	0	0	10	10	0	10

Ani^4= 65% amnesia (N=20)

Ani^5= 95% amnesia (N=20)

Ani^3 under the conditions of training did not cause amnesia as the Ani^3+P-ECS and $Saline^3$+P-ECS did not differ. It required at least 4 successive injections of Ani to get significant amnesia. Ani^3 treatment delayed the ECS amnesia gradient until recovery from inhibition of protein synthesis was occurring; the gradient was displaced at least 8 hr. Ani^3+P-ECS and $Saline^3$+P-ECS groups had N's of 10 at each treatment time. Ani^3+ECS groups had N's of 20 each. The $Saline^3$+ECS groups had N's of 20 for treatment times 1 and 30 min; the remaining times had N's of 10 per time.

Table 4

Effect of ECS and Anisomycin on Retention for Active Avoidance

Drug	ECS	Percent Amnesia Time of ECS Treatment after Training				
		1 min	30 min	5 hr	6 hr	7 hr
Ani[2]	ECS	85	85	80	40	15
Ani[2]	P-ECS	15	10	15	15	15
Saline[2]	ECS	75	25	15	10	15
Saline[2]	P-ECS	15	10	15	10	15

The tabled values are for percent mice classed as amnesic or having forgotten. Amnesia is defined as taking 4 or more trials to make an avoidance response. Naive subjects had a mean value of 5.8 trials to make their first avoidance response. For the 1 min treatment time, Ani[2]+ECS took 5.2 mean trials to make an avoidance, Saline[2]+ECS took 4.6 mean trials and Saline[2]+P-ECS took 2.6 mean trials.

TABLE 5a

Effect of ECS on Protein Synthesis

% inhibition (-) or % Stimulation (+)

Treatment Time of ECS or P-ECS after Saline	Injection Time After ECS	
	10 min Saline+ECS	30 min Saline+ECS
180 min	-28%	-18%
240 min	-21%	- 6%
	Injection Time After P-ECS	
	10 min Saline+P-ECS	30 min Saline+P-ECS
180 min	+19%	+ 8%

Results are expressed as a difference from control subjects injected with saline and given either ECS or P-ECS as appropriate.

Table 5b

Effect of Ani or Ani+ECS on Protein Synthesis

% inhibition (-) or % stimulation (+)

Treatment Time of ECS or P-ECS after Saline	Injection Time* After ECS					
	10 min			30 min		
	Ani	Ani+ECS	Diff.	Ani	Ani+ECS	Diff.
180 min	-48%	-53%	- 5%	-40%	-37%	- 3%
210 min	-35%	-50%	-15%	-25%	-32%	- 7%
240 min	-19%	-32%	-13%	- 8%	-33%	-25%

	Injection Time* After P-ECS					
	10 min			30 min		
	Ani	Ani+P-ECS	Diff.	Ani	Ani+P-ECS	Diff.
180 min	-48%	-31%	+17%	-40%	-22%	+18%

* Injection times refers to the time of ^{14}C-(U)-Valine administration after ECS or P-ECS as appropriate. The mice were sacrificed 10 min after the Valine injection.

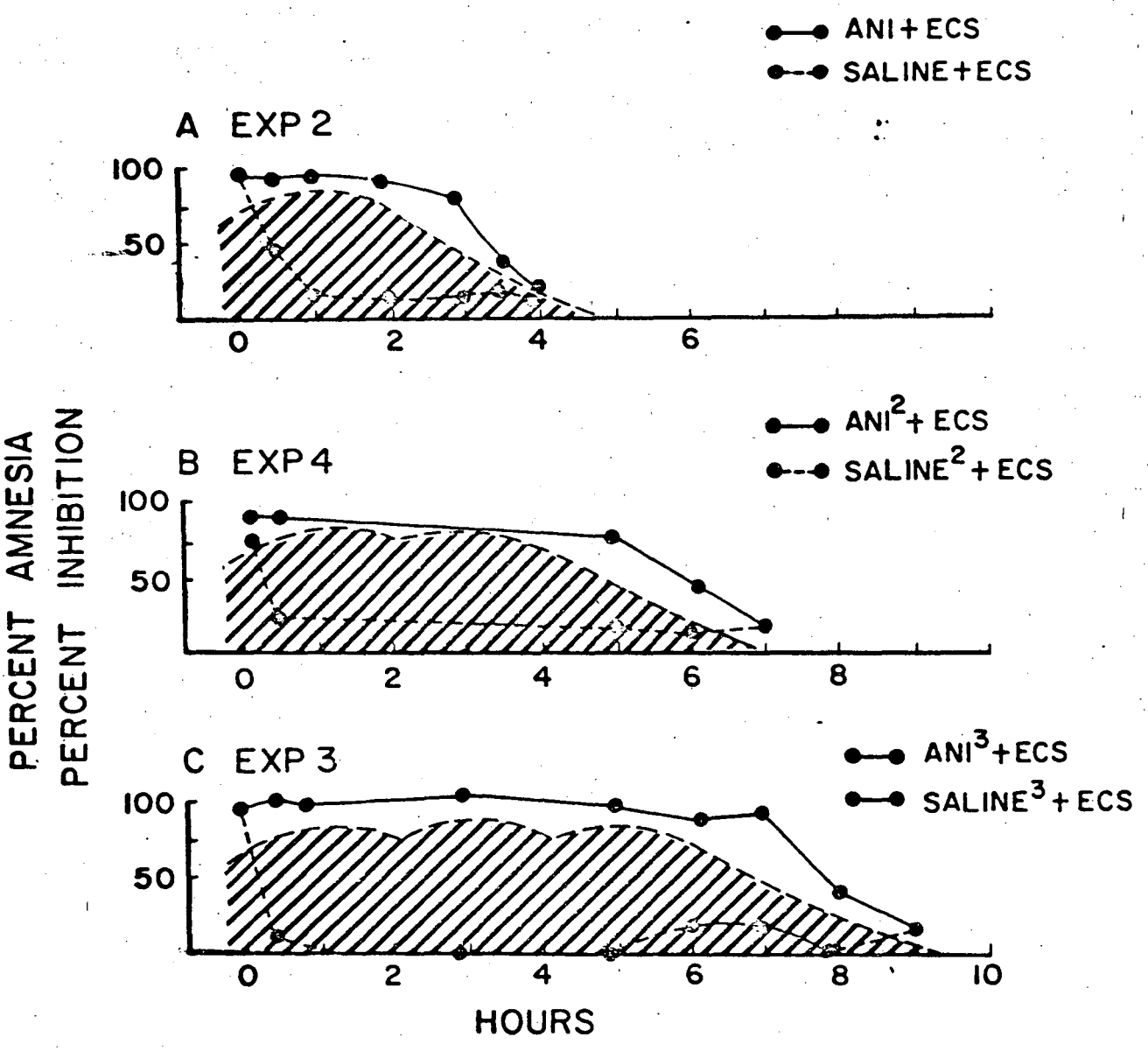
ANI + ECS
SALINE + ECS
A EXP 2
100
50
0
2
4
6
ANI² + ECS
SALINE² + ECS
B EXP 4
100
50
0
2
4
6
8
ANI³ + ECS
SALINE³ + ECS
C EXP 3
100
50
0
2
4
6
8
10
PERCENT AMNESIA
PERCENT INHIBITION
HOURS

FIGURE CAPTION

Figure 1. The relation between protein synthesis inhibition and the combined treatment of anisomycin(ANI) and ECS on the gradients of ECS-induced amnesia. Across the three experiments, one can see that the amnesic gradient for saline and ECS is always shorter than for ANI and ECS. Second, the three experiments are consistent in showing that the ANI+ECS condition results in decreasing levels of amnesia as the recovery of protein synthesis proceeds. This suggests that the shape of the ECS retrograde amnesic gradient is dependent on how much protein synthesis preceeds ECS administration.

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