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Anterograde and Retrograde Effects of Electroconvulsive Shock and of Puromycin on Memory Formation in the Goldfish

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Electroconvulsive shock [ECS] or puromycin administered prior to training did not significantly impair acquisition of shock-avoidance in goldfish. Significant retention deficits are observed on retraining 72 hr later in groups of fish that received ECS 2.5, 1 or 0.5 hr before training as well as in groups that received ECS 0, 4 or 24 hr after training. Puromycin produces significant retention deficits on retraining when given 24, 16, 8, 4 or 0 hr prior to, or 0 or 0.25 hr following training. A temporal course of development of the retention deficit that has been seen with puromycin was not observed with ECS as the deficit was maximal at the earliest train-retrain interval examined. ECS administered before both training and retraining did not relieve the deficit. Since performance was not diminished in fish retrained just after ECS, it appears that this proactive effect of ECS reflects disruption of memory rather than state-dependent learning.

Electroconvulsive shock or puromycin administered following training has typically been assumed to induce retrograde amnesia (RA) of training events and is presumed to be due to disruption of memory formation. Considerably less is known about anterograde amnesia (AA), i.e., memory impairment resulting from an amnesic agent delivered prior to training. Kopp, Bohdanecky and Jarvik (1968) demonstrated ECS-induced AA in mice using a one-trial passive avoidance task. A single ECS treatment delivered 1-4 hr prior to training caused retention deficits, but had no effect on memory when delivered 24 hr prior to training. Using mice in a one-trial passive avoidance task, Zerbolio (1969) found no significant retention deficit when ECS was given 60 min before training but it did affect memory when administered 5 min prior to training. Further support for time-dependent anterograde

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effects of ECS on retention in a one-trial learning paradigm is provided by Gardner, Glick and Jarvik (1972), who observed that ECS administered 25 min prior to training affected retention but had no effect when given 24 hr before training.

Although the results of the above studies are in agreement, it is difficult to conclude that pretraining ECS affected memory and not learning, since ECS may have produced a confusional state and poor learning. The time-dependency of pretraining ECS effects on memory could also be explained on this basis. Since a single training trial was used in the above studies, there was no measurement of acquisition. In the present experiments, we have examined the possibility of ECS-induced AA in a multitrial task in the goldfish and further explored the proactive effects of puromycin on memory (Agranoff, 1970).

EXPERIMENT 1

Method

Subjects. Common goldfish (*Carassius auratus*), 6-7 cm in length from snout to caudal peduncle, weighing 8-11 g were obtained from Ozark Fisheries, Stoutland, MO. Upon arrival, the fish were housed in 750-liter tanks for approximately 1-2 wk. Each subject was placed in a separate 1.5-liter tank for 1-2 days prior to the beginning of the experiment. The fish were housed in continuous light, were not fed and were maintained at $20 \pm 1^{\circ}$ C. All experiments were performed between January and April.

Apparatus and procedures. Fish were acclimated in individual shuttle-boxes for 5 min prior to 20 training trials (Agranoff, 1971). Each trial lasted 1 min and began with 20 sec of light presented on the side of the box occupied by the fish, followed by up to 20 sec of light paired with pulsed shock delivered through the water (3.5 V, 60 Hz, *rms*, 100 msec duration, 1.5 sec interpulse interval). An avoidance response was recorded when a fish crossed a barrier to the opposite side of the box within the first 20 sec of light onset, and an escape was recorded if the fish crossed once shock had ensued. Either avoidance or escape responses terminated the trial and initiated the intertrial interval of at least 20 sec of darkness. A failure to escape was recorded when a fish failed to cross into the safe compartment during the first 40 sec of a trial. Approximately one-fifth of the fish were eliminated on the basis of (a) making more than five avoidances in the first ten training trials, (b) having more than four failures to escape in either block of ten training trials or (c) failing to escape in more than four of the ten retraining trials (23 out of 732 fish randomly distributed across treatments).

The subjects were randomly divided into 23 groups. Sixteen groups were divided among four ECS treatments: ECS (0.1 sec, 30 mA, 60 Hz, *rms*, see

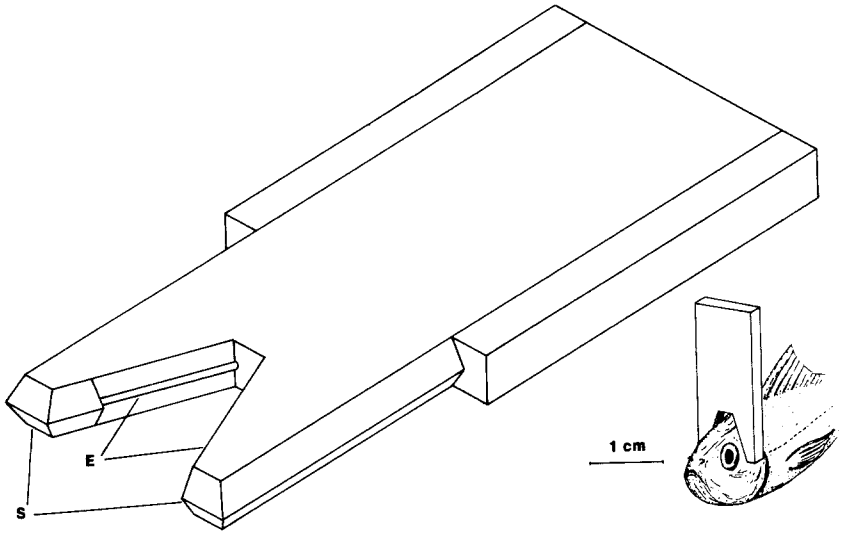

Fig. 1. ECS electrode. The body of the device was constructed of Plexiglas. All but the ends of the stainless steel electrodes (E) were covered with silicone rubber insulation (S) and Plexiglas. The ECS pulse was initiated with a foot pedal to protect the operator from line voltage.

Fig. 1) delivered behind the eyes 1.5 hr prior to training, 0.5 hr prior to training, immediately following training, and no-ECS treatment. The four groups within each of the above treatments were retrained at either 2, 6, 24, or 72 hr following training. Another three groups received ECS 4 hr following training and were retrained at either 6, 24 or 72 hr after training. The four remaining groups received ECS 4 or 2.5 hr before training or 24 or 48 hr following training and were retrained at 72 hr.

Results

A multiple regression based on the number of avoidances in the first block of ten training trials and second block of ten training trials (A_1, A_2), the total number of failures to escape in the 20 training trials ($F_1 + F_2$), and the number of shocks received in the first ten and second ten training trials (S_1, S_2) for 240 no-ECS control animals was used to determine an equation that predicted performance on the 10 retraining trials. This equation ($P = 6.31 + 0.51 \log (A_1 + 1) + 1.14 \log (A_2 + 1) + 0.15 \log (F_1 + F_2) - 0.02 S_1 - 0.02 S_2$) was then used to predict retraining avoidance for each fish of the experiment. The constants in the equation were derived separately for each of the experiments reported (1, 2 and 3) from no-ECS controls, as the experiments were performed at different times. Although failures to escape are positively correlated with predicted retraining performance, the weighting of

this factor is low and is balanced by the subtraction of shocks which vary directly with failures to escape. In addition, predicted and achieved scores are highly correlated ($r = 0.55$, $t < 0.01$).

Independent two-tailed t tests were performed comparing the no-ECS treatment mean P score with the mean P scores of four other treatments: ECS 4.0 hr, ECS 2.5 hr, ECS 1.5 hr and ECS 0.5 hr before training (Table 1). These tests served to ascertain that acquisition of the avoidance response was not affected in groups that received ECS prior to training. P scores were chosen for the analysis as they incorporate several response measures of acquisition level. Only the group that received ECS 2.5 hr before training differed significantly from no-ECS controls ($t(198) = 2.10$, $P < 0.05$). The enhanced acquisition in this group is not readily understood, however, the mean P score for the group that received ECS 2.5 hr prior to training was 6.52 as compared to the mean P of no-ECS controls of 5.76. Hence, the 2.5 hr group displayed better acquisition than the controls and any memory deficit in this group as compared to controls could not be explained by ECS having impaired acquisition.

Dependent, one-tailed t tests were used to determine the significance of the difference between the number of avoidances achieved in the ten retraining trials and the number of avoidances predicted to occur during the retraining for each treatment (A-P). These tests revealed significant deficits in memory at the 2, 6, 24 and 72 hr training-retraining intervals in the groups that received ECS 0.5 hr before or 0.0 hr after training (Table 1 and Fig. 2). Similar deficits were also evident at the 72 hr training-retraining interval in the groups that received ECS 2.5 hr before, 1.5 hr before, 4.0 hr before and 24.0 hr after training. The no-ECS control group demonstrated a significant deficit in memory when retrained 2 hr following training. This deficit is unexplained, but may be the result of fatigue of the subjects at this brief training-retraining interval. Alternative interpretations of a biphasic time course of performance in goldfish have been offered (Riege and Cherkin, 1971).

In order to determine whether amnesia developed over time (Fig. 3), one-way analyses of variance were performed within treatments on the retention scores of each group across training-retraining intervals. Of the treatments in which three or more groups were retrained at either 2, 6, 24 or 72 hr after training, only the group that received ECS 4 hr after training demonstrated a significant development of amnesia ($F(2,86) = 4.45$, $P < 0.025$); within each of the remaining treatments there were no significant differences between groups (P's > 0.10). The apparent development of amnesia in the 4.0 hr post-training ECS treatment was a function of a low retention deficit in the group retrained at 24 hr (Fig. 3). Two-tailed independent t tests performed on the retention scores of the groups receiving ECS 4 hr after training found that the group retrained at the 24 hr training-retraining interval

TABLE 1

Effect of ECS on Memory as a Function of Training-ECS Interval and Training-Retraining Interval[a]

Treatment	N	Trials: Training 1-10	Trials: Training 11-20	Time (hours between training and retraining)	Retraining 21-30 A (Achieved)	P (Predicted)	Retention score[b] A–P
No ECS	50	1.00 (0.21)	1.86 (0.35)	2	5.22 (0.46)	5.87 (0.21)	−0.65 (0.39)*
	23	1.00 (0.35)	1.83 (0.59)	6	5.70 (0.67)	5.58 (0.36)	0.12 (0.53)
	46	0.78 (0.17)	1.67 (0.34)	24	6.00 (0.40)	5.59 (0.22)	0.41 (0.34)
	51	1.14 (0.20)	2.29 (0.39)	72	5.61 (0.46)	5.89 (0.24)	−0.28 (0.37)
ECS 4.0 hr before training	26	1.42 (0.32)	3.00 (0.68)	72	7.04 (0.54)	6.35 (0.36)	0.68 (0.53)
ECS 2.5 hr before training	25	1.36 (0.33)	3.32 (0.64)	72	5.56 (0.58)	6.52 (0.34)	−0.96 (0.54)*
ECS 1.5 hr before training	29	1.14 (0.28)	1.86 (0.47	2	5.79 (0.59)	5.85 (0.30)	−0.06 (0.58)
	37	0.54 (0.14)	2.22 (0.50)	6	4.97 (0.58)	5.61 (0.28)	−0.64 (0.48)
	38	0.61 (0.19)	1.16 (0.35)	24	5.34 (0.54)	5.03 (0.24)	0.31 (0.50)
	28	0.64 (0.19)	1.89 (0.49)	72	4.46 (0.59)	5.50 (0.30)	−1.04 (0.57)*
ECS 0.5 hr before training	32	0.56 (0.16)	0.97 (0.22)	2	3.84 (0.59)	5.25 (0.17)	−1.41 (0.57)**
	33	0.85 (0.27)	1.51 (0.45)	6	3.67 (0.61)	5.43 (0.29)	−1.76 (0.50)**
	39	1.15 (0.22)	2.49 (0.50)	24	4.77 (0.54)	5.97 (0.29)	−1.20 (0.47)**
	25	0.52 (0.24)	1.20 (0.37)	72	2.84 (0.61)	4.98 (0.29)	−2.14 (0.57)**
ECS 0.0 hr after training	25	0.52 (0.17)	1.20 (0.40)	2	2.04 (0.59)	5.34 (0.27)	−3.30 (0.51)**
	28	0.68 (0.26)	2.00 (0.61)	6	2.96 (0.54)	5.45 (0.29)	−2.49 (0.38)**
	29	1.34 (0.25)	2.66 (0.56)	24	3.41 (0.64)	6.19 (0.33)	−2.78 (0.51)**
	27	0.82 (0.25)	1.96 (0.57)	72	2.59 (0.55)	5.59 (0.32)	−3.00 (0.51)**
ECS 4.0 hr after training	33	0.42 (0.14)	1.27 (0.39)	6	3.85 (0.54)	5.34 (0.25)	−1.49 (0.46)**
	27	1.22 (0.27)	2.89 (0.60)	24	5.67 (0.72)	6.28 (0.34)	−0.61 (0.58)
	29	0.72 (0.21)	1.69 (0.46)	72	2.76 (0.47)	5.45 (0.29)	−2.69 (0.40)**
ECS 24.0 hr after training	25	1.12 (0.29)	2.16 (0.56)	72	4.32 (0.70)	5.90 (0.29)	−1.58 (0.06)**
ECS 48.0 hr after training	27	0.93 (0.26)	1.82 (0.55)	72	4.96 (0.65)	5.65 (0.30)	−0.60 (0.52)

[a] The mean number of avoidance responses and the standard error, in parentheses, are given for each block of ten trials.

[b] A retention score of zero signifies normal memory.

*$P < 0.05$, one-tailed t test.

**$P < 0.01$, one-tailed t test.

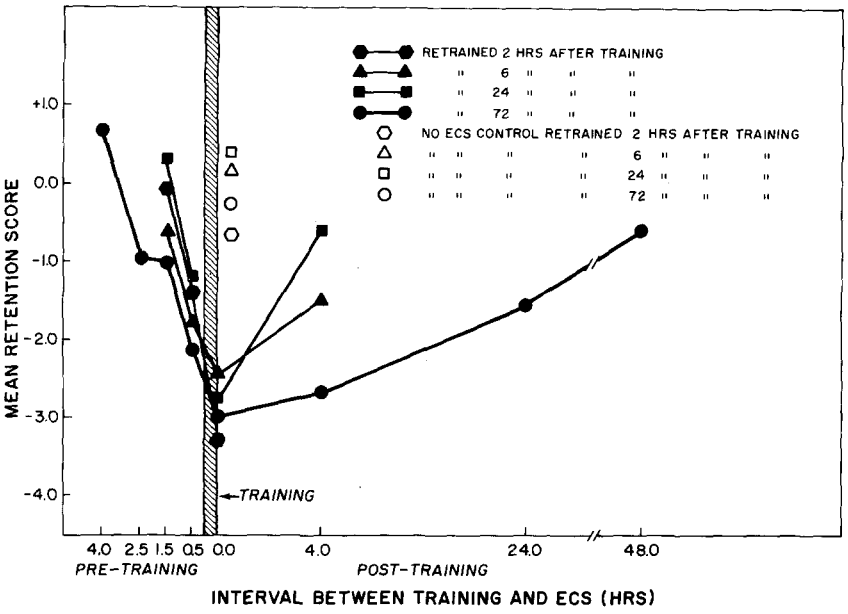

Fig. 2. Mean *A–P* retention score as a function of ECS-training interval and training-retraining interval.

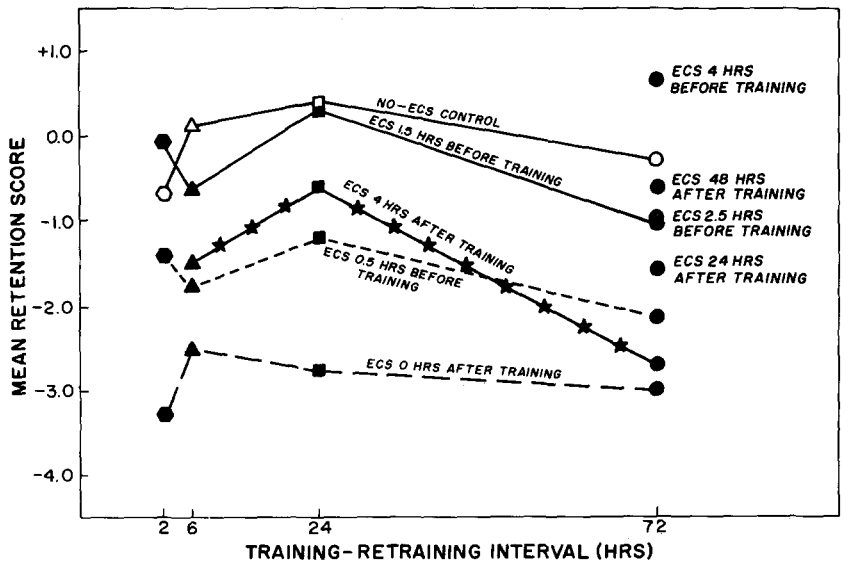

Fig. 3. Mean *A–P* retention score as a function of training-retraining interval.

differed significantly from the one retrained at the 72 hr interval ($t(54) = 2.94$, $P < 0.01$); however the groups retrained at 6 and 72 hr did not differ from one another ($P > 0.05$), thus suggesting that there was actually no development of amnesia with this treatment.

EXPERIMENT 2

The robust proactive amnesic effect of ECS in Experiment 1 may suggest that pretraining ECS interferes with memory fixation. Alternatively, a state-dependency model (Thompson and Neely, 1970) would propose that ECS delivered before training induces a unique brain state in which information is acquired and that this state must be reintroduced for learning to be manifest on a subsequent test trial. As the state induced by ECS is believed to wear off with time, the animal would appear amnesic at the time of testing despite completed memory fixation. Thus, reintroducing the ECS state prior to testing should lead to non-amnesic performance. This state-dependent model would further predict that animals trained in a normal (non-ECS) state and tested in an ECS state should show little evidence of prior memory fixation.

In order to determine whether the anterograde effect of ECS observed in the initial experiment was actually due to interference with consolidation or to state-dependent learning a 2 X 2 factorial study was performed.

Method

Subjects, apparatus and procedure. All apparatus and procedures were identical to those described earlier and similar subjects were used. Two groups of fish received no-ECS prior to training and were retrained 72 hr following training with one of these two groups receiving ECS 0.5 hr prior to retraining. Two additional groups received ECS 0.5 hr before training and were retrained 72 hr following training with one group receiving a second ECS 0.5 hr prior to retraining.

Results

Independent two-tailed t tests were used in comparing the mean P scores (Table 2) of the no-ECS controls against the remaining three groups. None of the groups differed from the no-ECS controls (P's > 0.05) indicating the failure of ECS to affect acquisition.

One-tailed, dependent t tests on A vs P retention scores (Table 2) revealed significant deficits in both groups that received ECS prior to training. A 2 X 2 ANOVA on the A-P scores of the four groups found a significant effect of ECS delivered prior to training ($F(1,108) = 20.23$, $P < 0.001$) while

TABLE 2

Effect of ECS on Memory When ECS is Administered Prior to Training, Retraining or Training and Retraining[a]

Treatment	*N*	Trials: Training 1-10	Trials: Training 11-20	Time (hours between training and retraining)	Retraining 21-30 *A* (Achieved)	*P* (Predicted)	Retention score[b] *A*–*P*
No ECS	36	1.50 (0.26)	3.06 (0.52)	72	6.22 (0.49)	6.44 (0.27)	−0.22 (0.38)
ECS prior to retraining	27	0.89 (0.28)	3.19 (0.69)	72	6.07 (0.62)	6.13 (0.39)	−0.06 (0.57)
ECS prior to training	26	0.62 (0.20)	2.35 (0.62)	72	3.42 (0.56)	5.67 (0.35)	−2.25 (0.55)**
ECS prior to training and retraining	23	1.00 (0.31)	2.96 (0.73)	72	3.39 (0.66)	5.78 (0.43)	−2.39 (0.41)**

[a]The mean number of avoidance responses and the standard error, in parentheses, are given for each block of ten trials.
[b]A retention score of zero signifies normal memory.
*$P < 0.05$ one-tailed t test.
**$P < 0.01$ one-tailed t test.

the effect of ECS delivered prior to retraining, as well as the interaction of ECS before training and retraining did not achieve significance (P's > 0.50). Independent two-tailed t tests found that the pair of groups that did not receive ECS before training did not differ from one another ($P > 0.50$) and that the two groups receiving ECS before training also did not differ ($P > 0.50$). Both groups receiving ECS before training differed significantly from the two groups not receiving ECS prior to training (P's < 0.01).

EXPERIMENT 3

Puromycin has been found effective in producing a retention deficit in goldfish when injected immediately before training (Agranoff, Davis and Brink, 1966) or within 30 min after training (Davis, Bright and Agranoff, 1965). As the inhibitory effects of puromycin on protein synthesis have been reported to last for 24 hr (Lim, Brink and Agranoff, 1970) the present experiment examines the effectiveness of puromycin in impairing memory when it is injected at various intervals before or after training. In addition, this study will permit a comparison of the anterograde and retrograde effects of puromycin with the effects of ECS reported in Experiment 1.

Method

The experimental apparatus were those described in Experiment 1 and similar subjects were used. The procedure differed from that described earlier in that the CS was presented for 15 sec, CS-shock for 20 sec and time out for 25 sec. A small change has been made in these parameters over the years that this apparatus has been used, and the effect of these differences on interpreting the data are discussed below. Two groups of fish received an intracranial saline injection (10 μl) either 24 or 16 hr prior to training. Six groups were injected intracranially with puromycin (130 μg in 10 μl of saline) at either 48, 24, 16, 8, 4, or 0 hr prior to training and seven groups of fish were injected either 0, 0.25, 1, 4, 8, 24 or 48 hr following training. All groups were retrained 8 days following training.

Results

An ANOVA on P scores (Table 3) across all groups did not achieve significance ($P > 0.1$) suggesting that the pretraining treatments did not affect acquisition. Dependent, one-tailed t tests were used to determine the significance of the difference between the number of avoidances achieved in retraining and the number of avoidances predicted to occur during retraining (A-P). These tests (Table 3) found significant retention deficits in the groups that received puromycin at either 24, 16, 8, 4 or 0 hr before training and in

TABLE 3

Effect of Puromycin on Memory as a Function of Training-Puromycin Interval[a]

Treatment	*N*	Trials: Training 1-10	Trials: Training 11-20	Trials: Retraining 21-30 *A* (Achieved)	*P* (Predicted)	Retention score[b] *A*–*P*
Saline 24 hr before training	37	0.89 (0.21)	1.76 (0.38	5.57 (0.49)	5.33 (0.17)	+0.24 (0.43)
Saline 16.0 hr before training	24	0.88 (0.32)	1.46 (0.44)	4.67 (0.59)	5.35 (0.15)	−0.68 (0.59)
Puromycin 48.0 hr before training	29	1.04 (0.27)	1.97 (0.37)	4.93 (0.63)	5.40 (0.18)	−0.47 (0.58)
Puromycin 24.0 hr before training	55	1.62 (0.23)	3.06 (0.39)	4.55 (0.36)	5.58 (0.14)	−1.03 (0.35)**
Puromycin 16.0 hr before training	47	1.32 (0.19)	2.68 (0.37)	4.30 (0.49)	5.27 (0.14)	−0.97 (0.45)*
Puromycin 8.0 hr before training	22	0.86 (0.19)	2.46 (0.52)	3.18 (0.52)	5.22 (0.19)	−2.04 (0.51)**
Puromycin 4.0 hr before training	17	0.65 (0.30)	2.82 (0.72)	3.47 (0.54)	4.96 (0.18)	−1.49 (0.52)**
Puromycin 0.0 hr before training	21	0.67 (0.22)	1.38 (0.41)	1.95 (0.41)	5.08 (0.17)	−3.12 (0.34)**
Puromycin 0.0 hr after training	31	0.84 (0.26)	1.77 (0.41)	2.23 (0.40)	5.13 (0.15)	−2.90 (0.33)**
Puromycin 0.25 hr after training	22	1.18 (0.30)	1.59 (0.47)	2.82 (0.54)	5.39 (0.22)	−2.57 (0.48)**
Puromycin 1.0 hr after training	22	1.64 (0.37)	2.82 (0.67)	4.64 (0.71)	5.68 (0.18)	−1.05 (0.62)
Puromycin 4.0 hr after training	23	1.09 (0.24)	1.87 (0.44)	4.96 (0.66)	5.36 (0.18)	−0.40 (0.64)
Puromycin 8.0 hr after training	24	1.21 (0.34)	1.75 (0.54)	4.42 (0.61)	5.31 (0.22)	−0.89 (0.64)
Puromycin 24.0 hr after training	22	1.41 (0.33)	2.32 (0.50)	6.05 (0.59)	5.61 (0.19)	+0.43 (0.55)
Puromycin 48.0 hr after training	26	1.58 (0.32)	2.69 (0.50)	5.58 (0.59)	5.67 (0.21)	−0.09 (0.53)

[a] The mean number of avoidance responses and the standard error, in parentheses, are given for each block of ten trials.

[b] A retention score of zero signifies normal memory.

*$P < .05$, one-tailed t test.

**$P < 0.01$, one-tailed t test.

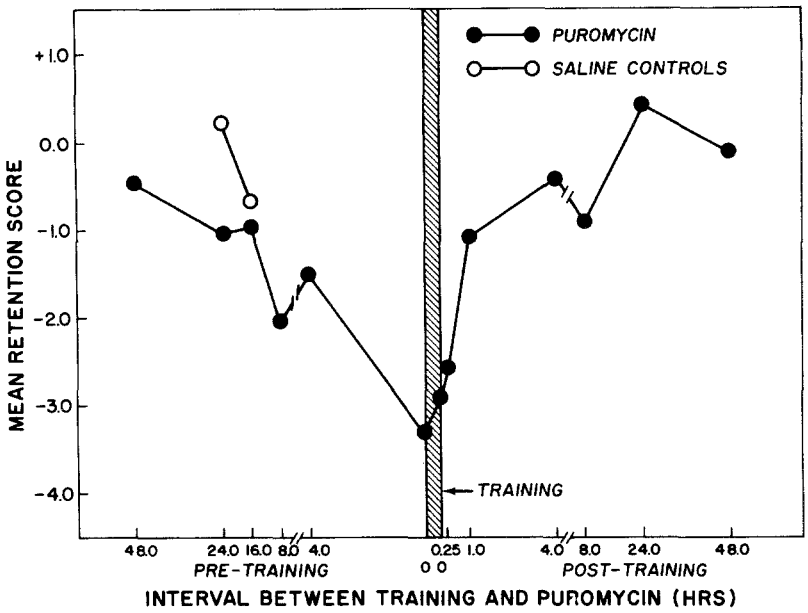

Fig. 4. Mean *A–P* retention score as a function of puromycin-training interval.

groups that were injected with puromycin 0 or 0.25 hr following training (Fig. 4).

GENERAL DISCUSSION

When a putative memory-blocking agent administered before or after training produces a retention deficit on retraining, alternative explanations that do not involve disruption of memory must be considered. For example, if the agent is delivered before training it could produce a confusional state that is not apparent from performance on acquisition, particularly in one-trial learning paradigms. The deficit could alternatively be the result of a state-dependent condition induced by the agent. If so, the deficit should be overcome by the administration of the same treatment just before retraining. In the present studies, we observed no performance deficits on acquisition with either puromycin or ECS administered before training, nor was there any evidence to support a state-dependency hypothesis for the action of ECS.

Impaired performance on retraining, seen in animals treated with a memory-blocking agent following training is usually interpreted as retrograde amnesia, particularly if a temporal gradient of growing insusceptibility to the agent is seen. The presence of this gradient rules out the possibility that the

performance deficit can be accounted for by lingering effects of the agent at the time of retraining. A number of alternative explanations of deficits resulting from post-training treatments generally deal with the idea that the agent has stimulus effects, i.e., is in some way aversive (Lewis and Maher, 1965). The best evidence that an agent is selectively blocking memory would appear to come from a demonstration of both proactive and retroactive effects. We have previously shown (Agranoff *et al.*, 1966) that various blockers of protein and RNA synthesis, administered immediately before or at varying times after training, produce both such performance decrements, consistent with specific memory disruption. We had not however systematically investigated the interval between injection of puromycin and initial training that would still permit a retention deficit at subsequent retraining. A single data point using a somewhat different task (Task I) had suggested a diminished effect of puromycin given only 20 min before training (Agranoff *et al.*, 1966). For these reasons, an extensive examination of the pre-trial effects of the agent was undertaken. The systematic study of pretrial effects of puromycin reported here in Task III with goldfish indicates clearly that the antibiotic can exert its effect as long as 24 hr after its injection. This result is generally compatible with the day long inhibition of protein synthesis in the brain seen after injection with puromycin (Lim *et al.*, 1970). The exact duration of inhibition of protein synthesis following its intracranial injection is difficult to estimate precisely and it would be misleading to make more than a qualitative comparison between the depth and duration of the inhibition of protein synthesis and the extent of amnesia produced. Measurement of inhibition of protein synthesis is at best imprecise, since it does not take into account differences in the relative inhibition of synthesis of various protein components or of brain regions, although we do have evidence that the drug penetrates the entire brain (Lim *et al.*, 1970). Also, corrections for the soluble precursor pool are approximations, since they measure only the amount of precursor present at the end of incubation (Agranoff, 1967). Similarly, referring to an amount of memory is inexact, since we can only guess at the associative strength of the learned habit from the measured performance. What is perhaps of greatest interest is the finding that pretrial injections at no time produce a significantly greater block of memory than posttrial injections. The result argues against hypotheses which suggest that the antimetabolite in some way exerts its effect via a process that requires time for accumulation of a toxic product or depletion of an existing protein population.

In comparing previous experiments (Davis *et al.*, 1965; Agranoff *et al.*, 1966) and the present ones with both puromycin and ECS, we must first consider differences in training paradigms used in this laboratory over the past 10 years. In the initial shuttlebox used (Task I), light and shock continue on the starting side of the shuttlebox until the fortieth sec regardless of whether the animals have avoided during the first 20 sec or escaped in the second

20 sec. Task II was essentially the same, except that animals were trained to swim into the light instead of into the dark. With the present task (III), light and shock are terminated whenever the animals cross the barrier. In addition, there is a gate over the barrier that must be deflected. This leads to much lower initial training scores but has relatively little effect on retraining scores. Also, in the initial studies with puromycin and ECS in task I, retraining was measured on Day 4. In the present experiment with puromycin, it was measured on Day 8. In the experiments reported here there are some differences within task III. In the puromycin experiment, the avoidance, shock and time out were 15, 25 and 10 sec respectively. In both ECS experiments the comparable intervals were 20, 20 and 20 sec. These differences must be considered when comparing the experiments, as discussed below.

We did not detect development of amnesia (short-term decay) following ECS, possibly because of the train-retrain interval used. Previous reports of amnesia developing in rodents following ECS (McGaugh and Landfield, 1970; Miller and Springer, 1971) suggest that a rapidly developing amnesia would have been seen in the fish had they been retrained at intervals of less than 2 hr following training. Shorter train-retrain intervals were not examined as no-ECS controls retrained 2 hr after training evidence a significant retention deficit. Consequently, the evaluation of any deficits in experimental groups retrained soon after training would be difficult. The rapid onset of amnesia is consistent with the idea that ECS produces an "electrical storm" which destroys memory before it is converted into a long-term form. The demonstrated proactive effects of ECS reported here contradict this argument. ECS administered 2.5 hr prior to training did not impair acquisition yet prevented the formation of long-term memory, much as had been described with various antibiotic agents. ECS thus produces changes which are still effective several hours after the animal appears to be fully recovered. The mechanism by which ECS produces its effect appears to be quite different than that of the antibiotics, since the latter agents do not produce gross behavioral or neurological effects. ECS results in some decrease in protein synthesis (Andry and Luttges, 1972), but not an amount sufficient to explain the block of memory, since moderate degrees of inhibition produced by the antimetabolites do not result in a measurable loss of memory (Agranoff *et al.*, 1965). Although pretrial ECS produces about the same degree of deficit as puromycin, its effect is gone within 2.5-4.0 hr, whereas that of puromycin continues for 24 hr.

The observed RA gradient of ECS was much longer than anticipated. Although we have observed in experiments using task I that there is a somewhat longer RA gradient with ECS than with puromycin (Davis *et al.*, 1965), it is much more pronounced in the present instance. It is not likely that the difference in the RA gradients between puromycin and ECS in the present experiments (task III) could be attributable to the 5 sec difference in

CS-US interval in the two paradigms, although the longer interval might be expected to result in a more sensitive measure of performance decrement. For example, the 4 hr RA gradient with puromycin compared with the 1 hr gradient seen in Task I, has also been seen in Task III when a 20 sec-20 sec-20 sec sequence is used for avoidance, shock and time-out respectively. More likely, differences in the difficulty or complexity of the two tasks account for the different RA gradients. The differences in the ECS gradients is more likely accounted for by the difference in the mode of administration of ECS in the two experiments. In the previous study, ECS was administered through the water. In the present one, it is localized and therefore probably more effective. Gold, Macri and McGaugh (1973) observed that the length of the observed RA gradient for ECS in mice trained in a one-trial passive avoidance task was related to the amount of current delivered to the brain, a conclusion consistent with the present observation.

Agranoff (1971) reported that the effects of puromycin administered before training could not be explained by a state-dependent model. In the present experiment, we find that this is also true for ECS. It appears now that puromycin and ECS each produces both anterograde and retrograde memory loss. A principal difference between the action of the two agents is that the antimetabolite does not produce gross convulsions. The possibility that puromycin exerts its action on memory by producing brain seizure activity (Cohen and Barondes, 1967) has been seriously questioned. While puromycin does potentiate pentylenetetrazol behavioral convulsions in fish, the glutarimide antibiotics do not potentiate convulsions, yet they produce amnesia (Agranoff, 1970). In addition, puromycin aminonucleoside, an analog of puromycin, also potentiates convulsions in the fish but does not impair memory and has no effect on protein synthesis (Agranoff and Klinger, 1964).

We had previously proposed that antimetabolites might exert their action on the brain either by blocking an information-specific process in the neuronal bodies or at synapses involved in the mediation of a learned behavior, or could alternatively be involved in a non-information-specific process, such as the release of a "fix" signal in the brain (Davis and Agranoff, 1966). It appears from the present studies that ECS may too exert its affect via a non-information-specific mechanism, possibly related to the known effects of ECS on turnover of certain neurotransmitters (Kety, *et al.*, 1967). The temporal courses of action of ECS and of puromycin may prove useful in the ultimate elucidation of the mechanism of action of the two different amnesic agents.

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