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BRIEF REPORT

Puromycin-Induced Retention Deficit in Goldfish as a Function of Attained Training Performance Level[1]

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Goldfish received either 20 or 50 active avoidance training trials followed by puromycin (or no-treatment) and 10 retraining trials either 1 or 7 days following training. While 50 trials resulted in significantly more training avoidances than 20 trials, the groups which received puromycin showed equivalent retention deficits on Day 7. A within-group comparison of fish whose training performance was high or low further revealed that the degree of the retention deficit was independent of achieved training performance level. These data support the hypothesis that puromycin interferes with a memory fixation process that is initiated only upon completion of the training session.

The suggestion that long-term memory formation depends upon synthesis of protein is supported by studies which have demonstrated that potent inhibitors of protein synthesis (e.g., puromycin and cycloheximide) are capable of disrupting memory fixation in various species (Agranoff and Klinger, 1964; Barondes and Cohen, 1968a). Nevertheless, several phenomena associated with antibiotic-induced amnesia remain poorly understood. Among them is a report that acetoxycycloheximide disrupts fixation in mice but fails to do so when subjects are given extended training (Cohen and Barondes, 1968). Since antibiotics do not completely block protein synthesis, the authors proposed that the residual protein synthesis in the presence of antibiotic can store memory if the training session is sufficiently prolonged. Previous experiments in our laboratory with goldfish suggested, however, that puromycin effectively impaired retention of shuttlebox acquisition whether subjects received 20 or 30 training trials. Consequently, the present study sought to determine whether additional training would prevent the retention deficit normally induced by puromycin, in analogy to the results reported in mice.

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METHOD

Subjects

Goldfish (*Carassius auratus*), 6 to 7 cm in body length and weighing 8.5 to 11.0 g, were obtained from Ozark Fisheries, Stoutland, Missouri. The fish ($N = 256$) were placed in separate 1.5 liter tanks for 1 to 2 days prior to training. They were not fed and were housed in continuous light at 20° ± 1°C. The study was performed in February and March.

Apparatus and Procedure

Active avoidance training was conducted in an aquatic shuttlebox divided into two compartments by a hinged gate which is described in detail elsewhere (Agranoff, 1971). Each trial lasted 1 min and was initiated with a 20 sec light presentation on the side of the box occupied by the fish. The trial was terminated whether a fish avoided during the first 20 sec or escaped in the second 20 sec light + shock period, leaving the fish in darkness until the beginning of the next minute. Shock (3.5 V, 60 HZ, *rms*, 100 msec duration, 1.5 sec interpulse interval) was delivered through stainless steel electrodes fixed on the side of the box. Failure to escape was recorded when a subject did not cross within the first 40 sec of a trial.

The fish were randomly divided into eight groups in a factorial design. Half the groups received 20 training trials while the remainder received 50 training trials. These two main groups were further subdivided into groups that received either an intracranial injection of 130 μg of puromycin in 10 μl of saline immediately following training or received no injection. The above groups were further subdivided into two groups given 10 retraining trials either 1 or 7 days following training (Table 1).

Sixteen fish, evenly distributed across groups, were discarded for either having made more than six avoidances or more than five failures to escape in the first 10 training trials, or more than five failures to escape during retraining.

RESULTS

A one-way ANOVA found that the four groups that received 50 training trials did not differ from one another in the number of avoidances made in the last 10 training trials ($P > 0.25$; Table 1). Similarly, the four groups that received 20 training trials also did not differ from one another ($P > 0.50$). As expected, the pooled 50-trial groups made significantly more avoidances in the last 10 training trials than did the pooled 20-trial groups [$t(254) = 7.57$, $P < 0.001$]. Analysis of the number of avoidances made in the 10 retraining

TABLE 1

Puromycin-Induced Amnesia as a Function of Number of Training Trials[a]

Treatment	Training trials	N	Mean training avoidances in blocks of 10 trials					Training-retraining interval (days)	Mean retraining avoidances
control	20	32	1.19 (0.29)	2.81 (0.51)				1	6.53 (0.43)
puromycin	20	28	1.61 (0.31)	3.36 (0.64)				1	4.18 (0.49)
control	50	33	1.30 (0.31)	2.73 (0.51)	4.12 (0.62)	4.76 (0.64)	5.76 (0.59)	1	7.97 (0.34)
puromycin	50	34	1.62 (0.30)	3.26 (0.55)	4.03 (0.55)	4.65 (0.54)	5.12 (0.56)	1	6.06 (0.49)
control	20	34	1.06 (0.24)	2.29 (0.46)				7	5.29 (0.50)
puromycin	20	30	0.80 (0.23)	1.93 (0.50)				7	1.77 (0.47)
control	50	32	1.16 (0.25)	2.69 (0.51)	3.97 (0.61)	4.88 (0.66)	6.16 (0.67)	7	7.75 (0.41)
puromycin	50	33	1.27 (0.27)	3.21 (0.50)	4.36 (0.66)	5.30 (0.67)	5.42 (0.61)	7	2.88 (0.42)

[a]The standard error, in parentheses, is given for blocks of 10 trials.

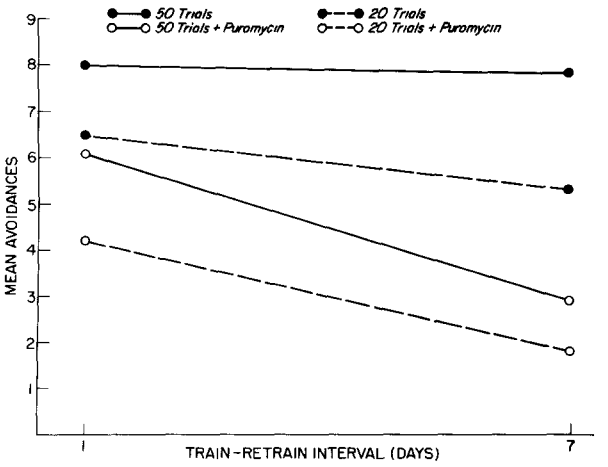

Fig. 1. Decay of memory over the 1-7 day train-retrain interval following puromycin injection.

trials revealed a retention deficit in the puromycin-treated groups, whether the fish received 20 or 50 training trials (see Table 1 and Fig. 1).

Retraining avoidances (see Table 1 and Fig. 1) were analyzed with a 2 X 2 X 2 ANOVA. The effect of puromycin proved significant [$F(1, 248) = 97.31$, $P < 0.001$] as did number of training trials [$F(1, 248) = 28.87$, $P < 0.001$] and train-retrain interval [$F(1, 248) = 30.22$, $P < 0.001$). The puromycin X train retrain interval interaction achieved significance [$F(1, 248) = 10.37$, $P < 0.001$] while the remaining interactions did not achieve significance ($Ps > 0.10$). The significant interaction was attributed to the decrease in retention over the 1 to 7 day train-retrain interval in those groups that had received puromycin and the absence of memory loss in control groups (see Fig. 1).

If puromycin impairs retention independently of performance level attained during training, separation of 50- or 20-trial puromycin-injected fish into high and low performers on the basis of training scores should result in amnesia for both subgroups. To examine this, mean number of avoidances during the last 10 training trials were computed for 50- and 20-trial groups. The means were applied to the individual groups and the fish in each group were divided into above- and below-mean avoiders. Data for this analysis are presented in Fig. 2. Separate 2 X 2 ANOVAs were used to examine retraining avoidances of high and low avoiders. In both cases the interaction between puromycin and number of training trials failed to achieve significance ($Ps > 0.10$).

It has been suggested that amnestic agents are ineffective in blocking retention when animals are "trained beyond the asymptote of the learning

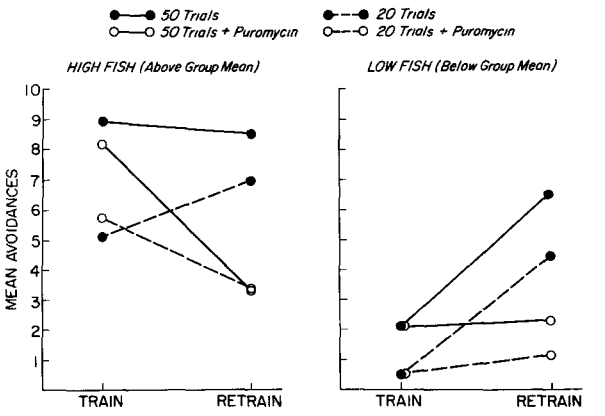

Fig. 2. Puromycin-induced retention deficit at 7 day train-retrain interval in groups divided into above- and below-mean avoiders.

curve" (Flood *et al.*, 1972). To examine this possibility, fish that had apparently reached asymptotic avoidance levels were selected from the above group mean avoiders of the 50-trial puromycin ($n = 13$) and control groups ($n = 10$) (see Fig. 2). These data are plotted in Fig. 3 and indicate that puromycin produces a deficit even when fish are trained beyond asymptotic levels of avoidance since the two groups differed in retraining [$t(21) = 8.01$, $P < 0.000001$].

DISCUSSION

The results indicate that puromycin injection immediately following training results in a greatly reduced responding rate upon retesting, whose

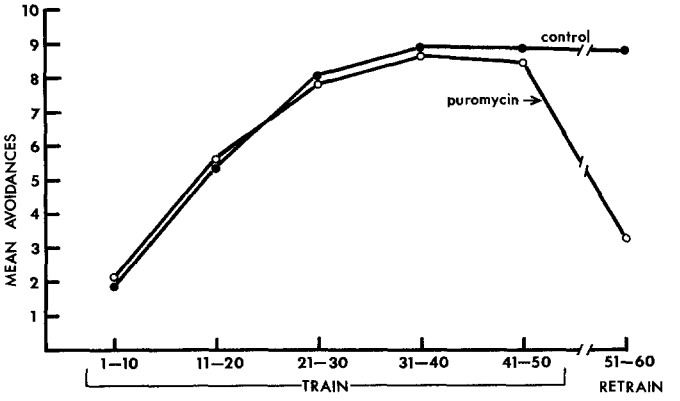

Fig. 3. Retention deficit in fish that achieved maximal avoidance responding in 50 training trials.

magnitude is independent of the previous number of acquisition training trials and level of avoidance responding. This is apparent in Fig. 1 and by the absence of an interaction between puromycin and number of training trials. The analysis of high and low avoiders in each group also demonstrated that the puromycin-induced impairment of retention was independent of the previously attained performance level (Fig. 2). A deficit was observed even in fish that had achieved what appeared to be their maximal avoidance level (Fig. 3). Progressive decay of memory between the 1-day and the 7-day train-retrain interval is evident in Fig. 1 and is consistent with previous findings in this laboratory (Davis and Agranoff, 1966).

The results of the present experiment are in contrast to a report that extension of training prevents puromycin-induced amnesia in goldfish. Potts and Bitterman (1967), using a paradigm similar to ours but involving, in addition, discrimination, showed that goldfish given 90 training trials had not yet reached a plateau level of avoidance responding. Intracranial injection of puromycin immediately following training did not result in memory loss. They concluded that fixation of memory had occurred during the training session. However, due to the nature of their paradigm they were unable to establish a significant deficit even after 20 training trials, a result that precludes comparison of their experiment with ours and mitigates their conclusions concerning 90 trial fish.

In contrast to the present data, studies with mice indicate that increasing number of training trials or intensity of the punishing stimulus can attenuate antibiotic-induced deficits (Barondes and Cohen, 1967; Cohen and Barondes, 1968; Flood *et al.*, 1972; Flood *et al.*, 1974; Flood *et al.*, 1975). The possibility that differences in experimental findings are a consequence of species or antibiotic used cannot be excluded. In related mouse studies, glutarimide antibiotics such as acetoxycycloheximide and anisomycin were used, while the present study employed puromycin. Nevertheless, both classes of antibiotics have similar behavioral effects in both mice and fish. It might be difficult to establish whether extension of training beyond 50 trials would prevent a puromycin-induced retention deficit in goldfish. A confounding effect of fatigue might result, particularly during times of the year when performance is suboptimal (Agranoff and Davis, 1974). In any event, there is no trend in the direction of lessened amnesia in fish whose acquisition levels are maximal prior to Trial 50 (Fig. 3).

Several hypotheses have been offered to explain the effect of attenuated amnesia following extended training in mice. Barondes and Cohen (1967) have argued that memory is encoded by the residual protein synthesis present following antibiotic administration. Flood *et al.* (1975) have shown that protraction of protein synthesis inhibition for long periods of time overcomes the tendency of extended training to prevent amnesia. The explanation offered by Flood *et al.* (1975) assumes that the capacity of the central

nervous system to synthesize memory fixation-related protein is somehow extended by prolonged training. Therefore, protein synthesis relevant to memory fixation is believed to occur when measurable brain protein synthesis has resumed.

An alternative and testable hypothesis to explain both the findings in mice and goldfish is based upon the previous observations that removal of goldfish from the training environment leads to the conversion of short-term memory (STM) to long-term memory (LTM) (Davis and Agranoff, 1966). In that study, puromycin produced a retention deficit in goldfish that had been detained in the training apparatus for 1 hr following training, while fish returned to the home tank immediately following training and injected 1 hr later had become insusceptible to the amnestic effects of the agent. It was postulated that detention, by preventing the lowering of arousal, delays the onset of conversion of STM to LTM. We propose that in the present experiment the additional training trials, by detaining the fish in the training apparatus, also serve to postpone the conversion of STM to LTM. The hypothesis assumes that ongoing protein synthesis is necessary for the establishment of LTM and that the lowering of arousal is necessary for the initiation of the process whereby STM is converted to LTM. Thus, lowering of arousal at a time when protein synthesis is inhibited should lead to the initiation of an abortive fixation process. Experiments in which amnesia appears to be counteracted by increased training may thus reflect the delay of initiation of LTM formation (as a result of longer-lasting arousal associated with a higher degree of aversive training) to a time when protein synthesis is sufficiently restored to permit LTM formation.

The injection of puromycin in the fish produces an inhibition of protein synthesis that lasts over a day, and it has been shown that injection of puromycin a day before training can still produce an amnestic effect (Springer *et al.*, 1975). In contrast, antibiotics administered to mice have a shorter duration of action, and there are no comparable reports of retention deficits for long injection-training intervals. In the mouse, extended training could serve to prolong arousal sufficiently long after they had been returned to their home cages, to delay initiation of the conversion of STM to LTM until brain protein synthesis has been restored. This interpretation could also explain the reported antagonism of the action of pretraining injection of cycloheximide on memory by amphetamine given to mice after training (Barondes and Cohen, 1968b), since amphetamine could delay the lowering of arousal to a time at which brain protein synthesis has recovered. The proposed delayed onset of LTM formation could also explain the report that the effects of extended training are counteracted by extended periods of protein synthesis inhibition (Flood *et al.*, 1975), since the lowering of arousal and the consequent initiation of LTM formation would now occur at a time that protein synthesis inhibition was still maximal.

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