presentation maintaining the most responding; moving model, an intermediate number of responses; and the stationary model associated with the lowest response output) are consistent with Forselius' observations on *Betta* and more recent work by Picciolo on *Trichogaster* (*5*). These workers report that agonistic display can most readily be released by a mirror, or by appropriately colored moving models. These findings suggest that the relative positive reinforcing properties of visual stimuli, similar to a male fish in aggressive display, covary with the degree to which stimuli will evoke unlearned display (*6*).

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6. Supported in part by research grants MY-1604 from the Institute of Mental Health, and NsG 189-61 from the National Aeronautics and Space Administration to the University of Maryland. I am indebted to Larry Potash and Barbara Seldeen for their assistance in conducting the first phase of this research.

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Memory in Mice as Affected by Intracerebral Puromycin

Abstract. *The antibiotic, puromycin, caused loss of memory of avoidance discrimination learning in mice when injected intracerebrally. Bilateral injections of puromycin involving the hippocampi and adjacent temporal cortices caused loss of short-term memory; consistent loss of longer-term memory required injections involving, in addition, most of the remaining cortices. Spread of the effective memory trace from the temporal-hippocampal areas to wide areas of the cortices appears to require 3 to 6 days, depending upon the individual animal. Recent reversal learning was lost while longer-term initial learning was retained after bilateral injections into the hippocampal-temporal areas.*

The suggestion has become increasingly frequent during recent years that nucleic acids or proteins may be concerned with learning and memory. We were led to investigate the effects of the antibiotic, puromycin, on these aspects of behavior by the discovery of Yarmolinsky and de la Haba (*1*) that puromycin produces profound inhibition of protein synthesis in a cell-free system and by the later demonstration that it efficiently suppresses protein synthesis in vivo (*2*). In an earlier paper with de la Haba and Roberts (*3*) we have reported studies on mice which received the maximum amount of puromycin which could be tolerated in a single subcutaneous injection. Although this treatment appeared to suppress the rate of protein synthesis in various parts of the brain to 80 percent of the control value for a period of 6 hours, it was without effect on the learning and retention of simple or discrimination avoidance responses.

The experiments to be reported here have been made with intracerebral injections of puromycin. The amounts injected were smaller than previously used (*3*) so that disorientation of the animal at the time of testing was avoided. With this intracerebral approach, we have found that memory can be consistently destroyed, difference in the effective loci of recent and longer-term memory apparently established, and the time factor concerned in modification of the effective locus determined. Upon recovery, animals were capable of learning again. We cannot now relate these behavioral effects to suppression of protein synthesis, since our biochemical studies are not yet complete.

Adult white mice were trained in a Y-maze with a grid floor through which shock could be applied. The animal was placed in the stem of the Y. To avoid shock the mouse had to move into the correct arm within 5 seconds. If it entered the incorrect arm, it received shock until it moved to the correct arm. Training was continued in one session of about 20 minutes to a criterion of 9 out of 10 correct responses, thus avoiding overtraining. The same procedure was used in testing for memory of the training experience, shock having been given for errors of performance except as noted. In this type of training we have found mice to behave essentially like rats and to retain excellent memory of the training for at least 5 weeks. Intracerebral injections of puromycin, each injection of a volume of 0.012 ml, were made through small holes in the skull, as previously described (*3*).

From one to three injections of puromycin were made into each hemisphere, all at a depth of 2 mm from the surface of the skull. Bilateral injections were made through holes placed (i) just above the angle between the caudal sutures of the parietal bones and the origins of the temporal muscles—these are here designated temporal injections; (ii) 2 mm lateral to the sagittal suture and 2 mm rostral to the caudal sutures of

Table 1. Effects of different sites of injection of puromycin on short- and longer-term memory. T, V, and F refer, respectively, to temporal, ventricular, and frontal injections, all given bilaterally.

Puromycin injections			No. of mice in which memory was:		
Site	Days after learning	Milligrams	Lost*	Impaired*	Retained*
		Short-term memory			
T + V + F	1	0.03 to .06	7	0	0
T	1	.09	8	0	0
T	1	.06	14	3	1
V	1	.09	0	0	5
F	1	.09	0	0	5
V + F	1	.09	0	1	2
		Longer-term memory			
T + V + F	18 to 43	0.03 to .06	7	0	0
T	11 to 35	.06 to .09	0	0	7
V	12 to 38	.06 to .09	0	0	3
F	16 to 27	.06 to .09	0	0	3
V + F	28	.06 to .09	0	2	2
V + T	28 to 43	.09	1	1	2
T + F	28	.09	0	0	3

* For the 37 mice with loss of memory, the means and standard deviations for percentages of savings of trials and of errors were respectively 1 ± 3 and 2 ± 6; for the seven mice with impaired memory, 26 ± 29 and 39 ± 12; and for the 33 mice with retention of memory, 90 ± 14 and 90 ± 9. Negative savings in the group with lost memory have been designated zero so that the mean for this group is an overestimation of savings.

Table 2. Effect of bilateral temporal injections of puromycin on memory of increasing age. Each injection contained 0.09 mg of puromycin.

Injections: days after learning	No. of mice in which memory was:		
	Lost*	Impaired*	Retained*
2	3	0	0
3	4	0	1
4	0	1	1
5	0	1	2
6	0	0	3

* For the seven mice with loss of memory, the means and standard deviations for percentages of savings of trials and of errors were respectively 1 ± 4 and 0 ± 0; for the seven mice with retention of memory, 85 ± 19 and 93 ± 7. In one mouse with impaired memory the percentages of savings for trials and errors were respectively 38 and 20; for the other, 39 and 55.

the parietal bones—these are here designated ventricular injections; and (iii) 4 mm rostral to these last holes and 1 mm lateral to the sagittal suture—these are here designated frontal injections. At the present time we are dependent upon control injections of a solution of fluorescein (*3*) to estimate the spread of puromycin; more refined studies, using other techniques, are not yet complete. Animals which receive injections of fluorescein were sacrificed 1 hour after the injection.

Results with four of the seven types of injections are shown in Fig. 1. This shows that the area around the caudal rhinal fissure was stained with temporal but spared with ventricular injections. The three types of injection not shown in the figure consisted of combined ventricular and temporal injections, of combined ventricular and frontal injections, and of combined temporal and frontal injections. The distribution of fluorescence in these combined injections was essentially the sum of the individual injections as shown in Fig. 1.

The effects of intracerebral injections of puromycin on memory of the training experience are given in Table 1, which shows the number of animals in which memory was lost, impaired, or retained after puromycin injection. In the legend of the table, the means and standard deviations of the percentage savings in retention are given for the three categories of memory. Percentage savings in retention tests were calculated for both trials and errors by subtracting the number to criterion in the retention test from the number to criterion in the learning experience, dividing by the number in the learning experience, and multiplying by 100. Retention tests were given usually 3 days after puromycin, to allow ample time for recovery of the animal. At the time of testing, any weight loss had commonly been regained and feeding, general locomotor activity, and reactions to the maze were normal.

Our first observations were made on mice trained to criterion on one arm of the maze and injected with puromycin 1 day later (Table 1, "Short-term memory"). After combined bilateral temporal, ventricular, and frontal injections, retention tests showed that memory of the training experience had been completely lost. An effort was then made to localize this effect. Memory was also completely lost with high consistency when puromycin was given in bilateral temporal injections. By contrast, bilateral frontal, ventricular, or combined frontal and ventricular injections were essentially without effect.

The next series of observations was made on mice trained to criterion and injected with puromycin 11 to 43 days later (Table 1, "Longer-term memory"). Only combined, bilateral temporal, ventricular plus frontal injections consistently destroyed memory in these animals. Bilateral temporal or frontal or ventricular injections were without effect. Three combinations of two injections (combined ventricular and temporal, or ventricular and frontal, or temporal and frontal) into each hemisphere were without effect in the majority of animals, even though the total amount of puromycin in all but two of eleven of these was at the maximum level tolerated and twice that amount injected in six of the seven experiments with combined temporal, ventricular, and frontal injections. There was consequently a clear distinction between recent and longer-term memory; recent memory was lost when puromycin was introduced through temporal injections into hippocampi and caudal cortices, including the entorhinal areas, while loss of longer-term memory required puromycin additionally in a substantially greater part of the cortex and possibly in the thalamus also.

How long does it require for this modification of the locus of the effective memory trace? As shown in Table 2, bilateral temporal injections consistently destroyed memory 2 days after training but were consistently without effect 6 days after training. Results were variable at 3, 4, and 5 days. It consequently appears that the enlarged locus of longer-term memory in the type of learning experience we have used with the mouse becomes completely effective in from 3 to 6 days, depending upon the individual animal.

We proceeded from these observations to experiments in which the animal received reversal learning 3 weeks after its first training in the Y-maze, that is, the mouse was first trained, for example, to move from the stem of the Y into its left arm; then 3 weeks later was retrained to move from the stem of the Y into its right arm. Was it possible to destroy memory of reversal learning 24 hours after reversal training, spare the longer-term memory of the initial training experience given 3 weeks earlier, and in consequence have the mouse perform the task for which it was first trained?

To test this possibility bilateral temporal injections were made 24 hours after reversal learning and 3 weeks after initial learning in seven animals. Shock

Table 3. Differential effect of bilateral temporal injections of puromycin on recent and longer-term memory. Each injection had a volume of 0.012 ml and contained 0.06 or 0.09 (experiment 71) mg of puromycin. Choices of the arm of the Y-maze by an animal after injection were scored as "1" if consistent with initial learning, and as "2" if consistent with reversal learning. For various reasons trials were continued irregularly beyond the ten originally planned.

Expt. No.	Animal No.	Initial learning: trials to 9/10 criterion	Reversal learning 3 weeks later: trials to 9/10 criterion	Choice of arm of Y-maze*
			Experimental animals	
86	26A	13	22	1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1
86	24A	7	10	1,1,2,1,1,1,1,2,1,1,1,1,1,1,1,1,1,1,1,1,1,2,1,1,1
86	25A	8	10	1,1,1,1,2,1,1,1,1,2,1,2,2,1,2
86	22A	9	8	1,2,2,1,1,2,1,2,1,2,1,1,1,1,1,1,1,1,1,1,1,1
86	23A	13	4	1,1,1,1,1,1,1,1,1,1,1,1
71	49	22	9	1,1,2,2,1,1,1,2,1,1,1,1,2,1,1,1,1,1,1,1,1,1,1,1
86	27A	12	5	1,1,1,1,1,1,1,1,1,2,1
			Control animals	
86	58A	10	14	2,2,2,2,2,2,2,2,2,2,2,2,2,2,2
86	60A	10	12	2,2,2,2,2,2,2,2,2,2,2,2

* The experimental animals made their choices 3 days after temporal injections of puromycin, which were given 24 hours after reversal learning. The control animals made their choices 4 days after reversal learning, no puromycin being injected. Neither group received shock.

was omitted in the retention trials 3 days after puromycin injection, since there was, within the design of the test, no right or wrong choice. As shown in Table 3, on testing for memory the first choice of all animals was consistent with the first learning experience, as were the large majority of subsequent choices. In view of consistent results with numerous untreated animals on various schedules of learning and reversal learning, only two control animals (Table 3) were used in this series. All these untreated animals, in sharp contrast to the experimental group, made choices consistent with their second, or reversal, learning. Because the experimental animals were able to perform the older position habit efficiently and consistently, this experiment offers strong evidence that the effect of puromycin in destroying a recent habit is not due to disorganization or incapacitation of the animal.

A beginning has been made in testing for the specificity and reversibility of the puromycin effect. Numerous control injections of saline, of subliminal concentrations of puromycin, and of puromycin hydrolyzed at the glycosidic bond were without effect on memory. Most animals treated with effective doses of puromycin were demonstrated to be capable of relearning after loss of memory, though the process of relearning, particularly with high doses of puromycin, often required considerably more trials than in the initial training experience. This aspect of the effects of puromycin will be reported more extensively at a later time.

Although the effective locus of short-term memory clearly appears different from that of longer-term memory we cannot now define the difference with precision. It does appear that the area around the caudal rhinal fissure, likely entorhinal cortex, carries the short-term memory trace, since short-term memory was retained with ventricular injections but lost with temporal injections. The part played by the hippocampus will not become evident until experiments are performed which provide for exposure of the entire temporal cortex to puromycin while the hippocampus is spared. Similarly, we cannot state whether the locus of longer-term memory is confined to the cortex or whether other parts of the brain, principally the hippocampus, are also involved. It can only be said that our observations are consistent with the evidence and conclusions of others (*4*), that the hippocampal zone is the site of recent memory and, that an extensive part of the neocortex is concerned with longer-term memory.

It must be emphasized that our results, although apparently clear-cut in important particulars, should be interpreted at this time with caution. We are in the process of obtaining more precise information, for example, on the localization of puromycin after intracerebral injection. Histological studies on the cells of the hippocampus and cortex must be completed. Determinations must be made of the degree of suppression of protein synthesis, and, particularly in view of the negative behavioral results with subcutaneous puromycin (*3*), the possibility must be kept in mind that loss of memory after intracerebral injection of puromycin may be owing to effects not related to changes in protein synthesis. Further, it remains to be shown that other learning situations, currently being investigated, and other animals are comparable to the mouse in the training experience we have used (*5*).

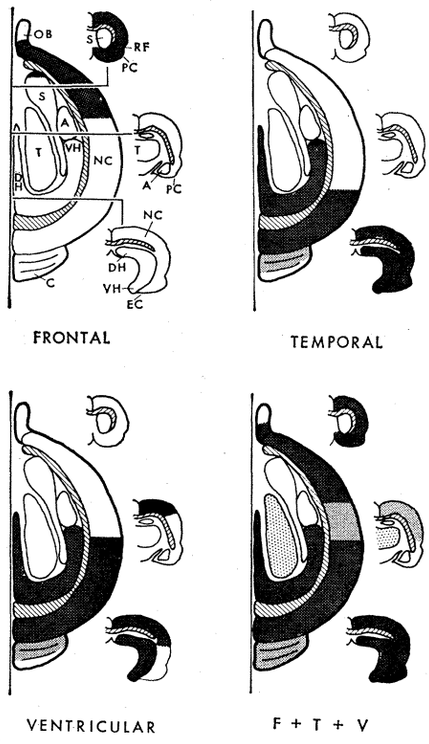

Fig. 1. Spread of fluorescein after intracerebral injection. The diagrams at the left indicate structures viewed from the top after removal of a horizontal section of the hemisphere; at the right, cross (frontal) sections of the hemispheres at the level indicated in the diagram for frontal injections. Relative intensity of staining is indicated by relative density of stippling. With all injections there was intense staining, not shown in the diagram, of the corpus callosum (cross hatched). Abbreviations: *A*, amygdaloid nucleus; *C*, cerebellum; *DH*, dorsal hippocampus; *EC*, entorhinal cortex; *NC*, neocortex; *OB*, olfactory bulb; *PC*, pyriform cortex; *RF*, rhinal fissure; *S*, corpus striatum; *T*, thalamus; *VH*, ventral hippocampus; *F* + *T* + *V*, frontal + temporal + ventricular injections.

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Fragment Sizes Produced from T5 Bacteriophage DNA Molecules by Acid Deoxyribonuclease

Abstract. *The action of acid deoxyribonuclease on T5 bacteriophage DNA results in a "random distribution" of sizes of duplex fragments as judged by electron microscopy. No preferred subunit size resulting from the "single hit" action can be detected down to lengths of 0.1 micron or less. The results are in agreement with the coexistence of a "single-hit" and a "double-hit" kinetics.*

An investigation (*1*) on the digestion of deoxyribonucleic acid (DNA) by acid deoxyribonuclease has shown that two different types of degradation take place at the same time, (i) a "single-hit" degradation causing the simultaneous breakage of both DNA strands at the same place, and (ii) a "double-hit" degradation of the type already known for pancreatic deoxyribonuclease (*2*). This second mechanism becomes effective in breaking DNA molecules only after a lag time. Results presented (*1*) suggest that in the case of DNA from chicken erythrocytes the "single-hit" degradation stops after a molecular weight of the order of 0.5×10^6 has been attained. This observation raises the possibility that the "single-hit" degradation is breaking the molecule at spe-