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Effect of Ribonucleic Acid on Pole Climbing Performance in Rats

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EFFECT OF RIBONUCLEIC ACID ON POLE CLIMBING PERFORMANCE IN RATS

by

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ABSTRACT

This study investigated the effect of daily intraperitoneal injections of yeast-RNA on the behavior of young and old rats in the acquisition of two tasks. The rats were initially trained to escape shock by climbing a vertical pole and were later trained to avoid the shock. The performance of the RNA injected rats did not differ from that of normal controls or from rats similarly treated with physiological saline in the number of trials or time to achieve learning criterion. The old rats who were treated with RNA showed a significant loss of weight. The results of this experiment suggest that learning or memory processes in the rat are not aided by chronic yeast-RNA treatments.

CHAPTER 1

INTRODUCTION

An important problem in physiological psychology is that of determining the nature of the physical-chemical changes underlying the acquisition and storage of information in a living organism. There seems to be general agreement that learning is the result of changes in the activity of the nervous system; however, the investigations of these changes have used different approaches.

One popular method of studying learning is to search for the structures and circuits in the brain which are involved in the acquisition and retention of individual experiences. Many experimenters claim to have found evidence in support of the idea that memory functions are mediated by specific brain structures. The strategies used for the identification of the neural structures and circuits have included the removal of certain structures and systematic severing of circuits to prevent the consolidation of memory traces. Another approach is to record changes in electrical activity in various parts of the brain during and after learning in an attempt to localize the areas involved in the formation of memory traces.

Hypotheses have also been derived asserting that the learning processes rely on synaptic connections between neural elements. As a result of environmental stimulation, new connec-

tions may be formed between motor and sensory components of the nervous system. Previously separated neurons are brought into contact with one another through the growth of new fibers and synapses. Thus, learning may be viewed as the formation of synaptic connections between afferent and efferent elements to establish new circuits.

Another approach for investigating the learning process is to study the physical or chemical changes that take place within an individual neuron. The neurological changes that occur during the recording and storage of information may be due to some molecular modification in the neurons themselves. This molecular modification could be the mechanism responsible for coding the experiences of the organism. Possibilities exist that the information could be encoded directly in the molecular structure of the neuron or that the new synaptic growths are a result of these structural modifications in the biochemical makeup of the individual neurons in the brain. Recent advances in biochemistry have suggested that several biochemical mechanisms may underlie the acquisition or storage of memory traces.

There has been much speculation as to what takes place at the molecular level in the central nervous system when an organism experiences something. The macromolecules which have been of chief concern to investigators of behavior are the lipids, proteins, and the nucleic acids.

One of the first hypotheses to account for learning and

memory as a biochemical mechanism was put forth by Katz and Halstead (1950). According to their theory, during learning events, a structural change occurs in the neurons of the brain resulting in a specific geometric patterning of protein molecules. Most likely, it is the nucleoproteins which are involved in the restructuring and they act as templates for subsequent protein synthesis. Although the protein templates can be formed in various parts of the neuron, including the synapse, they ultimately become part of the cell membrane. This specific molecular configuration is thought to affect other neurons by either diffusing across the synapse or as a result of some orienting force. Thus, one neuron would be capable of conducting impulses to adjacent neurons possessing similar membrane protein configurations.

Most of the recent biochemical research has been directed toward the role of RNA molecules in the acquisition and storage of memory traces. For example, Hyden (1965) believes that memory is the result of a change in the structure of RNA molecules. The incoming neural impulse is thought to produce a shift in the adenine to uracil ratio of the RNA molecule. This restructured molecule then serves as a template for proteins which will dissociate in response to the same type of stimulation which originally formed the specific RNA. The dissociation of the protein causes the release of a substance across the synapse. Hyden and Egyhazi (1963) stated that it is possible that

the glia and the glial RNA constitute the substrate for a short-term memory since the folded membranes of the glia would be well suited for very rapid processes.

Hyden states in a later work (1967) that the specific protein coded by the RNA may be stored in a more permanent form in the neuron cell membrane and this protein formation "would lead to an increased differentiation, a modulation of the protein pattern of the cells". This pattern would then determine whether the cell would respond to a particular pattern of stimulation and thus cause the next neuron in the chain to be excited.

Landauer (1964) proposes two hypotheses involving RNA in learning. The first suggests that when a neuron is excited, the cell membrane becomes more permeable allowing RNA to enter from surrounding glial cells. This glial RNA modifies the neuron so that it becomes more sensitive to the events going on in the brain at the time the RNA was transferred. The second hypothesis is that the information is coded in frequency characteristics of "spreading ac potentials to which a neural membrane can become tuned by alteration of its protein structure". The tuning of cell membranes is, therefore, dependent on their modified composition due to migration of RNA molecules from the glia into the neuron.

Gaito (1961, 1963, 1964, 1966, 1968) agrees somewhat with Hyden but goes on to suggest that changes in the DNA molecule may provide a more stable basis for memory storage. Gaito (1967)

also postulates that memory may be coded in the arrangement of the amino acids of certain protein molecules. Finally, Dingman and Sporn (1964) feel that more attention should be given to the possible role of the lipids of the nervous system in memory mechanisms.

This thesis will focus on the role of RNA in learning and memory. The above theories involving RNA in the memory process draw upon two main types of evidence. Researchers have studied the effects of behavior on the subsequent structure of RNA molecules and have also reversed the method of attack, that is, they have observed the effects of RNA molecules on behavior. Hyden (1963, 1965, 1967) has carried out much of the research which has examined the RNA content of the brain following a learning experience. For example, Hyden (1963) reports that there was an increase in the amount of RNA per nerve cell in rats who were required to learn to balance on a thin wire. The adenine to uracil ratio also increased significantly and similar changes were found in the glial cells.

The second line of evidence is of chief concern for this thesis. These studies investigated the behavioral effects following administration of RNA to animals and humans. The RNA most frequently used is derived from yeast. Cameron (1961) and Cameron et al. (1966) reported that oral administration of RNA improves the memory of aged patients suffering from brain arteriosclerosis and senile dementia. Withdrawal of RNA treat-

ment caused relapses in ¼ of the cases, but these were reversed by reinstitution of the treatment.

Even though young rats seem to have an adequate supply of ribonucleic acid (Adams, 1966), Cook et al. (1963), using relatively young rats (less than 90 days old), found that long term intraperitoneal injections of yeast RNA facilitate learning of pole climbing in response to shock. Corson and Enesco (1966) and Wagner et al. (1966) have recently obtained the same results on the pole climbing task but report negative findings on other tasks such as visual discriminations motivated by shock and food reward. Brown found no difference in the number of sessions it took RNA-injected rats and controls to achieve asymptotic behavior in pressing a lever for food reinforcement. There was, however, a higher response rate exibited by the RNA animals during acquisition and extinction of the barpress response. Using a Y-maze, Cohen and Barondes (1965) found no difference between RNA and saline-injected mice in the rate of learning a black-white discrimination to avoid shock. On the basis of these experiments, RNA seems to improve learning of a pole climbing response to shock while having no effect on some other tasks.

In this experiment, an attempt was made to examine the effect of RNA injections on rats in the pole climbing situation. A similar basic design to that of Cook et al. (1963) was used. They tested two groups of rats, RNA-injected and saline-injected

controls on their rate of escaping from shock by climbing a pole suspended from the center of a shock box. Their results indicated that the RNA animals learned to escape the shock in a fewer number of trials than did the saline control animals. A lower rate of weight gain by the RNA animals was also reported. As a further investigation of Cook's results, an additional control group receiving no injections was introduced in this study. This group was intended to serve as a comparison for the injected groups in both learning ability and weight gain tendencies.

In addition to the three groups of young rats receiving RNA, saline, or no injections, three groups of older rats treated identically were also included. According to Hyden (1967), brain RNA content in man fails to increase after the age of 40 and declines rapidly after age 60. Since Cameron's patients (1961) were above 60 years of age, it is possible to attribute his results to a replenishing of the deteriorating supply of RNA in these patients. Adams (1966) found a difference in the rate of RNA synthesis between young and adult rats suggesting a decline in rat-brain RNA after 6 months. Hyden (1967) also reports that, in old rats, the brain RNA content has decreased significantly and that during maturation there is a significant change in the RNA base ratios. On the basis of this evidence, it may be hypothesized that old rats would benefit from an increased supply of RNA. Thus, in a learning

situation, old rats may show improved performance following yeast-RNA injections.

To investigate further the type of task used in obtaining the results of Cook et al. (1963), an additional similar task was included in this study. After learning to escape the electric shock by climbing the pole, as in Cook et al. (1963), the rats were then required to avoid the shock during a neutral five-second light CS. This was thought to be a more complex response to acquire than the mere escape from shock. Thus, another task, yet similar, was provided with which to evaluate the seemingly task-specific results obtained by Cook et al. (1963), Corson and Enesco (1966), and Wagner et al. (1966).

CHAPTER 2

METHOD

Subjects and design

A 2x3 factorial design was used with the variables age (old or young) and treatment (RNA, saline, or non-injected). The subjects were 78 male Holtzman albino rats comprising the two age groups. Thirty-nine Ss weighed 150-160g (32-34 days old) at the start of experimentation while the remaining 39 Ss were older rats (between 6 and 16 months old) weighing 438-559g. Groups of 8 young rats or 6 old rats were housed in community cages measuring 26" by 9½" by 7½". Food and water were available at all times.

Members from each age group were randomly divided into one of three treatment groups. One of the groups received daily intraperitoneal injections of 160 mg/kg yeast-RNA in 10% aqueous solution. A second group received equal volumes of physiological saline injections and the third group consisted of non-injected controls. Due to severe respiratory infections it was necessary to discard 12 Ss prior to the start of testing. The final number of animals in each group was as follows:

	Non-injected	RNA	Saline
Young	12	13	12
Old	10	10	10

Apparatus

The testing chamber consisted of an electrified grid floor in a box measuring 17" by 18" by 16". The four side walls of the box were constructed of brown masonite and were covered by a sheet of transparent plexiglass. A 5/8" diameter wooden pole was suspended from the top center of the box through the bars on the floor. The bars were $\frac{1}{4}$" in diameter and spaced 3/4" apart and carried a shock intensity of 0.5 ma.

Testing was carried out in a dimly lit room producing an illumination inside the testing box of 0.15 units on a standard Sekonic light meter. During avoidance trials, a light stimulus was presented from the top of the box through a translucent sheet of plexiglass. The source of the light was a 25 watt bulb with a resultant illumination of 8.00 units on the Sekonic light meter.

During all testing, a tape recorder emitted continuous "random noise" in order to mask any extraneous sounds that might have interfered with testing. The "noise" from the recorder registered a 55 decibel reading inside the testing box as compared to less than 40 db in the "quiet" room.

The experimenter observed the Ss through a 2" by 6" plexiglass-covered slit centrally located at the base of one wall.

Procedure

The rats were injected daily with either the RNA solution or physiological saline. The normal control animals were simi-

larly handled but received no injections. Injections were started 14 days prior to testing and were continued daily at 5 p.m. until termination of experimentation. On each day of experimentation, the animals were tested prior to receiving injections.

Each animal received 1.60 ml/kg of body weight of solution with each intraperitoneal injection. For the RNA-injected rats, this amounted to approximately 160 mg/kg/day of yeast-derived RNA tetranucleotides. The tetranucleotide material was purchased in powder form from Pabst Laboratories under the name of "Ribonucleic Acid, RNA, 'Yeast Nucleic Acid'" (Cat. No. 3700). It appears to be an alkaline hydrolysate of yeast-RNA and may be characterized as relatively short chain tetranucleotides composed of the bases adenine, guanine, uracil and cytocine in random chemical combination (personal communication, Davidson). This material was used without further purification.

Following the procedure of the Cook et al. (1963) study, solutions of this material were prepared by suspending a weighed quantity of the powdered material in a volume of distilled water, slightly less than the required volume. This suspension was put into solution by bringing it to a ph of 6.5 to 6.7 by the dropwise addition of 1 N NaOH, with continuous stirring, in an ice bath. The resulting solution was then brought to a final concentration of 10% (w/v) by the further addition of distilled water. This solution was then stored frozen and brought to body

temperature before each daily use.

On the 13th and 14th injection days, each rat was placed in a screen-covered one gallon plastic bucket and transported to the testing room. The animal was placed in the testing chamber and allowed to freely explore the testing apparatus. These habituation sessions lasted for 15 minutes on each of the two days.

Testing began on the 15th day of injections with each rat receiving 5 escape trials per day. The entire experiment was run blind. The experimenter was not informed as to which group the animals belonged until the termination of the experiment. Each rat was placed in the box and two minutes later received electric shock until he escaped by climbing the pole or until 30 seconds of continuous shock had elapsed. Before a rat was considered to have climbed the pole all four feet must have been off the grid floor for a duration of 2 seconds. When the rat came down from the pole, he was placed in a corner of the box to await the next trial. On the last four trials the S was placed in a different corner of the box and 45 seconds later the current was again turned on. Testing continued until each animal was consistently escaping the electric shock. Each rat was tested daily until he climbed the pole within 5 seconds after the onset of shock on each of the 5 trials on a single day.

On the day following the completion of the escape training,

each animal began avoidance training. When the rat had been in the chamber for two minutes, the overhead light was turned on followed after 5 seconds by the electric current. The trial ended when the animal climbed the pole. If the rat climbed the pole within the 5 second interval between the onset of light and the onset of shock, he successfully avoided and therefore received no shock. Twelve avoidance trials per day were given with a variable intertrial interval ranging from 10 to 30 seconds with a mean of 20 seconds. This interval began when the rat returned at least his two hind feet to the grid floor and remained on the bars for the duration of the interval. In any instance that the animal climbed up the pole during this interval, the interval was repeated when he returned to the floor. The daily avoidance conditioning continued until each rat successfully avoided the shock on 16 of any 20 consecutive trials.

CHAPTER 3

RESULTS

Escape training

Four measures were used to determine the rate of acquisition of pole climbing in response to the shock; (a) the total time in seconds, summed across trials, that it took each S to reach criterion (total time to criterion); (b) the time in seconds before each S responded by climbing the pole for the first time (time to first response); (c) the number of trials, divided into blocks of five, for each S to reach learning criterion (number of days to criterion); (d) the number of trials before which each S responded correctly for the first time (number of trials to first response).

The means and standard deviations for the total time in seconds to learning criterion for the 6 groups of Ss are given in Table 1, and the results of the analysis of variance are presented in Table 2. There were no significant differences among the groups in the time required to consistently escape the shock.

Edwards (1966) suggests making reciprocal transformations of scores obtained in experiments using time units as a measure of performance. This will tend to stabilize the variance and decrease the skewness of such measures. Table 3 shows the means and standard deviations for the reciprocals of the total

TABLE 1

MEANS AND STANDARD DEVIATIONS FOR TOTAL TIME TO CRITERION

		Non-injected	RNA	Saline
Young	M	93.6	111.5	129.4
	SD	38.2	54.7	77.4
Old	M	71.1	85.0	84.2
	SD	33.7	24.7	25.6

TABLE 2

ANALYSIS OF VARIANCE FOR TOTAL TIME TO CRITERION

Source	SS	df	MS	F
Age	5287.50	1	5287.50	2.12
Treatments	6798.54	2	3399.27	1.36
Age X Treatments	1621.00	2	810.50	0.32
Error	152323.57	61	2497.10	

TABLE 3

MEANS AND STANDARD DEVIATIONS FOR RECIPROCAL TRANSFORMATIONS OF TOTAL TIME TO CRITERION

		Non-injected	RNA	Saline
Young	M	.01321	.01144	.01023
	SD	.0066	.0060	.0048
Old	M	.01597	.01449	.01321
	SD	.0056	.0084	.0047

time to criterion scores. Even with this transformation no significant differences were found (see Table 4).

The means and standard deviations of the time it took the Ss to make their first pole climbing response are contained in Table 5. Table 6 indicates the results of the analysis of variance for these data. Table 7 shows the means and standard deviations of the transformed scores, and the results of the analysis of variance are presented in Table 8. No significant differences among the groups were obtained in the time taken to make the first response when either the direct time-measure or the reciprocals were used.

The final two measures of escape performance deal with the number of trials it took each subject to reach criterion and the number of trials before each S responded for the first time. As mentioned earlier, a rat reached the criterion for escape learning when he climbed the pole within 5 seconds of the onset of shock on each of the five trials on a single day. Therefore all criterion scores must be in intervals of 5 trials; that is, a rat might reach criterion after 5, 10, 15,...trials. These trials were reduced by 1/5 for analysis in terms of days to reach criterion and the means and standard deviations are presented in Table 9. The analysis of variance on this measure indicates that there are no significant differences among the groups (see Table 10).

Table 11 gives the means and standard deviations for the

TABLE 4

ANALYSIS OF VARIANCE FOR RECIPROCAL TRANSFORMATIONS OF TOTAL TIME TO CRITERION

Source	SS	df	MS	F
Age	.000143	1	.000143	3.47
Treatments	.000099	2	.000050	1.21
Age X Treatments	0	2		
Error	.002514	61	.0000412	

TABLE 5

MEANS AND STANDARD DEVIATIONS FOR TIME TO FIRST RESPONSE

		Non-injected	RNA	Saline
Young	M	26.3	32.7	45.7
	SD	17.2	29.6	12.2
Old	M	22.3	29.0	33.4
	SD	13.7	14.8	19.5

TABLE 6

ANALYSIS OF VARIANCE FOR TIME TO FIRST RESPONSE

Source	SS	df	MS	F
Age	736.04	1	736.04	1.10
Treatments	2584.57	2	1292.29	1.93
Age X Treatments	262.97	2	131.49	0.20
Error	40770.61	61	668.37	

TABLE 7

MEANS AND STANDARD DEVIATIONS FOR RECIPROCAL TRANSFORMATIONS OF TIME TO FIRST RESPONSE

		Non-injected	RNA	Saline
Young	M	.05306	.04643	.03918
	SD	.028	.025	.028
Old	M	.04866	.05926	.04032
	SD	.029	.035	.023

TABLE 8

ANALYSIS OF VARIANCE FOR RECIPROCAL TRANSFORMATIONS OF TIME TO FIRST RESPONSE

Source	SS	df	MS	F
Age	.0001104	1	.0001104	0.13
Treatments	.0029800	2	.0014900	1.72
Age X Treatments	.0001104	2	.0000552	0.06
Error	.0529000	61	.0008672	

TABLE 9

MEANS AND STANDARD DEVIATIONS FOR NUMBER OF DAYS TO CRITERION

		Non-injected	RNA	Saline
Young	M	2.92	3.00	3.16
	SD	0.86	0.68	1.07
Old	M	2.50	2.80	2.50
	SD	0.50	0.60	0.50

TABLE 10

ANALYSIS OF VARIANCE FOR NUMBER OF DAYS TO CRITERION

Source	SS	df	MS	F
Age	.3014	1	.3014	0.49
Treatments	.4085	2	.2042	0.33
Age X Treatments	.5851	2	.2925	0.47
Error	37.8500	61	.6205	

TABLE 11

MEANS AND STANDARD DEVIATIONS FOR NUMBER OF TRIALS TO FIRST RESPONSE

		Non-injected	RNA	Saline
Young	M	0.42	0.69	1.00
	SD	0.64	1.06	1.29
Old	M	0.20	0.70	0.60
	SD	0.40	0.71	0.80

number of trials it took before the first correct response was made. The results of the analysis of variance for these data are presented in Table 12. There were no significant differences among the groups on this measure.

Avoidance training

The measure used in determining the rate of acquisition on the avoidance task was the number of trials it took each S to avoid the shock on 16 of any 20 consecutive trials. This proved to be a very strict criterion and many of the Ss showed no indication of learning after as many as 250 trials. For this reason, the level of avoidance performance was analyzed in terms of whether an animal performed above or below the median performance of the group as a whole. In other words, an animal was considered as having learned to avoid the shock if his criterion score fell below the median score of all the animals.

Table 13 shows the number of Ss in each group who scored above and below the median on the avoidance task. A median test was performed on these data and the results indicate no significant differences amomg the groups ($\chi^2 = 2.03$, df = 5, $p > .05$).

Weight changes

Each S was weighed on the first and twentieth day of injections. Table 14 indicates the mean weight gain in grams for the young group of Ss. The results of the analysis of variance

TABLE 12

ANALYSIS OF VARIANCE FOR NUMBER OF TRIALS TO FIRST RESPONSE

Source	SS	df	MS	F
Age	0.6845	1	0.6845	0.81
Treatments	2.9388	2	1.4694	1.73
Age X Treatments	0.4670	2	0.2335	0.28
Error	51.7900	61	0.8490	

TABLE 13

NUMBER OF RATS IN EACH GROUP WHO LEARNED OR DID NOT LEARN THE AVOIDANCE TASK

		Non-injected	RNA	Saline
Young	Learned	6	8	4
	Did not learn	6	5	8
Old	Learned	5	5	5
	Did not learn	5	5	5

TABLE 14

MEAN WEIGHT GAINS OF YOUNG RATS
(IN GRAMS)

	1st Day	20th Day	Gain
Non-injected	154.4	266.2	111.8
RNA	155.8	261.1	105.3
Saline	157.0	271.3	114.3

shows no significant differential weight gain among the three groups (see Table 15). The mean weight change for the old group of rats are presented in Table 16. The Table shows that the RNA rats lost much more weight than either of the other two groups. The results of the analysis of variance in Table 17 shows that the difference is significant ($F=30.51$, $df=2,27$, $p<.001$).

TABLE 15

ANALYSIS OF VARIANCE FOR WEIGHT GAINS OF YOUNG RATS

Source	SS	df	MS	F
Treatments	512.99	2	256.495	0.95
Error	8112.01	30	270.400	

TABLE 16

MEAN WEIGHT LOSSES OF OLD RATS
(IN GRAMS)

	1st Day	20th Day	Loss
Non-injected	495.4	493.6	1.8
RNA	519.3	471.5	47.8
Saline	511.7	508.7	3.0

TABLE 17

ANALYSIS OF VARIANCE FOR WEIGHT LOSSES OF OLD RATS

Source	SS	df	MS	F
Treatments	13748.27	2	6874.135	30.51*
Error	6083.20	27	225.304	

* Significant beyond .001 level.

CHAPTER 4

DISCUSSION

There were no significant differences in the rate of learning the escape task either between age groups or among the three treatments. These results contradict the findings of Cook et al. (1963), Corson and Enesco (1966), and Wagner et al. (1966). These three studies used only two treatment groups, RNA and saline, in finding that RNA injections enhanced pole climbing performance in response to shock. In the present study, the additional non-injected control group served as a standard as to whether the RNA rats actually do learn faster or that the saline rats learn slower than normal.

In examining Tables 1, 5, 9, & 11, one can see a difference in the performance of the RNA and saline groups. There seems to be a trend toward increased learning in the young RNA group. In the young group, the RNA animals did yield better learning scores than the saline animals, while the older saline-injected animals performed better on three of the four measures used in evaluating escape learning. However, for both age groups on all four criterion measures, the non-injected controls showed greater performance than either of the injected groups. Thus, the suggestion that RNA-injected animals learn faster than saline-injected animals is meaningless when it can be shown that normal animals perform better than either of

these groups. The results of the three studies mentioned above cannot be interpreted to mean that yeast-RNA injections enhance acquisition of the pole climbing response. The data of this study tend to indicate that normal animals show the greatest learning ability and that yeast-RNA injections, instead of improving learning and memory, may even have an adverse effect on pole climbing performance.

For the more difficult learning task, the results of the median test for the data in Table 13 indicate that there were no significant differences in the performance of the six groups of rats on avoidance acquisition. If the experimental treatments had no effect on learning, each of the six groups should have an equal number of Ss which learned and did not learn the task. This was true for the three groups of old rats. There was an equal 5-5 split in the learned-not learned categories suggesting that yeast-RNA injections do not improve learning ability on this type of avoidance task. For the young rats, however, there was a difference in the number of Ss within each group which learned the avoidance task. Eight RNA-injected rats learned the task and 5 failed while only 4 saline-injected rats learned and 8 failed. There seems to be a trend showing that more young RNA-injected animals learned the avoidance task, but a larger number of subjects would have to be tested before any conclusions could be made about RNA enhancing performance in the avoidance situation.

The results did not support the hypothesis that old rats would benefit from yeast-RNA injections in a learning situation. It is possible that the age range of the rats in this study (6 to 16 months) was too wide to constitute a uniform group. It was expected that old rats would not perform as well as young rats. However, the equivalent performance shown by the old rats could have been caused by a greater learning ability on the part of the younger of the old rats. As mentioned earlier there is a decline in rat-brain RNA after 6 months of age. Perhaps by using more older rats, the group performance would not be so high, and an improvement would be found following yeast-RNA injections.

An alternative explanation for the lack of improvement in the old RNA rats is based on the large weight losses incurred in the course of the experiment. The old saline and non-injected rats maintained a stable weight while the RNA rats lost an average of 47.8g (see Table 16). This substantial weight loss may have had detrimental effects which overshadowed any beneficial effects from the yeast-RNA injections.

Research in this area should continue, perhaps, in the direction of the types of learning tasks and reinforcement employed. The pole climbing situation may not be the proper test for determining the degree of acquisition and memory. At present, the pole climbing response motivated by shock is the only response that has been improved by yeast-RNA injections.

It would be of no importance if RNA had a unique effect on pole climbing ability.

Another limitation of this study is the type of RNA that was used. Eist and Seal (1965) found that yeast C^{14} tagged RNA does not pass the rabbit blood-brain barrier or blood-cerebrospinal fluid barrier. If this is true for the rat, then yeast-RNA would not affect the central nervous system. An explanation would have to be made for the learning improvement apart from any central biochemical storage mechanism. However, Sved (1965) reports a slight increase in protein synthesis in the mouse-brain following large amounts of yeast C^{14} tagged RNA injections. Perhaps a more positive effect could be found if species-specific RNA was used instead of yeast-RNA in investigating the connection of RNA with memory storage and learning.

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APPROVAL SHEET

The thesis submitted by Thomas J. Hinkel has been read and approved by two members of the Department of Psychology.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Arts.

Aug. 1, 1969
Date

Richard a Maier
Signature of Adviser