Cortical application of ribonuclease and amnesia for a visual discrimination

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After reaching a 9/10 criterion in a black-white T maze or after overtraining on the same task a solution of RNAase or an appropriate control solution was applied to the posterior cortex of rats. Testing after application of the enzyme revealed amnesia for the task for an average of 5.0 days. Control groups returned to criterion performance in 2.2 days on the average. These data are unlike those obtained either with functional ablation or with trauma-induced amnesia.

Hyden (1959) suggested that the RNA molecule might be capable of modification by cellular activity and thus have a possible role in memory storage. Many attempts to obtain such positive effects as enhanced acquisition and/or retention with injections of trained or naive RNA have generated data which are difficult to replicate or interpret (Jacobson et al, 1965; Byrne et al, 1966; Cameron & Solyom, 1961). More consistent results have been produced by attempts to interfere with the hypothesized RNA store.

Corning & John (1961) reported lack of retention of a light conditioned contraction in planaria regenerated in RNAase while control groups regenerated in pond water showed a significant saving.

Mei Chen-tung (1964) presented evidence that RNAase applied through cortical ducts to pigeon hyperstriatum resulted in loss of a conditioned response in pigeons beginning two days after application and lasting approximately 11 days. This loss was reportedly paralleled by a reduction in RNA in striatal neurons. The neuronal RNA returned to normal levels somewhat before the reappearance of the learned response.

Krylov, Danylova, & Tongur (1965) reported the loss of a conditioned reflex in mice on the day following temporal RNAase injections, injections of DNAase, serum albumin and trypsin had no effect.

The present study extends and partially replicates these findings in the albino rat.

METHOD

Forty-nine male Sprague Dawley rats, 110-130 days old at the time of training served as Ss. Ss were gentled for 5 min/day for four days prior to training. Twenty-three hour food deprivation was instituted two days prior to training, Ss being allowed access to a wet mash made up of finely ground Purina Labena and water for 60 min each day. Ss were run in groupings of 11-20 with both experimental and control Ss included in each grouping.

A T maze with a main alley 24 x 5½ x 6½ in. and one black and one white goal arm each 12 x 5½ x 6½ in. was used. All Ss were trained to enter the white arm, normally the non-preferred arm for albino rats. Reinforcement was 30 sec access to wet mash in the white arm. The intertrial interval was approximately 5 min. An error was recorded whenever half of S's body entered the black arm. Latencies were recorded both pre- and postoperatively. A correction procedure was used for running preoperatively. A modified Gellerman schedule determined the position of the two goal arms on each trial. Ten trials were given each day until S reached a criterion of 9/10 across days. Ten of the rats then received five days of overtraining at 10/day.

Four to 10 h after reaching criterion or after completing the overtraining schedule S was given Nembutal anesthesia (52 mg/kg). The scalp was opened and two trephine holes approximately 3 mm in diameter were made over the posterior portion of the hemispheres (see Fig. 1). The dura was slit and retracted to the edge of the openings, care being taken to minimize damage to the underlying cortex. Pads of reabsorbable Surgicel (Johnson & Johnson) approximately 3 mm in diameter were placed over each opening and soaked in .025 ml of active RNAase (10 mg/ml distilled water, Worthington pancreatic RNAase crystalized 3x), isotonic saline, or heat treated RNAase (raised to ph 12 and boiled 1 min). Fifteen of the rats trained to a 9/10 criterion received topical application of RNAase as did the 10 overtrained rats. Control solutions were used on the remaining 24 criterion trained rats. Eight received heat treated RNAase and 16 received isotonic saline. The scalp was sutured, .1 cc of Combiotic was injected intramuscularly and the animal was returned to its home cage. (An attempt was made to equate trials to criterion for experimental and control groups.)

All Ss were operated by CWH and tested in a blind fashion by RJK.

Testing was begun the following day, approximately 12-20 h after operation. Since we were interested primarily in an estimate of retention we attempted to minimize the Ss' opportunities to relearn the T maze during postoperative testing. Other data in our laboratory had suggested that Ss who have reached a 9/10 criterion on this task rarely make more than one mistake on subsequent trial blocks of 10 for at least 60 trials past criterion. Using this earlier data as a guide we decided to allow each S to run until he made two errors, an index of nonretention, or until he ran nine out of 10 correct trials on any given day. This index of nonretention may appear rather strict, but almost all Ss making two errors would do so in the first five trials and typically in the first three.

The postoperative procedure then went as follows: An S making two incorrect choices on the first two trials postoperatively would not be given any further trials until the next day and, again, would only be allowed to run until two errors were made or until nine out of 10 choices were made correctly. This procedure kept the number of experiences in the T maze postoperatively to a minimum while permitting an estimate of retention.

The first group of Ss (N = 11) to be run were given two additional days of testing after running 9/10 trials correctly. Their performance was so consistently good that this extra testing was eliminated for later groups. The noncorrection procedure was used during testing to further minimize any retraining effect but reinforcement was available in the T maze.

Postoperatively one control and one experimental S were discarded due to illness. Two additional Ss were discarded after histologies had been performed, due to the presence of large unilateral cortical lesions. All other Ss had gliosis in the upper cortical layers and limited superficial damage in the area of Surgicel application. No difference between groups in extent or nature of superficial cortical damage was seen.

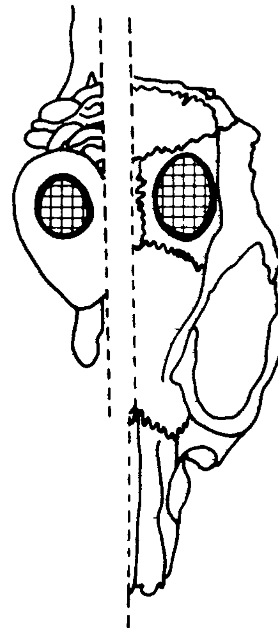

Fig. 1. The skull opening (r) and the cortical area (l) involved in topical application of experimental and control solutions.

Table 1
Mean and SD of Trials to Achieve the 9/10 Criterion Preoperatively and Mean and SD of Trials and Days to Again Achieve this Criterion Postoperatively

	Pre-Op		Post-Op			
Group	Trials to Criterion /	SD*	Trials to Criterion /	SD*	Days to Criterion /	SD
Saline Control	30.4	7.1	14.7	6.1	2.23	1.
Heat Treated Control	35.3	14.5	13.6	5.5	2.0	1.2
RNAase	29.7	6.1	22.6	9.4	4.57	2.
Overtraining	30.7	9.6	29.6	17.1	5.6	3.35

**Includes 9/10 correct trials*

RESULTS

Ss averaged 30.8 trials to learn the task to the 9/10 criterion preoperatively. On Trials 20-30 preoperative Ss were running with a latency of 10.8 sec from start to choice (N = 32). Postoperative average latency was 7.48 sec. There was no significant difference in latency between groups indicating retention, at some level, of the learning experience, e.g., habituation of emotional reactions. Consummatory behavior was normal in all groups. It was not possible to differentiate experimental and control groups on running behavior, consummatory behavior, or general appearance. Ss were evaluated in a blind fashion by RJK. Despite this similarity in gross performance the choice behavior of experimental Ss indicated a marked retention deficit (see Table 1). While saline controls ran to a 9/10 criterion postoperatively within 2.2 days on the average, and heat-treated RNAase controls ran to criterion within 2.0 days, criterion-trained rats with active RNAase took an average of 4.6 days to reach criterion and the overtrained rats with active RNAase took an average of 5.6 days to again achieve a 9/10 criterion. Since Ss were stopped after making two errors on any given day of postoperative testing, the number of trials to criterion postoperatively may not accurately reflect the number of trials necessary to re-establish the habit. It is noteworthy, however, that control Ss showed marked savings in the number of trials to again achieve the 9/10 criterion while Ss treated with active RNAase did not exhibit any apparent savings.

An analysis of variance was used to evaluate the differences in number of days required to postoperatively achieve the 9/10 criterion. Since the prediction had been made, based on Mei's (1964) finding and our pilot work that criterion-trained rats given RNAase would show a deficit in the retention of the T-maze task when compared with their control counterparts, a planned comparison was used to test this prediction (Hays, 1963). No prediction was made for Ss receiving overtraining. The predicted difference between RNAase criterion-trained Ss and control Ss was significant beyong the required .025 level. The F test for the remaining variance was also significant beyond the .025 level. A Scheffé contrast between control and overtrained Ss accounted for a major portion of this variance and was significant at the .025 level.

DISCUSSION

As compared with Mei's report the loss of a learned habit with RNAase in the present study had a more rapid onset and in most instances did not last as long. The motoric impairment which interfered with eating behavior in her study was never seen in our Ss. The appearance of a retention loss the following day is consistent, however, with Krylov et al's (1965) findings and the 4-5 day duration of loss was supported by pilot observations in our laboratory on rats similarly treated in an operant conditioning task.

While the degree of impairment was clearly less than in Mei's study the solution was considerably stronger. Conceivably the difference in result may be attributed to differences in species, method of application or extent of buffering. In the present study the active RNAase, the heat treated RNAase, and the isotonic saline were all assayed periodically throughout the experiment to check their effectiveness in breaking down RNA in brain tissue. Fourteen μ sections of brain tissue were soaked for 30min or 60 min in a dilute RNAase solution (1 mg/1 ml distilled water) or in isotonic saline and then stained for RNA with methyl green pyronin. This *in vitro* test demonstrated clearly the singular effectiveness of active RNAase in breaking down RNA. There were no apparent changes in RNA with either control solution. Assays 1 h, 12 h, and 24 h following *in vivo* application of heat treated RNAase to one hemisphere and active RNAase to the other were attempted on three Ss but the differences in RNA content between hemispheres were difficult to ascertain. Mei has published photographs from *in vivo* tests with her Ss which do demonstrate a reduction in RNA following a time course similar to, but briefer than, the time course of behavioral changes.

Dr. Mei suggested that her behavioral results might be seen as the effects of prolonged reversible ablation of the involved tissue. While this cannot be conclusively ruled out, the normal latencies in the T-maze of the RNAase groups were in marked contrast to the long latencies reported for Ss undergoing functional ablation induced by KC1 initiated spreading depression (Freedman & Lash, 1966). Moreover, the unresponsiveness as indexed by reduced exploration, reduced consummatory behavior, and general inactivity observed with animals under bilateral spreading depression (Delprato, 1965; Schneider, 1965), was not evident in the RNAase group. Finally EEG data on four anesthetized Ss from 0-4 h and at 12 h following RNAase application on one hemisphere and heat-treated RNAase application on the other revealed no gross differences in EEG activity between hemispheres (recorded from the areas of application) as is typical with the classical functional ablation techniques.

It might be suggested that the RNAase acted as a general trauma to the system producing retrograde amnesia in a way analogous to that of ECS, anesthesia or spreading depression when administered subsequent to learning. However, the time parameters involved here are quite unlike those involved in the typical disruption of consolidation (McGaugh & Petrinovich, 1965). Nor again does the apparent normalcy of electrical activity support such a notion.

It appears from the present data that some specific effect of active RNAase in this area of the cortex, was the critical factor in the disruption of learned habits.

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