Ribonucleic Acid: Effect on Conditioned Behavior in Rats

Abstract. *Acquisition of a behavioral response motivated by shock was enhanced in rats chronically treated with yeast ribonucleic acid, and resistance to extinction was greater in rats so treated than in controls. This extends the role of ribonucleic acid to include a behavioral effect in laboratory mammals treated with a purified preparation from yeast.*

Ribonucleic acid (RNA) derived from yeast, when chronically administered orally or intravenously, had a "favorable effect in general upon memory retention failure in the aged" particularly in patients with arteriosclerotic brain disease (*1*). Treatment improved retention in a counting test and retention and speed of reconditioning in a conditioned response procedure. In addition, this treatment increased alertness, interest, initiative, and confidence. Reduction of psychogenic confusion and improvement in memory have been reported in patients treated for cerebrovascular disease and confusional disorders (*2*). Tablets containing the ribonucleotides cytidylic acid, adenylic acid, uridylic acid, and guanylic acid were administered orally each day (total 1.05 g) for an average of 25 days.

Certain aspects of the relationship of RNA to behavioral processes in animals have been reported (*3*), but direct evidence that the administration of purified RNA can affect the behavior of animals is lacking. We recognize that our study may not be directly related to the reported clinical findings; however, it was designed to study the interaction of purified RNA with certain basic elements of behavior, that is, acquisition and extinction (*4*) of a conditioned response in rats.

Ribonucleic acid in powdered form (*5*) was administered as a 10 percent aqueous solution adjusted to *p*H 6.5 to 6.7. Tests were negative for pyrogenicity (by a rabbit assay) and for bacterial and mold contamination. In a preliminary investigation, rats were given 160 mg/kg doses of RNA intraperitoneally each day for 1 month; no overt symptoms were noted, and no gross pathological changes were seen in the peritoneal area upon autopsy.

Sprague-Dawley rats (*6*), 150 to 200 g at the start of the experiment, were divided by weight into two groups of eight. One group was injected intraperitoneally each day with a 160-mg/kg dose of RNA, and the other group was similarly injected with normal saline. No significant differences in appearance or general health were apparent between groups at any time throughout either procedure, that is, over a period of more than 10 weeks, except for a slightly lower rate of weight gain in the treated group. After 53 days the rats were trained to perform a conditioned response by an established procedure (*7*). Administration of RNA and saline to each group was continued throughout the tests. The rats were individually placed in the same position, in a chamber with an electrified grid floor, a pole suspended from the top center, and a buzzer. In unconditioned response trials the buzzer and electric shock through the grid floor were presented simultaneously; in conditioned response trials, only the buzzer was presented. Each trial was terminated either by a response (jumping onto the pole after onset of stimuli) or at the end of 30 seconds. Rats from each group were alternately tested in individual trials.

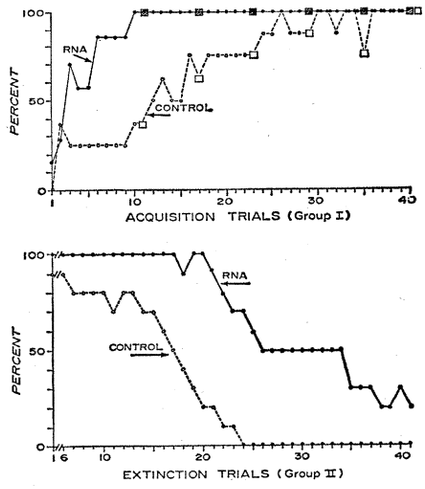

Fig. 1. Behavioral effects of RNA in rats. Dotted lines indicate averages for controls; solid lines indicate averages for treated group. (Top) Effect of 53 daily injections of RNA on acquisition of conditioned responses (group I). Circles indicate unconditioned response trials; squares indicate conditioned response trials. Percentage of animals of each group exhibiting the response is on ordinate; individual trials, over a period of 4 days, are on abscissa. All rats received the same number of trials each day—average ten trials per day. Differences between groups (χ^2) were significant at $P = .05$ or less (one-tailed). (Bottom) Effect on extinction after a total of 30 daily injections of RNA (group II). Conditioned stimulus was presented without shock reinforcement. Percentage of rats of each group responding to the conditioned stimulus is on ordinate; individual trials, over a period of 3 days, are on abscissa. Differences between groups (χ^2) were significant at $P = .05$ level or less (one-tailed).

Figure 1 (top) shows the acquisition performance curves measured by percentage of animals responding. Significant differences in performance between the treated group (group I) and the saline group were seen. In the treated group the rate of acquisition of the response was markedly faster ($P<.02$) than in the controls. Even after 100 percent performance was achieved in both groups, the response latencies of the treated group in subsequent trials (not shown in figure) were approximately half those of the saline group.

These results were confirmed with other groups of rats (group II). The treatment was similar to that in the first experiment; however rats were tested after only 1 month. Again, the rate of acquisition of the response was significantly faster ($P < .05$) in the treated group than in the controls. When the two groups (ten rats each) achieved maximum performance, both the controls and the treated rats responded to the buzzer alone. Extinction of this conditioned response was then carried out. The treated group (group II) was more resistant to extinction than the saline group (Fig. 1, bottom). The apparent difference in the extinction rate was also statistically significant ($P < .001$).

Similar results on acquisition were observed after daily treatment for 1 or 2 weeks. After 3 days of treatment no effect was observed. However, resistance to extinction was higher in the treated rats than in controls after 3 days, 1 week, 2 weeks, or 1 month of daily treatment.

These results demonstrate that rats injected daily with RNA exhibit significantly better performance during acquisition of a conditioned response and higher resistance to extinction than rats injected with saline. We are now attempting to determine whether RNA itself, one or more of its components, degradation products, some impurity, or some biochemically resynthesized molecule is responsible for the effects described.

It is premature to conclude that the administered RNA directly affected learning or memory processes. Perhaps these measured behavioral

changes are the result of the interaction of RNA with one or more of the experimental parameters utilized. However, the findings are generally consistent with some of the reported clinical results.

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4. Acquisition is the progressive incremental change in the proficiency of performance as a result of repetitive presentation of conditioned and unconditioned stimuli under controlled conditions. Extinction is the progressive decremental change in performance as a result of repetitive presentation of the conditioned stimulus without the unconditioned stimulus.
5. RNA was purchased from Pabst Laboratories. Preliminary studies were conducted with 10 cent aqueous solution of yeast RNA, kindly supplied by Dr. D. E. Cameron, Montreal, Canada.
6. Sprague-Dawley rats were obtained from Charles River.
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29 April 1963

Discovery of Right Whales in the Gulf of Mexico

Abstract. *Two whales were observed closely for an hour off Sarasota, Florida, by residents who provided observations of structural details which identify only the right whale,* Eubalaena glacialis, *a temperate and subpolar species previously known to range to the Florida east coast, but not to enter the Gulf of Mexico.*

On 10 March 1963, the Gulf of Mexico had a fresh wind and a chop off New Pass, Sarasota, Florida, when Ben B. Sanders and Paul Reeves, residents of Sarasota cruising in a 28-foot (8.4 m) boat, saw two whales swimming west in water only 30 to 34 feet deep (9.4 to 10.3 m). Together with Merton Wilcox, a precision instrument engineer and a consultant to Cape Haze Marine Laboratory, who joined them in another boat, Sanders and Reeves observed the whales from 3:30 to 4:40 P.M., approaching them as closely as 12 feet (3.6 m) in one instance. They described their experience to one of us within 22 hours. The only camera aboard took inadequate photographs, but the details reported to us seem to provide unassailable identification of the whales as right whales, *Eubalaena glacialis* and, thus, the first evidence of this species ranging into the Gulf of Mexico.

According to Sanders, Reeves, and Wilcox, these whales had (i) no dorsal fin; (ii) the mouth cleft in side view, high on the head and arched; (iii) a bumpy area in a ragged patch on the head forward of the blowhole; (iv) a length exceeding 40 feet (12.1 m), in the larger probably approaching 55 feet (16.7 m); (v) a color of charcoal gray and black; Wilcox and Sanders saw inconspicuous whitish patches low on the head near the eye; and (vi) a single spout 3 to 5 feet high (0.9 to 1.5 m). The whales created a slick in the choppy water above and around them, even when not breaking the surface. Most of the observations relate especially to the larger individual which showed itself more freely. The first five items identify only one species known to inhabit North Atlantic waters, the right whale, *Eubalaena glacialis,* and only one item could be construed as evidence against this: observed from behind, the blow or spout should have been double (or V-shaped) and higher (*1*). This incongruity may result from a defect in observation, or possibly from the whales' breathing less forcefully in relatively warm, shallow water. After corresponding on the diagnostic points, we double-checked these observations with the witnesses, and we see no cause to doubt that the animals described were right whales.

The right whale was the easiest and most lucrative species to catch, and by about 1750 it had been reduced in the North Atlantic to numbers too low for further economic exploitation (*2*). Their near extinction so long ago has severely limited scientific knowledge of the southern extent of their original range in the North Atlantic. One specimen from the eastern Atlantic, that would have passed as far south as 36°N. latitude in the Strait of Gibraltar, is known (*3*) from Taranto, Italy, and one from the western Atlantic was observed (*3*) near Charleston, South Carolina (just below 34°N.). One of us (*4*) recorded that a few individuals still reach the Atlantic coast of Florida in late winter, with one occurrence as far south as 26°15′N. Attainment of the upper Florida east coast by a few right whales seems now to be regular (*5*), but there is no previous evidence that this species ranges into the Gulf of Mexico (*2, 6*). By international agreement in 1929, the right whale was protected from commercial whaling (*1*) and since then its western North Atlantic population has evidently increased so that it is now straggling into the Gulf of Mexico.

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2 May 1963

Transpiration by Sudangrass as an Externally Controlled Process

Abstract. *Transpiration from a well-watered sudangrass stand in a highly evaporative environment (Tempe, Arizona, in July) can be considerably increased by exposing a small plot of about 1 square meter to radiative and convective heat input. Thus, the transpiration of sudangrass in a full stand appears not to be determined by any physiological factor during any time of the day.*

A transpiring plant cover may, for purposes of analysis, be compared to an open water surface. However, unlike evaporation from open water, transpiration can be determined or limited by availability of soil water, capacity of water-carrying tissues, and impedance to vapor diffusion in the leaf in interstitial and stomatal pathways.

In this report we give data indicating