# An Investigation of Maze Position Preferences of Naive Rats After Injection of an RNA Extract from Trained Rats

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Branch and Viney (1966) suggested that variables such as dosage level and amount of training may partially account for recent contradictory findings (e.g., Babich et al., 1965; Luttges et al. 1966) concerning the effect of ribonucleic acid (RNA) on transfer of learning phenomena. Babich et al. (1965) found highly significant memory transfer effects after injecting naive rats with relatively large concentrations of RNA extracted from donor rats which had been subjected to extensive training in a Skinner Box. Luttges et al. (1966), however, failed to find significant transfer effects, but most of their experiments did not use training criteria as rigorous and concentrated as those employed by Babich et al. (1965). Dosage level was also smaller in some of the experiments of Luttges et al. Branch and Viney (1966) reported transfer of position discrimination findings which, though statistically insignificant, nevertheless persisted to favor RNA-injected subjects as opposed to control subjects. Since the direction of results of this study consistently favored RNA recipients, the present study was undertaken in order to see if an RNA dosage level larger than that utilized in the previous study would effect significant transfer of learning.

## METHOD

*Subjects and Apparatus*—The *Ss*, 36 male Wistar rats approximately 120 days old at the beginning of the study, were randomly assigned to experimental conditions with 24 serving as recipients, 6 as trained donors, and 6 as untrained (control) donors. The animal housing unit was maintained under constant temperature (25C) and light (alternating but equal 12-hour periods of light and darkness) conditions throughout the experiment.

The apparatus was a double-alley maze similar to one reported by Logan (1965). The maze consisted of two parallel alleyways (34 × 3½ × 10 inches), a start box (8 × 4 × 10 inches), and a choice area (4 × 8 × 10 inches). A partition (30 × 10 inches) separated the two alleyways. The apparatus was constructed of 1-inch lumber painted a flat mid-grey. Guillotine doors separated the choice area from the runways and the start box from the choice area. The guillotine doors were yoked together and raised simultaneously so as to expose the choice area and the beginning of each alleyway. The alleyways, start box, and choice area were covered by black screen-wire doors. The floors were constructed of boards 1 inch thick, covered with a layer of cardboard and overlaid with black paper. Korotkoff sound microphones were inset in 1½-inch circles cut in the intermediate cardboard layer of the floor. The microphones were located 8 inches inside each runway entrance and 1 inch in front of feed troughs located at the terminal end of each runway. The goal area microphones in the terminal ends of the runways were connected to a common preamplifier and the output was connected to one channel of a Physiograph Four (E & M Instrument Co.). The microphones located in the runway entrances were connected to separate preamplifiers, each connected to separate channels of the physiograph. Thus, output appeared in separate channels, depending upon the runway selected by the *S*. The mechanically operated guillotine doors activated a microswitch connected to the time and event recorder of the physiograph.

*Procedure*—Training of donor *Ss* and testing of recipient *Ss* took place in a soundproofed room (9 × 14 ft) maintained at a constant (25C) temperature. A screen (4 × 6 ft) separated the maze from the experi-

menter and recording equipment. Light levels in the apparatus were less than 1 foot-candle.

All donor and recipient subjects were housed in individual cages in the animal unit. Water was present on an *ad lib.* basis. All donors received a reduced diet of Purina Rat Chow during the course of training. This was accomplished by having the Rat Chow available for 1½ hr of each 24-hr period. Food deprivation was identical for both trained and untrained donors.

Training sessions were conducted for a 15-min period each day until *S* reached a criterion of 15 successive correct choices. *Ss* were rewarded with two 0.45-mg Noyes pellets for running to the food trough in the right hand runway. *S* was considered committed to a runway when the entrance microphone was stimulated, at which time the guillotine door was lowered to prevent retracing. A response was counted following stimulation of the microphone in the goal area. Following criterional learning, all donors were given 70 overtraining trials in 3 additional sessions, consisting of 30 trials in session 1, 30 trials in session 2, and 10 trials in session 3. Each day the untrained donor (control) was given the same number of Noyes Pellets as the trained animal received in his training session.

Within a 15-minute period following the final overtraining session, the trained donor and his control were sacrificed. The brains were then quickly (3 to 4 min removal time) but carefully removed in a cold room (0 - 5 C). An anterior cut separated the frontal areas from the olfactory lobes and a posterior cut separated the cerebrum from the cerebellum. This brain tissue was placed in a solution consisting of 5 ml of 90% phenol and 5 ml of 0.25 M sucrose and homogenized in a Serval Omni-Mixer. The homogenate was then centrifuged at 10,000 × G for 20 min at 0 C. The aqueous phase (3.5 ml) was then carefully drawn off and placed in a clean test tube. A 0.25-ml volume of $MgCl_2$ (1.5M) was added to bring the final concentration of Mg to 0.1 M. Two volumes (7.5 ml) of cold ethanol were added to precipitate the RNA. The suspension was then centrifuged (10,000 × G, 0 C) for 20 min. Following centrifuging, the supernatant liquid was discarded and the precipitated RNA resuspended in 1.0 ml of 0.25 M sucrose.

Two recipient *Ss* were immediately given a 0.4-ml IP injection of RNA extracted from an untrained donor and two recipients were injected with 0.4 ml extracted from a trained donor. Recipients had been deprived of food for approximately 12 hr prior to the RNA injection.

Recipient subjects were tested in 15-min sessions at 10- and 20-hr intervals following injection. Each recipient received a code letter so that his testing could be on a "blind" basis. That is, the experimenter did not know the status of each recipient. Half of the recipients in each RNA group were rewarded for a right handed response (an acquisition procedure) and half were not rewarded (an extinction procedure). Otherwise, testing procedure for recipients were identical to training procedures for donors.

## Results and Discussion

The results for all recipient groups including total responses and directional responses are presented in Table I. An analysis of variance based on total responses indicated no significant main effects or interactions between any of the treatment groups. An analysis based on directional (right minus left) measures also indicated no significant differences. Thus, the treatments utilized in this experiment failed to produce statistical evidence of transfer of learning effects.

It is apparent at the present time that, if transfer of learning via RNA is a genuine effect, it cannot be accomplished without relatively

large concentrations of RNA. The present study with a donor-recipient rat'o of 1:2 and an earlier study (Branch and Viney, 1966) with a donor-recipient ratio of 1:4 failed to show transfer effects, although the direction of results of both studies favors subjects receiving RNA extract from trained donors. Other studies (Babich et al., 1965; Jacobson et al., 1965), reporting statistically significant transfer effects, have utilized donor-recipient ratios of 1:1, and a recent study (Byrne and Samuel, 1966) reporting significant transfer effects made use of a donor-recipient ratio of 2:1. That is, each recipient subject was injected with RNA extracted from the brains of 2 identically trained donors. The major implication for future research is that relatively large concentrations of RNA are apparently needed in order to effect significant transfer of learning phenomena.

SUMMARY

Albino rats given intraperitoneal injections of RNA extracted from the brains of trained donor rats failed to show better transfer of learning than control rats injected with RNA extracted from untrained donors. The failure was attributed to an inadequate dosage level of RNA.

TABLE I. ACTIVITY AND DIRECTIONAL SCORES FOR ALL RECIPIENT GROUPS

| Groups | | Total Responses | Right Responses | Left Responses | R-L |
|---|---|---|---|---|---|
| Experimental Reward | 10 hr | 25 | 13 | 12 | 1 |
| | 20 hr | 22 | 12 | 10 | 2 |
| Experimental No Reward | 10 hr | 21 | 13 | 8 | 5 |
| | 20 hr | 13 | 8 | 5 | 3 |
| Control Reward | 10 hr | 24 | 13 | 11 | 2 |
| | 20 hr | 14 | 7 | 7 | 0 |
| Control No Reward | 10 hr | 20 | 10 | 10 | 0 |
| | 20 hr | 6 | 4 | 2 | 2 |

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