Failure to transfer a learned response in rats using a brain extract containing RNA¹

R. F. HOFFMANN² AND C. N. STEWART, FRANKLIN & MARSHALL COLLEGE
H. N. BHAGAVAN, RESEARCH INSTITUTE, ST. JOSEPH HOSPITAL

An attempt was made to transfer a learned response between individual rats by means of brain extracts containing RNA. Donors were trained on a light-dark discrimination task with a food reward in a "Y" maze. Control groups consisted of donors given non-discriminative experience in the maze equal to that of the trained group, donors maintained in their home cages, and a saline control group. Recipient Ss did not differ significantly either in the number of errors made during training, or in the number of trials necessary to meet a criterion of five consecutive correct responses.

In several recent studies attempts have been made to "transfer a learned response" from one animal to another using a brain extract containing RNA. Some experimenters have reported positive results using brain homogenates (Albert, 1966; Ungar & Ocegüera-Navarro, 1965), while others have obtained positive transfer effects from RNA extracts (Babich, Jacobson, Bubash, & Jacobson, 1965; Jacobson, Babich, Bubash, & Jacobson, 1965). Jacobson et al (1965) have suggested that a specific response pattern can be transferred from one rat to another using IP injection of RNA.

Other experimenters (Gross & Carey, 1965; Kimble & Kimble, 1966), however, have failed to find the predicted "RNA transfer effect" in studies similar to those mentioned above. The present study was designed to retest the "RNA transfer hypothesis" after making several changes in the initial design by Babich et al (1965). Donor control groups with and without non-discriminative maze experience were included to assess possible transfer of nonspecific experiential factors, while responses were recorded mechanically by photoelectric cells in order to improve the accuracy of measurement of the dependent variable. These changes in the experimental design were included in order to show clearly the significance of any recorded transfer effect and to make the study more easily replicable.

Subjects

Sixty-eight naive male Long-Evans hooded rats (Rockland Farms), weighing 200-250 g, served as Ss in this experiment.

Apparatus

A light-dark discrimination Y maze with a screen floor was used for training. It was gray with a 12 in. long start alley followed by two 18 in. arms. At the end of each arm was a 5x10 in. Plexiglas panel which could be illuminated. Guillotine doors separated the start and goal boxes from the runway areas. Photoelectric cells were located at the entrances to each goal box for the purpose of recording running times to the nearest .01

sec. Food rewards consisted of five 45 mg Noyes food pellets presented in gray glass dishes in the goal boxes.

Procedure

Three RNA donor groups (Donors Trained (DT, N=10), Donors Untrained (DU, N=9), and Donors Cage (DC, N=10)) and four recipient groups (Recipients Trained (RT, N=10), Recipients Untrained (RU, N=9), Recipients Cage (RC, N=10), and Recipients Saline (RS, N=10)) were used in this study. All Ss were maintained on a 23 h food deprivation schedule beginning eight days before training and continuing until the end of the experiment.

The Ss in all groups except Group DC received light-dark discrimination training in the Y maze consisting of two days of adaptation to the maze followed by 72 training trials spaced over the next 12 days in the following manner: one trial on Days 1 and 2; two trials on Days 3 and 4; ten trials on Days 5 through 9; five trials on Days 10 and 11, and six trials (donors) or five trials (recipients) on Day 12. For the DU group, food was paired with light 50% of the time and with dark 50% of the time; for the other five trained groups, food was paired with light 100% of the time. Training trials were run following a correction procedure. If S made an incorrect (unreinforced) response, it was returned to the start box and allowed to run again under the same stimulus conditions. This was repeated until S ran to the correct side, thus ending the trial. Presentation of both food and light was done according to a predetermined changing order so that each was located on the left on 50% of the trials and on the right on 50% of the trials.

RNA was extracted from the brains of donor Ss on the day following the 12th day of training, 14 h after training had been completed. The extraction procedure was basically the same as that of Babich et al (1965), except that the RNA preparation was washed twice with 70% ethanol and finally dried in a vacuum dessicator in cold. It was then suspended in 1.5 ml of cold 0.9% NaCl for injection. Parallel extractions were carried out using brains from two additional donor rats and the RNA preparations obtained were used for RNA analysis, and tests for protein, DNA, and phenol.

Intraperitoneal injections of the RNA extract were made 14 h after the donors had been decapitated. Each rat received an RNA extract of one brain from a corresponding donor rat. Discrimination training for recipients was begun on the day following injection (16 h after injection), and was completed 12 days later. The

Table 1 Mean number of correct responses and errors and mean number of trials and errors to criterion

Group	Mean Correct Responses	Mean Errors	Trials to 5 Correct Responses	Errors to 5 Correct Responses
RT	53.8	21.7	21.5	16.1
RU	52.6	22.3	19.8	19.4
RC	55.2	19.8	14.9	10.6
RS	54.8	19.4	22.4	14.9

training was not run under a blind condition; however, the automatic recording of responses and the randomization of the order in which Ss were run reduced the possibility of E bias affecting the results.

Results

Comparisons were made between DT and DU groups for both the mean number of errors (22.7 and 5.7, respectively) and the mean number of correct responses (54.5 and 37.6, respectively) made for each group during training. These data yielded *t* values which indicated significant differences (correct responses: $t=13.54$, $df=17$, $p<0.01$; errors: $t=7.27$, $df=17$, $p<0.01$).

For the four recipient groups, RT, RU, RC, and RS, one-way analyses of variance were done on the mean number of correct responses and errors made during training, and on the mean number of trials and errors needed to reach a criterion of five consecutive errorless trials. These data, presented in Table 1, yielded no reliable group differences for any one of these four measures. Mean running times for all Ss receiving RNA did not differ significantly from those receiving saline.

Spectrophotometric analysis of the extract yielded a value of 0.621 mg RNA per brain. Most of the RNA was found to be in the native state as indicated by the alkaline denaturation test. Tests for protein, DNA, and phenol were all negative.

Discussion

The data show no difference between the recipients of RNA from the three groups of donors. Apparently, neither a specific nor a general response tendency was transferred from the donors to the recipients. One possible reason for these results is that intraperitoneally

injected RNA does not reach the brain. Luttges, Johnson, Buck, & Holland (1966), as well as Sved (1965), failed to show a significant incorporation of radioactivity in the brains of mice after IP injections of P^{32} labeled RNA or intravenous injections of C^{14} labeled RNA. Even if peripherally injected RNA does pass the blood-brain barrier, it still may not mediate the transfer of a response. Hyden & Egyhazi (1962) have shown a correlation between the acquisition of a response and changes in the RNA content of the brain. However, this does not necessarily imply that learning is coded in the RNA molecule. The change in the RNA content could merely be the result of some other yet unrecognized change within the cell. A much firmer foundation in our knowledge of the biochemical processes involved in learning is necessary before attempts to demonstrate such second-order phenomena as the biochemical transfer of learning are made.

References

- ALBERT, D. J. Memory in mammals: Evidence for a system involving nuclear RNA. *Neuropsychologia*, 1966, 47, 79-91.
- BABICH, F. R., JACOBSON, A. L., BUBASH, S., & JACOBSON, A. Transfer of a response to naive rats by injection of RNA extracted from trained rats. *Science*, 1965, 149, 656-657.
- GROSS, C. G., & CAREY, F. M. Transfer of learned response by RNA injection: Failure of attempts to replicate. *Science*, 1965, 150, 1749.
- HYDEN, H., & EGYHAZI, E. Nuclear RNA changes of nerve cells during a learning experiment in rats. *Proc. Nat. Acad. Sci.*, 1962, 48, 1366.
- JACOBSON, A. L., BABICH, F. R., BUBASH, S., & JACOBSON, A. Differential approach tendencies produced by injection of RNA from trained rats. *Science*, 1965, 150, 636-637.
- KIMBLE, R. J., & KIMBLE, D. P. Failure to find "transfer of training" effects via RNA from trained rats injected into naive rats. *Worm Runners' Digest*, 1966, 8, 32-36.
- LUTTGES, M., JOHNSON, T., BUCK, C., HOLLAND, J., & McGAUGH, J. An examination of transfer of learning by nucleic acid. *Science*, 1966, 151, 834-837.
- SVED, S. The metabolism of exogenous RNA injected into mice. *Canad. J. Biochem.*, 1965, 43, 946-958.
- UNGAR, C., & OCEGUERA-NAVARRO, G. Transfer of habituation by material extracted from brain. *Nature*, 1965, 207, 301-302.

Notes

1. This research was supported in part by National Science Foundation Undergraduate Research Participation Grant GY-136.
2. Now at the University of Wisconsin, Madison.