"Transfer of learning" by injection of brain RNA: A replication

RICHARD GAY[1] AND ALFRED RAPHELSON
UNIVERSITY OF MICHIGAN, FLINT COLLEGE

Brain RNA was extracted from two groups of rats one of which had learned a passive avoidance of a dark chamber previously preferred by both groups. Recipient groups received the two kinds of RNA extract via intraperitoneal injection. The group injected with the extract from the brains of the conditioned animals showed significantly greater avoidance of the preferred chamber.

Recent research has reported beneficial effects on memory due to ribonucleic acid (RNA) administration (Cameron & Solyom, 1961; Cameron et al, 1963; Cook et al, 1963; Wagner et al, 1966; Corson & Enesco, 1966). There have also been reports of "transfer" effects accomplished through RNA extraction from the brains of trained animals and its injection into naive animals (Babich et al, 1965; Jacobson et al, 1965; Fjerdingstad et al, 1965). These results are especially significant since they provide evidence of a direct relationship between nucleic acid and memory. Recently the generality of these findings has been seriously questioned (Luttges et al, 1966; Graves & Carey, 1965; Gordon et al, 1966; Byrne et al, 1966). These negative findings have weighed heavily against the RNA molecular theory of memory.

As Byrne et al point out, "failure to reproduce results is not after all, unusual in the early phase of research when all the relevant variables are as yet unspecified" (1966). In hopes of contributing toward the eventual uncovering of the significant variables involved in the "memory-transfer" phenomenon, the following experiments which obtained positive results are reported.

Subjects

Experiment 1. Ss (19 male hooded rats, approximately 65 days of age) were divided into four groups: Learned-Donors (N = 7); Control-Donors (N = 4); Learned-Recipients (N = 4); Control-Recipients (N = 4).

Experiment 2. Ss (22 female hooded rats, 100 days of age) were divided into four groups: Learned-Donors (N = 7); Control-Donors (N = 7); Learned-Recipients (N = 4); Control-Recipients (N = 4). The test trials were carried out by an assistant who did not know the designation of the individual Ss nor the purpose of the experiment. In all other respects the two experiments were similar.

Apparatus

A maze was constructed in which three boxes (11 in. x 12 in.) were connected by two runways, 24 in. x 6 in. The boxes were arranged with a white one in the middle of the runway which served as the start box (SB). A second white box (WB) was connected to SB by the left runway (LRW), and a third box, painted black, connected to the SB by the right runway (RRW) served as the goal box (GB). The whole apparatus, with the exception of GB, was covered by clear Plexiglas. A 25 W light was placed directly over WB. The GB was covered with a wooden top with a small dark blue Plexiglas window. The floor of GB contained a grid, and a guillotine door controlled the entrance.

Preliminary work demonstrated that rats would, when allowed to freely explore, show a definite preference for the darkest area. The Ss uniformly avoided the bright areas (SB, WB, LRW, RRW) and spent the greatest portion of the time in the dark GB. The learning task in these experiments, utilizing the passive avoidance paradigm, established the preference for the dark box as the positive response for both the Learned-Donor and Control-Donor groups and then had the Learned-Donor group acquire an inhibition of this response via avoidance training.

Procedure

Ss in the Learned-Donor and Control-Donor groups were individually placed in the Start Box (SB) and allowed to explore the entire apparatus for 180 sec. The amount of time each S spent in the dark GB and the bright WB was recorded. There was little exploration during this period with all Ss showing a definite preference for GB. The average time spent in GB for both groups was about 120 sec. Only one of the 25 Donor Ss entered WB during the exploration period.

After all Ss had demonstrated the preference for GB, the Ss in the Learned-Donor group were confined in GB and administered a 27 V shock for 5 sec, after which time, the gate was raised and the Ss were allowed to escape the shock by retreating up the runway. Four shock trials were given each day for four days. All Ss in this group demonstrated an inhibition of their original preference for GB along with signs of emotionality (e.g., urination, defecation, freezing). Two h after the last training session, all Ss were sacrificed and their brains removed.

Within each group the brains were pooled and processed together according to the following RNA extraction procedure: The brains were homogenized for 5 min in a 10 ml mixture of equal parts of 92% phenol and isotonic saline, then centrifuged at 15,500 rpm for 45 min. The aqueous phase was drawn off and the mixture centrifuged again at 6000 rpm for 5 min. The mixture was then brought up to a concentra-

Table 1.

Average Time (in sec) Spent in Goal Box and White Box by Learned-Recipients and Control-Recipients during the First Transfer Test

Experiment	Goal Box		White Box	
	Control Recipients	Learned Recipients	Control Recipients	Learned Recipients
One	123	0	0	62
	153	58	0	0
	126	54	0	0
	120	0	0	35
Two	83	0	0	45
	150	0	0	45
	117	0	0	2
	84	12	0	28
Mean	119.5	15.5	0.0	21.5

tion of .1 M MgC_{12} and two volumes of cold 95% ethanol were added to precipitate the RNA. The precipitation was enhanced by a final centrifugation at 6,000 rpm for 15 min. The remaining ethanol was evaporated off and the RNA dissolved in 1.25 ml of isotonic saline.

Within 1 h after the extraction was completed, the Recipient Ss received .25 cc intraperitoneal injections of the appropriate RNA extractate. In each of the two experiments, four Ss received RNA extractate from the Control-Donor group and four Ss received RNA extractate from the Learned-Donor group.

Two h after the injection, all recipients were individually placed in the Start Box (SB) and allowed to explore the entire apparatus for 3 min. The amount of time each S spent in GB and WB was recorded as well as the time it took each S to reach the GB after being placed in SB. Ss were tested in this manner five times at 2 h intervals.

Results

The results from both experiments for the first transfer test trial are presented in Table 1.[2] It will be remembered that both Donor groups showed a definite preference for GB initially, but the Learned-Donor group acquired an avoidance reaction to it. On the first transfer test, five out of the eight Ss who received the RNA extracted from the Learned-Donor group did not enter GB during the entire 3 min period. The Ss who received RNA extracted from the Control-Donor group spent, on the average, close to two-thirds of the period there.

In addition, none of the Control-Recipient Ss entered WB, whereas five of the Learned-Recipients entered this box.

The trends which appear on the first test trial continue throughout the later tests. The latency data as well as qualitative observations (e.g., defecation, crouching, grooming, freezing) also supported the observation that the Learned-Recipient Ss acted in a fearful, avoidant manner in relation to GB.

Discussion

It appears from the data presented that some type of "transfer" was obtained in these two experiments. However, in face of the large number of negative findings that have recently been reported, one can only be cautious in generalizing from these results. Rather it may be more appropriate to allow these results to highlight the conditions under which "positive findings" were obtained: Long-Evans rats, trained in a fairly simple passive avoidance task, with RNA extraction following the cold phenol procedure and recipients tested in the identical apparatus for the identical response.

Further research is underway to determine if the "transferred avoidance reaction" is situationally specific and if the extinction curve for the transferred avoidance reaction closely approximates the curve obtained for such a reaction under ordinary extinction conditions.

References

Babich, F. R., Jacobson, A. L., Bubash, S., & Jacobson, A. *Science*, 1965, 149, 656; Jacobson, A. L., Babich, F. R., Bubash, S., & Jacobson, A. *Science*, 1965, 150, 636; Fjerdingstad, E. J., Nissen, Th., & Roigaard-Petersen, H. H. *Scand. J. Psychol.*, 1965, 6, 1.

Byrne, W. L., Samuel, D., Bennett, E. L., Rosenweig, M. R., Wasserman, E., Wagner, A. R., Gardner, F., Galambos, R., Berger, B. D., Margules, D. L., Fenichel, R. L., Stein, L., Corson, J. A., Enesco, H. E., Chorover, S. L., Holt, C. E., Schiller, P. H., Chiappeta, L., Jarvik, M. E., Leaf, R. C., Dutcher, J. D., Horovitz, A. P., & Carlson, P. L. *Science*, 1966, 153, 658.

Cameron, D. E., & Solyom, L. *Geriatrics*, 1961, 16, 74; Cameron, D. E., Sved, S., Solyom, L., Wainrib, B., & Barik, H. *Amer. J. Psychiat.*, 1963, 120, 320; Cook, L., Davidson, A. B., Davis, D. J., Green, H., & Fellows, E. *Science*, 1963, 141, 268; Wagner, A. R., Carder, J. B., & Beatty, W. W. *Psychon. Sci.*, 1966, 4, 33; Corson, J. A., & Enesco, H. E. *Psychon. Sci.*, 1966, 5, 217.

Luttges, M., Johnson, T., Buck, C., Holland, J., & McGaugh, J. *Science*, 1966, 151, 834; Graves, C. G., & Carey, F. M. *Science*, 1965, 150, 1749; Gordon, M. W., Deanin, C. G., Leonhardt, H. L., & Gwenn, R. H. *Amer. J. Psychiat.*, 1966, 122, 1174.

Notes

1. Present address: Western Michigan University.

2. The significance of the data will be obvious from an inspection of the distributions of individual scores in Table 1. However, tests of significance were run on mean differences between the two groups in time spent in the Goal Box and the White Box and reached at least .05 level of significance in both instances.

Editor's Note

It is the usual policy of this journal to give full citations to literature. However, in this case, because of the controversial nature of the field and the limited space available, grouped and abbreviated citations have been permitted.