"Transfer of learning" by injection of brain RNA as a function of time

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Brain RNA was extracted from two groups of rats, one of which had learned a passive avoidance of a black chamber, and intraperitoneally injected into independent recipient groups. Significant avoidance of the previously preferred chamber was found at 8 h following injection but not at 1, 2, or 16 h.

Results of studies of "transfer of learning" effects by brain ribonucleic acid (RNA) extraction from trained rats and its injection into naive rats have been apparently conflicting. In a recent study by Luttges, Johnson, Buck, Holland, & McGaugh (1966) no difference was found in task performance between naive rats injected with RNA from trained rats and naive rats injected with RNA from naive rats. On the other hand, Babich, Jacobson, Bubash, & Jacobson (1965) found the task performance of naive rats injected with RNA from trained rats to be superior to that of naive rats injected with RNA from naive rats.

Studies supporting RNA "transfer of learning" effects have tested recipient rats 1 to 3 h after injection, whereas, studies disproving this transfer effect have tested the rats 18 to 24 h after RNA injection. In the present study, time between RNA injection and performance testing of recipient rats was varied in an attempt to verify "transfer of learning" effects and determine the optimal transfer retention interval for a passive-avoidance task requiring light-dark discrimination.

METHOD

The Ss were 80 naive, male, Sprague-Dawley rats, approximately 55 days old at the beginning of the study.

A Miller-Mowrer box (24 x 5 x 15 in.) was divided into a black and a white section of equal size by a guillotine door (5 x 15 in.). A 25-W light was placed directly above the white section and a shock source of 2.4 mA was wired to the grid of the black section. Electric clocks with photoelectric relays measured time (to the nearest .1 sec) spent in each section.

Previous work has demonstrated that rats show a definite preference for the darkest areas in a maze when permitted to explore freely (Gay & Raphelson, 1967). Avoidance training for this preference was carried out on 20 rats. These 20 rats and 20 others were decapitated and brain RNA extracted and injected into 40 rats. The recipients were tested for light-dark preference 1, 2, 8, or 16 h after injection.

On a 3-min preference trial all 80 rats showed the expected preference for the black compartment and were randomly divided into four groups of 20: Learned-Donor, Control-Donor, Learned-Recipient, and Control-Recipient. Each Learned-Donor rat was confined in the black compartment and administered a 2.4-mA shock for 5 sec, after which time the door was raised 3 in. and S permitted to escape to the white compartment. Three such shock trials per day for five consecutive days was sufficient for the Ss to exhibit an inhibition of their original preference for the black compartment.¹ During these sessions the Donor-Control Ss were administered an equal amount and duration of shock in a neutral environment. Two hours after the last training session both Donor groups were decapitated.

Within each group the Donor brains were pooled and processed together according to the following procedure:² The brains were homogenized for 5 min in a 40 ml H₂O solution (15 deg C). The mixture was added to 100 ml of 90% phenol (27 deg C) and stirred for 30 min. The suspension was cooled

in an ice bath for 5 min and then centrifuged at 48,500 x G for 10 min at 0-5 deg C. The cloudy supernatant, aqueous layer, and most of the intermediate layer were decanted from the brown phenol phase. The aqueous layer was centrifuged twice at 48,500 x G for 10 min at 0-5 deg C. One-tenth volume of 20% potassium acetate (pH 5) was added and two volumes of cold absolute ethanol was added to precipitate the RNA. The cloudy solution was cooled for 5 min in an ice bath and then centrifuged at 48,500 x G for 10 min at 0-5 deg C. The precipitate was kept overnight at 0-5 deg C. Then the precipitate was washed once with ethanol:water (3:1), absolute ethanol, and once with ether, and centrifuged at 48,000 x G, all at 0-5 deg C. The precipitate was dried and weighed, then dissolved in 6 ml isotonic saline.

The Recipient Ss received .3 ml intraperitoneal injections of RNA extractate. The Learned-Recipients received RNA from the Learned-Donor pool and the Control-Recipients from the Control-Donor pool. The Recipients were divided into four groups each consisting of five Learned-Recipients and five Control-Recipients. Each group was tested at one and only one time period following injection: 1, 2, 8, or 16 h. At the respective time period each rat was individually placed in the white compartment and allowed to explore both sections freely for 180 sec. The amount of time each S spent in the white and black sections was recorded.

No placebo control or noninjected control groups were used since previous research has found them unnecessary (Fjerdingsstad, Nissen, & Roigaard-Petersen, 1965).

RESULTS AND DISCUSSION

Each Recipient S's score was postinjection test time minus preinjection test time in the black compartment. A 4 by 2 analysis of variance showed no significance over the four injection-test delays. Significance was found only between the Learned and Control Recipients ($F = 5.467$, $df = 1/25$, $p < .05$). It was necessary to perform t-tests for each time level to determine when the differences between Learned-Recipients and Control-Recipients were significant (see Fig. 1). Significance was found only at the 8-h level ($t = 1.91$, $df = 8$,

Fig. 1. Mean difference scores (pre- to postinjection time in black compartment) of recipient groups, tested at 1, 2, 8, or 16 h following injection.

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Fig. 1. Mean reciprocal start, running, and goal times for 50% and 100% Ss for the 10 days of acquisition (eight trials/day).

any apparent CE for start scores is at variance with previous findings which indicate that, if the CE is obtained in an experiment for any response measure at all, it is invariably found in the measure taken earliest in the response chain (Amsel, Rashotte, & MacKinnon, 1966; Goodrich, 1959; Haggard, 1959; Surridge, Boehnert, & Amsel, 1966; Wagner, 1961, 1963; Weinstock, 1958). Perhaps low drive leads to less generalization of r_f to earlier portions of the runway. Although in most studies the CE is absent in the goal scores, Robbins & Weinstock (1967) found, as in the present study, a CE for goal speed.

The finding of a significant CE under zero deprivation suggests that r_f develops relatively independent of deprivation level. It seems that when incentive is sufficient to support learning, whether that incentive is compounded of deprivation-induced need and need satisfiers, "hedonic"

factors, or other variables, such incentive may also be sufficient to support certain frustrative nonreward phenomena.

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NOTES

1. Supported by PHS Research Grant No. MH12099 from the National Institute of Mental Health. Computer processing of data was supported by the Computer Laboratory of Tulane University.
2. We are somewhat dubious about the magnitude of the CE in the goal-speed data, since it seems at least partly attributable to the inexplicable sharp downturn for Day 10 of the 100% group and the upward spurt of the 50% group on that day. More stable curves based on a larger number of Ss, or additional training trials, might have clarified the matter.
3. Complete summary tables of the analyses of variance and tables of cell means may be obtained by writing to the first author.

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$p < .05$). The difference at 16 h did not reach significance ($t = 1.74$, $df = 8$, $p > .05$) because of large variance in the Learned-Recipient group.

The results showed an apparent "transfer of learning" at 8 h after injection, suggesting that previous negative results may have been due to nonoptimal intervals between injection and test.

Further investigations are in progress to study the variables of injection dosage and locus, specificity of transfer, and task difficulty, in addition to postinjection delay.

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NOTES

1. One Donor-Learned rat was discarded after failing to learn the passive avoidance.
2. Joseph Quashnock carried out the extraction procedure.