

Specific and general factors in biochemical transfer of memory¹

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Extract was collected from the brains of 18 discrimination-trained or handled-only rats (donors), and injected intracisternally into an equal number of naive rats (recipients). Mann-Whitney U tests showed no error-to-criterion differences between donors and recipients as a whole, but recipients of extract from handled-only donors made fewer errors than all other recipients ($p < .025$), whereas recipients trained to the discrimination identical to that of their donors made more errors than all other recipients ($p < .01$). Latencies and errors of recipients showed a significant negative correlation ($r = -.46, p < .05$). The major conclusion was that brain extracts may contain both treatment-specific and general facilitating factors.

Concurrent with recent attempts to provide procedures for obtaining independent replications of chemical transfer of training (Golub & McConnell, 1968; Ungar, Galvan, & Clark, 1968), a good deal of attention has been directed toward variables influencing the effect at both the chemical and behavioral levels. Reports of much of this work are contained in a bibliography compiled by Jacobson (1967).

Many transfer studies have shown enhancement of performance in animals injected with brain extract, but a form of negative transfer has also appeared in several

laboratories under various labels, cf. "reversed" transfer (Nissen, Røigaard-Petersen, & Fjerdingsstad, 1965), "inversion" (Rosenblatt & Miller, 1966), "antagonistic transfer" (Rucker & Halstead, 1967), and "reversal" (Ungar, 1967). In these studies there is less learning in animals which receive a "trained" extract than in animals receiving control extract. Consistently similar preliminary results led to an experiment which would shed light on this negative transfer as well as the problem of whether it is specific memory or general activation which is transferred.

Support is given to the argument for specific transfer by numerous studies using a variety of training procedures (e.g., Fjerdingsstad, Nissen, & Røigaard-Petersen, 1965; Rosenblatt & Miller, 1966; Ungar, 1967; and Dyal & Golub, 1968). A relatively difficult simultaneous visual pattern discrimination was used in the present study to provide evidence of specificity of transfer and extend research beyond the simpler learning studied previously.

Some investigators have found positive transfer from control donors receiving the same handling but not the specific training given an experimental group, providing evidence for a general activation factor (Dyal & Golub, 1968). A handling-control group was therefore included in the present study.

METHOD

The discrimination apparatus used in this experiment is a modification of the Thompson "Y" maze (Thompson & Bryant, 1955), using horizontally and vertically striped doors as the cues. The rat traveled from a start box in the leg of the Y through a hinged swinging door to the junction of the

arms, where it was confronted by the striped doors. Upon pushing through the correct door it received a water reward of 1.6 ml in the goal box. Hunter photo-relays (Model 335F) and counters (Model 120A) measured errors and latencies. The box for handled-only control rats had identical grid floor area, wall height, and wall color as the maze.

Subjects were 36 male Long-Evans rats, 70 days of age and weighing between 200 and 250 g. Half of the animals were randomly assigned to the donor group, and half to the recipient group. The donors were divided into three subgroups balanced on the basis of pretraining activity levels. These groups were designated Horizontal (H), Vertical (V), and Handling-Only (HA), according to whether they were trained to obtain reward at the horizontal door, vertical door, or were merely exposed to the handling-control box. In this control box the HA group was yoked to the groups given discrimination training (H and V) on all variables except the actual presentation of the cues.

After the donors reached the criterion of learning, or had completed handling in the control box, chemical extracts from their brains were injected into one of six recipient subgroups as follows (see also Table 1): (1) Two subgroups were designated as "Same" (S) and consisted of animals receiving an extract from the donor group which had been taught the same discrimination as the recipients were to learn. (2) Two "Reversal" (R) subgroups consisted of animals receiving an extract from a donor group which had been taught the discrimination with cues reversed. (3) The "Handling-Control" (HC) group consisted of animals trained on either horizontal or vertical discriminations after receiving an extract from the HA group.

Prior to training, each group was placed on a water-deprivation schedule of approximately 12 min per day ad lib drinking for 9

Table 1
Donor-Recipient Design

Donors	Extract to	Recipients
Horizontally Trained (H) (N = 6)	→	Horizontally Trained [Same (S)] (N = 3)
		Vertically Trained [Reversal (R)] (N = 3)
Vertically Trained (V) (N = 6)	→	Vertically Trained [Same (S)] (N = 3)
		Horizontally Trained [Reversal (R)] (N = 3)
Handled Untrained (HA) (N = 6)	→	Horizontally Trained [Handling Control (HC)] (N = 3)
		Vertically Trained [Handling Control (HC)] (N = 3)

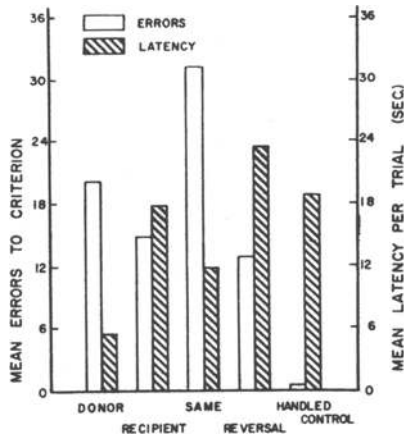


Fig. 1. Mean number of errors to criterion and mean latency in completing a response.

days. During deprivation, the animals were gentled for 2 min per day to reduce stress of subsequent training and handling. Pretraining consisted of 20 rewarded trials, 15 without cues present and 5 with cues present and the incorrect door blocked. Pretraining and training for the recipient groups was identical to that of the trained donors. An error was recorded when an animal entered the incorrect arm of the maze and triggered the photo-relay; position of cues was varied randomly across trials, and a noncorrection procedure was used. The E was ignorant of group membership during training. After 5-10 days all animals reached the criterion of 9 out of 10 correct responses on any one day of training. They were then returned to their cages and kept at the same level of deprivation they had experienced prior to training. One day after all donor rats had reached criterion, they were given five more trials to confirm that the response had not been forgotten.

Subjects were sacrificed with ether immediately after completing the five posttraining trials. Their brains were removed within 3 min to be quick-frozen and stored over dry ice for 24 h. The RNA extraction procedure was slightly modified from that of Babich, Jacobson, Bubash, & Jacobson (1965). The initial centrifugation was done at 15,500 rpm for 43 min at 0 deg C using a Sorval RC-2 centrifuge with a SS34 rotor. The resulting extracts were stored over dry ice for 24 h, at the end of which time the extract from the six animals in each donor group was pooled and dissolved in an amount of bicarbonate solution (.145 M Na⁺ and .005 M K⁺) to bring the total volume to .35 ml. Immediately after pooling of the extracts, intracisternal injections were given under light ether anesthesia, each animal receiving 50 μ l of the solution. The E giving the injections was ignorant of both donor and recipient group membership.

RESULTS

Figure 1 shows the mean number of errors to criterion and average latency for culminating a response. A Kruskal-Wallis analysis of variance showed that the three recipient subgroups (S, R, and HC) were significantly different in errors to criterion [$H(5,5,5) = 8.61$, corrected for ties, and $p < .009$]. Mann-Whitney U test comparisons of errors as presented in Table 2 revealed no significant difference between donors and recipients overall, but comparisons between recipient subgroup errors disclosed important differences. The HC group was clearly the superior one, performing significantly better than the donors ($p = .01$), the combined S and R groups ($p < .025$), and the S group ($p = .004$). Performance of the S group was insignificantly poorer than that of the donors, and significantly poorer than that of the R group ($p = .028$).

Latencies based on time elapsed before leaving the start box (SB), time elapsed after having left the start box in reaching the water cup (WC), and total time elapsed (TT) during a trial (latency shown in Fig. 1), were compared with error scores using the Spearman rank correlation. The SB and TT latencies of recipients showed significant negative correlations with errors (for SB, $r = -.44$, $p < .05$; for TT, $r = -.46$, $p < .05$). Correlation of the WC latency with errors was also negative, but not significantly. Mann-Whitney U tests revealed that recipients as a whole, and each recipient subgroup had significantly longer SB, WC, and TT latencies than the donors, with significances ranging from $p < .001$ [$U(12, 25) = 14$] to $p < .025$ [$U(12, 5) = 9$].

DISCUSSION

The most profound effect appeared when the errors of the rats injected with handled-only extract (HC) were compared with the errors of the trained donors. The superiority of the injected animals' learning performance supports the contention that brain extracts effect a change in the behavior of injected recipients. However, because the HC group received only handling, it is clear

that the transfer effect was not specific in this particular case.

Although it is difficult to make comparisons with studies using different Ss, tasks, and types of extract, the present finding supports that of Dyal & Golub (1968), who obtained results showing improved performance with injection of extract from yoked control rats during tests on bar-pressing and magazine entry (ME). Studies by Essman & Lehrer (1967), using mice in a water maze, and Hartry, Keith-Lee, & Morton (1964), using planaria, also obtained positive transfer in both groups, but Dyal and Golub also found significant superiority in recipients of trained extract over recipients of yoked control extract. Dyal and Golub suggested that the MEs of the yoked control group may have increased as a result of transfer of general activation, whereas the still greater increase in MEs of the recipients of trained extract may have been augmented by transfer of a specific factor.

Since the present study made use of both a discrimination task and a yoked control, strongest support for the Dyal and Golub interpretations would have resulted if the S group (extract from donors trained to same cue) had performed significantly better than the HC group, and if the R group (extract from donors trained to reverse cue) had performed significantly worse. Contrarily, the S group made significantly more errors than the HC group, but the results still offer support for a specificity hypothesis. As the training milieu of the recipients approached that of the donors, recipient performance became poorer. Thus the three extracts had specific effects on the subgroups. The fact that negative transfer was obtained in the S group makes the data more difficult to interpret. In addition to possible transfer of general activation and specific memory, a concept of reactive inhibition may be relevant to the negative transfer effect. The R group may have been subjected to specific negative transfer, which may well have been overcome by transfer of such factors as general activation, cue sensitization, and favorable reactive inhibition. However, in considering the positively or negatively

Table 2
Mann-Whitney U Tests Applied to Errors to Criterion

Comparison ^a and (N)	U	p
HC ^b (5) vs Don (12)	8	.01
Don (12) vs S (5)	14	>.05
R (5) vs Don (12)	22	>.05
HC (5) vs S (5)	0	.004
R (5) vs S (5)	3	.028
HC (5) vs R (5)	7	.155
HC + R (10) vs S (5)	3	<.01
HC (5) vs S + R (10)	7	<.025
HC + S (10) vs R (5)	25	>.05
Rec (15) vs Don (12)	74	>.05

^a First member of each comparison made fewer errors.

^b Don = Donor, Rec = Recipient, S = Same, R = Reversal, HC = Handling-Control

transferring factors to which recipients may be subject, it is difficult to find a weighting of such factors which would result in the obtained ranking of performances among the groups.

Although the possibility of negatively and positively transferring factors is manifest in discrimination tasks, these factors, more subtly present, may account for some failures to demonstrate either transfer effect in other studies. As in the present study, combined negative and positive transfer may result in no overall differences between donors and recipients.

Evidence that any activation effect in this experiment was not of the simple motor sort, but perhaps of a general alerting nature, is found in the latencies, which actually increased in all recipient groups. Furthermore, the significant negative correlation of latency with errors illustrates the importance of multiple measures of the dependent variable and the limited comparability of studies using different measures.

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NOTE

1. The authors gratefully thank Allan L. Jacobson for his insightful comments on this paper.

behavior during a stimulus which precedes it (Leitenberg, 1966).

Although differences in methodology make comparisons and interpretations difficult to integrate at present (cf. Herrnstein, 1955; Leitenberg, 1966; Pliskoff, 1961), the view expressed by Herrnstein (1955) has established a basis for the prediction of various TO effects. In general, operant behavior will be accelerated during a stimulus which precedes TO from a schedule of infrequent reinforcement potential. Operant suppression usually occasions a stimulus which precedes TO from a schedule of frequent reinforcement. A notable exception was the recent report (Leitenberg, Bertsch, & Coughlin, 1968) of response suppression for rats during a stimulus signaling TO from a schedule of infrequent reinforcement potential (VI 7). The Leitenberg et al (1968) study employed the conditioned suppression paradigm and TO was retraction of the response lever from the experimental chamber. The present study employed the same TO technique within the conditioned suppression paradigm, but investigated the effect of TO upon performance during a stimulus which signaled the interruption of a frequent (VI 30-sec) reinforcement maintenance schedule.

METHOD

Six male hooded rats, 120 days of age, were reduced to and maintained at 85% ad lib body weight through the experiment. Individual experimental sessions were preceded by 18-23 h food deprivation. The experimental chamber was a Scientific Prototype rodent test cage equipped with a retractable response lever and enclosed in a sound attenuated box.

All Ss were shaped to lever-press under CRF and gradually shifted to a VI 30-sec reinforcement schedule for 1½-h daily sessions. Ss were then divided into two groups, A and B. On Day 3 of the VI 30-sec training, Ss of both groups were exposed to four 1-min presentations of a 1000-Hz tone, 32 dB above a 50-dB white noise background. Response rates during the tone pre-TO stimulus (P-TOS) and during the preceding 1-min interval, comparison interval (CI), were recorded. The VI 30-sec reinforcement schedule remained in effect during these and all CI and P-TOS periods. Following P-TOS adaptation, Ss of Group A were given the following training for 14 days. Fourteen minutes after S was placed in the experimental chamber the P-TOS was presented for 1 min and terminated coincident with the retraction of the lever from the experimental chamber for a 10-min period. During the 10-min TO ambient illumination was decreased from approximately 10 c/m² to virtual darkness (stimulus change, SC). The TO and SC terminated

Time-out from a short mean-interval variable-interval reinforcement schedule¹

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decrease in ambient illumination.

Within the conditioned suppression paradigm, the lever-press behavior of rats was attenuated in the presence of a tone which preceded a 10-min time-out (lever retraction) from VI 30-sec reinforcement. The degree of response suppression controlled by the pre-time-out stimulus was only slight and did not occur for a second group when time-out was accompanied by a

Positively reinforced operant behavior will be attenuated by a signal which precedes electric shock. First demonstrated by Estes & Skinner (1941), conditioned suppression is often attributed to the incompatibility of "fear" respondents and on-going operant behavior. Time-out (TO) from positive reinforcement may, under certain conditions, have accelerative effects upon