

Biochemical Transfer of Relational Responding (Transposition)

Abstract. Hooded rats were trained to choose the larger of two circles in order to obtain a liquid reward. Recipients of brain extract from these trained donors showed a significant preference for an even larger circle when given a non-reinforced test for several days after the injection of extract. The substrate for relational learning may be biochemically transferred.

The injection of brain homogenates or extracts from trained donor animals into untrained recipient animals modifies recipient behavior so that it resembles that of the trained donors (1). Tasks in which an animal learns a discrete response to a discrete, concrete stimulus have been described, but recipients have seldom (2) been tested in a situation other than that in which the donors had been trained. No one has attempted to induce complex, patterned behavior with brain extracts, nor has this behavioral bioassay method been attempted with anything approaching relational or conceptual behavior. If one assumes that relational or conceptual behavior occurs in subhumans (3), and that such behavior has definite biological substrates, it is not unreasonable to conclude that the substrates that allow a trained animal to perform appropriate responses in a somewhat altered situation might also be transferable and bias the recipient animal in such a way that it, too, might make appropriate responses in a somewhat different environmental context. We are not suggesting that the appropriate behavior itself is transferred, but rather a substrate that makes various kinds of behavior possible or probable, provided some environmental inducement is present at the time of testing.

We chose transposition as one of the simplest relational or conceptual phenomena with which to test our hypothesis. In a transposition experiment, an animal might be trained to respond to the larger of two stimuli; in testing, the animal chooses between the previously larger stimulus and a new, even larger, stimulus. If the animal chooses the latter, it is said to have transposed (that is, to have shown apparently relational responding); if the animal chooses the former, it is said to respond in a concrete way, choosing the physical stimulus that had been reinforced during training. If donors are trained with one set of stimuli representing a relation, can the training be transferred so that the recipients of their brain extracts, given different stimuli that reflect the same relationship, respond appropriately?

Eight 80-day-old male Long-Evans

hooded rats (Blue Spruce Farms) were deprived of food (until they reached 85 percent of their initial body weight) and then trained in a Y-maze for a reward of water enriched with protein and vitamins. The saccharin in the vitamin (Vy-Daylin) solution produced a highly palatable reinforcer. Concentrated protein powder (80 percent amino acid) provided each animal with proteins above its daily requirements, without satiation. Eight control donors remained in their home cages and were given equivalent amounts of the reward solution.

The apparatus consisted of an unpainted wooden Y-maze with a hinged clear Plexiglas top (4). The discrimination stimuli were white cardboard circles glued onto black baffles that were staggered 10 cm from each other on opposite sides of the arm so that the animals had to run between the baffles and behind the second baffle to reach the goal. The goal cup contained 0.7 ml of the reward solution. The stimuli were glued on both sides of both baffles. One arm contained white circles with diameters of 3.2 cm while the other arm contained white circles with diameters of 7 cm. The reward was always behind the larger circles, but the arm position of the baffles was varied randomly to prevent the animals from responding merely to position. Each of the eight donors received ten trials of discrimination training, without correc-

tion, each day for 12 days. This training produced a 90 percent or greater correct response rate for the last 2 days of training for all animals. Twenty-one hours after the last training trial, the animals were exposed to ether for 3 minutes, and then decapitated. Their brains were removed immediately, placed in powdered Dry Ice until frozen, and then removed and stored at -15°C until they were processed 2 days later. Eight control donors that received no training were also killed, and their brains were processed in the same manner.

The whole brains were homogenized in six volumes of saline solution (0.85 percent NaCl) for 3 minutes in a glass homogenizing tube, with a motor-driven Teflon pestle. This mixture was then stirred magnetically for 4 hours at 4°C , and then centrifuged for 1 hour at $40,000g$ at 4°C . The clear supernatant was then lyophilized, and the resulting dry powder was mixed with enough $10^{-3}M$ NaCl solution so that an injection of 0.7 ml (per recipient rat) would contain the equivalent of material from 1.3 donor brains.

Twelve recipients (80-day-old male Long-Evans hooded rats) were deprived of food as described above for the donors, and used in the transposition testing phase of the experiment. Six recipients (experimental animals) received extracts from the brains of trained donors, while six recipients (control animals) received extracts from the brains of untrained animals. Each animal was given a 0.7-ml intraperitoneal injection of the appropriate extract. The experimenter was unaware of the group to which these animals belonged while testing them in the same Y-maze without reinforcement. However, the baffles now contained transposition stimuli that were circles with diameters of 7 cm and 12 cm. In the learning phase, the donors had been reinforced for choosing the 7-cm circle, because it was the larger circle. Therefore, in the transposition testing phase, a response to the 12-cm circle was considered to be a correct response because it was now the larger circle. Transposition testing consisted of five nonrewarded trials per day at 12, 24, 48, 72, 96, and 120 hours after injection. The number of trials per day was reduced in order to retard the extinction effect brought about by nonreinforced trials.

Three types of scores were recorded, and each was weighted differently. Standing in front of one of the maze

Table 1. Comparison of composite and complete difference scores for experimental (E) and control (C) recipients at various times after injection. N.S., nonsignificant difference.

Time (hours)	Difference scores (means)		Probability,* C vs. E
	C	E	
<i>Composite scores</i>			
Pretest	- 0.11	0	N.S.
24	- 0.77	+ 6.30	$P < .001$
48	+ 0.44	+ 5.80	$P < .001$
72	- 0.77	+ 2.60	$P < .05$
<i>Complete scores</i>			
Pretest	- 0.11	- 0.11	N.S.
24	0	+ 1.20	$P = .05$
48	+ 0.11	+ 1.00	N.S.
72	- 0.22	0	N.S.

* Determined by the Mann-Whitney U test.

arms (with nose within 5 cm of the arm) was scored as 1 point, having head and forepaws inside the arm was scored as 2, and a complete response (running behind the second baffle) was scored as 3 points. This scoring system provided a more complete record of a response tendency. Since testing was done without reinforcement, fewer complete, but many more partial (1- and 2-point) responses, were made. On each trial, the animal was removed from the apparatus after making either a complete correct or a complete incorrect response, or after 1 minute had elapsed, whichever occurred sooner. Experimental and control groups were randomly constituted, and then tested for circle size preference; they were found not to differ with respect to the test before injection. A recipient's circle preference was measured by calculating two kinds of difference scores: a composite score that included both partial (1- and 2-point) and complete (3-point) responses, and a complete score that included only full (3-point) responses. In each case the difference score was calculated by subtracting the number of weighted "smaller than" responses from the "larger than" responses.

At every test period after the injection, recipients of brain extracts from trained donors made more responses to the larger test stimuli than did the recipients of control extract (Fig. 1). The difference in composite scores between the experimental and control recipients was significant at 48 and 72 hours after injection (Mann-Whitney $U = 5.5$ and 3 , $P < .03$ and $< .008$, for six subjects each). Differences in complete responses were also significant at the 48- and 72-hour tests ($U = 6.5$ and 5 , $P < .047$ and $P = .021$). For all other test periods, both complete and composite scores indicated a preference for the larger circles. The performance of the control recipients was never significantly different from chance (50 percent preference, zero difference score).

Following nonreinforced transposition testing, all recipients were given 3 days of rest, and then a nonreinforced pretest (five trials) with the original stimulus circles (diameters, 3.2 and 7 cm). The controls made a few more correct responses, but this difference was not significant. It would thus appear that all the transfer effect, as measured by nonreinforced testing, was gone. However, we felt that there might still be some residual effect that

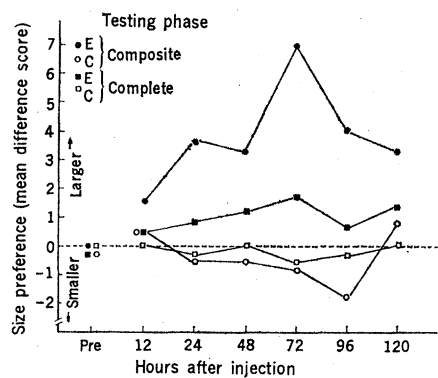


Fig. 1. Mean difference scores for experimental (E) and control (C) recipients for a test given before injection of extract, and for six nonreinforced transposition tests given after injection.

might facilitate learning, and that could be detected by reinforced training trials. Therefore, the animals were given ten reinforced training trials per day for 10 days. By using the original stimuli, we could compare the performance of the experimental group directly to the control animals, and indirectly to the donor group that was trained on the same stimuli. This learning phase was begun 220 hours after injection. The extract from brains from trained donors was able not only to produce a "transfer of transposition" but was able also to facilitate learning of the original task (Fig. 2). The experimental animals made more correct responses (both complete and composite) than did the control animals on every day of the learning phase.

Difference scores for complete approaches were calculated and these scores were significantly greater for experimental animals on days 2, 3, 5, 6, 7, 8, and 9 (Mann-Whitney $U = 5.5$, 6 , 2.5 , 1 , 4.5 , 3 , and 5 ; $P < .032$, $P =$

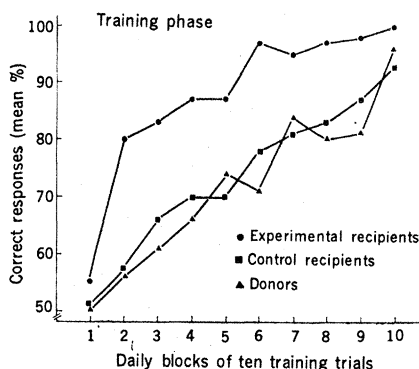


Fig. 2. Comparison of correct responses (mean percent) on daily reinforced training sessions of the original discrimination problem, for donors, experimental recipients, and control recipients.

$.032$, $P < .008$, $P = .002$, $P < .021$, $P = .008$, and $P = .021$; for six subjects each). Differences between groups at all other days indicated a preference of the animals for the larger circles. Composite difference scores closely paralleled complete scores, but were, in general, even more significant; they were significant for days 2, 3, 5, 6, 7, 8, 9, and 10. At no point did the scores of the control recipients differ significantly from those of the original donor animals; this was true for both complete and composite scores. The superiority of the experimental recipients relative to the donors cannot be accounted for in terms of increased familiarity with the apparatus, as the control recipients, which shared this familiarity, did not differ from the donors.

Experiment 2 was a systematic replication of the first part of experiment 1 except that the sample size was larger and there were fewer days of training. Twelve 80-day-old male Long-Evans hooded rat donors were trained for 10 days on the original pair of stimuli (3.2- and 7-cm circles) for the reward of water enriched with protein and vitamins. This training produced an 80 percent or better response rate during the last 2 days of training. These experimental animals were killed 21 hours after the last training trial, and their brains were removed and processed. Twelve control animals that received no training were also killed at the same time, and their brains were removed and processed.

Eighteen recipient animals were randomly assigned to two groups, and were then pretested on the test stimuli (7- and 12-cm circles). No difference in preference was found. Nine experimental and nine control animals were then given an intraperitoneal injection (0.7 ml) of the appropriate brain extract. Recipients were tested without reinforcement at 24, 48, and 72 hours after injection. Testing was done by an experimenter who was unaware of the group to which the animals belonged. The recipients of brain extract from trained donors transposed significantly more than did control recipients at 24, 48, and 72 hours after injection, as judged by the composite difference score (Table 1). Recipients of the extract from trained donors were significantly superior to controls on the 24-hour transposition test, as judged by the complete difference score.

The behavior of these experimental recipients on the transposition test trials

was modified so that appropriate transposition behavior was seen after injections of brain extracts from donors that had been given relational training, even when these donors had never experienced the newer (correct) transposition test stimulus. Brain extracts from untrained donors did not alter circle preference in any way, and this preference was remarkably close to the chance, 50 percent, choice of each test stimulus.

The second phase of experiment 1 indicated that extracts significantly modified behavior under nonreinforced test conditions for 72 hours, but under reinforced conditions even at 248 hours after injection; a large increase in the performance of the experimental recipients could be seen with only 2 days of reinforced training on the donors' original discrimination problem. This suggests that extracts may facilitate learning of a task long after any effect of these extracts can be detected with nonreinforced test trials.

Finally, the effects in these experiments were strong and enduring. This could have been due to (i) optimization of behavioral and biochemical parameters; (ii) the high incentive value of the protein and vitamin solution used as the reinforcer; (iii) possible nutritional or metabolic effects of the protein and vitamin solution; (iv) the nature of the task, that is, perhaps relational or conceptual tasks produce especially strong and persistent learning and memory (as they certainly seem to do in studies of human memory processes); or (v) various combinations of the foregoing factors.

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5. We thank R. Hoffman for his assistance.

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Evaporation Retardation by Monolayers

Wu (1) has conjectured that "the retardation of evaporation due to monolayers in the field under turbulent wind may actually be due to the wave-damping effects of these layers," rather than to their influence on the passage of water through the liquid-air interface. In fact, it has already been noted (2) that some reduction in evaporation should follow from the changes in air flow associated with wave damping. In light of Wu's comments, however, it seems desirable to stress that the effect is not "significant enough to constitute the major mechanism responsible for evaporation retardation by monolayers in the field" (1).

At Lake Hefner, near Oklahoma City (3), it was found that evaporation rates E' (in centimeters per 3 hours) could be well predicted from the relation

$$E' = 1.214 \times 10^{-3} U_s (e_s - e_8) \quad (1)$$

where U_s (in meters per second) is the wind velocity 8 m above the surface, e_s (in millibars) is the vapor pressure at the surface, and e_8 (in millibars) is the vapor pressure at 8 m. This formula has been found to be widely applicable (4). During the experiments at Lake Hefner it was found also that

$$(U_s/U_*)^2 \approx 3.4 \times 10^{-3}$$

where U_* is the friction velocity (3). Incorporation of this and other conversions gives

$$E = 2.63 \times 10^{-2} U_* (c_s - c_8) \quad (2)$$

where E has the dimensions of kilograms per square meter per second, and c_s and c_8 , the vapor concentrations, are in kilograms per cubic meter.

Now Eq. 2 may be written as

$$\Omega_s = \frac{1}{2.63 \times 10^{-2} U_*} \quad (3)$$

where Ω_s (in seconds per meter) is the resistance to the transfer of water vapor from the surface to a height of 8 m, where it may be assumed that the water vapor is effectively mixed with the atmosphere.

In Wu's analysis (1) the greatest reduction in U_* resulting from wave damping is from about 0.2 to 0.1 m sec⁻¹. If this reduction is applied to Eq. 3, this gives an increase in the aerodynamic resistance Ω_s from about 190 to 380 sec m⁻¹. This is distinctly the maximum increase in Ω_s predictable from Wu's data; all other decreases

in U_* due to smoothing are much less than 0.1 m sec⁻¹. A film spread from mixtures of 1-hexadecanol and 1-octadecanol has a typical resistance of 300 sec m⁻¹ (5). Thus in this extreme instance a film increases the original resistance to evaporation from 190 sec m⁻¹ to about 680 sec m⁻¹. If we ignore thermal compensation (5), this indicates a total reduction in evaporation of about 72 percent. Without the change in Ω_s the total resistance is 490 sec m⁻¹, corresponding to a reduction in evaporation of 61 percent. Altogether, in this most favorable instance the aerodynamic effect is not significant.

Apart from this point, it is by no means certain that the transport coefficient of water vapor is lowered linearly with those friction velocity reductions due solely to smoothing. Studies on momentum transfer (6) to rough and smoothed surfaces show that the surface traction imparted to a water surface by a given wind is independent of the surface roughness. With this division in momentum transfer it is difficult to generalize about mass transfer.

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In a recent report (1) Wu analyzes in detail the effect of wind upon the retardation of evaporation by monolayers and concludes that this retardation, in the field and under turbulent wind, "may actually be due to the wave-damping effects of these layers." Furthermore, he argues that the wave-damping effect operates through a change in the detailed flow pattern of the air near the surface. This last argument is based on the partially implicit