C00-825-35

The following is a revised version of a paper read by the senior author at the annual meeting of the American Association for the Advancement of Science in New York City on the 30th of December, 1967. The research reported in this paper was supported by contract AT(11-1)-825 from the U.S. Atomic Energy Commission, by grants NIH K3 MH 16697 and MH-13151, by a grant from the Michigan Memorial Phoenix Project and by the Mental Health Research Institute at The University of Michigan, Ann Arbor.

MASTER

ATTEMPTS TO TRANSFER APPROACH AND AVOIDANCE
RESPONSES BY RNA INJECTIONS IN RATS¹

CONF-67/208--4

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ABSTRACT

Three different experimental paradigms were used to study the so-called "memory transfer" phenomenon. In the first set of studies, donor rats were trained to approach a cup to get a pellet of food when a buzzer sounded. RNA from the brains of these animals was injected intraperitoneally into untrained animals that were subsequently given unreinforced test trials in the same chamber. Recipients that received RNA from trained donors approached the food cup significantly more often than did recipients that received RNA from untrained control donors or that received hydrolyzed RNA from trained donors. In the second paradigm, food-deprived rats were trained to approach a milk dipper in an operant conditioning chamber in response to a distinct "click" made by the dipper when it was elevated into the chamber. An RNA extract was prepared from the brains of these animals, as well as from the brains of control animals that had received no such training. The RNA was injected into the brains (subarachnoid space) of naive recipients, who were then required to learn to press either of the two bars in the chamber in order to elevate the dipper and get milk. Recipients injected with RNA from trained donors

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typically learned the problem within 5 days. Recipients that received RNA from untrained donors or hydrolyzed RNA from trained donors typically did not learn the problem until after 5 days, if at all. In the third paradigm, donor animals were trained to avoid drinking a saccharin solution by pairing consumption of the saccharin solution with injections of apomorphine hydrochloride. An RNA extract was obtained from the brains of some of the donors and injected into the brains (subarachnoid space) of naive recipients; the homogenized brains of other trained donor animals were put through dialysis, and either the dialysate or the non-dialysable fraction was injected into the peritoneum of naive recipients. Following injection, the consumption of saccharin solution by the recipients was measured. While some statistically significant results were obtained, the magnitude of the "transfer" effect was considerably smaller than in the first or second paradigm.

INTRODUCTION

The first studies suggesting that an inter-animal "transfer of acquired behavioral tendencies" was possible were conducted in this laboratory in 1960 (Humphries & Jacobson, 1961; McConnell, Jacobson, Humphries, 1961; McConnell, 1962). Planarians given classical conditioning training in which light was the conditioning stimulus (CS) and electric shock the unconditional stimulus (UCS) were, following training, chopped in pieces and fed to untrained, cannibalistic flatworms. The latter were then given conditioning trials. The response rates to light (CS) of these experimental animals were, from the first set of training trials, significantly greater than the response rates of control cannibals fed the bodies of untrained victims. In 1962, we were the first to show that a crude RNA mix extracted from the bodies of conditioned planarians and injected into the bodies of untrained flatworms apparently caused an elevation in response rates in the experimental animals, as

compared with the behavior of several groups of control animals injected with a crude RNA mixture extracted from the bodies of various groups of untrained planarians (Zelman et al. , 1963). In recent years, these early experiments have been replicated in several other laboratories under more controlled conditions than obtained in the original research (Fried & Horowitz, 1964; Fuchs et al. , 1966; Jacobson et al. , 1966a, 1966b; John, 1964; Kabat, 1964; McConnell, 1966, 1967a, 1967b; Pickett et al. , 1965; Ragland & Ragland, 1965; Rieke & Shannon, 1964; Westerman, 1963a, 1963b). While there remains some argument as to what it is (behaviorally) that transfers in the case of planarians, it seems relatively clear that the "transfer effect" itself is a valid one, at least in flatworms.

The first experiments suggesting that a similar "transfer effect" could be obtained in higher organisms, such as rats and hamsters, were performed in Czechoslovakia by Reiniš (1965, 1966), in Copenhagen by Fjerdingsstad, Røigaard-Petersen & Nissen (1965), in Houston by Ungar and his associates (Ungar & Ocegüera-Navarro, 1965), and in Los Angeles by Jacobson and his colleagues (Babich et al. , 1965a, 1965b, 1965c; Jacobson et al. , 1965, 1966). Our own work with rats began in 1965 when we attempted to repeat the experiments reported by Jacobson and his colleagues showing that if one extracted an RNA mixture from the brains of rats trained to approach a food cup when a click sounded, and then injected this mix intraperitoneally into untrained animals, the recipients tended to approach the food cup more than did control animals when presented with the click. Our work yielded statistically significant results, and the use of additional control groups indicated that the "transfer molecule" was probably RNA.

In our first experiments, we used a training apparatus built as closely as possible to the one employed by Jacobson and his associates (Babich et al. , 1965a), a masonite box (26 cm³) with a grid floor. In one corner of the box was a metal food

cup into which food pellets could be dropped from a tube outside the box. A buzzer was mounted in the exact center of the bottom of the box, beneath the grid floor. The walls of the box were painted gray; however, an area of 63 cm² around the foodcup was white and constituted the "food cup area." The subjects were 64 male hooded rats (Long-Evans) from Maxfield Farms in Cincinnati. The animals were approximately 60 days old, and weighed approximately 250 grams, when used. Both donor and recipient animals were put on a food deprivation schedule and maintained at approximately 90% body weight. The recipients were 48 hours deprived when tested. Twenty-four of the donors were trained 5 days by hand-shaping to approach the food cup to get a pellet of food whenever the buzzer sounded. By the end of training, all the animals were responding appropriately at least 95% of the time. The 8 untrained donors were handled and deprived in the same fashion as were the trained donors but were not put in the training box. Immediately after their last training session, the donors were dropped into liquid nitrogen and frozen. The whole cerebrum (i.e., the portion of the brain posterior to the olfactory lobes and anterior to the cerebellum) was used for extraction purposes. The brains were individually placed in a cold homogenizer and ground with 10 volumes of phosphate buffer for approximately 2 minutes. An equal volume of 88% phenol was added to the homogenate and the mix stirred for one hour at 2° C. The mixture was then centrifuged at 20,000 x g for 5 minutes at 2° C. The phenol layer was carefully removed and discarded, and an additional half-volume of phenol was added and the mix was stirred for 30 minutes. The aqueous phase was carefully drawn off and centrifuged at 3500 rpm for 15 minutes. The RNA was precipitated by addition of .1 volume of 20% potassium acetate and 2.5 volumes of 95% ethanol. The RNA was separated by centrifuging at 3000 rpm for 15 minutes, washed with ethanol-water (3:1) twice, and then was dissolved in one ml of isotonic saline after the remaining ethanol was evaporated off. This preparation constituted the "unpurified" or "crude" RNA mixture.

Some of the "crude" RNA was then hydrolyzed with ribonuclease. This RNA was dissolved in a solution of (1 mg/ml) ribonuclease (bovine pancreatic, phosphate free and lyophilized) instead of the isotonic saline and was incubated at 37°C for 5 hours. Ten µg/ml bentonite were added to the incubated solution, and the clear supernatant was drawn off after the mixture had been centrifuged at 10,000 x g for one hour. This preparation constituted the hydrolyzed "crude" RNA mixture.

For the preparation of "purified" RNA from both trained and untrained donors, the "crude" precipitated RNA was dissolved in 3 ml of distilled water. To this solution, one volume of 2.5 M K_2HPO_4 , 0.05 volume of 33.3% H_3PO_4 , and one volume of 2-methoxyethanol were added, in that order. The mixture was shaken vigorously and allowed to stand at room temperature for 15 minutes; it was then centrifuged at 3000 rpm for 15 minutes. The top layer (containing most of the RNA) was then carefully drawn off. A small amount of the top layer was poured back into the lower layer and this mixture was shaken vigorously and then allowed to stand for 5 to 10 minutes so that separation into two layers again occurred. This procedure was repeated 3 times. Then the combined top layers were centrifuged at 10,000 x g for one hour to remove any remaining 2-methoxyethanol. The RNA was precipitated from the clear supernatant obtained by this centrifugation. RNA yields were determined from the optical density at 260 mµ with 10 mm cell length. For the "crude" RNA preparation, the average yield was .96 mg per gram of brain tissue; the yield was .62 mg/gm for the purified" RNA from both trained and untrained donors.

Four groups of recipients were used. The 9 animals in Group I were injected with the "purified" trained RNA, the 8 animals in Group II received "crude" trained RNA, the 7 animals in Group III received hydrolyzed "crude" trained RNA, while the 8 animals in Group IV received "purified" untrained RNA. Each recipient was injected intraperitoneally (without anaesthetic) with an amount of the appropriate preparation that represented the RNA from one donor brain. The animals were

assigned code numbers, and injection and testing were performed "blind."

Six test sessions of 5 trials each were given. The first such session occurred 6 hours after injection; additional test sessions were given once each 24 hours thereafter for five days. Each animal thus received a total of 30 test trials. A test trial was not begun unless (1) the animal was at least one body length distant from the food cup, (2) unless the animal was turned at least 90° away from the cup, and (3) unless the animal was not making any gross locomotor movements. At the start of a test trial, the buzzer was sounded and if any part of the animal's body crossed into the white "food cup area" within 5 seconds, the response was scored as an "approach." No food or other reward was given to any of the recipients while they were in the test chamber. Two observers recorded all responses independently.

One week after the recipients had been given the 30 unreinforced test trials, each animal was given 100 reinforced trials during which a pellet of food was dropped into the food cup immediately after the buzzer sounded. If the animal reached the food cup in 5 seconds after the buzzer sounded, the response was considered "correct."

The results of this first set of experiments are shown in Tables I, II and III. As the data in Table I indicate, the animals that received either "crude" or "purified" RNA from the brains of trained donors were significantly superior to the animals in both control groups in terms of the number of approaches they made to the food cup during the 30 unreinforced test trials. The data in Table II show that the animals receiving the "purified" untrained RNA made significantly fewer errors during the subsequent 100 reinforced trials than did the animals in any other group, while the animals receiving the hydrolyzed "crude" trained RNA were significantly the poorest group. If we look at the last trial on which each animal failed to respond correctly during these 100 reinforced trials (Table III), we find much the same picture.

I	II	III	IV
Purified Trained RNA	Crude Trained RNA	Hydrolyzed Crude Trained RNA	Purified Untrained RNA
Ss			
1	1	0	1
3	2	0	1
3	3	2	2
5	3	2	3
5	4	3	3
5	5	3	4
6	5	3	4
6	7		4
7			
MEAN 4.6	3.8	1.9	2.8

By the Mann-Whitney U-Test,

Group I is significantly superior to Groups III and IV

Group II is significantly superior to Group III

TABLE I. Number of approaches to the food cup for four groups of rats injected with various substances.

	I	II	III	IV
	Purified Trained RNA	Crude Trained RNA	Hydrolyzed Crude Trained RNA	Purified Untrained RNA
MEAN	4.0	3.6	5.1	2.0

By the Mann-Whitney U-Test,

Group IV is significantly different from all three other groups.

Group II approaches significant difference from Group III ($p = .076$)

TABLE II. Number of incorrect responses out of 100 reinforced trials for four groups of rats injected with various substances.

	I	II	III	IV
	Purified Trained RNA	Crude Trained RNA	Hydrolyzed Crude Trained RNA	Purified Untrained RNA
MEAN	36	27.9	46	7.7
MEDIAN	31	27.5	44	6

By the Mann-Whitney U-Test,

Group IV differs significantly from all other Groups.

Group II differs from Group III at the $p = .168$ level of confidence.

TABLE III. The last trial on which each animal failed to respond correctly during 100 reinforced trials for four groups of rats injected with various substances.

These data suggest to us that one must measure several different "transfer" effects: There seems to be a "non-specific", excitatory, perhaps pharmacological effect on performance from injecting any kind of an RNA mixture into untrained animals. This non-specific effect shows up during reinforced training trials, but is not apparent during unreinforced test trials. In addition, it would seem that one can obtain both facilitatory and inhibitory "transfer" effects from injecting material from trained brains into recipients. The facilitory effect shows up most clearly during unreinforced trials, but to some extent also during subsequent reinforced trials, while the inhibitory effect shows up most clearly during the reinforced trials.

In point of fact, these findings should not be too surprising. For decades now, learning theorists have emphasized that in any learning situation, the animal surely acquires both approach and avoidance tendencies whether the reinforcement used to control the animal's behavior is positive or negative. Thus the animal's behavior is seen as reflecting some kind of "algebraic sum" of several different excitatory and inhibitory processes. In our opinion, the outcome of any "transfer" experiment hinges on the balance obtained among several different "transfer" factors, at least one of which is inhibitory. We believe it likely that one reason that several laboratories have been unable to replicate the many successful "transfer" experiments now in print stems from their use of procedures that maximize the "transfer" of inhibitory factor(s) rather than excitatory or facilitatory factors.

OPERANT CONDITIONING STUDIES

Although we were successful in replicating the work of Jacobson and his colleagues in our first experiments, several considerations prompted us to switch to a different experimental paradigm. To begin with, the Jacobson procedure involved the use of observers who watched the animal and merely recorded whether or not it moved to the food cup within 5 seconds after the buzzer had sounded. While we obtained

high inter-judge reliability in these studies (96%), we believed that use of a procedure in which the recipient's responses were automatically recorded might free us from any lingering suspicion that "experimenter bias" was somehow affecting the results of our experiment. Then too we wished to find a paradigm that would let us determine what experimental conditions would maximize the facilitatory factors and minimize the inhibitory factors described above. The use of an operant conditioning chamber (Skinner box) and a specific experimental technique called "magazine training" seemed to fulfill our requirements best. The next eight studies were then begun using this technique.

In the standard operant conditioning apparatus, the lever or bar that the rat must depress is always located near the food cup that delivers the positive reinforcement the animal gets when it presses the bar. In general, the further away the food cup is from the lever, the more difficult it is for the animal to associate pressing the bar with the appearance of the food. If the cup is directly opposite the lever in a fairly large chamber, most rats learn the problem fairly slowly. One may make the task even more difficult by substituting a dipper arrangement of some kind for the food cup. Usually, when the animal presses the bar, a pellet of food drops into the cup and remains there until the animal finds it; the experimenter has no way of removing the pellet once it has been dropped into the cup. The dipper, which typically contains a liquid reinforcer such as sweetened milk, can be elevated into the chamber automatically, but if the animal does not find and drink the milk within a period of a few seconds (the exact time being specified by the experimenter), the dipper is automatically withdrawn until the animal presses the bar again. The solenoid operating the dipper typically makes a loud and rather distinct "click" noise as it elevates the dipper, a fact that makes most rats avoid the vicinity of the dipper at least at the beginning of training. Under these conditions, a great many rats fail to learn to bar press since they must somehow learn to rush from one side of the chamber to the other in

order to claim the reward.

If one wishes to teach a rat to bar-press under these conditions, one typically must break the training up in two parts. At the beginning of the experiment, the rat is put in the chamber and the machinery set so that the lever is non-functional and the dipper is automatically elevated into the chamber at irregular intervals. The rat soon learns to sit by the dipper and wait for its daily ration of "manna from heaven" to appear. This first part of the procedure is called "magazine training," because what the animal learns to do is to approach the food magazine on signal. Once the animal knows the significance of the dipper, and associates the loud "click" with the presence of a reward, the second stage of training begins. In the second part of training, the dipper is set to operate only when the animal presses the bar. A rat beginning this part of training typically sits quietly by the dipper for a while, waiting for it to operate automatically. When, after a few minutes, no food appears, the rat often begins wandering about the chamber and, presumably by accident, presses on the lever. The dipper elevates into the chamber, and the loud "click" made by the apparatus reminds the rat that food is now present. The second stage of training usually proceeds very rapidly, and the animal learns to press the bar to get the milk without further difficulty. However, if the "magazine training" sessions are omitted, the rat learns the task slowly if at all.

In the present series of experiments, the donor animals were always trained to go to a dipper to obtain milk when a click sounded; the recipients were forced to learn to press a bar in order to activate the dipper, but the recipients were never given the all-important first-stage "magazine training." It was our belief that if the tendency to approach the milk dipper when the click sounded could be transferred chemically, the recipients that were injected with "trained" brain materials would learn the bar-press task rapidly, while the control recipients injected with "untrained" brain materials would learn slowly, if at all.

It was our opinion also that injecting the RNA directly into the brains of the recipient animals was more likely to yield success than the intraperitoneal injections used in earlier experiments. We chose the technique of injecting into the subarachnoid space of the forebrain described by Lindberg & Ernster (1950) since these authors present evidence that materials injected by their method diffuse rapidly to different parts of the brain and because the RNA thus injected is presumably "inside" the "blood-brain barrier."

An extraction procedure similar to the one that yielded the "crude" RNA in the first experiments was used throughout this second set of studies. Some modifications were introduced, however: During homogenization of the brains, isotonic saline was used rather than phosphate buffer, the homogenate was stirred with phenol but once, for 30 minutes, then centrifuged at 20,000 x g for 60 minutes; 0.1 vol. MgCl_2 (1 M) and ethanol were added and the RNA was precipitated overnight in a freezer. The RNA was collected after centrifugation at 2500 rpm for 30 minutes and was washed with 75% ethanol three times or more. Yields of RNA were approximately 1.5 milligrams per gram of brain tissue, but measurable amounts of DNA, protein and polysaccharides were present in the mix. Rather than freezing the whole animal in liquid nitrogen and removing the brain afterwards, as we did in the first studies, we decapitated the animals and removed the brain and froze it by placing it in powdered dry ice. Decapitation took place 8 hours after final training of the donors. All experiments save the first one reported below were performed "blind"; in all experiments, however, the data were recorded automatically.

The subjects were male hooded rats of the Long Evans strain (obtained from Maxfield Animal Supply Co., Cincinnati, Ohio, and from Blue Spruce Farm Inc., Altamont, New York) having an average body weight of 250 gm and were 50-60 days old at the beginning of the handling and deprivation schedules. The animals were maintained in group cages and received food (Rockland Diet) and water ad lib. for

5 days. On the sixth day after arrival, each animal was moved to a metal individual cage and handling and deprivation begun. Except as noted below, all animals were brought down to 80% of their body weight and maintained there for the balance of the experiment; the handling schedule was maintained for more than 10 days in the donors before their training began and for more than 20 days in the recipients before pre-injection testing (except for Experiment MT-2a, described below).

Apparatus for Training and Testing

All training (donors) and testing (recipients) was conducted in two identical wooden two-bar operant conditioning chambers (Fig. 1) with a 16 x 14 in. mesh floor and with 7 in. high walls one of which, a sliding door, consisted of a one-way glass. The chambers were housed in separate sound-insulated enclosures covered with fiber glass. The interior walls and floors of the chambers were painted flat black. The ceilings were glass through which the inside of the chambers were illuminated by 14-W cool white bulbs (fluorescent).

The manipulanda, two square bars (1 x 1 x 0.25 in.) protruded, 8 in. apart, into the chambers through vertical slits in one of the walls 2 in. above the floor. A vertical force on each bar of approximately 24 gm. triggered a solenoid that raised a metal dipper which delivered 0.08 ml of milk through the hole in the bottom of the dipper area. A cavity was made 2 in. above the floor so as to form the dipper area (2.5 in. in diameter, 1.25 in. deep) in the wall opposite to the one in which the bars were set. A solenoid, placed behind the dipper, generated a distinct click (which was much louder than the usual clicks in conventional Skinner boxes) and lifted up the dipper through the hole. A milk dish which was placed directly under the dipper area was sufficiently large to contain enough milk for 180 continuous reinforcements and deep enough to prevent the recipients from stealing the milk by stretching their fore-legs through the hole.

FIGURE 1

Fig. 1. Apparatus used in the magazine training and the pre- and post-injection tests. (S = solenoid; D = dipper; D.A. = dipper area; B = bar.)

In our later experiments, a photo-cell device was attached to the dipper area so as to record the number of times the animal stuck any part of its body inside the dipper area.

A jet of air was introduced into the sound-insulated enclosure through a small hole (0.5 in. in diameter) made in an upper part of a wall. Another small hole was connected with a vacuum device. These two attachments provided cool fresh air, constant white noise, and removed warm stale air from the inside of the chamber throughout the experiment. The temperature inside the chambers was kept at approximately 70-75° F.

The milk dish, dipper and the dipper area (inside and outside of the chamber), bars and bar area were cleaned and fresh sawdust was placed in the tray under the mesh floor of the chamber whenever necessary (usually every 4 hours). Condensed milk to which was added 10% sucrose was used as a reinforcer.

During the donor training, both bars were disconnected from the dipper mechanism so that a bar-press did not activate the click noise or dipper. During the recipient testing, the bars were connected to the dipper mechanism so that each bar-press activated the dipper (which made a "click" as it elevated into place).

Training and testing of both donors and recipients proceeded automatically throughout all experiments. The animals were put into the chamber when it was dark. Immediately after the door was closed, the house light was turned on and the first dipper presentation occurred (in donor training).

Training of Donors

Each donor animal was trained to approach the dipper under a variable interval (VI-45 seconds) schedule. The animal was given 60 trials per session for 5 days (plus one extra session at the start for hand-shaping). During the first two days, the dipper was elevated for 20 seconds during each training trial. On day 3, the

time was cut to 10 seconds for each presentation, to 7.5 seconds on day 4, and to 5 seconds on the final day of training. The donors were maintained at 80% body weight and were trained when 23 hours deprived. After each training session, the animal was weighed and given enough Rockland Diet to bring it back up to 80% of body weight.

During the preliminary shaping session, the dipper (with milk in it) was held up by E's hand from the beginning of the session until the animal found it and consumed the milk. The animal was given the milk 10 times in this manner whenever it approached the dipper. No click was sounded at this stage of shaping. Next, the click was sounded while the animal was sticking its nose inside the dipper area deep enough so that it could very easily find the dipper with milk immediately after the click in spite of any emotional response the animal might evince to the onset of the click. This click-dipper presentation was repeated successively more than 10 times until the rat consumed the milk within 20 seconds 10 times in succession. Then, the click-dipper presentation was withheld until the animal moved away from the dipper area an inch or two. After the click-dipper presentation was repeated for 5-10 times under this condition, the inter-trial interval was gradually increased up to 60 seconds, and then it was gradually shifted to the VI-45 seconds dipper-presentation schedule. Total number of dipper presentations per day during this and subsequent days was approximately 60. Almost all animals established a dipper-approach response during this shaping session.

Untrained donor animals were treated similarly to the trained donors outside of the chamber situation. These animals had been handled, deprived and maintained at 80% body weight throughout. During the last 6 days prior to sacrifice, untrained donors were given extra handling equivalent in time to the trained donors and weighed, fed, and maintained at 80% body weight. The untrained donors did not experience the chamber situation, however, and were not fed milk.

Trained and untrained donors were sacrificed in pairs.

Injection Procedure

Fourteen hours prior to its first post-injection test session, the recipient animal was injected with RNA from a trained or untrained donor. The injection was made into the space between the olfactory bulbs so as to facilitate rapid diffusion of the injected materials into the subarachnoid space of the brain.

Prior to injection of the RNA, each animal was anaesthetized with an intra-peritoneal injection of sodium-pentobarbital in a manner that minimized emotional disturbance. A cut was made along the line of suture sagittalis between the animal's eye balls with a sharp knife, the remaining muscles were pushed aside and the skull was exposed. The incision area was thoroughly sterilized (with zephiran-chloride: ca. 0.2%). A dental drill with a one-mm-wide tip was used to make a hole in the suture sagittalis directly between the eye balls (i.e., above the space between the olfactory bulbs). The drill tip was placed on the suture sagittalis so as to form an angle of 45° with the frontal plane (Lindberg & Ernster, 1950).

The hole was drilled gently only about 2/3 of the way through the skull, to prevent mechanical leakage of the RNA solution after injection. A 27-gauge needle was placed in the hole and was rotated several times under moderate pressure for 10-15 seconds to pierce slightly the remaining bone. This needle was then removed, and a 250 µl tuberculin syringe with a 30-gauge 1/4-inch-long needle was used to inject the RNA. This syringe had been kept in a refrigerator and was loaded with 200 µl of cool RNA solution immediately before the injection. The tip of the needle was introduced by rotation under minimum pressure. The needle was kept at a 45° angle with the frontal plane, 2 mm deep from the outside surface of the skull. Two hundred µl of the cool RNA solution were injected into each recipient over a 5 minute period.

The needle was withdrawn slowly after the injection was made so that leakage of the injected solution was minimized. After complete withdrawal of the needle, the incision was sterilized and the epidermal tissues were dried slightly with cotton for 30 seconds. The incision was closed without suturing by pressing the skin edges together with moderate pressure from a pair of forceps.

Testing Procedure

(A) Pre-Injection Test. Each recipient animal was tested 10 hours before injection (i. e. , 14 hours before the first session of the post-injection test) under the same experimental conditions as obtained during the post-injection test. Animals were then assigned to experimental or control groups according to baseline response tendencies during the pre-injection test.

The pre-injection test lasted for 60 minutes, during which time a bar-press elevated the dipper for 20 seconds. It was typically the case that few if any animals actually obtained the milk during the pre-injection test, however.

In several of our early experiments in this series, we found a significant correlation between the number of bar-presses the rat made during pre-injection testing (regardless of whether or not it obtained reinforcement) and the probability that it would eventually learn to press the bar to obtain the milk. In general, it appears that animals with a high pre-injection response rate (more than 10 bar-presses during the hour's test) will learn the task no matter what one injects them with, while animals with a very low response rate (0-2 bar-presses) fail to learn no matter what one injects them with. The reasons for this fact are obvious and have nothing to do with "memory transfer" per se. Rats with a very high initial response rate are highly active animals, hence much more likely to explore the box immediately after a bar press, hence more likely to discover the connection between the milk and pressing the bar. Rats that are (for some reason) low responders tend to be frightened animals

that cower in the corner of the box and do not explore; this high emotionality appears to overwhelm the effects of injection of trained material.

It is important to note, then, that the "transfer effect" can best be demonstrated in this paradigm when one uses animals with a "medium" pre-injection response rate (2-10 bar-presses) and when one makes sure that experimental and control groups are balanced in terms of this factor.

It has been our experience that the average pre-injection response rate varies widely in animals obtained from the same supplier at different times. We found it expedient to order at least twice as many recipient animals as we planned to use so that we could discard the very high and low responders prior to injection.

(B) Post-Injection Test. Following the pre-injection test, each animal was weighed but was not fed; thus when it was given its first post-injection test 14 hours after the injection, the animal had been food-deprived for 48 hours. The post-injection testing procedure was the same as was used for the pre-injection test. The dipper was elevated for 20 seconds after each bar-press, the inter-session interval was 24 hours, the animals were food-deprived for 23 hours (after the first session) and were maintained at 80% of body weight across sessions.

Criterion for learning was specified as being 60 "correct" bar-press responses within 30 minutes. "Correct" bar-press responses were defined as those responses that actually actuated the dipper. Any responses the animal made during the 20 second dipper-holding period (after a "correct" response) were thus not counted. The animals were tested for 10 sessions, or until they met the criterion.

Results

Experiment MT-1. The results of this experiment are shown in Table IV. Although the data were recorded automatically, the experiment was not run "blind." All of the rats injected with "trained" RNA learned within 9 days; none of the rats

Day	Rat #	Injected With RNA From Brains of Trained Donors					Injected With RNA From Brains of Untrained Donors				
		1	2	5	7	10	3	4	6	8	9
1		11	6	26	9	2	2	2	4	2	0
2		55	13	55	2	46	0	3	0	0	1
3		60	60	60	5	60	3	3	2	1	4
4		60*	34	60	3	60	1	1	0	1	6
5			60*	60*	5	60*	1	4	2	2	4
6					28		1	4	1	0	0
7					60		1	4	2	0	0
8					60		0	4	2	0	0
9					60*		0	1	8	0	2
10							0	3	6	1	4

*Criterion is 60 responses in less than 30 minutes.

TABLE IV. Number of bar-press responses during each one-hour daily test session for naive rats injected with RNA from trained and untrained donors (Experiment MT-1).

injected with RNA from untrained donors learned within 10 days. Rat #7 in the Experimental Group was in poor health during the first five days following injection. The two groups differ significantly ($p < .01$).

Experiment MT-2. Because Experiment MT-1 was not performed "blind," we attempted an immediate replication using "blind" techniques. In our rush to get the experiment underway, however, we gave the recipients but 3 days' handling prior to injection and did not bring them down to 80% body weight. They were not 48 hours deprived when given their first test, but were maintained on a 24 hour deprivation schedule thereafter. The experiment (MT-2a) was halted after four days' training for obvious reasons, and all the animals were given a week of handling and were brought down to 80% body weight. They were then reinjected with additional "trained" and "untrained" RNA. Since one of the control animals had reached criterion and two others died, two of the animals that were injected with "trained" RNA the first time were injected with "untrained" RNA the second time. After the second injection, the animals were again assigned code numbers and the data gathered "blind" (MT-2b).

The results of Experiment MT-2, shown in Table V, strongly suggest that the recipients must be well-gentled and deprived if the "transfer" phenomenon is to be demonstrated in a food-approach situation, a fact first pointed out by Reiniš (1966). The fact that animals #14 and #18, which received "trained" RNA on the first injection and "untrained" RNA the second, did not reach criterion rapidly suggests that there were few if any "lingering" effects of the first injection still present by the time the second injection was made. During the second part of Experiment MT-2, the experimental animals were significantly superior to the controls ($p < .01$).

Day	Rat #	Injected with RNA From Brains of Trained Donors									Injected with RNA From Brains of Untrained Donors					
		1	3	6	11	13	14	18	19	20	2	4	10	15	16	17
1		12	7	4	7	10	3	3	5	3	3	3	6	5	6	41
2		3	2	3	1	5	0	1	6	0	7	2	3	4	3	60
3		3	2	0	0	2	2	1	2	1	1	0	1	9	3	60*
4		7	3	2	12	1	1	3	6	3	2	2	0		5	

Day	Rat#	Re-Injected with RNA From Brains of Trained Donors					Re-Injected with RNA From Brains of Untrained Donors				
		1	3	11	13	19	4	10	14 ^a	16	18 ^a
1		1	60	60	60*	60	7	3	1	2	4
2		9	60*	60*		60*	13	0	2	1	3
3		60					20	0	1	2	1
4		60*					5	0	1	5	7
5							8	0	1	3	4
6							15	0	0	2	2
7							60	-----			
8							60*				

*Criterion is 60 responses in less than 30 minutes.

^aNote that rats #14 & #18 were injected with mix from Trained Donors the first time but with mix from Untrained Donors the second.

TABLE V. Number of bar-press responses during each one-hour daily test session for naive rats injected and then reinjected with RNA from trained and untrained donors (Experiment MT-2a and 2b).

Experiment MT-3. The first two experiments suggested strongly that some kind of "transfer" effect could be obtained under the conditions we had employed, but the results of these two studies could easily be attributed to some kind of non-specific, pharmacological agent in the RNA mix. For example, one possible explanation would be that the "trained" RNA contained some kind of "general activator" substance, and that the experimental recipients were therefore more active than the control animals, hence more likely to learn the bar-press task. Also, we had no assurance that, even if we had discovered a genuine "transfer" of acquired behavioral tendencies, RNA was the molecule that mediated the "transfer." Experiment MT-3 was designed to test both these possibilities.

Donor animals were trained as before. Prior to injection, however, the recipients were given two hours of testing. During the first half-hour, each recipient was put in a specially designed activity chamber that had the same internal dimensions as the operant test chamber. Rather than having two bars at one side and a milk dipper at the other, however, the activity chamber had built into its wooden floor two metallic plates measuring 3.5 x 5 cm. The plates were on opposite sides of the chamber. During all tests in the activity chamber, we measured the total number of times that the animal pressed on either plate, as well as the number of times that it pressed both plates within 20 seconds. Our belief was that these two measures would tell us something about the gross activity levels of the recipients, as well as whether any of them showed a tendency to move rapidly from one side of the chamber to the other (as would be the case if the animals learned during the post-injection tests in the operant chamber).

Following the first 30 minutes in the activity chamber, each recipient was put for an hour into the operant conditioning apparatus for its pre-injection test. Then the animal was given a second 30-minute period in the activity chamber. The animals were then divided into four groups that scored roughly the same number of

bar-presses during the pre-injection test. Some 14 hours after the conclusion of the pre-injection tests, the animals were injected with one of four different solutions: Group A received RNA from trained donors, Group B was injected with hydrolyzed RNA from the brains of trained donors, Group C received RNA from the brains of untrained donors, while Group D received hydrolyzed RNA from the brains of untrained donors. Hydrolysis of the RNA was accomplished as it had been during our first series of experiments, described above.

About 14 hours after injection, the entire pre-injection series of tests was given again to each animal; about 23 hours later, this procedure was repeated. Thereafter, the activity measures were dropped and only the daily training session was given. At the end of the fifth day, all of the animals in Group A, but none of the animals in any other group, had reached criterion, so the training was terminated.

The bar-press data for experiment MT-3 are shown in Table VI. Only the animals in Group A, which received "trained" but unhydrolyzed RNA, reached criterion in 5 days. Since the animals in Group B, which received hydrolyzed "trained" RNA, were if anything somewhat inferior to all other groups, we have tentative evidence that the "transfer factor" in this situation is indeed RNA.

There were no significant differences between the four groups in terms of activity levels before or after injection. The highly significant differences between Group A and the other three groups in bar-press activity following injection do not seem to be the result of any non-specific, pharmacological activator.

Experiment MT-4. An inspection of the data from Experiment MT-3 suggested to us that, within any one of the four groups, there was a correlation between the animals' pre-injection response rates and the speed with which they reached or approached criterion after injection. In Experiment MT-4 we hoped to maximize the differences between experimental and control recipients by selecting only the very

	Group A				Group B				Group C				Group D			
	Injected With RNA From Brains of Trained Donors				Injected With Hydrolyzed RNA From Brains of Trained Donors				Injected With RNA From Brains of Untrained Donors				Injected With Hydrolyzed RNA From Brains of Untrained Donors			
Rat #	14	9	6	2	13	10	17	16	15	11	3	12	1	7	4	5
Pre- Injection Responses	2	2	4	7	1	2	4	8	1	2	6	6	0	3	4	9
Day																
1	5	8	5	4	0	1	0	4	0	2	3	1	0	1	1	0
2	8	7	3	22	1	2	0	5	0	3	0	3	1	0	1	2
3	60	15	3	60	0	0	0	4	0	1	1	6	1	1	3	16
4	60*	60	60	60*	0	0	0	8	1	2	2	47	0	2	3	19
5		60*	60*		0	1	0	24	0	2	1	60	1	44	4	51

*Criterion is 60 responses in less than 30 minutes.

TABLE VI. Number of bar-press responses before injection and during each one-hour daily test session for naive rats injected with hydrolyzed or unhydrolyzed RNA from trained and untrained donors. (Experiment MT-3).

lowest responders on the pre-injection response test.

In order to decrease emotionality in the recipients, they were all given 5 days of pre-injection (but reinforced) testing in the chamber. Seven animals were injected with "trained" RNA, six animals with "untrained" RNA. The two groups were balanced in terms of their responses to the lever on the final pre-injection day of testing. The average pre-injection response rate on the 5th day was less than 2 responses in both groups.

The subjects were all Long Evans hooded rats from Blue Spruce Farm Inc., Altamont, New York; the recipients were younger and smaller than usual since the supplier could not provide us with animals of the weight and age we had used in previous experiments.

The yield of RNA was 1.0 milligrams per gram of brain tissue, somewhat smaller than usual.

No measurable "transfer" effect was recorded in Experiment MT-4. Only one animal (one that received "trained" RNA) reached criterion within 5 days. By the end of 10 days' training, 3 experimental animals and 3 control animals reached criterion, and a fourth experimental animal was close to criterion. Five of these seven animals were the highest responders during the 5 days of pre-injection testing.

The data from Experiment MT-4 suggest that the "transfer" effect is difficult to demonstrate in animals with very low pre-injection response rates since almost none of the animals reach criterion within 5 days no matter with what substance they are injected.

Experiment MT-5. Since Experiment MT-4 failed to yield a "transfer" effect, we believed we should attempt to repeat our original three experiments using the magazine training paradigm.

The animals were from Blue Spruce Farm, Inc., but were of the same size and age as were the animals in the first three studies. Only those animals with pre-injection response rates of 4-14 bar-presses were used. We had hoped to use animals with response rates of 2-10 bar-presses, but there were not enough animals with these response rates.

The results of this experiment, which are shown in Table VII, are encouraging but must be taken with a grain of salt since the two groups were not adequately balanced for pre-injection response rates. However, it is pertinent to note that the fastest learners among the experimental animals were not those with the very highest response rates, and that control rats with response rates greater than many experimental rats failed to learn at all.

Experiment MT-6. Donor training and recipient testing in this experiment were the same as in previous experiments, but the recipients received two injections 24 hours apart. Each injection was the equivalent of the RNA extracted from one-half a cerebrum, hence these animals all received about twice as much RNA as did the animals in the earlier experiments. The animals came from Maxfield Animal Supply Co., and were of the same age and weight as those used in the first experiments. The two groups were well-balanced in terms of their pre-injection test scores (2-11 bar-presses).

The results of Experiment MT-6 appear in Table VIII. The two groups differed significantly on the first day of post-injection training ($p < .05$) and in when they reached criterion. Four of the five animals in the experimental group reached criterion before any of the 5 animals in the control group did. Since the "transfer" effect was not significantly stronger than that found in prior studies, we conclude that a double injection of "trained" RNA does not necessarily yield a greater effect than does a single injection as administered in this situation.

Rat #	Experimental rats						Control rats				
	1	4	5	6	8	11	2	3	7	9	10
Pre-injection session	5	8	14	14	5	11	4	9	6	5	5
Post-injection session											
1	0	32	2	4	33	42	3	6	2	1	1
2	1	60	5	3	60	60	1	2	1	1	0
3	1	60*	60	53	60*	60	4	1	0	2	3
4	0		60*	55		60	5	0	2	1	2
5	2			60*		60*	7	1	1	0	2
6	1						2	1	1	0	2
7	0						4	3	1	2	2
8	0						5	1	1	15	5
9	0						1	1	1	60	60
10	0						1	1	2	60	60*

*Criterion is 60 responses in less than 30 minutes.

TABLE VII. Number of bar-press responses during each one-hour daily test session for naive rats injected with RNA from trained and untrained donors (Experiment MT-5).

Rat #	Injected with RNA From Brains of Trained Donors					Injected with RNA From Brains of Untrained Donors				
	2	5	7	8	9	1	3	4	6	10
Pre- injection	7	10	4	8	4	11	7	6	7	2
Post- injection										
1	23	30	11	22	8	5	7	1	10	6
2	60	60	60	11	2	3	7	3	60	5
3	60*	60*	60*	60	2	13	4	1	60	1
4				60*	2	41	3	6	60	0
5					0	60	4	21	60*	2

*Criterion is 60 responses in less than 30 minutes.

TABLE VIII. Number of bar-press responses during each one-hour daily test session for naive rats injected with RNA from trained and untrained donors (Experiment MT-6).

Experiment MT-7. One of the variables Ungar (1967) reported as influencing the strength of the "transfer" effect was the amount of training given donor animals. In this experiment, we tested the potency of this variable by giving donor animals varying degrees of training prior to sacrifice. One group of donors was given 10 days of magazine training, a second group was given but the usual 5 days we have employed in all previous experiments. Deprivation, handling, extraction, injection and training were all as before. Five animals that were given 10 days' training and 10 animals that were given 5 days' training were used as donors. Six recipients were injected (subarachnoid space) with RNA from the 10-day-trained donors, while 12 animals were injected with RNA from the 5-day-trained donors. The groups were balanced in terms of their pre-injection response scores. Both injection and training of recipients were performed "blind."

The curves showing the average number of bar-press responses per session for the recipients are shown in Figure 2. As can be seen, the animals receiving RNA from the 10-day-trained donors had a significantly higher response rate than did the animals receiving RNA from the 5-day-trained donors.

Experiment MT-8. In an attempt to extend the findings of Experiment MT-7, we gave 5 groups of donor animals different experiences. Group 1D donors received 5 days' magazine training, Group 2D received 10 days' magazine training, Group 3D received 20 days' magazine training, Group 4D donors were not given any training but were housed under normal laboratory conditions, while Group 5D donors were kept under conditions of moderate sensory deprivation for a period of 30 days from age 60-90 days, being housed in a light-proof box that had constant white noise present to mask external sounds. Deprivation, handling, extraction, injection and training were all as above. We did make one additional change in procedure, however: A photocell device was added to the cup containing the milk dipper. Each time an animal

Figure 2

Fig. 2. Number of bar-press responses during each one-hour daily test session for naive rats injected with nucleic acid mix (RNA) from 10 days trained and 5 days trained donors. (Mean of each group).

put its nose (or any other part of its body) into the cup, a relay was broken and a "magazine entry" was recorded automatically. There were 6 animals in Group 1R (that received subarachnoid space injections of RNA extracted from the Group ID donors), 5 recipients in Group 2R, 5 in Group 3R, 6 in Group 4R and 6 in Group 5R. The groups were balanced in terms of their pre-injection response levels. Injection and testing were performed "blind."

The mean number of bar-press responses per session for each of the 5 groups is shown in Figure 3. The Group 2R animals were significantly superior to all other groups for the first two days following injection and from Groups 3R, 4R and 5R for the final three days. The Group 1R animals were no different from Groups 3R, 4R and 5R on the first two days, but were significantly superior to these groups on days 3-5.

It would appear that, as in one case described by Ungar (1967), the relationship between amount of donor training and the strength of the "transfer" effect is U-shaped, with a peak near 10 days' training, a result consistent with our findings in Experiment MT-7. The fact that there were no significant bar-press differences between the animals in Groups 4R and 5R suggests that RNA extracted from the brains of untrained donors does not affect speed of learning a bar-press response in recipients whether or not the donor animals had undergone moderate sensory deprivation prior to sacrifice.

The average number of "magazine entries" recorded by the photocell device on the cup containing the milk dipper are shown in Figure 4. As will be seen, the Group 1 and Group 3 animals showed more magazine entries during the pre-injection test than did the animals in the other three groups (it is difficult, if not impossible, to balance 5 groups of recipients in terms both of their bar-press responses and of their magazine entries prior to injection). Since the Group 2 animals were inferior to Groups 1 and 3 on this measure prior to injection, yet were significantly

FIGURE 3

Fig. 3. Number of bar press responses during each one hour daily session for naive rats injected with nucleic acid mix (RNA) from 20, 10, and 5 days trained, untrained, and sensory deprived donors.

FIGURE 4

Fig. 4. Number of dipper approach responses during each one hour daily test session for naive rats injected with nucleic acid mix (RNA) from 20, 10, 5, days trained, untrained, and sensory deprived donors.

superior to Groups 1 and 3 immediately after injection (in terms of the actual number of bar-press responses made), we may conclude that "magazine entries" are not as meaningful a measure of the "transfer effect" in this situation as are "bar-press responses" following injection. This conclusion is supported by an inspection of the data yielded by individual animals. Almost all rats that eventually learn to press the bar to get milk show a peak number of magazine entries two to three days prior to reaching criterion. As they approach criterion, however, the number of magazine entries drops significantly. Animals that maintain a very high number of magazine entries for several days often do not learn to press the bar at all, but rather spend all their time exploring the food cup area and trying with paw or nose to reach the milk in the reservoir below. The rat that actually learns the problem spends a large proportion of its time shuttling back and forth from the bar to the dipper and in pressing the bar; its magazine entries tend to come only during the period immediately after it has pressed the bar.

Discussion

The "magazine transfer" paradigm has yielded highly significant "transfer" results in our laboratory for some two years now, and we have performed enough parameter studies that we begin to appreciate what the critical factors are that influence the success of these experiments. The factors we believe are critical include the following: (a) the amount of RNA injected should be at least the equivalent of that taken from half a donor brain; (b) the recipients should be well gentled and well habituated prior to injection; (c) the recipients should be well deprived prior to injection; (d) the donors should be given an optimal amount of training prior to sacrifice, and this optimal amount of training must (at the moment at least) be determined empirically in any given training situation; (e) and perhaps most important of all, the pre-injection response tendencies of the recipients must be

measured carefully and taken into account if the transfer effect is to be maximized. Additionally, we recommend that the donor animals be sacrificed within minutes after their last training session in a situation such as the one that we have employed, and that the injections be intra-cranial if RNA is used as the transfer material (McConnell, 1967a).

We have had little success in attempting to use dialyzed materials in the magazine-approach experiments; indeed, we have some tentative evidence that use of dialysate from the brains of animals trained in an operant conditioning chamber causes what appears to be a "negative" transfer effect. In one piece of pilot work (not reported above), we trained donor animals to press the lever in order to activate the dipper (on a fixed ratio schedule of 1 reinforcement for each 50 bar-presses); recipients injected with dialysate from the brains of these trained donors learned significantly more slowly than usual, while animals injected "untrained" dialysate were not retarded in their speed of learning. Such results are consonant with our findings (in our early attempts to replicate the procedure used by Jacobson and his colleagues described above) that intraperitoneal injections of materials from trained brains could significantly retard learning when food reinforcement was given the animals during training, even though a positive "transfer" effect could be demonstrated in the same recipients during unreinforced trials. It is fascinating to speculate that "approach" tendencies may be mediated by large molecules, such as RNA, while "avoidance" tendencies may be mediated by small molecules, such as would be found in a dialysate. The fact that most successful "transfer" studies in which dialysate was used were, in fact, "avoidance" learning paradigms, lends some further credence to this kind of speculation. The "saccharin avoidance" studies to be described below are, in part, an attempt to test this hypothesis.

The magazine training paradigm we have employed in these experiments does seem to yield striking results, although somewhat more sophisticated equipment is

required than has been used in most other laboratories working in this field. We do not as yet know whether or not the "transfer" achieved is stimulus-specific, nor can we guarantee that it is "learning" or "memory" (as opposed to some motivational component) that is being "transferred," but we are relatively sure that the effect is not one that involves a mere change in the recipients' activity level. All in all, the "magazine training" paradigm seems worthy of considerable further study.

ATTEMPTS TO TRANSFER AN AVOIDANCE RESPONSE

In the past three years (1964-1967), there have been reports from several laboratories of successful attempts to transfer acquired behavioral tendencies from one rat or mouse to another, by extracting substances from the brains of trained animals and injecting these extracts into untrained recipients. In almost all cases, the training given to the donor animals in these experiments has been the sort that lasts over many trials and usually over several days; to the best of our knowledge, no one has yet been able to transfer anything like "one trial" learning, although several investigators have made the attempt. Indeed, in both our laboratory and in Georges Ungar's, evidence has been obtained that suggests that the more training one gives the donor animals, at least up to a certain maximum (as we showed above), the stronger the resulting transfer (Ungar, 1967). In some ways, such a finding is rather surprising, for many of the "one trial" learning situations employed by experimental psychologists yield rather strong and long-lasting effects and the "one trial" situation has proved an effective tool in studies of memory consolidation. One might intuitively think that the stronger the learning, and the more easily it comes about, the more likely it is that it could be transferred chemically from one animal to another. (Another charm of a one-trial learning situation, of course, is that it leads to considerable savings of the experimenter's time.) This section of our paper, then, is a progress

report of a new series of experiments we have recently begun in which we attempt to transfer an easily-established conditioned aversion to a novel-tasting food in rats.

In the spring of 1967, Samuel H. Revusky visited the Mental Health Research Institute at The University of Michigan and described some research that he and Frank DeVenuto had performed at the U.S. Army Medical Research Laboratory at Fort Knox, Kentucky. Briefly stated, they trained rats to avoid a saccharin solution by giving x-irradiation to the animals shortly after the animals were given the saccharin solution. After the saccharin aversion had been established, they extracted RNA from the brains of the donor animals using a cold phenol technique, then injected the material sub-cutaneously into 40 recipient animals. Another 40 rats received RNA injections from control donors. Both groups of recipients were then tested under 24 hour liquid deprivation to see if any part of the saccharin aversion had transferred chemically. The results of the study by Revusky and DeVenuto were equivocal, but they did find some statistically significant results in the predicted direction (Revusky & DeVenuto, 1967).

As Revusky and DeVenuto point out, the experimental paradigm they employed is interesting in a great many ways. In the first place, it is a simple matter to train large numbers of donors. Only two or three pairings of the saccharin solution with x-irradiation, or with an injection of a nauseating agent such as apomorphine, are necessary for a very strong and long-lasting aversion to take place. The control donor animals can be given exactly the same experiences as the experimental donors, but if the x-irradiation is paired with tap water rather than with saccharin, the donor animals will actually show an increased consumption of the saccharin solution rather than developing an aversion towards it. We thought that this paradigm had a great deal to commend it, so we set out to replicate the experiment.

The subjects in our first experiment were 18 adult male Sprague-Dawley rats; 6 of these animals were donors, while 12 were recipients. We began by giving

the donor animals 10 days of restricted access to water. During these 10 days, they were allowed to drink all they wanted during a ten-minute period each day, but were not given water at any other time. After these 10 days, the saccharin aversion training began. On days 11, 13 and 15, the donors were given 10 minutes' access to a solution of saccharin in drinking water. Fifteen minutes after they had drunk the saccharin solution, the donors were injected intraperitoneally with apomorphine hydrochloride (15 mg/kg) (Garcia et al., 1966). On days 12 and 14, the donors were given plain tap water and were not injected. As we expected, their response to this training was a dramatic aversion to the saccharin solution. On day 15, the donors drank about one-third as much of the saccharin solution as they did on day 11. Their consumption of plain drinking water on days 12 and 14, however, remained the same as before the injections began--obviously it was the saccharin flavor, and not liquid per se, that the rats were avoiding.

Four hours after the apomorphine hydrochloride injection on day 15, the donors were sacrificed by decapitation and their brains immediately frozen. Three of the brains, chosen at random, were used for RNA extraction; the other three donor brains were put through dialysis. The RNA extraction method we employed was the same as that described above. The brains that underwent dialysis were homogenized with 5 volumes of saline and then dialyzed against 20 volumes of distilled water for 48 hours. The volume of the dialysate was then reduced to 1 ml per brain by lyophilization. As many of you will recognize, this is the dialysis procedure used by Ungar and his associates (Ungar, 1967).

Our 12 recipient animals were given 14 days of restricted water intake. During this period of time, they were allowed 10 minutes of free access to liquid daily, the 10 minutes being divided into two contiguous 5-minute periods. During the first 8 days, the recipients were given ordinary tap water during both of the 5-minute periods. On the 9th day, we established a preference baseline for all the

recipients. Half of them were given saccharin during the first 5-minute period and water during the second; the other half of the animals were given water and saccharin solution in reverse order. In all cases, the recipients consumed more liquid during the first 5-minute period than during the second 5-minute period. This is, of course, just the sort of behavior you'd expect from a very thirsty rat.

For the next five days following the preference test, all recipients were given ordinary water during both 5-minute periods of time. On the 14th day, the 12 recipients were split into three groups of 4 animals each; the groups were balanced for baseline saccharin preference and matched for weight. The 4 animals in Group I received 200 μ l of RNA extract injected into the subarachnoid space of their brains; the animals were injected under pentobarbital anaesthesia. The 4 animals in Group II received 1 ml of dialysate injected intra-peritoneally without anaesthesia, while the 4 animals in Group III received 1 ml of non-dialyzable material intraperitoneally, also without anaesthesia. About 14 hours after injection, each of the animals was given a repeat of its preference baseline test--that is, the rat received water during one 5-minute period and saccharin solution during the other 5-minute period. Half the animals received the water first, while the rest of the animals received the saccharin solution first. For the next three testing days, the order of presentation was reversed daily. Thus half the recipients received water first on test days 1 & 3, while the other half received water first on test days 2 & 4, and the experiment was balanced throughout.

Our analysis of variance did show a triple interaction between extracts and solution between days that was significant at the .025 level, and inspection of the behavior of the animals in the various sub-groups could lead one to speculate about interesting processes going on here and there, but frankly, if there was a transfer effect, it wasn't what you might call outstandingly noticeable.

Perhaps these results were only to be expected. For one thing, we made the classic mistake of trying to "improve" on the paradigm used by Revusky and DeVenuto. These experimenters failed to find a strong transfer effect with RNA injections when their recipients were run under 24 hours water deprivation. However, when they offered one group of animals continual access to both water and saccharin solution, they found a very significant drop in saccharin solution consumption on the second day of testing. It is possible that we would have found significant differences had we also tested our animals under conditions of reduced drive and when they had free access to both water and saccharin solution. We intend to do so immediately.

It is also true that we had fewer donors per recipient than did Revusky and DeVenuto. While this fact might not have mattered as far as the RNA group was concerned, for we injected as much RNA as we have in some two dozen past experiments that yielded positive transfer results, we were certainly using far fewer donors in the dialysate groups than Ungar and his associates find necessary. And I regret to report that we have some evidence that our RNA extract may not have contained very much intact RNA. Analysis of an RNA extract we obtained on a subsequent experiment showed no intact RNA present at all, just break-down products; we are not entirely sure why there was no intact RNA present. This finding suggests strongly, however, that one should save a portion of the RNA extract from each donor group and analyze it afterwards to make sure how much RNA the extract actually contained.

We are now in the process of repeating the saccharin aversion work with a larger group of animals and making every effort to correct the mistakes that we made in our first experiment. It is our opinion that the evidence obtained so far is sufficiently encouraging to warrant further work on the saccharin avoidance paradigm. At the moment, however, we cannot recommend it as a paradigm in which it is easy to demonstrate the chemical transfer of acquired behavioral tendencies.

SUMMARY AND CONCLUSIONS

During the past 30 months, we have used more than a thousand rats in a dozen or more experiments to test whether or not acquired behavioral tendencies can be transferred chemically from one animal to another by extracting various substances from the brains of trained animals and injecting them into untrained recipients. In general, our results strongly support the hypothesis that some kind of informational "transfer" can indeed be achieved, and we believe we now know what some of the critical factors are that affect the "transfer" phenomenon. We are not as yet sure what types of "information" can thusly be passed from one animal to another, nor how "stimulus-specific" the effect is, but it does appear that the storage and "transfer" of different types of information may well be mediated by different kinds (or at least by different sizes) of molecules. Hopefully, further research in our own and other laboratories will help answer some of the knotty problems as yet unsolved.

We would like to close by emphasizing a point too often forgotten in the controversy that the "transfer" idea has engendered, namely, that our primary interest is in how memories are encoded, stored, and decoded in the brains of the donor animals. The transfer technique is primarily useful as a kind of "behavioral litmus paper" to tell us more about the processes going on in the brains of the donors during the learning process. We know by now from the large number of successful reports from laboratories all around the world that we can get an effect of some kind by injecting untrained recipients with chemicals taken from the brains of trained animals. Even if subsequent experiments should prove conclusively that the effect is not really that of an implantation of "memories" into the brains of the recipients, it is still quite likely that the "transfer" technique itself will be a useful tool in our attempts to learn what goes on in the brains of the donor animals when they learn whatever task we set them.

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