The room-temperature $\beta$ proton coupling constants of the ethyl heptanoate, ethyl octanoate, and ethyl nonanoate radicals differ slightly in the two hosts. The two $\beta$ protons of each of these radicals in PHTP are magnetically equivalent whereas in urea the two $\beta$ protons of at least one radical, the ethyl octanoate radical, are magnetically distinguishable. This suggests either the deviations from a time averaged all-trans ester radical conformation are greater in urea than in PHTP or the motion of the guest radical in PHTP is slightly less hindered than it is in urea. This, however, remains only a suggestion until extensive low-temperature data become available. The $\beta$ proton coupling constants of the octyl propionate radical in the two hosts are, as expected, the same within experimental error.

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**CROSS-SPECIES TRANSFER OF LEARNING: EFFECT OF RIBONUCLEIC ACID FROM HAMSTERS ON RAT BEHAVIOR**

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In two previous reports, we showed that if ribonucleic acid (RNA) was extracted from the brains of trained rats and injected into untrained rats, the latter then evidenced a marked tendency to perform the originally trained response, even though no reward was provided for this behavior. The response involved was approach to the food cup in a Skinner box upon presentation of a distinctive stimulus. The first of these papers showed that the transfer effect was strong and consistent; the second showed that the approach response transferred was specific to the stimulus (click or blinking light) employed during training of the donor animals and was not attributable to differential handling or box adaptation of the donor animals.

Ribonucleic acid has been implicated in memory functions in organisms as disparate as man and planarian. If the mechanism of memory is indeed this parsimonious, one might be able to transfer learning from one species to another. The present experiment investigated this hypothesis. In the fashion used in our earlier papers, one group of animals was trained, another group injected and tested. In this study the donor animals were hamsters and the recipient animals rats.

Subjects were 16 adult male hamsters weighing approximately 100 gm and 16 adult male Sprague-Dawley rats weighing approximately 220 gm. The rats were fed Purina Lab Chow for 1 hr per day during the course of the experiment. Hamsters received one hundred 45-mg Noyes pellets per day (as described below) plus an hour of wet mash every few days and occasionally lettuce and carrots.
Eight hamsters received magazine training in a standard Grason-Stadler Skinner box; that is, they were trained to approach the food cup upon hearing the distinct click produced by operation of the pellet dispenser. Magazine training of hamsters proved to be somewhat more difficult than is magazine training of rats; and accordingly, the Skinner box was modified slightly to facilitate the process. Instead of being released by the food magazine, pellets were dropped by hand down a polyethylene tube to which a funnel was attached. The food magazine itself was emptied, but the click it produced was employed as the discriminative stimulus for approach to the food cup. Thus, the two components of training, click delivery and pellet delivery, could be controlled independently. A final modification of the box consisted of placing an aluminum floor over the grid bars to enable the hamsters to locomote more easily.

Magazine training was accomplished as follows. On the first day, a given hamster, deprived of food for 48 hr, was placed in the Skinner box and allowed to eat two 45-mg Noyes pellets which had been placed in the cup. Then, while the hamster was investigating the cup, the food magazine was operated a number of times in succession, producing a distinct click each time, and immediately after each click a pellet was dropped into the food cup. As training progressed, the click was withheld until the hamster moved first a short, and later a longer distance from the cup. During this time, the hamster was permitted a number of interspersed cup investigations which were not preceded by the click and were not rewarded with food. On a few occasions, the hamster did not approach the food cup promptly when the click was sounded; in this case, no food was delivered.

Each hamster was given 100 food-reinforced approaches to the food cup per day for 5 days. On the whole, hamsters are more sluggish than rats, and a slightly lower level of performance was achieved than is typically obtained with rats. Still, by the end of training, each hamster approached the food cup from most parts of the box when the click was presented, and the approach behavior of the animals was clearly under the control of this discriminative stimulus.

A control hamster was matched to each experimental hamster and was run simultaneously in an adjacent identical Skinner box. This yoked control animal received the click whenever the experimental animal did, but was not fed in the box. Instead, each control animal was given 100 pellets in a glass dish immediately upon being returned to its home cage at the end of a session.

On the day of completion of magazine training, each of the 16 hamsters was sacrificed with ether and the brain was taken out as quickly as possible. A cut was made on a line joining the superior colliculus to the rostral end of the pons. The tissue posterior to this cut was discarded, as was the tissue of the olfactory bulbs. The average weight of the tissue retained was 0.8 gm. RNA was then extracted from this tissue by the following procedure. The tissue was placed in a cold mortar with 5 ml of phenol (90%) and 5 ml of isotonic saline, and was ground with purified sand for approximately 3 min. The mixture was then centrifuged at 18,000 rpm for 30 min at 0°C. The aqueous phase was carefully drawn off to avoid contamination with phenol or with the interphase. The aqueous phase was then brought up to a concentration of 0.1 M MgCl₂ and 2 vol of cold ethanol were added to precipitate the RNA. Precipitation time was 15 min. The suspension was centrifuged at 6000 rpm for 15 min, after which the supernatant liquid was poured off.
The remaining ethanol was evaporated off, and the RNA was dissolved in 2.0 ml of isotonic saline. The amount of RNA was determined from the optical density at 260 μm (ε = 7450 in 0.2 M NaCl). The average yield was 2.0 mg/1.0 gm of tissue. In our earlier use of this extraction procedure, tests for protein and for DNA were negative.1

Approximately 8 hr after extraction, the RNA from each of the hamsters, experimental or control, was injected intraperitoneally with a 3/4-in. 22-gauge needle into an untrained rat (the xiphoid process was used as a guide for the injection).4 Prior to injection, each of the rats had been adapted to the Skinner box (without the aluminum floor) for 4 days, 15 min per day, and during each session the magazine had been operated two separate times, producing a distinct click each time. No food was ever given to these animals during the adaptation series, although food powder was sprinkled lightly over the grid floor to keep the animals active and to counteract any tendency on the part of the rats to approach the food cup on the basis of residual odor.

The 16 injected animals, then, consisted of 8 rats which received RNA from trained hamsters and 8 rats which received RNA from untrained hamsters. These 16 rats were assigned code letters. All testing from this point on was conducted “blind”: the testers did not know the group membership of any animal until the completion of testing. The experimental and control rats were tested in a random sequence; a different sequence was used for each session.

A session of testing for a given rat consisted of placing that animal in the Skinner box, permitting 30 sec to elapse, and then delivering a series of five clicks (produced by operation of the food magazine), spaced no less than 30 sec apart. Five such testing sessions were given, at 6, 8, 10, 22, and 24 hr after injection. Each test animal thus received a total of 25 trials. At the beginning of testing, all rats were approximately 24-hr food-deprived. After the third test session, each rat was fed 4–5 gm of Purina Lab Chow.

A response on a test trial consisted of the rat’s placing its nose inside a demarcated 63-cm² area surrounding the food cup, within 5 sec of click delivery. The food cup was located in one corner of the box, the floor of which had an area of 670 cm². That is, the rat had to approach to within a certain specified distance of the cup in order for a response to be counted. Further restrictions were placed upon the test trials as follows: two judges scored all trials independently, and a response was counted only if their tallies agreed; and trials were given only when the animal was facing away from the cup by more than 90°, was located at least a body length from the cup, and was not making gross locomotory movements. During testing, as during adaptation, food powder was sprinkled lightly over the grid floor.

A comparison of the two judges’ tallies revealed that they agreed on 398 out of 400 trials, i.e., on 99.5 per cent of the judgments.

Table 1 presents the score for each test animal in terms of the number of cup approaches, as defined earlier, out of the 25 click-presentation trials. The mean number of responses for the experimental rats was 7.9; the mean for the control rats was 0.6. By a Mann-Whitney U test,5 the difference between the groups was significant at well beyond the 0.001 level. Total scores of the eight experimental rats for the separate test sessions, in order, were: 16, 12, 13, 12, 10.
TABLE 1

TOTAL NUMBER OF RESPONSES FOR EACH EXPERIMENTAL ANIMAL AND ITS MATCHED CONTROL ON THE 25 TEST TRIALS

<table>
<thead>
<tr>
<th>Pair no.</th>
<th>Exptl. rats</th>
<th>Control rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>2</td>
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<tr>
<td>5</td>
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<tr>
<td>6</td>
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<td>1</td>
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<tr>
<td>7</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>11</td>
<td>1</td>
</tr>
</tbody>
</table>

Experimental animals, then, showed a significantly greater tendency than controls to approach the cup area when the click was presented. Since the control group was equated to the experimental group in terms of feeding, handling, and adaptation to box and click, these factors cannot be invoked to explain the results. "Learning" is sufficiently ill-defined that the possibility of a different interpretation of these results cannot be categorically rejected. Nonetheless, our several studies taken as a group suggest strongly that the effect being transferred is a specific learned response, and thus strengthen the hypothesis that RNA is an important element in the process of memory storage. Further, to the extent that a specific learned response is involved, the present experiment supports the notion that the mechanism of memory storage may be essentially identical in different species. This appears to be the case for at least two related species, the hamster and the rat.

Finally, although our experiments do not conclusively demonstrate that RNA is the effective agent in the transfer effect, this would certainly appear to be the most tenable hypothesis at present. Additional experiments with purified RNA preparations and with ribonuclease should answer this question.

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4 The injections were not done "blind." The tests were. The injectors and testers were the same two people (A. J. and F. B.). However, we do not believe any bias was introduced by this procedure because so many similar-looking animals were injected at one time and because the responses on testing were so characteristically either positive or negative.