

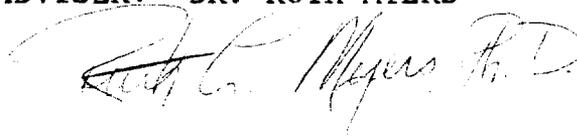
CHEMICAL TRANSFER OF LEARNING IN  
SUBHUMAN ORGANISMS

ID 499 HONORS THESIS

BY

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A handwritten signature in cursive script, reading "Ruth C. Myers, Ph.D.", is written over the printed name of the adviser.

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The area of learning has intrigued men for centuries. This is quite understandable in light of the fact that most human behavior is learned. Just what is learning? Learning is the process of acquiring a new skill on the basis of practice or experience. In the past, there have been many theories of how learning takes place. Two of the more prominent theories were that learning took place by electrical transmissions or by new synaptic connections. These theories were strongly supported until the 1950's when the discovery of DNA and RNA and their role in genetic information storage and recording gave rise to the idea that the basis of learning and memory could be molecular. With the growth of molecular biology came the conjecture that RNA, besides encoding genetic structure, might also encode individual experience. It was these molecular biologists whose research dealt with information coding in single molecules who first conceived the idea of possibly transferring information from one organism to another with chemical extracts.

The first concrete attempt to chemically transfer learning from one organism to another was made by James McConnell in 1962.<sup>1</sup> His research, like most of the initial

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<sup>1</sup>G. M. Cartwright, "Use of a Maze Habit as a Test of the Specificity of Memory Transfer in Mice," Journal of Biological Psychology, 12(1): 53, 1970.

work in this area, was primarily concerned with learning transfer in invertebrates. In particular, his subjects were planaria, a type of flatworm. Their primitive nerve system plus their amazing ability to regenerate made them ideal candidates for experimentation.

In McConnell's initial experiment, planaria were conditioned using a classical conditioning model. An electrical shock served as the unconditioned stimulus, a light as the conditioned stimulus, while body contractions made by the planaria were the unconditioned responses. When the trained planaria reached the designated criterion for learning their bodies were ground up and fed to an untrained group of similar planaria. The control group consisted of untrained planaria that had been fed the bodies of other untrained planaria. When put in a learning situation identical to the original, planaria who had ingested the trained worms gave half again as many conditioned responses as did the control group. This seemed to indicate that transfer of learning had indeed occurred.

In an experiment using similar training techniques, the team of McConnell, Allan L. Jacobson and Daniel Kimble trained planaria to a criterion of twenty-three correct responses out of twenty-five trials. When the criterion had been met, the subjects were cut in half laterally and allowed to regenerate, the tails forming new heads and the heads forming new tails. Upon completion of regeneration, each half was again tested by the savings method to

establish the amount of retention of the original training. Again, a control group was provided which consisted of the regenerated halves of untrained worms. In the original test, the mean number of trials it took the planaria to reach criterion level of responding was 134. However, it took the regenerated head an average of only 40 trials and, surprisingly enough, the regenerated tails required an average of only 43.3 trials. The control heads and tails required an average of 248.6 and 207.8 trials respectively. Here again, the experimenters conclude that this experiment gives evidence for a chemical basis of learning. Learning that spread in particular from the regenerated tail of the trained worm to the untrained head.<sup>2</sup>

It has been demonstrated that habituation too can be transferred cannibalistically. Also, papers by such men as Hartry et al., and Walken and Milton, combined with the great quantities of experiments which show that learning can be transferred suggest that all the simple behaviors which an animal learns can be transmitted to another chemically.

As interest in the chemical transfer of learning grew, more research was directed toward the transfer of learning in vertebrates. Subjects for these experiments included rats, mice, birds, goldfish, turkeys, and chicks. Inherent with this shift in interest came the problem of how to attempt a

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<sup>2</sup>James McConnell, Allan Jacobson, and Daniel Kimble, "The Effects of Regeneration Upon Retention of a Conditioned Response in the Planarian," Journal of Comparative and Physiological Psychology, 52:1-5, February, 1959.

chemical transfer in vertebrates. Attempts to find a solution resulted in several means of extracting brain chemicals from trained animals. One of the first such methods was that using crude brain homogenates. In this method, whole brain homogenates are injected intraperitoneally into recipient animals. While this technique did show significant positive results, it unfortunately resulted in high mortality rates among the recipients.

A second method utilizes RNA extracts. These are obtained from a cold phenol extract of the brain which is centrifuged. The aqueous phase is then retained and precipitated with magnesium chloride or potassium acetate and ethanol which is removed by evaporation. The resulting solution is then taken up in saline for injection.

Aqueous extracts are a third possibility. In this method, aqueous extracts of brain homogenates are used without any treatment other than centrifugation. Two final possibilities are extracts made with other reagents and precipitates from aqueous extracts made by partial purification of an aqueous extract using organic solvents.<sup>3</sup> Thus the first problem in studying learning transfer in vertebrates had been solved.

Regarding actual research, the first published account of learning or memory transfer in vertebrates was

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<sup>3</sup>George Ungar, Molecular Mechanisms in Memory and Learning, pp. 134-136.

made by Reinis in 1965.<sup>4</sup> In his experiment, rats were trained to approach a food cup and, upon reaching criterion level of performance, were sacrificed. Food-deprived rats were then injected intraperitoneally with brain homogenate from the trained rats. Two control groups were utilized. One consisted of rats injected with extracts from untrained rats and the other group received no injection. Results showed that in a retest situation the experimental group had an increased percentage of correct conditioned approach responses to the food cup. They also exhibited a decrease in orientation responses and consummatory latencies. These results were not true of the two control groups. This does seem to suggest a transfer of learning from one rat to another.

In an experiment utilizing goldfish as subjects, William G. Braud also demonstrated a transfer of learning.<sup>5</sup> Goldfish acquired a light-signaled avoidance to shock in an aquatic shuttle box. The avoidance response was then extinguished and an RNA rich extract taken from the brains of the trained organisms. When injected intracranially with the extract, recipient goldfish extinguished the avoidance response significantly faster than those injected with extracts from naive donors.

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<sup>4</sup>G. M. Cartwright, "Use of a Maze Habit."

<sup>5</sup>Braud, William G., "Extinction in Goldfish: Facilitation by Intracranial Injection of RNA from Brains of Extinguished Donors," Science, 168:1234-1236, June, 1970.

William G. Braud, Porter Laird, and Steven Richards used a between-subjects design to test for learning transfer in goldfish.<sup>6</sup> Goldfish were trained to avoid shock in a shuttle box and sacrificed twenty hours after completion of the task. A mixture of RNA and Protein extracted from the brain was injected into the brain of recipient goldfish. Performance of this group was consistently better than that of a control group which had been injected with extract from untrained subjects. In this experiment the greatest difference between experimental and control groups occurred after a seventy-two hour time interval. This suggests that the transfer effect may be time dependent.

While there have been many experiments successfully demonstrating a transfer effect, there are others which do not support this theory. The primary objections of this last group of researchers to experiments which do suggest a transfer effect are that (1) the injection of a foreign substance into the brain of a recipient animal does not transfer a specific bit of learning--instead it tends to raise the general activation level of the organism; (2) there exists experimental bias; (3) replicability; and (4) statistical treatment of data.

The inability to replicate experiments is no longer a valid charge. With refined techniques and procedures,

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<sup>6</sup>William G. Braud, Porter Laird, and Steven Richards, "Facilitation of Avoidance Behavior in Goldfish by Injection of Brain Material from Trained Donors: Effect of Injection-Testing Interval," Journal of Biological Psychology, 13(1):6-8, 1971.

experiments have been highly replicable. The statistical treatment of data is also a minor charge and one which can be easily corrected.

In answer to the charge of experimental bias, Frank Babich, Allan Jacobson, Suzanne Bubash, and Ann Jacobson conducted an experiment using "blind" judges.<sup>7</sup> In this experiment, there was .002 degree of significance between the experimental group which received injections from trained rats and the control group which received injections from naive rats. Thus in this experiment it was shown that experimental bias need not be a factor.

The charge that transfer effects are general and not specific has drawn the most attention. Numerous experiments have been conducted that demonstrate a transfer of specific rather than general learning. Notable among these experiments is one conducted by H. P. Zippel and G. F. Domazk.<sup>8</sup> Goldfish, in a no-shock training program, were conditioned to prefer a combination of green light plus acetic acid, both of which they normally dislike. Recipients of extracts from the trained fish showed a positive reaction to these previously disliked stimuli. This change in behavior noted between the second and seventh day after injection strongly suggests that

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<sup>7</sup>Frank Babich, Allan Jacobson, Suzanne Bubash, and Ann Jacobson, "Transfer of a Response to Naive Rats by Injection of Ribonucleic Acid Extracted from Trained Rats," Science, 149:656-657, June, 1965.

<sup>8</sup>H. P. Zippel and G. F. Domazk, "Transfer of Color and Taste Preference from Double-Trained Goldfish into Untrained Recipients," Journal of Biological Psychology, 13(1):28, 1971.

specific information was transferred.

Allan H. Schulman conducted an experiment in which young turkeys were operantly conditioned to peck a key to view an imprinted stimuli.<sup>9</sup> Results of the experiment show that key pecking was greater in turkeys receiving injections from extracts taken from the trained turkeys than in turkeys receiving control injections. There was no general activity increase, indicating that transfer was specific, not general.

In the same area, George Ungar demonstrated transfer to be specific by testing without reinforcement on recipient training.<sup>10</sup> Similar results have been obtained by McConnell, Jacobson, Babich and many others.

Although the original criticisms of transfer experiments have been met satisfactorily, there are many other factors that must be controlled. Among these are biochemical, physiological and psychological factors. Biochemical factors include obtaining brain extracts which are pryogin free, controlling PH level, obtaining pure extracts, replicating operations, and others. Physiological factors include the deprivation state of the donor, the part of the brain used for extraction purposes, the technique for sacrificing the subject and removing the brain, the temperature

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<sup>9</sup>Allan H. Schulman, "Transfer of Behavior Controlled by an Imprinted Stimulus Via Brain Homogenate Injections in Turkeys," Psychonomic Science, 27:48-50, April, 1972.

<sup>10</sup>George Ungar and L. N. Irwin, "Transfer of Acquired Information by Brain Extracts," Nature, 214:453-455, April, 1967.

at which the brain is stored, and, finally, the species, age, weight and sex of the organisms. The most crucial factors are the ones which are psychological. Very important in this area is the type of donor used; that is, whether the donor is "clever" or "dumb," or a slow or fast learner. A second factor is whether learning takes place in a one-trial learning situation or is spread over several days. It is also necessary for the recipients to be free of fear and emotionality.<sup>11</sup>

Not included above, factors relating to dosages, time, and incubation periods have also been shown to affect results significantly. Frank R. Masiarz, Arnold M. Golub, Trudy Villars and James McConnell, in research conducted with rats, found that rest periods inserted during the donor's training program significantly enhance the memory transfer effect.<sup>12</sup>

In an experiment aimed at examining the relationship of time to learning transfer Schad, Rollins and Snyder injected recipient rats with extracts from rats trained to avoid a dark chamber.<sup>13</sup> A significant avoidance of the

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<sup>11</sup>James V. McConnell, "Factors Affecting the 'Transfer of Training' Effect in Rats," Journal of Biological Psychology, 9(1):46-47, 1967.

<sup>12</sup>Arnold Golub, Frank Masiarz, Trudy Villars, and James McConnell, "Incubation Effects in Behavior Induction in Rats," Science, 168:392-394, April, 1970.

<sup>13</sup>Lester J. Schad, James E. Rollins and Charles W. Snyder, "Transfer of Learning by Injection of RNA as a Function of Time," Psychonomic Science, 14:112-113, February, 1969.

previously preferred chamber was found at eight hours following the injection. No such result was obtained at one, two, or sixteen hours after injection. This suggests that time is indeed a factor in the transfer effect.

Finally, dosage has been shown to exert a powerful effect on the transfer of learning, particularly its polarity. When the recipient behaves in a manner similar to that of the donor, the polarity is positive. When the behavior is in the opposite direction, the transfer is negative. Rosenblatt found that as doses increase from .0004 to 4.000 donor brains per recipient there are peaks of positive and negative transfer which alternate successively. Maximal positive transfer occurs when the dose is 0.025 of a donor brain and maximal negative transfer takes place when the dosage is 0.013 or 0.050 of a donor brain.<sup>14</sup>

In a similar experiment conducted by Bisping, Zahl-Begnum, Longo, Niemoeller and Reinaver, goldfish were injected with extract doses of 1 milli-gram, 10 milli-grams, 30 milli-grams, and 200 milli-grams. The experimental groups performed significantly better than the control group at 1 milli-gram, but as the doses increased the control groups performance equalled and finally surpassed that of the experimental groups.<sup>15</sup> A plausible explanation for this

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<sup>14</sup>Marilyn Stewart, Doreen Strackman, and William Rucker, "Interanimal Transfer of Training: A Pilot Study of Direction of Effect," Journal of Biological Psychology, 13(2):29, 1971.

<sup>15</sup>R. Bisping, O. H. Zahl-Begnum, N. Longo, U. Niemoeller, and H. Reinaver, "The Effects of Nucleic Acid Concentration on Biochemical Memory Transfer," Journal of Biological Psychology, 13 (2):32-35, 1971.

result is that the inhibitory effect may be caused by such a vast amount of information which the large doses contain. Whatever the reason, dosage does play a role in transfer of learning.

While a majority of researchers agree that there is a transfer of learning, there is still disagreement on what element is responsible for the transfer. At present, researchers are divided in their support for RNA, protein, peptides, or other small molecules.

In an effort to prove RNA was the element responsible for the transfer of learning, experiments were performed using inhibitors. The first study of the effect of mitabolic inhibitors on memory was made by Dingman and Sporn (1961) using 8-azaguanine, an inhibitor of RNA synthesis.<sup>16</sup> Although the results of the experiment were not conclusive, the researchers concluded that RNA may be involved in the acquisition but not in the retention of learned information.

Golub, Epstein and McConnell performed an experiment in which rats were trained to avoid a black box.<sup>17</sup> The rats were destroyed and from their brains extracts were made consisting of either whole brain homogenates, RNA or peptide fractions. Animals who received injections from trained, but

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<sup>16</sup>Ungar, Molecular Mechanisms, p. 69.

<sup>17</sup>Arnold M. Golub, Leon Epstein, and James McConnell, "The Effect of Peptides, RNA Extracts, and Whole Brain Homogenates on Avoidance Behavior in Rats," Journal of Biological Psychology, 11(1):48, 1969.

not untrained animals, reversed their natural preference for the dark box. The results suggest not only a transfer effect but that whole brain homogenate, RNA, and peptide fractions are effective in mediating the effect.

One of the most noted research attempts in this area was conducted by ChecRashin and Sheimann.<sup>18</sup> The inhibitor was RNA-ase, a chemical which inhibits production of RNA. Planaria were conditioned using light or vibration as the conditioned stimulus and shock as the unconditioned stimulus. If RNA-ase was added to the water during training, learning was inhibited. If it was added after training had been completed, recall was inhibited but the conditioned response returned when the RNA-ase was removed. RNA-ase also affected retention after regeneration. It is thought that RNA-ase probably selectively inhibits the integrating activity of the nervous system as well as the interaction of two stimuli. The authors concluded that RNA must play a particular part in the processes of formation and recall of the memory trace.

Hyden and his co-workers have a different version of how RNA may act as a mediator. They feel that the changes in amount and composition of RNA in the brain of trained animals may reflect intracellular redistribution of the present RNA rather than cellular changes in synthesis or other aspects of RNA metabolism.<sup>19</sup>

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<sup>18</sup>A. N. ChecRashin and I. M. Sheimann, "Conditioning in Planarians and RNA Content," Journal of Biological Psychology, 9 (1):6, 1967.

<sup>19</sup>Ungar, Molecular Mechanisms, p. 43.

While most original researchers believed RNA was the element behind the transfer effect, this has never been clearly shown. Other researchers, those who supported the protein hypothesis, pointed out that virtually all RNA-extracts were impure. They contain other substances, some of which is protein. To test for the effect of protein on learning again inhibitors were used.

Flexner and his co-workers used the protein inhibitor puromycin and tested its effect on learning.<sup>20</sup> Mice learned a Y maze by learning to avoid a shock. One day after training, the mice were injected with puromycin in the temporal area of the brain. This injection resulted in loss of the previous learning. However, if injections were given three to six days after learning took place, they had to be given in all six areas of the brain to disturb memory. This and other studies suggest that as time passes after learning, memory of that learning becomes less susceptible to the effect of puromycin.

In studies using substances related structurally to puromycin but which were not protein synthesis inhibitors there were no effects on memory. Cycloheximide and acetoxycycloheximide are also protein synthesis inhibitors but without some of the defects of puromycin and with greater potency. They too disrupt memory, providing training is not overly extensive.

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<sup>20</sup>Ibid., p. 60.

Bogock, Belval, and Chung trained pigeons in discrimination approach tasks and then fractionated the birds' brains.<sup>21</sup> One group was sacrificed immediately after training and another several months after training. There was in both groups a correlation between the amount of protein peaks in an individual brain and the proportion of correct pecks made by that pigeon in the last two or three training sessions. This seems to suggest that this group of proteins which are acidic and rich in carbohydrates have some role in the maintenance of a memory trace.

Frank Rosenblatt, John Farrow, and William Herblem say the transfer element cannot be RNA but could possibly be a protein or polypeptide.<sup>22</sup> They came to this conclusion when they found the molecule which transfers information to have a molecular weight between 1,000 and 5,000 as measured by syshadix fractionation. This hypothesis would exclude any known RNA form.

The series of experiments pertaining to the effect of protein on learning transfer led to the following hypotheses. First, learning and "short-term" memory are seemingly independent of cerebral protein synthesis. Excess training may hide the protein synthesis inhibition effect. Protein synthesis is evidently required for storage of "long-term"

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<sup>21</sup>ibid., p. 49.

<sup>22</sup>Frank Rosenblatt, John Farrow, and William F. Herblem, "Transfer of Conditioned Responses from Trained Rats by Means of Brain Extracts," Nature, 209:48, January, 1966.

memory and occurs concurrently with learning and/or within minutes following learning. And finally, since "short-term" memory is independent of protein synthesis and may last up to six hours, the lingering effect of "short-term" memory may mask the effect of inhibitors on "long-term processes."<sup>23</sup> Thus, as in the case of RNA, while research is promising, results are far from conclusive.

The final elements that have received much attention in the study of learning transfer are peptides. They are the newest elements to be studied and research to date has proved quite promising. A group of researchers for several years has shown that the transfer element has the properties of a peptide but no concrete results were available till very recently.

Ungar et al. isolated a 15-amino acid peptide responsible for the passive transfer of dark avoidance.<sup>24</sup> This peptide, called scotophobin, has been synthetically produced and when injected into recipient rats produces the same effect as the original peptide. Scotophobin has a molecular weight of 1,600 and only one aromatic residue, very similar to peptides isolated in other experiments. With this

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<sup>23</sup>William L. Byrne, Molecular Approaches to Learning and Memory, p. 32.

<sup>24</sup>M. H. Kleban et al., Influence of Donor Recipient Brain Transfers on Avoidance Learning," Psychological Reports 23:51-56, August, 1968.

and similar results, it is becoming clear that a family of peptides with a molecular weight near 1,500 is active in passive transfer of simple responses. While again it cannot be said definitely that peptides are the mediators for transfer of learning, it is now clear that they are involved in a major role.

Thus, in the search for an element responsible for the transfer of learning from a trained organism to an untrained organism, RNA, proteins, and peptides have been studied. Research designed to prove that each alone is the sought-after element have always been inconclusive. This has led to a somewhat obvious hypothesis, the hypothesis being that all three are involved in learning transfer. This is the assumption behind present research and only time will provide the exact answer.

From the previous material, it can be seen that in the few years since the transfer of learning studies began, amazing progress has been made. Not only has it been demonstrated that learning transfer actually does take place, but the elements which mediate the transfer have also been studied. These elements, RNA, peptides, and protein, have been studied individually and in combinations. The results indicate that the elements do work in combination and all three are necessary for learning transfer to take place. The work in this area is far from complete and continued research will undoubtedly bring more conclusive evidence concerning the transfer of learning in subhuman organisms.

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