

THE EFFECT OF PUROMYCIN AND ELECTROCONVULSIVE SHOCK
ON RETENTION OF SHOCK AVOIDANCE
TRAINING IN THE GOLDFISH

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CHAPTER I

INTRODUCTION

The capacity of organisms to record experiences and to modify their behavior according to the nature of the experience clearly ranks as one of the most exciting phenomena of the behavioral sciences today. The rapid and outstanding developments in the molecular sciences have had tremendous impact on the thinking and experimental approaches in practically all scientific disciplines. As a result of these significant trends in molecular psychobiology, we are in a period in history of translating abstract psychological terms into physiological realities; however, this period is in an infant stage, and the complexity of it demands an interdisciplinary approach.

General Purpose of Study

In general, the objective of the present study was to incorporate some of the new psychobiological approaches, with the theories propagating their use, in studying the phenomena of long-term memory in the goldfish. Several investigators have used physical and chemical agents believed to impair the formation of memory as tools in studying the nature of the mechanisms involved. Such an approach in studying memory was adopted for this study.

Survey of the Literature

Few behavioral scientists of any theoretical persuasion would deny that a learning experience produces some kind of neurological change. Freud, who was a neurologist, while deploring the total absence of evidence in this field, mentioned the possibility that memory rested on anatomical modifications of the nervous system (16). Koffka also stressed that learning be thought of in terms of configurations responding to an organization of the nervous system (16).

D. O. Hebb has been credited for the formulation of the hypotheses that experience brings about permanent modifications of the brain's "conducting channels". Hebb also emphatically maintained the idea that a persistent relationship could be established between certain data from the outside world and certain durable modifications of the neural groups (16).

According to Barbizet, much is due to Lashley and to his statement of the problem in "The Search of the Engram" (16). Lashley has given impetus to and focused the attention of many experimenters on the value of the concept of "engram" as a working hypothesis for the nature of the correlation between learned behavior and electrical and cellular activities in the brain. The term "engram" is used extensively in the neurological literature, usually as a verbal tool in discussing memory as material traces involved in storing and retrieving memory. Memory storage (long-term memory) has been explained by many prevailing theories of memory consolidation.

Support for a Consolidation Theory

The consolidation theory of learning was first proposed by Muller and Pilzecker (1900) in order to account for the forgetting of verbal material (25). They knew that a list of verbal material interpolated between the learning of an original list and its recall would result in interference with the retention of the original list -- the phenomenon of retroactive inhibition. Muller and Pilzecker hypothesized that the engrams produced by the recitation of the items from the original list were not yet consolidated, and thus, were susceptible to disruption produced by citing items from the interpolated list. In essence, Muller and Pilzecker postulated the existence of a neural perseverative process, subject to external interference and requisite to the consolidation of the memory trace for recently acquired material (22).

Shortly after the publication of Muller and Pilzecker's work, attention was called to the applicability of their perseveration theory to the explanation of retrograde amnesia resulting from cerebral trauma. It had been known for many years that human patients who had suffered head injuries tended to have difficulty in recalling events that occurred shortly before the injury.

Cerebral trauma. --During the first four decades of this century, the phenomenon of retrograde amnesia constituted the only direct physiological evidence for the existence of a neural fixation process. There

have been several studies exploring the nature of retrograde amnesia as related to cerebral trauma. Many of these studies are general in their findings; therefore, it would seem sufficient to present a more representative and comprehensive study done by Russell and Nathan (31). In a survey of 1029 cases of cerebral trauma (head injury), only 133 were found to have experienced no retrograde amnesia (RA) whatsoever.

Seven hundred and seven reported amnesia for events occurring from several seconds to 30 minutes preceding the injury, while 133 reported RA of more than 30 minutes duration. Records were unavailable with 56 patients in the sample. Since the use of barbiturate hypnosis reduced the period of RA in only 6 of the 40 cases, and produced no data for hysterical repression, the authors concluded that loss of the material was due to a blocked perseveration process.

Electroshock therapy. --The introduction of electroshock therapy in 1937 provided both the impetus and the technical apparatus for the laboratory study of RA. Immediately after its introduction, many practitioners observed that electroconvulsive shock (ECS) produced a temporary postshock amnesia which eventually shortened to a genuine RA for events immediately preceding the shock treatment. Following these observations, several investigators subjected these observations to systematic study. These various investigators, using human patients in study after study, were successful in employing ECS to interfere with

memory; however, they had not attempted to define adequately the time relations of such interference.

Experimental electroconvulsive shock. --The interval between the end of the learning trial and the ECS is a crucial one, because it is during this period that the engram is presumed to consolidate (25). Duncan (1949) manipulated this variable in a classic ECS experiment that gave support to a consolidation theory (15). Using a shuttle box, he gave his subjects one trial a day, followed at different time intervals by ECS. The intervals between the learning trial and the ECS for the various groups were: twenty seconds, forty seconds, four minutes, fifteen minutes, one hour, four hours, and fourteen hours. He found that the relationship between the number of successful avoidance responses and the time intervals was sharply negatively accelerated. There was no evidence of learning with the shortest interval, indicating total RA, but if one hour or more elapsed between the end of a trial and the convulsion, there was no apparent memory loss. This general finding has since been confirmed by Ransmeier (1953), Thompson and Dean (1955), and Leukel (1957) (22). All of the findings are compatible with the view that a single ECS can produce deficits in retention if delivered within fifteen to sixty minutes following a learning trial. Most recent authorities agree that this time period is quite short. Pearlman, Sharpless, and Jarvik (30, p. 109), for example, say, "It is agreed that the interval during

which such agents can exert significant retrograde effects is relatively short, a few hours at most." Deutsch (25) places it about one hour, while John (25) reduces it to between one-half to one hour.

Opposition to Consolidation Theory

These experiments, providing strong evidence for the general hypothesis that memory trace consolidation processes are time dependent, did not completely rule out the possibility that the results were due to some other effect or effects of electroshock. For example, it was suggested that punishment rather than amnesia, might be the basis of the retrograde effect of electro-shock treatments (28). That is, since electro-shock was administered immediately after each training trial, it seemed at least possible that the animals were merely learning to avoid making the responses that were followed by electroshock treatment. According to this view, the failure of the animals to perform under such conditions is not due to a memory loss but to an experimentally induced fear.

Conflict interpretation. --The most serious alternative to a consolidation interpretation of the ECS results has been offered by Coons and Miller (7). These investigators trained rats to eat in a runway and then shocked them while eating there. Avoidance was measured by an increased latency of approach to the eating place. ECSs were delivered to the animals

at varying intervals after shock to the mouth. Miller and Coons reasoned that any aversive qualities of the ECSs might be expected to produce increased avoidance. On the other hand, if the ECS really interrupted consolidation, the subjects would show the opposite behavior, namely, approaching the food without hesitation. In this experiment no evidence was found for an attenuation of the avoidance response by the ECS, leading the authors to argue that the retardation in learning by Duncan and others was simply a function of placing the rat in a conflict situation.

Recent Evidence for a Consolidation Theory

One-trial learning. --Recent evidence does not support this alternative view on the conflict and consolidation interpretations of "forgetting." In a one-trial learning experiment done by Madsen and McGaugh, (26) rats were placed on a small platform and were given a mild shock to the feet as they stepped from the platform on to the floor. The control group received only the foot shock, while the experimental group received foot shock and ECS on a one-trial learning basis. On a retention test given the next day, the experimental group (ECS) stepped off the platform significantly more times than the control group which received only the foot shock. In other words, the rats given only the foot shock tended to remain on the platform, that is, they appeared to remember the shock, while those given electroshock after the foot shock gave no evidence of

remembering either the foot shock or the electroshock. In subsequent experiments by Madsen and McGaugh (26), they found that electroshock treatments are aversive only if they are given repeatedly. Rats can learn to avoid making responses that are repeatedly followed by electroshock, and they can learn to avoid going to a place in a maze where they have received several electroshock treatments. However, in all of their experiments, aversive or punishing effects were observed only after several electroshock treatments had been administered, while retrograde amnesia was readily obtained with a single treatment (26).

Anoxia effect. --In a general way, the findings of the effects of electroshock stimulation are highly similar to results obtained with other treatments which have found to produce retrograde amnesia. Hayes (23) demonstrated equivalent retroactive effects of anoxia and ECS on maze learning in rats. He used a distributed practice procedure and administered the experimental treatment one hour after each trial. The experimental rats showed similar retardation in learning when their acquisition curves were compared with normal control animals. Hayes reports that histological examination of the brain produced no clear evidence of brain damage for any of the animals. Using a discrimination learning procedure, Thompson and Pryer (32) showed that anoxia, produced by placing rats in a decompression chamber during the postlearning period, could lead to decrements in retention analogous to those

produced by ECS. In a later study, Thompson found that a ten-minute exposure to a simulated 30,000-foot altitude produced deficits equivalent to those resulting from ECS.

Temperature effect. --A number of investigators have studied the effects of postlearning temperature on retention. A study by Cerf and Otis (6) indicated that temperature may have some effect on processes related to consolidation. Goldfish were given 10 massed trials in an avoidance situation using a shifting light as the conditional stimulus. At varying intervals following the trials, (zero minutes, fifteen minutes, sixty minutes, or four hours) the body temperature of different groups of fifteen to nineteen subjects was raised briefly to a point sufficient to induce heat narcosis (36.5° - 37.0° C). In retention tests carried out the next day, the criterion of five consecutive correct responses in ten trials was met by only 10.5 percent of the group narcotized immediately after learning, while 56.2 percent of the subjects paralyzed four hours following learning met the same criterion. The remaining groups occupied intermediate positions. Thus, the temperature induced narcosis produced much the same effect in the goldfish that ECS and anoxia have been found to produce in rodents.

Anesthetic effect. --Several studies have used anesthesia in determining possible relationships with retrograde amnesia. Leukel has reported that sodium pentothal, injected intraperitoneally (IP) after

each learning trial, impaired acquisition in a maze in experimental rats when their time or error scores were compared with any of three control groups (24). Subjects in the three control groups received either an intraperitoneally injection of water following each trial; an IP injection of pentothal thirty minutes following each trial, or no injection. The scores did not differ among these latter groups. Leukel interpreted his results in terms of interruption of consolidation of the memory trace in those subjects receiving pentothal one minute after each trial. Pearlman et al. (30), in a recent experiment, showed that an anesthetic itself can produce RA. They bar-trained rats to a stable rate of responding by a single shock delivered through the reinforcing apparatus. Either twenty seconds, five minutes, ten minutes, or twenty minutes after the CER, the animals received a sodium pentobarbital anesthetic through chronically implanted catheters. The experimenters found that the anesthetic produced RA as a function of the time between CER and the administration of the anesthetic. They concluded that the sodium pentobarbital produced RA by interfering with a consolidating engram.

Glickman suggested that if anesthetics can be shown to exert reliable retroactive effects on learning, they may eventually prove useful in the localization of the neural structures crucial to consolidation (22). Techniques have recently been developed which permit the delivery of small quantities of drugs to restricted sites within the brain. Glickman goes

on to say that it should be possible to selectively and temporarily block activity in various cerebral structures during the period immediately following exposure to the learning situation and thereby determine which structures, if any, are crucial to the consolidation process.

Stimulant effect. --Recent experimental findings have provided strong evidence that central-nervous-system stimulants can facilitate learning by enhancing memory consolidation. In several experiments McGaugh et al. (28), and subsequently others, have found that the learning of a variety of tasks in rats and mice is facilitated by injection of strychnine shortly after training trials. According to McGaugh, no facilitation is obtained if the strychnine is administered more than thirty seconds after the training is terminated. Other investigators gave other interpretations stressing possible motivational effects of the drug. However, in most of these experiments, the retention tests were given at least twenty hours after the injections, so that it would seem that the animals were never tested while drugged. Consequently, the post-trial injection studies are difficult to interpret in terms of motivational or perceptual effects.

The effect of drugs. --The list of drugs found to facilitate learning in laboratory animals has increased considerably through recent findings. Learning facilitation has been found with pre-trial injections of amphetamine, nicotine, and magnesium penioline (28). Facilitation of learning

has also been found with post-trial injections of caffeine, physostigmine, and amphetamine (28).

Morgan states (29, p. 548) that any physiological theory of the memory trace must meet two requirements. The first is to provide an explanation for the simple phenomena of learning itself. The second requirement is that for a memory trace to be complete and permanent.

The Contribution of Neurochemistry

Neurobiotaxis theory. --In the last 20 years much attention has been given to neurological and chemical bases for learning. The earlier systematic investigations utilized neurological concepts (8). A possible explanation for the basis of learning was offered by Hebb, who suggested a neurobiotactic basis for learning (29). Under this theory, connecting parts of a neuron grow closer together under the influence of stimuli involved in learning. The theory goes on to state that when the parts have grown closer together, a connection is made that enables a conditioning stimulus, for example, to evoke a response formerly called forth only by the unconditioned stimulus. The exact nature causing the neurobiotactic growth to take place is not explained, but Morgan presumes that perhaps this neurobiotactic growth to be a response to chemical or metabolic traces left by the learning experience.

Brain chemistry as related to learning and memory. --Following the neurological approach, a systematic set of chemical hypotheses and approaches were introduced for the exploration into the nature of learning. One prominent and systematic chemical approach was begun about 1953 at the University of California at Berkeley (8). Krech and his colleagues concerned themselves with chemicals involved in intercellular communication, acetylcholine (ACh) and acetylcholinesterase (AChE). Although their interest began in neurochemistry, their most important contributions appear to be of an anatomical nature. A significant finding was that varied experiences result in a thickening of the cortex in certain parts of the brain. Hyden (16) conducted a similar study on sensorily deprived and sensorily overprivileged rats. One set of rats were kept by themselves in dark, quiet boxes, so that only minimal visual or auditory stimulation was available. Another group of rats were put in a common box together. These were able to play with one another, and they were taught to perform additional tasks, requiring visual and auditory discrimination. At the end of the experimental period, the rats were sacrificed and their brains morphologically and chemically examined. Structurally, they were found to be similar. Compositionally, however, the two kinds of cortices were quite different. The sensorily deprived rats had cortical areas impoverished both in RNA and protein as compared with the similar cortical areas of rats which had led sensorily enriched lives. In a subsequent experiment, Hyden (16) taught a group of rats to

climb and balance upon a wire in order to reach their food. After sacrifice, the content of the vestibular geniculate was compared with rats that had neither learned or performed the task. The result was clear: RNA content was increased in that part of the brain concerned with balance, when the learning of balance was acquired.

Protein chemistry. --A second chemical approach was precipitated by the rapid and exciting developments in molecular biology (8). With advancing knowledge in protein chemistry and the role played by enzymes in nervous function, attempts have been made within the last twelve years to develop a protein theory of events taking place in learning. In investigating the possible connection between memory and protein synthesis, a quick summary from Gaito (21) on the role of nucleic acids is presented. A molecule of protein is made from twenty different kinds of amino acid molecules, strung together in a polypeptide chain. The stringing is done in the small bodies in the living cell called ribosomes. Each amino acid molecule is brought to the ribosome by a molecule of transfer RNA, a form of ribonucleic acid. The instructions, according to which the amino acids are linked in a specific sequence, are brought to the ribosome by another form of ribonucleic acid -- messenger RNA. These instructions have been transcribed by messenger RNA from deoxyribonucleic acid (DNA), the cell's central library of information.

RNA and learning. --An RNA theory for learning, based on firm knowledge of biochemical genetics and protein chemistry, is still relatively new; however, since the middle 1950's, several studies via different approaches and techniques have provided solid evidence for a RNA-learning relationship. In 1955 Jacobson, Kimble, and McConnell (27) performed the first experiment that suggested that memories might be stored chemically in planarians. In their experiment planarians were classically conditioned by using light as a conditioned stimulus and shock as an unconditioned stimulus. When the animals had reached the criterion of twenty-three responses out of twenty-five consecutive trials, they were cut in half and allowed to regenerate for a month. A second group was trained to criterion, then set aside to regenerate without being cut in half. A third group was first cut in half and allowed a month's regeneration, then conditioned for the first time. The second group which was never cut, took about 180 trials to reach criterion the first time around; a month later they retrained in forty trials. In the first group the regenerated heads reached criterion also in forty trials; the tails, in forty-three trials. Both these groups showed significant savings, while the third group took significantly longer to learn than had the original animals. Subsequent studies in this laboratory have provided evidence that two types of learning in planaria, light-shock conditioning and habituation, are capable of being transferred from conditioned planaria to experimentally

naive planaria. This transference has been demonstrated by cannibalization and direct RNA injection.

Use of Agents to Prevent Protein Synthesis

With the increasing knowledge of protein synthesis many investigators have focused on ways to interfere with the RNA synthesis process. The use of antibiotics as blocking agents on memory has come into prominence because of the known selective interference with RNA synthesis. For example, the antibiotic puromycin simply stops the growth of the polypeptide chain in the ribosome by replacing an amino acid (9). Another antibiotic, acetoxycycloheximide simply slows down the rate at which the amino acids are linked together.

Actinomycin D. --One means of inhibiting RNA synthesis was found with the antibiotic, actinomycin D, which binds specific sites on DNA molecules (8). In an experiment with this chemical, Barondes and Jarvik (4) found that mice were able to learn a shock-avoidance task even though RNA synthesis was inhibited 83 percent throughout the brain. Other research, however, has shown a deleterious effect on learning after actinomycin injection. Gaito (8) says that such inconsistent results may be due to the toxicity of actinomycin and its possible effects on general functions of the cell.

Puromycin. --In another research effort Barondes and Cohen (3) found that inhibition of protein synthesis by puromycin injections in the lobe of albino mice did not affect the acquisition of a shock-avoidance response five hours later. However, forty-five minutes after acquisition, retention was decreased by more than fifty percent. This result and other work by Agranoff and Klinger (1), Davis and Agranoff (11), and Davis (10), suggest that RNA and protein synthesis are not required for learning to occur, but that protein synthesis is necessary for the maintenance of memory traces.

Flexner and his associates (18) found that intracerebral injection of puromycin into mice after a training session causes loss of memory of avoidance discrimination learning. In an earlier investigation, Agranoff and Klinger (1) found that puromycin injected intracranially into goldfish produces memory loss for a shock-avoidance response.

In a subsequent study, Agranoff, Davis and Brink (13) investigated memory deficits obtained by injecting different amounts of puromycin at different times after training. In this study, after goldfish were given twenty shock-avoidance trials, they were given an intracranial injection of puromycin in saline solution. Each of the experimental groups received different dosages of puromycin at specified times on Day 1. On Day 4 they were given ten trials and scored for retention. The results indicated that memory fixation was insusceptible to puromycin one hour after training on Day 1. More specifically, ninety micrograms of

puromycin was found to be effective for less than sixty minutes, and fifty micrograms had no effect on memory. A dose of 170 micrograms was found to remain effective on memory for slightly more than one hour.

In another investigation, Davis and Agranoff (11) attempted to determine how long memory persisted in fish after an injection of puromycin immediately following training. The performance (retention) of puromycin fish appeared normal at one, three, and six hours, but responses decreased in twenty-four hours, and the decrease continued for more than forty-eight hours. The difference between retention scores at forty-eight and seventy-two hours was found to be significant to the 0.05 level. Agranoff has attributed this observed latent effect of puromycin on the perseveration of short-term memory.

Davis, Bright, and Agranoff (13) investigated the effects of electroconvulsive shock (ECS) compared with injection of puromycin introduced after one minute, thirty minutes, sixty minutes, ninety minutes, 120 minutes, 150 minutes, and 360 minutes after shock-avoidance training. Significant deficits occurred when ECS was given up to ninety minutes after training, whereas puromycin produced significant deficits when injected up to thirty minutes after training. It should be pointed out that this study used a smaller dosage of puromycin compared with previous studies, and this may have caused the ineffectiveness of puromycin injected thirty minutes or more after training.

In a more recent investigation, Davis (10) used a different training method and yielded a fixation time from 1.5 hours to three hours as compared to one hour using the original method. The change in method consisted of: (a) increasing the training trials from twenty to thirty, and (b) presenting the stimulus light in the goal compartment instead of the start compartment. This difference obtained in fixation time seems to warrant further investigation on the effects of different training techniques on memory consolidation.

Use of puromycin and other protein inhibitors in mice and rats. --

There have been several studies with mice and rats using various agents known to inhibit the synthesis of protein. Dingman and Sporn (14) used 8-azaquanine as their analogue and found the capacity to learn was severely retarded in rats in a maze-learning task. In an earlier study by Flexner and his associates, puromycin was shown to block out memory when injected directly into various areas of the brain of a mouse. Flexner (19) showed that retention of the original maze learning, which had been learned three weeks earlier, could be severely impaired by bilateral injections into the frontal and temporal regions. These results indicate susceptibility of the labile stage in memory to puromycin long after the time when most investigations have indicated that stable storage of information has been achieved.

Flexner (19) also measured the inhibition of protein synthesis in the rat brain after six injections (bilaterally in the temporal, ventricular, and

frontal regions) each consisting of thirty micrograms of puromycin. The inhibitory effects of these combined injections on protein synthesis was found to be most pronounced in the hippocampus and temporal regions. In these two areas, inhibition exceeded eighty percent for more than eleven hours after the injection. Inhibition of the cortex was somewhat less over this period. The parietal cortex, thalamus, and corpus striatum showed a larger decrement with time, reaching thirty-five to fifty percent inhibition 11.7 hours after the injection. The conclusion drawn was that long-term memory was destroyed by injections which inhibit protein synthesis in the hippocampus and temporal cortex by at least eighty percent for eleven hours, and in a substantial part of the remaining neocortex to a minimum of seventy percent for the same period of time.

In studying subcutaneous injections of mice, Flexner could not detect any interference with memory. It was concluded that protein synthesis was inhibited at a substantially lower level and for a shorter time with subcutaneous than with the effective intracerebral injections.

More recently, the use of acetoxycycloheximide (AXM) has come into focus. Davis, Agranoff, and Brink (5) have found that AXM, a potent inhibitor of protein synthesis in vivo, blocks both protein synthesis and memory formation in goldfish. In contrast to these results, Flexner and Flexner (17) have found that large amounts of AXM block mouse brain

protein synthesis without affecting memory formation. Furthermore, it was found that puromycin injected together with AXM did not block memory even though a pronounced deficit in protein synthesis was seen.

In recent experiments, Barondes (3) has shown that pre-trial injections of cycloheximide blocks protein synthesis without affecting memory. This is analogous to the Flexner findings with AXM. According to the knowledge of these two drugs, they both antagonize the action of puromycin.

As a consequence of these and comparable findings, Flexner no longer feels that protein synthesis is directly related and involved in memory formation. He has suggested that memory is more directly related to a stable messenger RNA, which codes for a protein, serving in turn as the inducer of its messenger (20). In other words, puromycin could block formation of its protein and allow its messenger to decay. Acetoxycycloheximide, on the other hand, would preserve the messenger on the polysome. In a more recent experiment in Flexner's laboratory, AXM produced a temporary memory loss in mice, thus, providing evidence for the hypothesis concerning the role of puromycin.

More recently however, Barondes (4) found that actinomycin D, an RNA blocking agent, did not block acquisition or memory formation at concentrations that block RNA synthesis. This finding contradicts the hypotheses postulated by Flexner, because actinomycin D should have

lead to the depletion of messenger RNA, thus, blocking protein synthesis which would in turn have lead to a loss in memory. Appel (2) has suggested that antibiotics might produce temporary lesions in areas of the brain related to a specific function. Some of Flexner's studies have indicated toxic effects produced by puromycin. In one study, mice receiving intracerebral injection of puromycin showed toxic symptoms. There was lethargy and loss of alertness followed by hyperexcitability, as well as loss of weight due to the failure to eat and drink normally. Flexner has suggested that in studies using protein inhibitors, the indicated memory loss may have been due to ill-producing effects of the inhibitors rather than an essential effect on the memory process.

Synthesis of Literature

The concept of memory consolidation implies that memory is made stable through changes in the brain which continue after the learning experience. A fixation process lasting minutes to hours after the learning experience has been indicated by several studies which have shown retention of an experience capable of being blocked by various physical and chemical agents administered shortly after learning. Most attempts to interfere with memory have used infra-human organisms, and the interfering agent most often used has been electroconvulsive shock (ECS). Most of the studies using ECS have reported significant memory impairment with ECS administered within one hour after learning; furthermore,

administration of the ECS after the consolidation phase (one hour or more), had no effect on memory of the learning experience.

Two major hypotheses concerning the effect of ECS stem from the above literature survey: (a) ECS produces a disruption in the consolidation phase, producing, in turn, an impairment in the formation of long-term memory; (b) ECS is a kind of punishment, and if it comes close enough to the completion of learning, it produces a fear-induced response when the subject is confronted with the same situation at a future time.

With the profound advances made in the knowledge of protein chemistry, biochemical correlates of memory have been possible through the use of antibiotics known to interfere selectively with RNA and protein synthesis. Puromycin and acetoxycycloheximide have been shown to inhibit protein synthesis as much as ninety percent in the goldfish and rat brain. Actinomycin D, another antibiotic, has been shown to inhibit mRNA synthesis in vivo. In general, these antibiotics have been found, presumably, to interfere with the formation of long-term memory if injected intracranially or intracerebrally into goldfish or rat, respectively. The general assumption is that these antibiotics have to be administered during the consolidation phase of memory in order to effectively block out memory. The time required for consolidation is generally agreed to be within two hours after learning. It should be pointed out that the consolidation phase seems to vary with the dosage of antibiotic given and the species of animal used.

Caution in interpreting the effects of these antibiotics has been emphasized by several investigators. Davis has pointed out the possibility that these antibiotics may serve as aversive stimulation to the central nervous system, and instead of impairing the actual memory processes, may cause fear-induced and/or ill-motivated responses. Flexner has reported ill-observed effects in mice injected with puromycin, and he has mentioned the possibility of confusing memory impairment with physical impairment.

Statement of the Problem

Agranoff, Davis, and their co-workers have found that goldfish injected with puromycin immediately after a session of shock-avoidance training in a shuttlebox, after three days show performance inferior to that of those receiving no puromycin, to those receiving only saline, and to those receiving puromycin after some delay. A reasonable conclusion to these results may be that the drug somehow interferes with retention; however, the question may be asked, "Is it possible that puromycin and other agents believed to interfere with the memory process, instead, render unaccountable or subtle physical effects, giving an experimental impression of memory impairment?" The goldfish studies by Agranoff and Davis have provided the strongest indication of relating the formation of long-term memory with the synthesis of protein. Unfortunately, however, the experimental designs utilized by these investigators have not

lent themselves to the complete functional analysis of the effects of puromycin, electroconvulsive shock, and other agents believed to disrupt the memory process. Invariably, the criterion used in these studies for inferring the effectiveness of these agents in blocking out memory has been the inferior performance of goldfish in a retraining shock-avoidance situation. This criterion used in gauging memory or retention is the crux of the problem presented here. At this point the question is asked, "Will a study using a criterion measure of superior performance as an analogue for memory impairment, support previous studies that have suggested the effectiveness of puromycin and ECS in blocking out memory?"

Specific Purpose of Study

The purpose of the present study was to determine the effectiveness of puromycin and electroconvulsive shock (ECS) in blocking out memory in the goldfish; moreover, in determining the effectiveness of these agents, an experimental procedure was designed for obtaining evidence leading to the resolution of the problem stated above, namely, the inconclusive evidence for the assumed effects of puromycin and ECS in goldfish. The criterion measure is described in Chapter II, but briefly, superior performance instead of inferior performance in a shock-avoidance retention situation was used as the correlate for memory impairment.

Hypotheses

It will be recalled that the findings by Agranoff and Davis have indicated the capacity of puromycin and ECS for blocking out long-term memory of a simple avoidance training in goldfish. Their findings have also indicated the ineffectiveness of these agents if administered more than ninety minutes after the training session. In order to clarify the implications of the hypotheses for testing the stated relations, a brief description of the training schedule is presented. An A-B-A learning paradigm was used in which subjects on Day 1 (designated A) were trained to swim to the light stimulus. On Day 2 (designated B) the subjects were trained to avoid the light stimulus, a reversal of A. On Day 5 (designated A) subjects were tested for retention of Day 1 (A) training. A more thorough description of the training procedure and its relation to the experimental design is presented in the next chapter.

Stated Relations

In keeping with the experimental findings of Agranoff, Davis, and their co-workers, and with the general theories presented above, this study was designated to test empirically the following hypotheses:

Hypothesis 1. --Those subjects receiving puromycin one minute after reversal training B will, on retention of A, perform significantly superior to (a) those subjects not receiving puromycin and ECS; (b) those subjects receiving puromycin and ECS 100 minutes after training.

Hypothesis 2. --Those subjects receiving ECS one minute after reversal training B will, on retention of A, perform significantly superior to (a) those subjects not receiving puromycin and ECS; (b) those receiving puromycin and ECS 100 minutes after training.

Hypothesis 3. --Those subjects given puromycin and ECS 100 minutes after reversal training B will not, on the retention of A, differ significantly from those not receiving these agents.

Hypothesis 4. --Those subjects receiving puromycin one minute after reversal training B will not, on the retention of A, differ significantly from those receiving ECS one minute after reversal training B.

Hypothesis 5. --Those subjects not receiving reversal training B will not, on the retention of A, differ significantly from (a) those receiving puromycin one minute after reversal training B; (b) those receiving ECS one minute after reversal training B.

CHAPTER BIBLIOGRAPHY

1. Agranoff, Benard W., and Paul D. Klinger, "Puromycin Effect on Memory in the Goldfish," Science, CXLVI (1964), 952-953.
2. Appel, S. H., "Effect of Inhibition of RNA Synthesis On Neural Information Storage," Nature, CCVII (1965), 1163-1166.
3. Barondes, Samuel H., and Harry D. Cohen, "Comparative Effects of Cycloheximide and Puromycin on Cerebral Protein Synthesis and Consolidation of Memory," Brain Research, IV (1967), 44-51.
4. Barondes, S. H., and M. E. Jarvik, "The Influences of Actinomycin-D on Brain RNA Synthesis and on Memory," Journal of Neurochemistry, XI (1964), 594.
5. Brink, J. J., R. E. Davis, and B. W. Agranoff, "Effects of Puromycin, Acetoxycycloheximide and Actinomycin D On Protein Synthesis in Goldfish Brain," Journal of Neurochemistry, XII (1966), 889-896.
6. Cerf, J. A., and L. S. Otis, "Heat Narcosis and Its Effect on Retention of a Learned Behavior in the Goldfish," Federation Proceedings, XVI (1957), 20-21.
7. Coons, E. E., and N. E. Miller, "Conflict Versus Consolidation of Memory Traces to Explain "Retrograde Amnesia" Produced by ECS," Journal of Comparative and Physiological Psychology, LIII (1960), 524-531.
8. Corning, W. C., and S. C. Ratner, editors, Chemistry of Learning, New York, Plenum Press, 1967.
9. Darken, Marjarie A., "Puromycin Inhibition of Protein Synthesis," Pharmacological Review, XVI (1964), 223-243.
10. Davis, Roger E., "Environmental Control of Memory Fixation in Goldfish," Journal of Comparative and Physiological Psychology, LV (1968), 72-78.

11. Davis, R. E., and B. W. Agranoff, "Stages of Memory Formation in Goldfish," Proceedings from the National Academy of Science, USA, LV (1966), 555-559.
12. Davis, R. E., J. J. Brink, and B. W. Agranoff, "Memory Fixation in the Goldfish," Proceedings from the National Academy of Science, USA, LIV (1965), 788-793.
13. Davis, R. E., P. J. Bright, and B. W. Agranoff, "Effect Of ECS and Puromycin on Memory in Fish," Journal of Comparative and Physiological Psychology, LX (1965), 162-166.
14. Dingman, W., and M. B. Sporn, "Molecular Theories of Memory," Science, CXLIV (1964), 26-29.
15. Duncan, C. P., "The Retroactive Effect of Electro-Shock on Learning," Journal of Comparative and Physiological Psychology, XLII (1949), 32-34.
16. Ehrenpreis, S., and Othmar C. Solnitzky, editors, Neurosciences Research, New York, Academic Press, 1968.
17. Flexner, L. B., and J. B. Flexner, "Effect of Acetoxycycloheximide and of an Acetoxycycloheximide-Puromycin Mixture on Cerebral Protein Synthesis and Memory in Mice," Proceedings from the National Academy of Science, USA, LV (1966), 369-374.
18. Flexner, L. B., J. B. Flexner, G. de la Haba, and R. B. Roberts, "Loss of Memory as Related to Inhibition of Cerebral Protein Synthesis," Journal of Neurochemistry, XII (1965), 535.
19. Flexner, L. B., J. B. Flexner, and G. de la Haba, "Loss of Recent Memory in Mice as Related to Regional Inhibition of Cerebral Protein Synthesis," Proceedings from the National Academy of Science, USA, LII (1964), 1165-1169.
20. Flexner, J. B., and R. B. Roberts, "Memory of Mice Analyzed with Antibiotics," Science, CLV (1967), 1377-1383.
21. Gaito, John A., "A Biochemical Approach to Learning and Memory," Psychological Review, LXVIII (1961), 288.
22. Glickman, Stephen E., "Perseverative Neural Processes and Consolidation of the Memory Trace," Psychological Bulletin, LVIII (1961), 218-233.

23. Hayes, L. J., "Anoxia and Convulsive Amnesia in Rats," Journal of Comparative and Physiological Psychology, XLVI (1953), 216-217.
24. Leukel, F. P., "The Effect of ECS and Pentothal Anesthesia on Maze Learning and Retention," Journal of Comparative and Physiological Psychology, L (1957), 300-306.
25. Lewis, Donald J., and Brendan A. Mahrer, "Neural Consolidation and Electroconvulsive Shock," Psychological Review, LXXII (1965), 225-239.
26. Madsen, Millard C., and James C. McGaugh, "The Effect of Electroconvulsive Shock on One-Trial Avoidance Learning," Journal of Comparative and Physiological Psychology, LIV (1961), 522-523.
27. McConnell, J. V., A. Jacobson, and D. P. Kimble, "The Effects of Regeneration Upon Retention of a Conditioned Response in the Planarian," Journal of Comparative and Physiological Psychology, LII (1959), 1.
28. McGaugh, James L., "Time-Dependent Processes in Memory Storage," Science, CLIII (1966), 1351-1358.
29. Morgan, Clifford T., Physiological Psychology, New York, McGraw-Hill Book Company, 1965.
30. Pearlman, C. A., S. K. Sharpless, and M. E. Jarvik, "Retrograde Amnesia Produced by Anesthetic and Convulsive Agents," Journal of Comparative and Physiological Psychology, LIV (1961), 109-112.
31. Russell, W. R., and P. W. Nathan, "Traumatic Amnesia," Brain Research, LXIX (1946), 280-300.
32. Thompson, R., and R. S. Pryer, "The Effect of Anoxia on the Retention of a Discrimination Habit," Journal of Comparative and Physiological Psychology, XLIX (1956), 297-300.

CHAPTER II

METHOD

Subjects

The subjects in the experiment proper were 99 experimentally naive goldfish, Carassius auratus, obtained at Reeder's Fish Farm, Seagoville, Texas, and were selected by length (3-4 inches from snout to caudal peduncle), with a total weight variation of 11.5-15.0 grams. A total of 106 fish were obtained in two equal shipments separated by two weeks of experimentation. The fish of each shipment were randomly chosen and separated in a 120-gallon tank (2'x8'x1') made from 1/2-inch plywood, and lined with 6-mil polyethelene sheeting. The tank was divided into 60 equal compartments by 1/4-inch mesh hardware cloth. The water was aerated and filtered with a 1/2-hp piston air pump and dynaflo motor filter. The water temperature was kept at $64 \pm 2^{\circ}\text{F}$ with a 100-watt heater. The subjects were fed at noon daily.

Plausibility of Studying Goldfish

The goldfish as an experimental animal is ideally suited for studies on biochemical correlates of behavior. They are easily housed, learn quickly, and retain what they have learned for a long period of time. Agranoff (1) has found significant retention of learning for at least one

month after the original training. The brain is small and easily penetrated with a thin needle, making it suitable for local hypo applications. The goldfish brain is a primitive brain devoid of a cerebral cortex, and, it seems, would constitute a simple system for the study of memory.

Apparatus

Training Apparatus

As shown in Figure 1, the apparatus was an aquatic shuttle box consisting of 1/8-inch crystal plastic tank measuring 4.5 x 10 x 4.5 inches deep and was divided into two equal compartments by an opaque underwater hurdle two inches in height. The water depth was 3.5 inches in each compartment and 1.5 inches over the barrier.

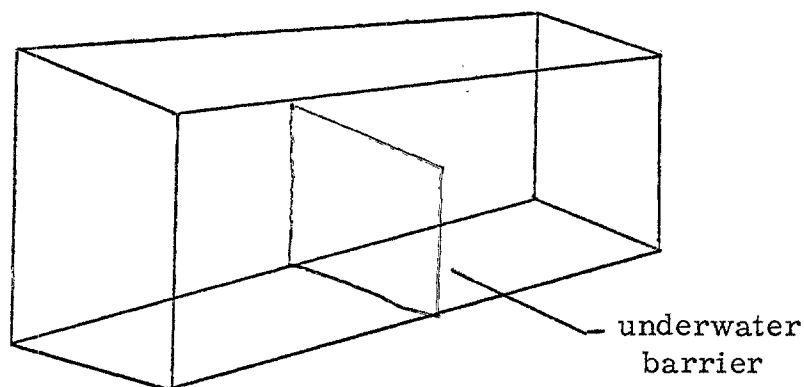


Fig. 1. Aquatic Shuttlebox

Each compartment had separate shocking grids of stainless steel screen on the inner surface of the sidewalls and a stimulus lamp (Sylvania 120

PSB) two inches outside each end wall. The shuttle was housed in the center of a larger 1/4-inch plywood box measuring 15.25 x 19.5 x 4.5 inches deep as diagrammatically shown in Figure 2. The complete training apparatus was set up in an enclosed cubicle measuring 4.5 x 4.5 x 4.5 feet in order to provide training devoid of any light.

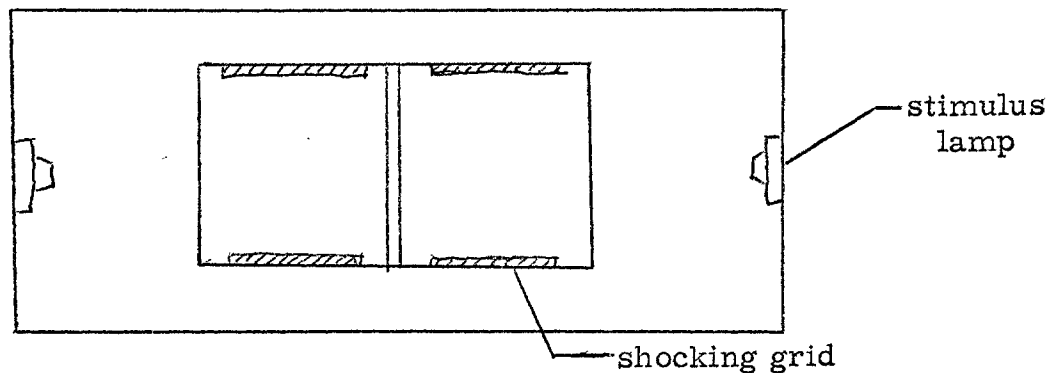


Fig. 2. Top view of shuttlebox

Timing Apparatus and Program

The timing for training was automatically programmed through two Lafayette decade interval timers and a Lafayette time programmer. Four silent toggle switches were used to manually open and close the circuit leading to the shocking grids and stimulus lamps. This apparatus was programmed so that a training trial cycle consisted of 20 seconds of light, 20 seconds of light coupled with shock, and 20 seconds of darkness. The electrical pulsating shock (3-v. ac, at 0.1 ma) and the stimulus light could be relayed to either side of the shuttle box by manipulating the four toggle switches.

Experimental Procedure

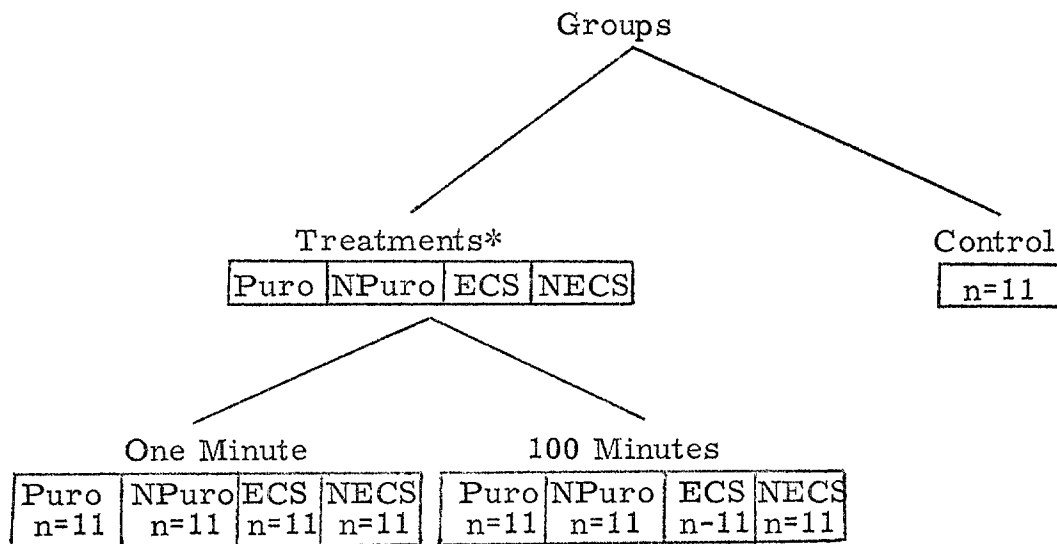
Pilot Study

A pilot study was performed in order to determine the feasibility of using a shock-avoidance training schedule patterned after a retroactive inhibition paradigm. Also, the pilot study was employed to test the efficacy of the apparatus and instruments designed for the study. A total of thirty goldfish were used, and each was conditioned by one of the two following training schedules: (a) thirty shock-avoidance trials followed twenty-four hours later by ten retention trials; (b) thirty shock-avoidance trials followed twenty-four hours later by thirty reversal trials, and followed seventy-two hours later by one of the following: (1) ten retention trials on the reversal training; (2) ten retention trials on the original training. Examination of the retention scores for all subjects clearly indicated the propensity for each subject to respond in a way more compatible with the last training. The favorable findings gleaned from this study provided the procedural grounds for the main study.

Experimental Design

A total of ninety-nine experimentally naive goldfish were randomly assigned to one of eight experimental groups and one control group. The experimental groups were subdivided into one-minute and 100-minute treatment classifications. The control group was differentiated from the

treatment (experimental) groups by the shock-avoidance training schema in which the reversal training was eliminated for the control group. A schematic presentation of the experimental design is found below.



- *Puro - Puromycin dihydrochloride
- NPuro - No puromycin dihydrochloride
- ECS - Electroconvulsive shock
- NECS - No electroconvulsive shock

Criterion Measure and Treatment of Data

The criterion measure used was the number of correct responses (avoidances) of each subject in the ten retention trials. The number of avoidances was defined as retention scores. A 2 x 4 factorial design with a single control group described by Winer (4) was employed. By this method, an analysis of variance of treatment vs. time was determined. This method of analysis also allowed the control group to be contrasted with all the experimental groups combined.

Training Schedule

In keeping with the objectives of this study and the findings from the pilot study, a paradigm for retroactive inhibition (A-B-A) was incorporated into the training sequence. This learning model is described by Hilgard (3). A detailed description of the training schedule is presented below.

Training A

In the original training A, each subject, after being placed in the shuttle box for one minute, was given thirty shock-avoidance trials in thirty minutes. The intertrial was twenty seconds. A trial consisted of twenty seconds of light in the goal compartment (the empty compartment), and then, paired with the light, twenty seconds of pulsating electrical shock in the darkened start compartment. The light and shock were terminated at the end of the forty-second trial. The twenty-second intertrial immediately followed. The subject could avoid or escape the shock by swimming over the barrier from the dark to the light compartment. The responses of each subject were recorded by direct observation. A correct response (avoidance) was recorded when the subject avoided the shock by crossing the barrier and remained in the light compartment during the trial. A few of the fish neither avoided nor escaped the shock during this training phase and were replaced.

Training B

Twenty-four hours after the completion of training A, each subject, except for those in the control group, underwent thirty trials of training B which was a reversal of A. Thus, the fish were retrained to avoid the shock by swimming over the barrier to the dark side of the shuttlebox. At the appropriate time after Trial 30 in this training phase, the experimental treatments were administered as described below.

1-min. Puro. --Eleven subjects were given 170 micrograms of puromycin intracranially exactly one minute after Trial 30. The fish were hand-held for injection and then immediately placed back in their individual home compartment.

100-min. Puro. --Eleven hand-held subjects were given 170 micrograms of puromycin intracranially exactly 100 minutes after Trial 30 and immediately placed back in their home compartment.

1-min. NPuro. --Eleven subjects, at exactly one minute after Trial 30, were hand-held in simulated fashion but were not given an injection.

100-min. NPuro. --Eleven subjects, at exactly 100 minutes after Trial 30, were hand-held without injection.

1-min. ECS. --Eleven subjects, at exactly 1 minute after Trial 30, were placed in the ECS box and given 15 seconds of electroconvulsive shock after which followed immediate placement in the home compartment.

100-min. ECS. --Eleven subjects, at exactly 100 minutes after Trial 30, were given 15 seconds of electroconvulsive shock and then immediately returned to their home compartment.

1-min. NECS. --Eleven subjects, at exactly one minute after Trial 30, were placed in the ECS tank in a simulated manner and were left for 15 seconds without shock. They were then immediately placed back in their home compartment.

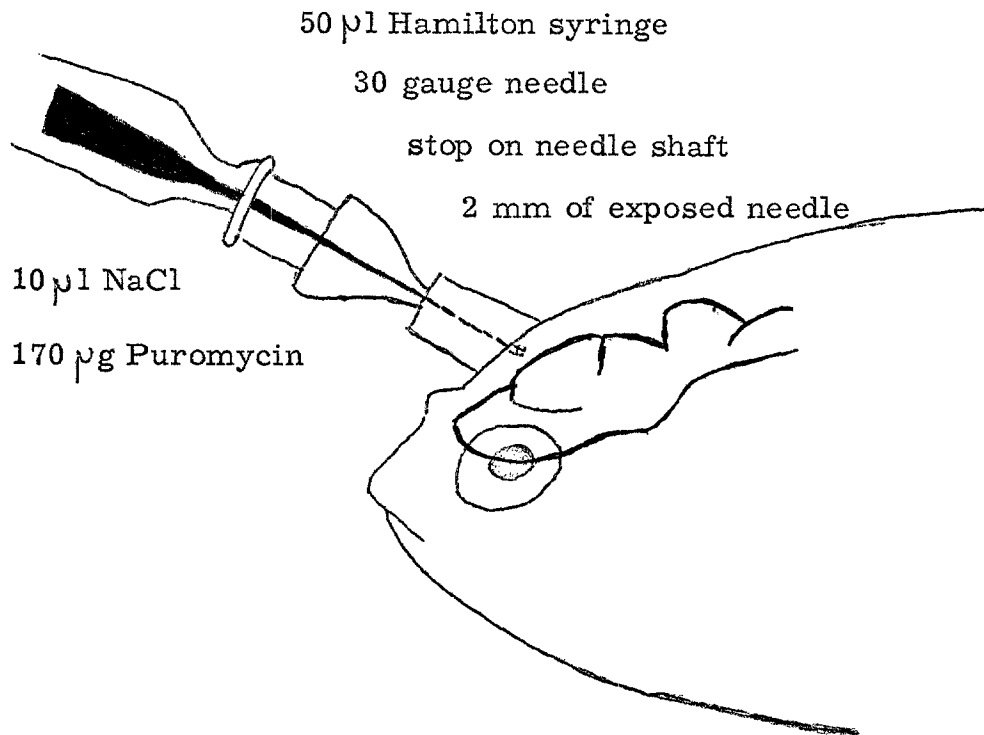
100-min. NECS. --Eleven subjects, at exactly 100 minutes after Trial 30, were placed in the ECS tank without receiving the ECS treatment. They were then immediately returned to their home compartment.

Retention A

Retention of the initial training A was tested ninety-six hours after Trial 30 of training A by giving all subjects, including those in the control group, ten retention trials. Retention was defined as the number of correct avoidances achieved in this retraining phase.

Injections

Puromycin dihydrochloride (Nutritional Biochemicals Corporation), 170 micrograms in 10 microliters of isotonic (0.15 m) sodium chloride, was injected into the cranial cavity over the midbrain with a No. 30 gauge needle and a 50-microliter Hamilton syringe (1). This method of injection is illustrated in Figure 3. The cranium was penetrated at the medial suture in line with the posterior margin of the orbits. The needle was inserted 2 mm at an angle of about 45 degrees to the surface and directed posteriorly, and the depth of penetration was limited by a 22-gauge needle stop on the 30 gauge needle shaft. By this method, the needle tip overlies the tecta and does not touch the brain substance. Goldfish could be injected rapidly without apparent brain damage. Sterilization of the instruments used for injection was maintained by autoclaving.



Penetration at medial suture in
 line with posterior margin of
 the orbits

Angulation is 45 degrees directed
 posteriorly

Depth is 1-2 mm so that tip is over
 tectum of midbrain

Fig. 3. Schematic illustration of intracranial injection of Puromycin into the cranial cavity of the goldfish.

Electroconvulsive Shock

The ECS was given in a plastic tank 3 x 9 x 3 inches deep, which was filled to a depth of 1.5 inches. The power of the ECS tank was delivered through two stainless steel plates suspended at each end of the tank. The ECS was 15 sec. of 60 cps, sine wave, 26-v. ac at 5 ma. This was in accordance with the study by Davis, Bright, and Agranoff (2).

Control for Various Order and Time Effects

Shock-avoidance training during a day lasted approximately ten hours, and completion of experimentation on each shipment of goldfish lasted two weeks. Consequently, it was necessary to devise a system for controlling time of training effects in relation to feeding, water temperature fluctuation, and time of training and treatment after shipment. Two subjects were selected from each of the nine "home" groups of fish, and these constituted a training group for a given day. The time of training was systematically varied for the different training groups so that the experimental and control groups were represented by fish trained at various times. This method was adopted for each of the training groups in an attempt to keep all possible training and treatment deviation at a minimum.

CHAPTER BIBLIOGRAPHY

1. Agranoff, Benard W., and Paul D. Klinger, "Puromycin Effect on Memory in the Goldfish," Science, CXLVI (1964), 952-953.
2. Davis, Roger E., Patricia J. Bright, and B. W. Agranoff, "Effect of ECS and Puromycin on Memory in Fish," Journal of Comparative and Physiological Psychology, LX (1965), 162-166.
3. Hilgard, Ernest R., and Gordon H. Bower, Theories of Learning, New York, Meredith Publishing Company, 1966.
4. Winer, B. J., Statistical Principles in Experimental Design, New York, McGraw-Hill Book Company, 1962.

CHAPTER III

RESULTS

Performance Gradient

With the purpose of investigating the effects of puromycin and electroconvulsive shock (ECS) on memory retention in the goldfish, a paradigm for retroactive inhibition was adopted for shock-avoidance training. All subjects were subjected to an initial training designated as training A. Except for eleven subjects serving as the control group, all subjects underwent a reversal of training A, and this was designated as reversal training B. Figure 4 represents the mean number of avoidances for all subjects that underwent training A, and all that underwent reversal training B. As will be recalled, "avoidances" (correct responses) referred to the number of times the subject avoided the shock by swimming over the barrier. These means are presented in blocks of ten trials.

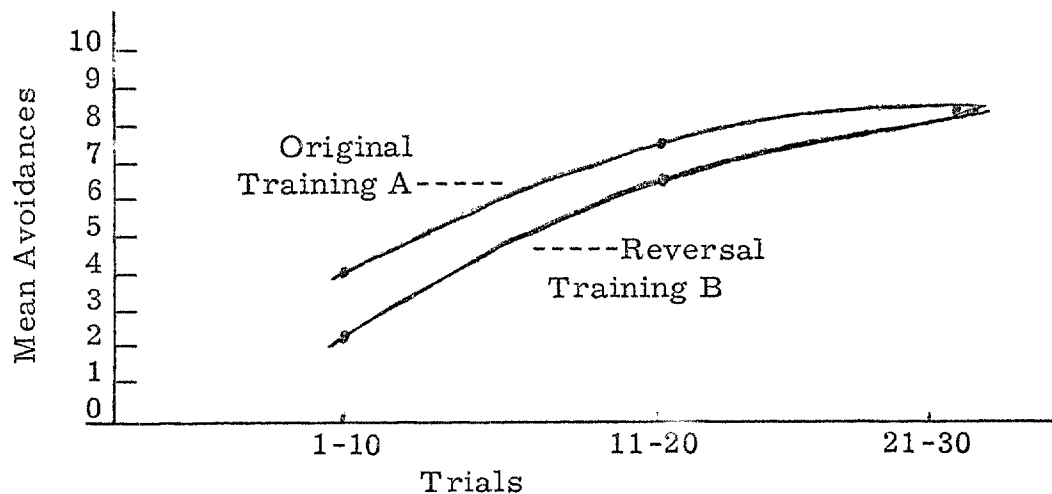


Fig. 4 -- Mean avoidances for all subjects participating in training A

As shown in Figure 4, the interfering effect of the reversal training B is quite obvious when the means for the first ten trials are compared. The subjects avoided the shock approximately 20 percent of the time in the reversal training B, as compared with 37 percent in the original training A. The performance gradient for the thirty trials is consonant with Agranoff's findings. Agranoff (1) found that naive fish, not previously exposed to the apparatus, avoided the shock up to 30 percent of the first ten trials and continued to improve in subsequent trials. If they were allowed to perform the task day after day, the performance curve flattened out at about 80 percent correct response.

Observed Treatment Effects of Puromycin and ECS

The subjects injected with puromycin did not demonstrate observable evidence of transient drowsiness or any other usual disorder; rather, they appeared to swim normally after injection.

The subjects receiving electroconvulsive shock showed immediate stiffening and abnormal posture during the 15-second convulsive period. These subjects invariably rotated on their sides with the body straight or curved and fins and opercula extended. Immediately at the termination of the shock period, the pectoral and pelvic fins showed slight quivering movements. Within approximately 25 seconds after the administration of ECS, respiration movements were restored. These observations are similar to those reported by Davis, Bright, and Agranoff (2).

Statistical Analyses

In retrospect, the statistical plan was to ascertain the effects of puromycin and ECS by analyzing the retention scores for all groups. Retention scores, as will be recalled, referred to the number of shock avoidances of each fish in the ten retention trials. Eight experimental (treatment) groups consisted of four major treatment groups which were classified into one-min. and 100-min. levels.

Central Tendency and Variability of Criterion

The basic data entered into the analyses of variance are presented in Table I in the form of means, standard deviations, and number of observations (N). The Mn, SD, and N for the control group are noted at the bottom of Table I.

TABLE I
MEANS AND STANDARD DEVIATIONS OF RETENTION
SCORES FOR TREATMENT GROUPS

Time	Statistic	Treatment				Combined Time
		Puro	No Puro	ECS	No ECS	
1-min.	Mn	5.00	2.27	7.36	2.54	4.30
	SD	1.48	.84	1.45	1.24	5.66
	N	11	11	11	11	44
100-min.	Mn	2.36	2.73	2.73	2.46	2.57
	SD	.88	.86	1.54	1.37	1.42
	N	11	11	11	11	44
Combined Treat- ment	Mn	3.68	2.50	5.05	2.50	
	SD	2.67	.76	7.64	1.69	
	N	22	22	22	22	

Note. --Control group... Mn=7.64; SD=1.03; N=11

In reference to Table I, the combined mean for groups under the 1-min. treatment (4.30) is considerably higher than the combined mean observed for the 100-min. groups (2.57). Also noteworthy, the means for the four groups receiving puromycin and ECS are relatively higher than the corresponding groups not administered these agents. Further inspection suggests that the means for the 1-min. puromycin and ECS groups are considerably higher than the corresponding means of the 100-min. puromycin and ECS groups. The control group yielded the highest group mean.

Homogeneity of variance was tested by Hartley's (4) F_{\max} statistic, and since the observed value ($F_{\max} = 3.4$) did not exceed the critical value ($F_{.95}(8, 10) = 7.87$), the hypothesis of homogeneity of variance was considered tenable. With this assumption of homogeneity of variance, an analysis of variance seemed statistically in order.

Factorial Analysis

It will be recalled that a factorial design was adopted by which the control group could be contrasted with all the treatment groups. In the analyses of variance for the factorial part of the experiment, the control group was disregarded in order to determine treatment and time effects on the retention scores. To reiterate, all analyses were performed on the retention scores obtained from the final ten training trials. A summary of the analysis of variance performed on the data is presented in Table II.

TABLE II
SUMMARY OF ANALYSIS OF VARIANCE OF THE RETENTION
SCORES FOR THE TREATMENT AND TIME EFFECTS
AND THESE COMBINED EFFECTS CONTRASTED
WITH A CONTROL GROUP

Source of Variation	SS	df	MS	F
Control vs. all	190.14	1	190.14	162.51**
Time	65.64	1	65.64	56.10**
Treatment	96.85	3	32.29	27.60**
Interaction	92.00	3	30.67	26.21**
Within	105.09	90	1.17	...
Total	359.59	97

**Significant at .01 level.

By using the $P=.01$ level of significance, the critical value for the test of interaction was $F_{.99}(3, 90)=4.04$. Since the observed F ratio ($F=26, 21$) was larger than the critical F value, the evidence contradicted the statistical hypothesis of zero interaction. The main effects for Time and Treatment also exceeded their critical values, ($F=4.04$ and $F=6.97$ respectively), thus, indicating significant difference between at least two of the treatment groups. The obtained F ratio for the control group contrasted with the combined treatment groups ($F_{.99}(1, 90)=162.51$) was also statistically significant beyond the one per cent level of significance. In order to examine the nature of the interaction and trend in the analysis, a graphic presentation of all experimental groups is given in Figures 5 and 6.

Presented in Figure 5 are the profiles of the treatment group and control group means.

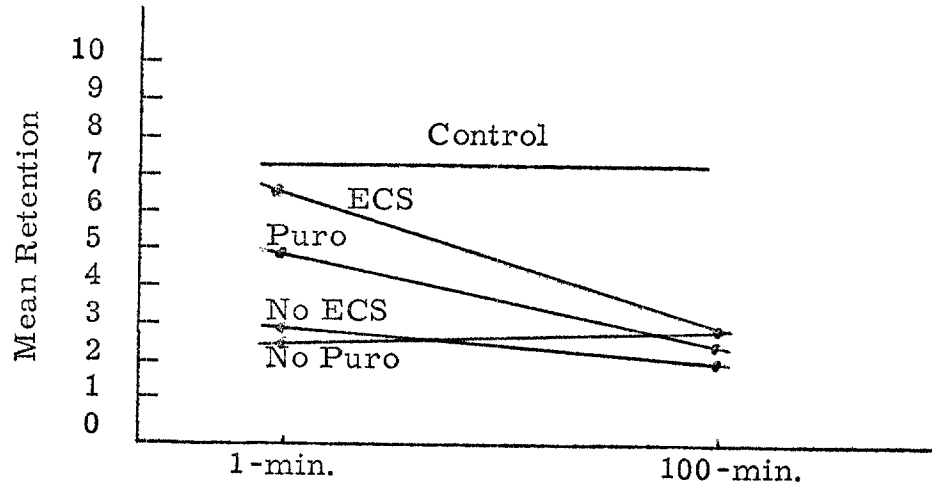


Fig. 5--Mean profiles for different treatments at 1-min. and 100-min. levels.

In the interest of clarity, Figures 5 and 6 contain the same means, but in Figure 5 the treatments are in the body of the figure and the time on the abscissa. This is reversed in Figure 6. Presented in Figure 6 are the profiles of the time of treatment.

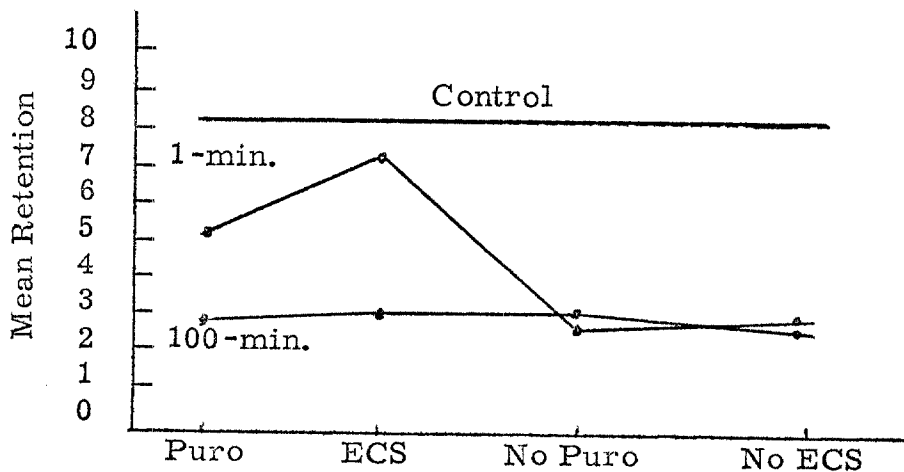


Fig. 6--Mean profiles for different time of treatment.

Inspection of Figure 5 and Figure 6 profiles indicates relatively large differences between the control group and the groups which did not receive puromycin or ECS and all the groups at the 100-min. level. There appears to be an intermediate difference between the control group and the 1-min. puromycin group. Relatively little difference between the following groups is observed: 1-min. NPuro; 1-min. NECS; all groups at the 100-min. level. The linear interaction is indicated by the lack of parrallelism of the profiles.

Post-hoc Examination of 1-Min. and Control Groups

In order to examine all possible comparisons at the 1-min. level with the control group included, a multiple comparisons procedure developed by Scheffé (3) was used. A simultaneous confident coefficient of .95 was chosen, and the derived confidence interval was $-1.320 \leq r_1 - r_2 \leq +1.320$. The comparisons are presented in Table III.

TABLE III

MULTIPLE COMPARISONS OF RETENTION SCORE MEANS OF
1-MINUTE GROUPS AND A CONTROL GROUP

Group	Puro	NoPuro	ECS	NoECS
Control	2.64*	5.37*	.28	5.10*
Puro	...	2.73*	2.36*	2.46*
NoPuro	-7.36*	-.27
ECS	4.82*
NoECS

*Significant at .05 level.

Two major observations can be made from Table III: (a) All comparisons were significant except for the Control-ECS comparison and the NPuro-NECS comparison; (b) Puro was significantly different from all groups. The latter observation warrants the rejection of Hypothesis IV, since there was a significant difference between 1-min. Puro and 1-min. ECS. Part A of Hypothesis V was also rejected because of the significant difference found between the Control and Puro groups.

Analysis of 100-Minute Groups

The profiles of Figures 5 and 6 indicated little difference among the 100-min. groups. In statistical manner, this indication was examined and supported by analysis of variance. The summary table is presented in Table IV.

TABLE IV

SUMMARY OF ANALYSIS OF VARIANCE OF RETENTION
SCORES FOR 100-MINUTE TREATMENT GROUPS

Source of Variation	SS	df	MS	F
Treatment	1.36	3	.4533	N.S.*
Error	43.64	40	1.1160	...
Total	45.00	43

*Nonsignificant at .05 level.

The 100-min. ECS group had the highest mean at the 100-min. level; consequently, a Fisher t test was computed to determine the significance of the difference between 1-min. Puro and 100-min. ECS. A highly significant difference was found ($t=375.07$), thus, indicating significant differences of the Control, 1-min. ECS, and 1-min. Puro groups with all 100-min. groups. On the basis of this and the above analyses, the tenability of the aforementioned hypotheses are presented below.

Tenability of Hypotheses

Since a significant difference was found between the 1-min. Puro group and 100-min. ECS group, it was concluded that the 1-min. Puro group differed significantly from all 100-min. groups, since the 100-min. ECS group represented the high mean; furthermore, since the 1-min. Puro group differed significantly from the 1-min. NPuro and 1-min. NECS, Hypothesis 1 was judged tenable, as follows: Those subjects receiving puromycin one minute after reversal training B will, on retention of A, perform significantly superior to (a) those subjects not receiving puromycin and ECS; (b) those subjects receiving puromycin and ECS 100 minutes after training.

Since the 1-min. ECS group mean was greater than the 1-min. Puro group mean, the rationale for supporting Hypothesis 1 is apropos for accepting the tenability of Hypothesis 2 as follows: Those subjects

receiving ECS one minute after reversal training B will, on retention of A, perform significantly superior to (a) those subjects not receiving puromycin and ECS; (b) those receiving puromycin and ECS 100 minutes after training.

Table IV indicates no significant difference between the 100-min. groups, and since Table III indicates no significant difference between the 1-min. NPuro and 1-min. NECS groups, Hypothesis 3 was judged tenable as follows: Those subjects given puromycin and ECS 100 minutes after reversal training B will not, on the retention of A, differ significantly from those not receiving these agents.

Table III indicates a significant difference between the 1-min. Puro group and the 1-min. ECS group, thus, providing evidence for the rejection of Hypothesis 4 which stated: Those subjects receiving puromycin one minute after reversal training B will not, on the retention of A, differ significantly from those receiving ECS one minute after reversal training B.

Table III revealed a significant difference between the 1-min. Puro group with the control group and no significant difference between the 1-min. ECS group and the control group; therefore, part (a) of Hypothesis 5 is rejected, and part (b) is accepted, as follows: Those subjects not receiving reversal training B will not, on the retention of A, differ significantly from (a) those receiving puromycin one minute after reversal training B; (b) those receiving ECS one minute after reversal training B.

CHAPTER BIBLIOGRAPHY

1. Agranoff, Benard W., and Paul D. Klinger, "Puromycin Effect on Memory in the Goldfish, " Science, CXLVI (1964), 952-953.
2. Davis, Roger E., Patricia J. Bright, and B. W. Agranoff, "Effect of ECS and Puromycin on Memory in Fish, " Journal of Comparative and Physiological Psychology, LX (1965), 162-166.

CHAPTER IV

DISCUSSION OF RESULTS

The results of the present study appear to be in line with previous studies using puromycin and electroconvulsive shock in indicating a blocking-out of long-term memory fixation. The general finding was that ECS and puromycin administered shortly after training improved performance in the retention trials by blocking out the interfering effect of the reversal training. However, when compared with a control group which did not receive the reversal training, the ECS and puromycin groups performed fewer avoidances (correct responses) in the retention trials, indicating that neither treatment produced a complete memory loss of the reversal training. The ECS was more effective, and the retention scores for this group did not differ significantly from the control group as did the puromycin group.

An interesting observation should be mentioned at this point in reference to the retention trials. It will be recalled that the retention trials consisted of ten trials in which each subject was retrained to avoid the shock by swimming to the light. Many of the subjects that received no treatment, and many that received ECS and puromycin 100 minutes after training B, showed strong resistance to the extinction of the reversal

training. This was clearly indicated by the number of times they would swim back to the shock (dark) side after escaping it during the twenty seconds of shock time. It was characteristic for these fish to shuttle back and forth across the barrier for as many as five times during the shocking period. Those receiving 1-minute treatments appeared naive with respect to the reversal training.

The theory proposed by several investigators that deficits in retention, which have been attributed to interference with memory formation, are rather an effect of a response to ECS or puromycin which competes with avoidance in the retention trial, is not tenable here. If there was a fear-induced element or performance depression by the subjects treated with ECS and puromycin, there would have been a decrement in the retention trials. However, by the relatively more active avoidances of these subjects in the retention trials, the logical conclusion would be that there was less fear, if any, involved. The A-B-A learning situation described here should commend itself as a promising tool in studying the biochemical correlates of learning and memory.

Fixation time in the present experiment was less than 100 minutes, as indicated by the inferior performance of those subjects receiving ECS and puromycin at 100 minutes after training. In other words, the reversal training of B had been consolidated within the 100 minutes, therefore, having a retroactive inhibiting effect on the retention of A. The indication

that consolidation took place within 100 minutes is consistent with findings by Agranoff and Davis. In a study by Davis, Bright, and Agranoff (4) memory was disrupted with ECS administered at ninety minutes but not at 120 minutes after training. Consequently, the indication in the present study is that memory consolidation is not disruptible by ECS between ninety and 100 minutes.

Puromycin in this study was found to be less effective than ECS in blocking out memory. Even though there was significant difference of 1-min. Puro with 100-min. Puro, 100-min. ECS, NPuro, NECS, there was still a significant difference when compared to the control group. This suggests that puromycin given shortly after training does inhibit long-term memory but does not appear to obliterate it completely, as stated by Agranoff (10). A discussion on the amount of memory impairment caused by puromycin seems relevant here. Even though the studies by Agranoff (1, 2) and Davis (5) indicated puromycin-injected fish did not differ significantly between the ten retention trials and the first ten trials, further inspection of the data consistently revealed that in absolute magnitude, puromycin fish avoided the shock relatively more. In other words, the assumption that puromycin fish are completely naive on retraining, when compared to the original training, should be re-evaluated.

The observation that puromycin does not produce complete memory deficit may also be accounted for by the concept of short and long-term memory. Memory is thought to consist of overlapping stages (9). In the

first stage the mechanism is believed to be bioelectric in that the electrical activity of those nerve cells participating in the learning process reverberate for a period of time. During this period the electrical activity is believed transformed into a more permanent record by a chemical transformation. It has also been suggested that this reverberation continues for some time after consolidation takes place. In a study by Agranoff and Davis (6), goldfish were tested at various times after immediate injection with puromycin. The general finding was that significant retention was demonstrated up to forty-eight hours. At seventy-two hours, however, the mean number of avoidance responses (2.35) in the retention trials was not significantly different from the mean number of avoidance responses (1.82) in the original first ten trials. The conclusions from this study implicated the persistence of short-term memory for at least forty-eight hours after training. Again, it should be noted that there was some difference between the original training and the seventy-two hour retraining, indicating a possibility of short-term memory continuing for more than seventy-two hours. If this is the case, this could explain the finding in this study that puromycin-injected shortly after the reversal training causes less than complete impairment of memory.

If the assumption is that some short-term memory is still occurring at seventy-two hours after training, the question may be asked as to why more severe impairment is caused by electroconvulsive shock. In answer to this, it will be recalled that short-term memory is considered to be of

an electrical nature. When a convulsive producing current is introduced during this phase, the electrical activity is apparently disrupted and impaired, preventing transformation into a more permanent record.

Theoretical Implications

This study, as well as others utilizing blocking agents in studying correlates of memory, indicates that the processes involved in the establishment of long-term memory are in some way related to the synthesis of proteins. Hyden (10) has compared the phenomena of memory with genetic expression in which the patterns of gene expression are dictated by the sequence of nucleotides in the DNA and are manifested during the complicated and mysterious processes known as differentiation. Roberts and Flexner (8), in a quantitative description, analyze the processes involved in terms of a self-inducing system. This assumes that the initial learning experience triggers the synthesis of one or more species of messenger RNA. This mRNA alters the synthetic rate of one or more proteins which are essential for the expression of memory; and these proteins are thought to modify the characteristics of synapses concerned in a learning process so that the passage of impulses between nerve cells is facilitated. In turn, the proteins, or their products, act as inducers of their related mRNA. Loss of this mRNA would lead to loss of essential protein with consequent permanent loss of memory.

A molecular theory of memory storage, as described by Hyden (10), states that the memory trace is stored in large RNA molecules. This requires a mechanism for the rapid transfer of chemical constituents. Deutsch (7) says that good candidates for this job are the neuroglial cells, the satellite cells that surround the nerve cells, outnumbering them about ten to one and making up about half the total volume of the brain. Most investigators believe that substances cannot pass from the blood into the neurons without first, passing through the glia. In a number of publications, Hyden has stated that the adenine-uracil ratio of the nuclear RNA increased in rats subjected to a learning experience, during which, a pattern of sensory and motor abilities was established (10). More significantly, in the last five years, the work by Hyden (10) has given the most direct evidence for a biochemical basis of memory. Learning has been shown to be related with a shift or change in base ratios of nuclear RNA in neurons involved in learning.

Based on the Hyden experiments, the general hypothesis has been that learning and memory are dependent on neuronal and glial synthesis of RNA, which eventually gives rise to protein (10). The present study, as well as others using protein inhibitors, support this hypothesis. Even though these antibiotics have been useful in differentiating different stages in memory, the experiments utilizing them throw very little light on the unique mechanisms by which memory occurs. Yet, with the advancing techniques of Hyden and others, molecular analyses have been applied at

a more molecular level. Hyden (7) has developed some amazing dissection, extraction, and analytical procedures for studying RNA in amounts as small as 20 micrograms. John (9) and his associates have provided techniques for studying bioelectrical activity at different brain sites in the cat. At the present stage of technological competency, these types of approaches may be one of the best ways to make any sense out of the diffuse and complicated activities of the brain in its natural state.

In conclusion, it should be emphasized that efforts in searching for the memory engram are in a primitive stage, and the fate of this search will depend upon interdisciplinary attacks. The brain is becoming an object of study in all scientific disciplines, and as Corning (3, p. 20) states, "This is not surprising, since the brain is common to all men."

CHAPTER BIBLIOGRAPHY

1. Agranoff, Benard W., Roger E. Davis, and John J. Brink, "Chemical Studies on Memory Fixation in Goldfish," Brain Research, I (1966), 303-309.
2. Agranoff, B. W., and Paul Klinger, "Puromycin Effect on Memory in the Goldfish," Science, CXLVI (1964), 952-953.
3. Corning, William C., and S. C. Ratner, editors, Chemistry of Learning, New York, Plenum Press, 1967.
4. Davis, Roger E., P. J. Bright, and B. W. Agranoff, "Effect of ECS and Puromycin on Memory in Fish," Journal of Comparative and Physiological Psychology, LX (1965), 162-166.
5. Davis, R. E., "Environmental Control of Memory Fixation In Goldfish," Journal of Comparative and Physiological Psychology, LV (1966), 72-78.
6. Davis, R. E., and B. W. Agranoff, "Stages of Memory Formation in Goldfish," Proceedings from the National Academy of Science, USA, LV (1966), 555-559.
7. Deutsch, Sid, Models of the Nervous System, New York, John Wiley and Sons, Inc., 1967.
8. Flexner, L. B., J. B. Flexner, and R. B. Flexner, "Memory of Mice Analyzed with Antibiotics," Science, CLV (1967), 1377-1383.
9. John, E. R., Mechnisms of Memory, New York, Academic Press, 1967.
10. Quarton, Gardner C., T. Melnechuk, and Francis O. Schmitt, The Neurosciences, New York, Rockefeller University Press, 1967.

CHAPTER V

SUMMARY AND CONCLUSIONS

Ninety-nine experimentally naive goldfish were used to determine the effectiveness of puromycin and electroconvulsive shock in blocking out long-term memory of shock avoidance learning. In order to control for possible fear and/or motivational effects, a retroactive inhibition model (A-B-A) was used for investigating the retention of training A after an interpolated reversal training B. The subjects were randomly assigned to one of nine groups of which one (control) did not receive the reversal training. The groups receiving the reversal training consisted of: (1) Puromycin given 1 minute after training; (2) Puromycin given at 100 minutes after training; (3) ECS at 1 min.; (4) ECS at 100 min.; (5) No Puromycin at 1 min.; (6) No Puromycin at 100 min.; (7) No ECS at 1 min.; (8) No ECS at 100 min.

All subjects underwent thirty shock avoidance trials in training A, followed twenty-four hours later by thirty trials of reversal learning. A control group missed the reversal learning. At the end of the reversal learning, the appropriate treatments were given at the designated times to each of the subjects in the remaining eight groups. Seventy-two hours later, all groups were retrained on A in the retention trials. The data

obtained in this study appeared to warrant the following conclusions and recommendations:

1. Subjects receiving puromycin or ECS one minute after reversal training B, on retention of A (original training), showed a significantly superior performance over those subjects receiving puromycin and ECS 100 minutes after the reversal training and those subjects that did not receive administration. This indicated that the 1-min. treatment with puromycin and ECS blocked out the reversal effect of B.

2. There was a significant difference in the retention trials between puromycin given one minute after training and ECS given one minute after training. This suggested that ECS produced a more extensive damage on long-term memory of the reversal trials. However, it seems quite possible that this difference is not attributable to a differential degree of impairment, but rather, to the perservation of short-term memory in the puromycin-injected fish. Furthermore, ECS has long been known to totally disrupt this short-term memory process.

3. There was also a significant difference between the 1-min. puromycin group and the control group which was not given the reversal training. This would also imply some retention of the reversal training by the puromycin fish. Again, it cannot be precluded that short-term memory was absent in the puromycin fish seventy-two hours after the reversal training. There was no significant difference between the ECS and control

groups, again, suggesting the capability of electroconvulsive shock in disrupting the short and long-term memory processes.

4. There was no significant differences between any of the following groups: Puromycin at 100 min.; ECS at 100 min.; No Puromycin at 100 min.; No ECS at 100 min.; No Puromycin at 1 min.; No ECS at 1 min. The null hypothesis was accepted and explained in terms of memory consolidation. Memory of the reversal training was apparently consolidated in these groups causing comparable inferior retention.

In final conclusion, it should be emphasized that the results of this study, as well as all others investigating the processes of memory, only indicate possible avenues for further research. With this in mind, the following recommendations are made in reference to this study:

1. It may be desirable to use a regression analysis of training scores in order to determine retention scores more reliably by using a large number of control fish. This may reduce any discrepancies due to individual differences in fish.

2. Future studies investigating the effect of blocking agents on memory should consider using a design comparable to the one used in this study in order to be sensitive to fear, motivational, and other inherent factors which could lead to error in interpretation.

3. The concept of short-term memory and its duration should be investigated more thoroughly in the goldfish in order to eliminate complication of possible perseveration of this process in the measurement of long-term memory.

4. The results of this study, in comparison with previous findings, suggest that memory consolidation is taking place somewhere between ninety min. and 100 min. after training. Further delineation of this time interval might prove beneficial in future studies.

BIBLIOGRAPHY

Books

- Corning, William C. and Martin Balaban, The Mind: Biological Approaches to Its Function, New York, Interscience Publishers, 1968.
- Corning, W. C. and S. C. Ratner, editors, Chemistry of Learning, New York, Plenum Press, 1967.
- Deutsch, Sid, Models of the Nervous System, New York, John Wiley and Sons, Inc., 1967.
- Ehrenpreis, S. and Othmar C. Solnitzky, editors, Neurosciences Research, Vol. I, New York, Academic Press, 1968.
- Gaito, John, editor, Macromolecules and Behavior, New York, Meredith Publishing Company, 1966.
- Hays, L. William, Statistics for Psychologists, New York, Holt, Rinehart and Winston, Inc., 1963.
- Hilgard, Ernest R., and Gordon H. Bower, Theories of Learning, New York, Meredith Publishing Co., 1966.
- John, E. Roy, Mechanisms of Memory, New York, Academic Press, 1967.
- Morgan, Clifford T., Physiological Psychology, New York, McGraw-Hill Book Company, 1965.
- Morrison, Donald F., Multivariate Statistical Methods, New York, McGraw-Hill Book Company, 1967.
- Quarton, Gardner C., T. Melnechuk, and Francis O. Schmitt, The Neurosciences, New York, Rockefeller University Press, 1967.
- Winer, B. J., Statistical Principles in Experimental Design, New York, McGraw-Hill Book Company, 1962.

Articles

- Agranoff, Benard W., Roger E. Davis, and John J. Brink, "Chemical Studies on Memory Fixation in Goldfish," Brain Research, I (1966), 303-309.
- _____, and Paul D. Klinger, "Puromycin Effect on Memory in the Goldfish," Science, CXLVI, (1964), 952-953.
- Appel, S. H., "Effect of Inhibition of RNA Synthesis on Neural Information Storage," Nature, CCVII, (1965), 1163-1166.
- Barondes, Samuel H. and Harry D. Cohen, "Comparative Effects of Cycloheximide and Puromycin on Cerebral Protein Synthesis and Consolidation of Memory," Brain Research, IV (1967), 44-51.
- _____, and M. E. Jarvik, "The Influences of Actinomycin-D on Brain RNA Synthesis and on Memory," Journal of Neurochemistry, XI (1964), 594.
- Brink, J. J., R. E. Davis and B. W. Agranoff, "Effects of Puromycin, Acetoxycycloheximide and Actinomycin D on Protein Synthesis in Goldfish Brain," Journal of Neurochemistry, XIII (1966), 889-896.
- Cerf, J. A. and L. S. Otis, "Heat Narcosis and Its Effect on Retention of a Learned Behavior in the Goldfish," Federation Proceedings, XVI (1957), 20-21.
- Cohen, H. D. and S. H. Barondes, "Further Studies of Learning and Memory After Intercerebral Actinomycin-D," Journal of Neurochemistry, XIII (1966), 207-211.
- Coons, E. E. and N. E. Miller, "Conflict Versus Consolidation of Memory Traces to Explain 'Retrograde Amnesia' Produced by ECS," Journal of Comparative Physiological Psychology, LIII (1960), 524-531.
- Darken, Marjorie A., "Puromycin Inhibition of Protein Synthesis," Pharmacological Review, XVI (1964), 223-243.
- Davis, Roger E., "Environmental Control of Memory Fixation in Goldfish," Journal of Comparative and Physiological Psychology, LV (1966), 72-78.
- _____, and B. W. Agranoff, "Stages of Memory Formation in Goldfish," Proceedings from the National Academy of Science, USA, LV (1966), 555-559.

- Davis, Roger E., John J. Brink, and B. W. Agranoff, "Memory Fixation in the Goldfish," Proceedings from the National Academy of Science, USA, LIV (1965), 788-793.
- _____, Patricia J. Bright, and B. W. Agranoff, "Effect of ECS and Puromycin on Memory in Fish," Journal of Comparative and Physiological Psychology, LX (1965), 162-166.
- Duncan, C. P., "The Retroactive Effect of Electro-Shock on Learning," Journal of Comparative and Physiological Psychology, XLII (1949), 32-44.
- Flexner, L. B., J. B. Flexner, "Effect of Acetoxycycloheximide and of an Acetoxycycloheximide-Puromycin Mixture on Cerebral Protein Synthesis and Memory in Mice," Proceedings from the National Academy of Science, USA, LV (1966), 369-374.
- _____, J. B. Flexner, G. de la Haba and R. B. Roberts, "Loss of Memory as Related to Inhibition of Cerebral Protein Synthesis," Journal of Neurochemistry, XII (1965), 535.
- _____, J. B. Flexner, R. B. Roberts and G. de la Haba, "Loss of Recent Memory in Mice as Related to Regional Inhibition of Cerebral Protein Synthesis," Proceedings from the National Academy of Science, USA, LII (1964), 1165-1169.
- _____, J. B. Flexner and R. B. Roberts, "Memory of Mice Analyzed with Antibiotics," Science, CLV (1967), 1377-1383.
- _____, J. B. Flexner and R. B. Roberts, "Stages of Memory in Mice Treated with Acetoxycycloheximide Before or Immediately After Learning," Proceedings from the National Academy of Science, LXV (1966), 730-735.
- Gaito, John A., "A Biochemical Approach to Learning and Memory," Psychological Review, LXVIII (1961), 288.
- Glickman, Stephen E., "Perseverative Neural Processes and Consolidation of the Memory Trace," Psychological Bulletin, LVIII (1961), 218-233.
- Hayes, K. J., "Anoxia and Convulsive Amnesia in Rats," Journal of Comparative and Physiological Psychology, XLVI (1953), 216-217.

- Leukel, F. P., "Effect of ECS and Pentothal Anesthesia on Maze Learning and Retention," Journal of Comparative and Physiological Psychology, L (1957), 300-306.
- Lewis, Donald J. and Brendan A. Maher, "Neural Consolidation and Electroconvulsive Shock," Psychological Review, LXXII (1965), 225-239.
- Madsen, Millard C. and James C. McGaugh, "The Effect of Electroconvulsive Shock on One-Trial Avoidance Learning," Journal of Comparative and Physiological Psychology, LIV (1961), 522-523.
- McConnell, J. V., A. Jacobson and D. P. Kimble, "The Effects of Regeneration Upon Retention of a Conditioned Response in the Planarian," Journal of the Comparative and Physiological Psychology, LII (1959), 1-10.
- McGaugh, James L., "Time-Dependent Processes in Memory Storage," Science, CLIII (1966), 1351-1358.
- Pearlman, C. A., S. K. Sharpless, and M. E. Jarvik, "Retrograde Amnesia Produced by Anesthetic and Convulsive Agents," Journal of Comparative Physiological Psychology, LIV (1961), 109-112.
- Russell, W. R. and P. W. Nathan, "Traumatic Amnesia," Brain Research, LXIX, (1946), 280-300.
- Thompson, R. and R. S. Pryer, "Effect of Anoxia on the Retention of a Discrimination Habit," Journal of Comparative and Physiological Psychology, XLIX (1956), 297-300.