A Study of the Effect of Magnesium Pemoline on the Avoidance Conditioning of Several Strains and Genera of Mice

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A Study of the Effect of Magnesium Pemoline
on the Avoidance Conditioning of Several
Strains and Genera of Mice

by
Denis S. Avery

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of the Graduate School
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BIOGRAPHY

Denis S. Avery was born in Los Angeles, California on February 22, 1942. He graduated from Loyola High School (Los Angeles) in 1959 and, subsequently, from Loyola University (Los Angeles) in 1963. During the last four years, he has been a student at Stritch School of Medicine (Chicago).

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PUBLICATION

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CHAPTER I

SURVEY OF LEARNING AND MEMORY THEORY

A. HISTORICAL

1. Theories of Learning

Since 1855, when Ebbinghaus published on the memorization and recall of verbal material, many theories have developed to explain learning and/or memory. Also there are many schools of learning, each favoring particular interpretation of these phenomena. It is extremely difficult to formulate a satisfactory definition of learning so as to include all the activities and processes already surmised to be intimately associated with the phenomenon, and to exclude all those which are nonessential. In general, however, learning may be understood to be the process by which a behavior of a system originates or is altered by a reaction between the system and an encountered situation, provided that the alteration cannot be explained on the basis of an innate direct response or maturation. Learning is not instinct, which is generally held to be complex, genetically determined, species-characteristic activity which is expressed in toto. Also just as growth leads to maturation, behavior matures through regular states irrespective of intervening practice, and this development is not learning. Perhaps less broad definitions of learning will be presented in reference to particular experiments or viewpoints.
Fatigue results in a loss of efficiency. In this sense then, both learning and fatigue are affected by previous performances: fatigue curves tend to show decreasing proficiency with repetition and recovery with rests, while learning curves show gains with repetition and forgetting over rests. More recent theories have been developed to include the possible molecular aspects.

Until recently, the bulk of work on the behavioral analysis of learning was conducted by psychologists. Psychological theories attempted to explain learning of an organism by observing changes in its gross behavior. These theories are molar rather than molecular. The molecular aspects of physics or physiology upon which behavior probably is based have identifying properties of their own, which are not the properties of behavior as molar. More recent theories have been developed to include the possible molecular aspects.

Psychological theories are basically of two types: Stimulus-Response and Cognitive. Of these, the Stimulus-Response (SR) group is more easily correlated with the molecular theories.

The Stimulus-Response (SR) group includes Thorndike, Guthrie, Skinner, Hull, and others. All tacitly assume an inherent capacity of the organism to experience, comprehend and react adaptively to the environment. Their theories embrace the notions of understanding, motivation, and forgetting, as well as the results of practice, and the
transference of association. Each has written considerably on these subjects.

Thorndike holds that learning is the result of an automatic strengthening by trial and error of specific hypothetical connections, directly. Guthrie's major emphasis is on the shifting of associations by formation of new connections, as seen in his recency principle. Skinner developed the terms operant and respondent conditioning, which have found a broad application in the theories and terminologies of behaviorism. Hull's systematic behaviorism in common with the above three, is one of the classic expressions of "black box" thinking and set the form which was followed by attempts to construct possible models for the black box.

The Cognitive group (Lewin, Tolman, Koehler and Koffka) was interested in insightful behavior and/or purposive behavior. These men introduced Gestalt theory and the trace hypothesis regarding memory and past experience, which is very near the present feeling about memory storage.

The mathematically oriented model builders approach learning as a system of probabilities which algebraically sum to determine the behavior of the system. A representative thinker is Ashby. By the pure use of the deductive method, he has described a homeostatic machine to elucidate his theory.
His central deduction was that self-programmed reactivity constitutes learning. Survival in a darwinian world has produced a learning process whose behavior is homeostatic. The organization of the brain may be so complex that no theory based on contemporary mathematics or pure mechanics can predict its behavior. The fact that the stability of a system is a property of the system as a whole is the result of the fact that the presence of stability always implies co-ordination of the homeostatic interaction among the parts. The constancy of some variable or systems may involve the vigorous activity of others to maintain stability. From such principles as these he described the ultrastable system, as follows:

Two systems of continuous variables (that we call 'environment' and 'reacting part') interact, so that a primary feedback (through complex sensory and motor channels) exists between them. Another feedback, working intermittently and at a much slower order of speed, goes from the environment to certain continuous variables which in their turn affect some step-mechanisms, the effect being that the step-mechanisms changes value when and only when these variables pass outside given limits. The step-mechanisms affect the reacting part; by acting as parameters to it they determine how it shall react to the environment.

He built a functioning homeostatic machine from hardware which, considered as a black box, shows purposeful behavior.

Molecular theories popular today are more susceptible to basic research than the molar approach employed by the psychologists and
more biological than the deductive model building of Ashby. These theories are open to investigation by physical and physiological means.

In 1949 Hebb proposed a speculative neuropsychological model of brain engrams introducing the terms "cell assembly" and "phase sequence." A cell assembly arises through frequently repeated particular stimulation. It corresponds roughly to the persisting neural counterpart (engram) of a simple association. It is a diffuse structure comprising cells in the cortex and subcortical centers. When a particular stimulation occurs, the cell assembly is aroused and it facilitates other systems and motor responses. A cell assembly can thus be activated by another cell assembly, by sensory stimulation, or by both at once.

A phase sequence is constituted by the arousal of a series of cell assemblies. For example, looking at the three corners of a triangle arouses the cell assemblies appropriate to each corner, and these facilitate each other. Thus the phase sequence is analogous to the thought process. The first stage, ontologically, of learning is the establishment of cell assemblies and their phase sequences. Further learning consists in interaction and modification of these basic relationships.

Gradually a clearer picture of the complex nature of learning has begun to emerge, and has stimulated further interest in the molecular activities of the nervous system. By 1959, Hebb stated that learning
consisted "of a modified direction of transmission in the central nervous system (CNS) so that, in the clearest example, a sensory excitation is now conducted to effectors to which it was not conducted before." A new physiological rather than psychological SR connection was established with a definition not too different from our earlier one. Hebb felt that the neurophysiological basis for persisting reverberating circuits would lie in changes in the synaptic knobs to alter the areas of contact between an axon or dendrite and the tissue with which it is associated.

Hebb's brain engrams represent an integration of psychological concepts with a more apoditic approach to the fundamentals of neural processes. Recently Mowrer, Sutherland and Krech have broadened our knowledge in several aspects. Mowrer, a psychological theorist, introduced a revised two-factor theory of kinesthetics in 1955, which holds that stimuli acquire the power to evoke affective states through contiguity conditioning and instrumental responses occur because the feedback stimuli from them evoke positive affective states. Sutherland, a physiologist using ablation techniques, has studied invertebrate learning in great detail and has introduced (1964) his own model of discrimination learning. Krech has been one of the major workers recently elucidating the anatomy and pharmacophysiology of the memory trace. The molecular theory of the genetic control of learning
mechanisms is well stated by Schmitt:

It may be characterized thus: information vital to life can be stored, transferred, and retrieved in systems containing large polymerizing molecules, through the virtually limitless repertoire of structural variants available in the tertiary conformation of protein molecules, by specific recognition and catalytic properties these molecules can utilize the information in the DNA and RNA and carry out the phylogenetic and ontogenetic instructions implicit in the DNA code... Allosteric modulations of repressor molecules probably play a major role in adapting genetic function to physiological needs at each particular time and place during the development and life of the organism.

The above attitude toward molecular modulation and repression is the basis of current speculation on memory and learning mechanisms.

2. Experiments of Others

This section is intended to cite briefly either techniques or the findings of others which provide a rationale for the work presented here. There has been no attempt made to be all-inclusive but rather I hope to show typical means by which the psychologist or the neurophysiologist tests his theories.

Pavlov. Years before his celebrated demonstration of the salivary reflex conditioned by a buzzer associated with food, Pavlov showed the remarkable adaptability or purposeful character of the salivary reflex to food. The physical and chemical qualities of the juice, as well as its quantity, are adapted to the physical or chemical characters
possessed by the particular substance initiating the reflex. He used clean pebbles, pebbles ground to a powder, strong acid, chunks of meat, biscuits, fried and powdered meat, and milk. Different secretions were obtained for each, and they reflect responses gauged to the need in quality and quantity. This built-in adaptability of a central reflex is perhaps similar to the adaptive change of motor patterns to afferent stimuli seen in true learning.

Thorndike. In a typical experiment a hungry cat is confined to a box with a concealed mechanism operated by a latch. Escape is possible only by proper manipulation of the latching mechanism. The first trials are accompanied by clawing, biting, etc., before the latch is moved. The escape latency (in seconds) is high. On succeeding trials the latency becomes less, slowly and irregularly. This increment suggests that the cat does not really "catch on" to the manner of escape but learns it by the gradual incorporation of correct responses and/or the removal of incorrect ones.

Guthrie. In a similar problem box, the cat was fully observed during the latency periods and its exact posture recorded photographically as it activated the release. It was observed that the cat learns the method of escape in the first successful trial and repeats what is essentially the same solution time after time. The cat exhibited stereotypy because stereotypy was a successful solution.
Skinner. 4 Skinner's experiments with schedules of reinforcement include fixed interval reinforcement, e.g., reinforcement is delivered at 3, 6, 9, or 12-minute intervals. It results in response rates that are proportional to the interval between reinforcements, the shorter interval yielding more rapid response rates, although each rate is relatively uniform for each interval.

Koehler. 6 In a single-box situation, a reward, such as a banana, is attached to the top of the chimpanzee's cage, and the animal is supplied with a box which may serve as a ladder if properly placed. Care was taken to prevent the problem's solution by direct imitation of others. And when the problem was mastered, an animal alone in a cage with box and banana would turn away from the fruit to seek the box and to move it into position. This demonstrates the detour character of insightful behavior.

Briggs and Kitto. 15 Briggs and Kitto were among the first theorists to suggest that structural changes occur within molecules during learning, and are responsible for memory and learning.

Hebb. 8 In studying memory imprinting, Hebb presented verbally a series of digits, and the subject was asked to reproduce them in the same order. After the subject attempted one series, the experimenter presented a second series and the subject forgot the preceding series completely. He did not, however, confuse the two, but resembled a
calculating machine, punching a second set of numbers and erasing the preceding set completely.

Eccles. 16 It is difficult to isolate any given experiment, but it is imperative to mention his long contribution to neurophysiology. His book, "The Physiology of Synapses," is a sine qua non of neurophysiology.

Sutherland. 11 On evidence derived from experiments with cephalopods, Sutherland proposed a model of discrimination learning which comprised five levels, each with different functions. They are: (1) stimulus input, (2) analysers, (3) outputs, (4) response attachments, and (5) responses. He was able to localize certain of these levels to discreet regions of the brain. He believes discrimination learning involves two separate processes: learning to switch in the analyser whose outputs differentiate appropriate stimuli and learning which response is best suited to those outputs.

Krech. 12 Since 1950, Krech has studied the effects of environment on future learning ability in rats. Rats given enriched experience develop, in comparison to restricted littermates, greater weight and thickness of cortical tissue and an increase in total acetylcholinesterase activity of the cortex. Such rats are less emotional and more intelligent.

Agranoff. 17 By using a hurdle task in goldfish, he was able to show the deleterious effects of electroconvulsive shock (ECS), 8-azaguanine and puromycin on the processes of recent and permanent memory, and
to relate this with the uptake of labeled leucine into the brain, indicating
a relationship between memory and protein synthesis.

Lashley. Lashley pioneered the experimental demonstration of
pharmacological activity in the nervous system. His studies of the
effects of strychnine on the spinal cord directed attention to the importance
of the spinal cord in the modulation of behavior.

B. PRESENT STATUS OF LEARNING AND MEMORY THEORY

1. Mechanisms

Contrary to Thorndike, who felt learning varied only in degree,
Bitterman has shown that there are real and qualitative differences
in types of intelligence among phylogenetically different species. By
means of habit reversal techniques, he was able to show qualitative
difference in intelligence among monkeys, rats, pigeons, turtles and
fish. Many workers have described learning in other phyla. In
chordates intelligence is associated with the cerebral cortex and the
species of animal with the most complicated cortex are in general the
most intelligent; and when the cortex is damaged through disease or
accident, intelligence is absent, too.

Only the centrally implemented ANS reflexes can be conditioned
(Gantt); and the prevalent opinion today (Gaddum, Eccles) is that
the seat of learned reflexes is in the cortex, whereas the brain stem is
the seat of consciousness.
The mechanism of learning may be a molecular adaptation within cells to their environment, which results in an altered cellular reactivity to some stimuli and differential reactivity to the others. Thus the present status of learning theories is built on a foundation of biochemistry. The work of Krech\textsuperscript{12} and his group is illustrative. By focusing on the possible anatomical changes of environmentally controlled animals they have first shown changes in the weight of brain tissue, and secondly in the specific activity of certain enzymes. The molecular basis of learning is also under investigation via protein synthesis (Agranoff\textsuperscript{17}), genetics (King and Weisman\textsuperscript{22}), and anatomical and hematological changes (King and Eleftheriou\textsuperscript{23}).

The factors controlling the spread of impulses through a nerve net have been analyzed by Beurle (1957).\textsuperscript{24} His model, simple compared with what must occur in living brains, is presented here. A neuron is affected by impulses arriving at synapses on the dendrites and cell body. Each impulse causes a local change often involving partial depolarization of the membrane (a post-synaptic potential). When a certain threshold is attained, there is a short delay known as the operating time ($T$ 0.5 msec), after which the cell rapidly depolarizes and an all-or-none impulse passes down the axon, followed by the refractory period. The branches of each neuron act on a large number of synapses in other neurons and the rate at which the concentration of these cells
falls off with their distance \( d \) from the original cell may be expressed as a function of the activity at any given place \( x \) and the time \( t \), which is measured by \( F \), the proportion of cells becoming active per unit time. The rate at which impulses arrive at secondary cells depends on the total activity in the neighborhood and is calculated by integrating a function of \( F \) with respect to both \( x \) and \( t \). The size of the wave \( M \) of activity passing through the cells is equal to the proportion of all the cells used during its passage. Beurle suggested there were two kinds of nerve fibers -- (E) and (I) -- which are of opposed function and are responsible for uncontrolled \( M \) waves. The (E) fibers increase \( M \) by subthreshold stimulation and the (I) fibers cause actual discharges of cells and so produce areas of refractory nerve nets. This theory is a more elegant expression of the same idea proposed by Hebb.

Memory may be classed as to its sensory source (auditory, visual, kinesthetic) or its permanence (recent or remote, momentary or fixed). In reference to the persistence of memory, the first hypothetical stage is the "short-term memory." Broadbent concluded that there must be a filter which selects one signal (input) for attention before either of other stimuli reach the place where memory takes place. There is some evidence that the frontal lobes play some part during the first few seconds in fixing patterns in the brain (Jacobsen). Memories may become fixed during the next 20 seconds (Malmo). The surface
positive discharge studied by Burns may last 0.5 to 5 seconds and spreads throughout the slab of cortex in one layer of cells, and it has been suggested that this electric change due to the initial spread of sensory patterns is responsible for immediate memory. Reverberating circuits may play a part in the further fixation.

The second hypothetical state of memory depends on the ability of the cortex to recapitulate the past. This process may be aided by proprioceptive reflexes and feedback from the sense organs, i.e., one pattern provides the proprioceptive response which is the CS for the next pattern.

Plastic nerve nets are unsuited for the prolonged storage of memories. There must be some mechanism by which a record is kept in the brain of the transient patterns which have formed in neural network. The amount of information is very large and is stored in some form of code. This code is chemical and depends on the structure of specific proteins (Schmitt), or of molecules of RNA, which might then determine the structure of specific proteins. The gross difference between two memories is analogous to the differences among cells; that is, every cell in the brain may be antigenically different from every other cell. Long-term memories could perhaps be roused by the release of suitable antibodies by some cells which would impose the appropriate pattern through affects on other cells. The actual stores of memories could
be kept in code as molecules of protein or of RNA; but it is difficult to postulate how this code is formed. It is likely to be a slow process and involved only in long-term memories. The anatomical location of long-term memory may lie in the temporal lobe (Penfield). 29

The structural changes relative to memory may be intracellular or intercellular. The intracellularists feel that the secret lies in the nucleoproteins (Gaito, 1961). 30 The intercellularists assume that a synaptic mechanism underlies all the theories to date. Eccles 16 feels that activation of synapses increases their efficacy by some enduring change in their fine structure; and post-synaptic inhibitory action is far more powerful and prolonged in higher centers than in the spinal cord. Presumably, inhibition would be concerned in the repressions of irrelevant responses. For example, strychnine acts directly at inhibitory synapses (pg. 191) to suppress them. This may explain its facilitation of motor conditioning when given in small (sub-convulsive) doses. When we consider the possible mechanism of action of Magnesium Pemoline, we will again refer to memory storage and fixation and enzymatic activity.

Perhaps the inter- and intracellular views are not mutually exclusive. Schmitt 13 feels that the primary role of molecular recognition of coded information stored in the macromolecules of the brain cells is expressed in the protein-protein molecular recognition in the
membranes of the axonal terminals and dendritic receptors. This could conceivably be altered by changes in RNA that produce new or different protein complexes within the cells and at the cell borders. It is important here to recall that the endoplasmic reticulum forms a communication between the nucleus and the cell membrane. It is a technical problem at present to study the effects of RNA on memory and learning, as shown by Cohen and Barondes, because RNA and its degradation products are almost completely excluded from the brain by the blood brain barrier.

2. Animals (except mice) used by others in experimental research

By far the greatest number of experiments have employed rats. Krech and his colleagues have regularly released the findings of their experiments in the Brain Chemistry and Behavior Research Newsletter, as well as the standard journals. They have dealt with strain differences, littermate behavioral studies, and especially the chemical and anatomical changes resultant from blindness and environmental complexity and training. Others have also studied strain differences and chemical changes in rat brains with learning. Rats selected for high and low rates of avoidance conditioning, punishment, stimulus generalization, and extinction have been studied. Also rats have been used in studies involving the effects of hypothermia on learning.
learning, even without running. 52

Behavioral drug studies of learning in rats have encompassed almost all varieties of psychoactive compounds: sedatives, 53 tranquilizers, (54, 55, 56) cholinomimetics, (57, 58) anticholinomimetics, (59, 60) CNS stimulants, (61, 62, 63, 64) hallucinogens, (58, 65) morphine, 66 RNA preparations31 and brain extracts. 67 Some have correlated their behavioral data with chemical analyses. 68

Invertebrates such as insects, (69, 70) worms, (71, 72, 73) planaris, (74, 75, 76) and octopi (77, 78, 79) have been used and exemplify simple learning models. Sutherland and his group, working with Octopus vulgaris, have been especially productive.

Many vertebrates have been studied: fish, (80, 81, 82) turtles, (83, 84, 85) birds (86, 87) and marsupials, (88) in addition to the better known mammals, such as dogs, 89 cats, 11 monkeys, 90 and humans. (91, 92)

Bitterman has compiled a table of learning that characterizes species as being either rat-like or fish-like, 19 and strongly advocates the validity of studying many types of animals. It is much easier, however, to adapt learning tasks (and derive usable data from the experiment) to simple learners than to more complex organisms.

3. Mice used by others in behavioral research

Mice have proven to be as satisfactory as rats as experimental animals for varied experiments. Along with ethological species
studies, discussed in relation to the strains used in this experiment, much research has been on the learning in mice. (a) For example the genetics of learning in mice have been revealing. Using six strains of inbred mice, Thiessen\(^93\) concluded that the genotype sets the limits for both behavioral and endocrine measures, although these appear to be regulated independently. However, density of population acted in the same direction and in a proportional manner for all genotypes. Kimura and Crow\(^94\) described three mating techniques for the avoidance of inbreeding, and advocated the use of the system which has the smallest drift variance at any generation. Bruell\(^95\) has shown that hybrids are not simply more vigorous than their parents, but they have the advantage of some traits showing heterosis while others show inter-
mediacy in inheritance. Intermediate inheritance is characteristic of traits which do not confer a selective advantage, while heterotic in-
heritance occurs in traits which had been subjected to selection. Collins\(^96\) demonstrated that the strain of the parents considerably affected the level of performance of \(F_1\) crosses, and the overall mode of inheritance of avoidance conditioning in \(F_1\) crosses was dominant, and heterotic. Reports from the Jackson Memorial Laboratory indicate that EEG varies as a function of strain, and corresponds more to innate behavior patterns than to gross anatomical differences,\(^97\) suggesting a relationship between EEG and motor function, and younger animals are
more sensitive to the effects of Actinomycin D than were older or mature animals, suggesting that learning is related to genetics and development. Feral mice tend to show greater spontaneous activity which is persistent even though unrewarded, and which is felt to be causally related to their better performance in exploration, learning, running and climbing situations.

One of the great advantages of using mice in behavioral analyses is the feasibility of studying both wild and laboratory strains. This behavioral comparison is not as easily made with other types of animals such as rats or monkeys.

(b) Social and environmental factors have also received study. Investigations have included social stress, social reinforcement, critical infantile periods, initial contact with strong stimuli, activity rates as social phenomena, modification of behavior by rearing mice with rats, and other behavioral differences between lines or strains of mice. Williams and Scott describe the very earliest perceivable appearance of learning and social behavior during the transition period, days 5 to 11 inclusive, between the infantile and juvenile periods.

(c) Drug studies with mice are numerous. Emlen has stressed the fact that their validity depends in part on the time of administration, since circadian rhythm has considerable influence on the rate of
recovery from certain drugs. Tedeschi et al\textsuperscript{113} demonstrated the usefulness of mice in differentiating the effects of specific drugs, showing that Meprobamate was unique in suppressing fighting behavior while exhibiting only a mild degree of anticonvulsant activity and mild depression of spontaneous activity. Zemp et al observed the increased incorporation of radioactive uridine into RNA isolated from brain nuclei and brain ribosomes, with no accompanying increase in liver or kidney RNA, in trained mice.\textsuperscript{114} They postulated a probable role for RNA synthesis as the molecular basis of learning. Furthermore, Puromycin\textsuperscript{115} was found to adversely affect both memory and protein synthesis. Everett\textsuperscript{(116, 117)} classified mice according to their catecholamine levels above and below the values of untreated mice with normal activity. By means of monoamine oxidase inhibitors (MAO'I's) both alone and in combination with deoxyphenylalanine (DOPA), he produced a scale of graded increases in alertness, responsiveness, irritability and aggressiveness, and correlated it with the increasing degree of MAO inhibition, and the concomitant increase of the catechol amines, including both dopamine and norepinephrine. Scudder\textsuperscript{118} has also studied similar behavioral modalities. Meier\textsuperscript{119} demonstrated the suppressive effect of physiological saline solutions upon activity in mice, implicating as key variables the ionic balance and the relative volume of the injections. And McKeever\textsuperscript{120} showed microscopically the differences in sizes and cell proportions of various endocrine glands between the sexes.
C. PHARMACOLOGY OF LEARNING AND MEMORY

1. Variables and Mechanisms of Action on Learning as Exemplified by Drugs

Drugs may enhance the health of an animal from one suffering from a deficiency state, disease state or senility, and thereby may affect learning; or other actions not on the central nervous system may affect behavior. Curare is an example. The significance to theory of the effects of a given drug on learning and memory depends on whether the primary action of the drug is on the receptor or effector systems or on the CNS. Any drug that has only peripheral effects may cause an indirect alteration in the CNS function by modifying input to the CNS. Scudder and Richardson have shown the inhibition of learning produced by glossopharyngectomy in the mouse. Conversely, any drug which has only central effects can produce alterations in peripheral nervous system function because of the general dominance of the CNS. For example, if a drug, such as serotonin, increases receptor sensitivity, then drug-induced differences in the rate of learning would relate in part to the varying intensity or quality of input into the CNS. On the other hand, if a drug, such as strychnine, is known to enhance the sensitivity of the CNS synaptic mechanisms, then drug-induced differences in learning would relate directly to the organization of the CNS itself.

Among the first psychoactive drugs discovered to affect behavior was ethanol or perhaps marihuana.
Both of these drugs have dose dependent action which may vary among individuals. This points up the possible importance of dosage levels. The choice of a dosage level of a psychoactive drug sometimes is based on determinations made in some other laboratory where the strain and/or species of animals is different. Different strains can vary greatly in sensitivity to various drugs. In the same strain, the behavioral effect of a drug can be completely different, depending on the dosage chosen; for example, Stratton and Petrinovich\(^{124}\) have reported that the effect of physostigmine on the rate of alley-maze learning depends on the dosage level. Small doses have no effect on learning, larger doses enhance learning, and still larger doses disrupt learning.

It is now widely recognised that great caution must be exercised when generalizing from either pharmacological or behavioral results obtained from only one species. Less widely recognised, however, are the less obvious but equally important differences which exist between strains of the same species, e.g., *Peromyscus maniculatus Bairdii* and *Peromyscus maniculatus gracilis*, and the daily fluctuations of a species or strain in drug sensitivities.\(^{112}\)

Another pitfall in behavioral research is dissociation. Dissociation is the condition in which habits learned by animals in a drugged state do not transfer to the normal state, but can be evoked again whenever the animal is drugged.\(^{125}\) It must always be watched for.
As discussed above (see Chapter I, Section B) memory storage involves processes which are active for some time after the termination of an experience.\(^{(24, 126)}\) Hebb's proposal\(^8\) is that short-term memory is based on transient neuronal activities, such as graded dc potentials or reverberations in networks of cells, and more lasting memory is based on further changes initiated or produced by these transient neuronal activities. CNS stimulants and depressants might be expected to exert different effects on learning by directly modifying the specific activities involved in the formation of permanent traces. The hypothesis that memory storage involves RNA and protein synthesis depends on changes in enzyme concentrations in brain cells.\(^{127}\) The recent evidence of Krech's group\(^{38}\) regarding brain acetylcholinesterase activity supports this hypothesis.

Mechanisms of action important in psychopharmacology include:

1. regulation of enzymatic activity, e.g., physostigmine;
2. alterations in protein synthesis, e.g., puromycin and actinomycin;
3. metabolic state, e.g., thiamine deficiency; and
4. modification of synaptic transmission, e.g., strychnine, scopolamine, etc.

A functional or behavioral classification of drug effects on learning may be formulated on a stimulation-inhibition basis, as discussed by McGough and Petrinovich.\(^{128}\) (See Table I) Sometimes the appearance of an agent in both impairment and facilitation groups may appear
confusing, e.g., cholinomimetics. This results from the variables noted above.

2. Pemoline

Pemoline, 5-phenyl-2-imino-4-oxo-oxazolidine, was originally manufactured by the reaction: $\text{CH}_2\text{BrCH}_2\text{COCH}_2\text{NHCOCH}_3 + \text{urea (dry)} 
\rightarrow \text{CH}_2\text{BrCH}_2\text{COCH}_2\text{NHCONHCH}_3 + \text{Pemoline.}$ It is slightly soluble in water and the usual organic solvents, and has a melting point of 256°C. It was manufactured by more conventional chemical methods in 1913, and was termed phenylisohydamtoin at that time. Pemoline is a stable, powdery, white crystal when dry. Little is known about absorption rates, metabolic utilization or excretion of Pemoline. It is readily absorbed from the GI tract.

The magnesium salt of Pemoline is pharmacologically classed as a mild CNS stimulant, and is devoid of sympathomimetic effects.

Schmidt reported it had no effect on circulation, could abolish the effects of minimal anesthesia and stimulated appetite in his volunteers. Although normal respiration was little affected by the drug, morphine-depressed respiration was strongly stimulated. He reported small toxicity and no addiction; and the drug appeared to be directly more effective if the subject were tired ("...umso deutlicher, je stärker die Ermüdung war...). Lienert using a total dose of 10 mg reported that Pemoline: (1) increased comprehensive performance more than did 200 mg. caffeine in humans; (2) had less subjective effects;
(3) lowered performance at higher doses, and (4) was more effective in fatigued subjects.

Glasky and Simon presented evidence that there was preferential stimulation of true-RNA polymerase over pseudo-RNA polymerase in rat brain homogenates, at a dose of Pemoline of 20 mg/kg IP. In the fresh preparation true-RNA polymerase/pseudo-RNA polymerase ratio was approximately 1.0; but this ratio approached 6.0 when a dose of 20 mg/kg Magnesium Pemoline was administered for 24 hours in vitro.

Plotnikoff has demonstrated an enhanced acquisition rate and retention of conditioned avoidance performance in rats treated with 5, 10, and 20 mg/kg doses orally of Magnesium Pemoline. These doses gave no stimulation of spontaneous motor activity. This was in contrast to metamphetamine and methylphenidate effects. Plotnikoff also showed that animals treated with 5, 10 and 20 mg/kg IP of Magnesium Pemoline recovered faster from retrograde amnesia of electroshock effects and regained jump-out behavior to pre-shock levels in a dose-response relationship. He postulated that magnesium pemoline is perhaps preventing the depletion phenomenon of ECS by accelerating nucleic acid synthesis.

More recently, several reports have appeared in the literature which have questioned the usefulness of Magnesium Pemoline in en-
hancing learning and memory. Smith\textsuperscript{135} reported that the performance of human subjects given Magnesium Pemoline was inferior to that of control subjects given a placebo in a double-blind study which employed drugs levels of 25 mg and 37.5 mg total dose. The only statistically significant effects suggested that the 37.5 mg dose was deleterious to verbal and motor learning in normal, adult men (p < 0.01). Beach and Kimble\textsuperscript{136} found that Pemoline caused less change in activity level and a more sustained response to a buzzer in treated rats than tragacanth-treated controls. The treated animals had shorter average response latencies, and no significantly changed retention. The 20 mg/kg dose caused in general longer response latencies. Drugged rats did not avoid a foot shock more often than control rats. Beach and Kimble decided that this alteration in responsivity and in activity level could account for the shorter latencies of Pemoline treated rats.

Burns et al.\textsuperscript{137} stated from their studies on human volunteers that the higher the dose of Pemoline, the slower the mean rate of learning. Morris et al.\textsuperscript{138} measuring the content of RNA in rat brain homogenates and calculating the specific activity of the RNA were unable to find any statistically significant increase of either the RNA content of the brain or the incorporation in vivo of H\textsuperscript{3}-uridine into brain RNA in treated animals or controls.
Frey and Polidora studied the effects of a 20 mg/kg intraperitoneal dose of Magnesium Pemoline on avoidance conditioning in rats in an apparatus similar to the one employed by Plotnikoff. Their results were on rats designated "slow learners" by Plotnikoff and found to freeze in response to the jump-out apparatus. Although the rate of acquisition was generally increased by Magnesium Pemoline, the absolute magnitude of the facilitatory effect of the drug was directly related to the amount of freezing behavior each shock condition produced. The administration of the drug before acquisition training had no effect upon retention when the level of initial learning was controlled.

Plotnikoff (personal communication) has agreed with this finding which was due to the stimulus parameters used by Frey and Polidora.

Talland and McGuire have recently reported poor results using Pemoline in learning and memory tasks for humans. However, their study is poorly controlled and statistically weak.

Magnesium Pemoline appears to be a drug with disputed behavioral activity and unproven biochemical effects. Continued work on this drug is presented in this paper.
CHAPTER II

EQUIPMENT, ANIMALS, AND EXPERIMENTAL DESIGN

A. INTRODUCTION: PURPOSE OF THE PRESENT STUDY

The purpose of the present study is to explore the intergeneric effects of Magnesium Pemoline on learning behavior in mice. By choosing strains and genera which are dissimilar in their modes of social and adaptive behavior and which differ in their undrugged performance in the climbing screen, I intend to explore further the behavioral effects of this drug.
B. EQUIPMENT

An automated avoidance conditioning climbing screen was built. It was constructed of plexiglas and consisted of a series of inclined runways (D1-5, Fig. 1) which connect by means of solenoid-operated gates (j) with small chambers (A1-5, Fig. 1). Each runway, inclined at an angle of 35°, was 12-in. long and 3-in. wide. Each base chamber was 3" x 2" x 3". The floor of the base chamber and of the runways was composed of 2012-12 gauge bus-bar wire fastened 1/8-in. apart forming grids (cf. also Bourgault et al., 1963). The grids of the base chambers as well as the four divisions of each runway could be electrified separately.

The programming controls consisted of elapsed time meters reading in tenths of a second (B1-5 and C1-5, Fig. 1) suitable delay timers (E, F, G, H, I) which allow the operator to set up a time schedule, and stimulus parameters. Two timers served to set the time a mouse must remain in a base chamber (E and F); two other timers were used to set the time interval between opening of a base chamber door and the occurrence of shock in the base chamber floor (G & H). A selector switch (I) was employed for varying the stimulus parameters; the speed of the distributor which randomized the shocking current to the grid floor could also be controlled. Ten elapsed time meters (B & C) were calibrated in tenths of a second; they were activated by the passage of a
mouse through light beams which impinged on photocells near the doorways to and from each chamber. These meters provided a record of the time spent by the mouse in each base chamber ("base time") as well as of the rate at which the mouse climbed from one chamber to the next ("climbing times").

The circuitry for the avoidance conditioning climbing screen is shown in Figure 2, and has been described in detail elsewhere.

The entire machine was operated on a 60 cycle/second, 110 volt current.

In a typical experiment, the stimulus parameters were set at 1.3 ma, 800 V; the two intervals until the moment the chamber exit gate opened and from that moment until the grid shock was applied were set at 60 sec. and 5 sec., respectively. The mouse was placed in the lowest chamber, the cycle initiated, and the exit gate opened 60 seconds later; the chamber floor shock was applied to the four runway sections at ten second intervals per section. Immediately, when the animal entered the next base chamber, the entrance gate closed behind it and the cycle was repeated. As a linear series of five chambers alternated with five ramps, a total of ten readings representing the progress of conditioning were derived from a single trial. The experiment entailed ten consecutive trials for each mouse of each of three groups for each of the genera or strains. For a typical group, ten mice, each processed
ten times through the machine, gave 1,000 readings for analysis, 500 base times and 500 climbing times.

C. MICE

The mice employed in the present study include three strains of Mus musculus (M.m. C57Bl/6J, M.m. CF-I, and M.m. "Missouri"), Microtus ochrogaster, Onychomys leucogaster, Perognathus penicillatus and Peromyscus maniculatus. Of these, the domestic and feral strains of Mus are well known to the behavioral field\(^{142, 143, 144, 145, 146, 147, 148, 149}\). They are generally docile and readily adaptable to laboratory situations. Mus musculus C57Bl/6J is a timid and avoiding inbred strain, which is slightly more intelligent (Scudder et al.)\(^{150}\) than Mus musculus CF-I, an aggressive inbred strain. Mus musculus "Missouri", a feral strain, is a good avoidance animal with good performance and learning.

Microtus ochrogaster (the meadow vole) is a large and heavy animal which was shown to be a poor learner in a previous paper from this laboratory (Scudder et al.)\(^{150}\). This genus is found\(^{151}\) in sparsely covered areas, undergoes a social female/male-dominant/male-subordinate interaction\(^{152}\) when exposed to crowding without sufficient water, and does not derive a beneficial effect from supplemental food\(^{153}\) in either greatly increasing its numbers or preventing decreases in numbers. It shows in the laboratory enhanced fertility with green plant extract, either
sprouted wheat or acetone-ether extracts of sprouted wheat, \(^{153}\) which
effect cells in the anterior pituitary gland. \textit{Microtus ochrogaster} is a
hoarding communal species.

\textbf{Onychomys leucogaster} (the Northern Grasshopper mouse) was first
described in detail by Bailey. \(^{155}\) It is a carnivorous, insectivorous
animal which is not colonial but is readily sociable. It will seldom
fight members of the same species and will either submit well to
captivity or make violent attempts at escape. Clark\(^{156}\) noted that, as
an alternative to aggression, it would cease attacking, go on the defensive,
or withdraw into catatonic immobility. Aggression was strongly in-
fluenced by learning. Ruffer described the male-female social order
in its burrow-digging\(^{157}\) and features of its behavioral development. \(^{158}\)
Ruffer\(^{159}\) has also studied its interaction with other species, showing
that it has numerous violent encounters with other animals until a
dominant-subordinate relationship is established after which it can be very
sociable. Schmidt-Nielsen and Haines\(^{160}\) reported on the survival effects
of group social order of these mice when exposed to water restriction.

\textbf{Onychomys leucogaster}, a carnivorous rodent from the Western States,
with its range of response from aggression to catatonia, was previously
noted not to learn avoidance (Scudder et al.). \(^{150}\)

\textit{Perognathus} has received little study. Tucker has performed
experiments on its oxygen consumption and torpor, \(^{161, 162}\) and
Scudder et al. have employed it in behavioral analyses. It is a jumpy, timid and feral mouse which has not yet reproduced in our laboratory, and is consequently always a wild animal in all our experiments. This genus has been studied in most detail in reference to serotonin levels and radiation resistance. *Perognathus penicillatus* is a solitary form captured in Northern Arizona.

*P. m. Bairdii* is both a feral animal and one that has been highly inbred in the laboratory. We have used an inbred *P. m. Bairdii* in the present study. Terman showed that the factors controlling laboratory populations of *Peromyscus* are basically behavioral, which means that our data may not correlate with that derived from recently captured animals. Olgie and Stinson report that *P. m. Bairdii* prefers a low mean ambient temperature and shows a large amount of variance in its responses. Bronson and Clarke have noted the relationship between the adrenals and coat color in these mice, and McKeever's study of other endocrine glands has already been cited. Wecker demonstrated that the choice of field environment by *P. m. Bairdii* is normally predetermined by heredity, but confinement to the laboratory for 12 to 20 generations results in an apparent reduction of the hereditary control over the habitat selection response; and laboratory mice retained the innate capacity to utilize early field experience in learning to respond to stimuli associated with the environment. Emlen showed the importance
of a circadian rhythm in this subspecies. The ecology of *Peromyscus* has been investigated by Rawson and Hartline and King et al. Species and subspecies studies have been conducted by Dice and Clark and by Wolf et al. Wolf showed that *P. m. gracilis*, a semiarboreal subspecies in its natural habitat, learned a response more environmentally adaptable for the organism more rapidly, and this response was more resistant to extinction and less susceptible to suppression by drugs. In contrasting *P. m. Bairdii* with *P. m. gracilis*, King demonstrated further that *P. m. Bairdii* matured more rapidly and was better able to employ experience in the avoidance conditioning situations. King and Eleftheriou have compared it to *P. m. gracilis*. *P. m. Bairdii* was described as a wild, timid and jumpy creature no matter what amount of handling it received, and it exhibited spontaneous activity more than *P. m. gracilis*, but was a poorer animal for conditioning experiments, and it tended to adapt by becoming more emotional. Bronson and Eleftheriou studied the density, subordination, and social timidity of *Peromyscus* with an interacting *Mus musculus* strain. King has also demonstrated the interrelationships influencing later behavior by maternal reactions during development. Brant and Kavanau and Kavanau noted that when given no other alternative, *Peromyscus* will quickly explore a maze which they had previously avoided, and also that exploration, learning, and running are self-rewarding activities in this species.
All mice except genus *Perognathus* were known to be between thirty and sixty days of age. Since *Perognathus* was captured in Arizona, and used within one week of arrival, no accurate ages were known. All mice were fed on standardized Purina pellet diet, and were given access to food and water *ad libitum* prior to testing in the machine. The nature of the machine and the testing schedule precluded access to either food or water for the duration of the test, a one-hour period.

Variance in weight was a genetic factor and large differences (20 gms) noted only in *Microtus* and *Perognathus*. Within a genus or strain weight varied little. Some mice weighed relatively little (*M.m. C57Bl/6J* -- 10-12 gms.), and others were large and heavy (*Microtus* and *Perognathus* -- 40+ gms.). All mice were housed in a constant temperature room at 25°C, and were tested at an ambient temperature of 22°C.

**D. DRUGS**

A saline control of animals randomly chosen from the population was run for each genus or strain. The saline solution was a commercial preparation 0.9% NaCl and distilled water mixture, a new vial opened each day and the solution kept in an air-tight jar between injections. The dose was calculated to be equivalent in volume to the Pemoline solutions used, on a 10 cc/kg volume to weight basis. Two doses of
Magnesium Pemoline (Cylert)* were employed: 3 mg/kg, and 12 mg/kg. These doses represent an intermediate and a moderately high dose. Other investigators have employed higher levels (Plotnikoff, Glasky), while others have used lower levels (Lienert). In our laboratory, preliminary tests in the climbing screen using M. m. CF-1 animals provided a pilot study on dose effects.

E. EXPERIMENTAL DESIGN

Each animal was weighed, injected, and given fifteen minutes in isolation, with food and water ad libitum, before testing in the climbing screen. The mouse was placed in the first base chamber to begin the first trial. The Pemoline treated animals were chosen in a random fashion from stock. Times of administration were also randomized to avoid a circadian rhythm-drug interaction. All tests were run between 12:00 PM and 9:00 P.M, seven days per week. Mice from all twenty-one groups (seven strains or genera, three drug categories) were run at random each day. Analysis of the data was performed on a 1620 I. B. M. Computer. **

* We are deeply indebted to Dr. N. Plotnikoff, Dept. of Neuropharmacology, Abbott Laboratories, North Chicago, for our supply of Cylert.

** We are indebted to the Dept. of Biostatistics, Veterans Administration Hospital, Hines, Illinois, for the use of the computer.
CHAPTER III

RESULTS

A. AVERAGE BASE TIMES

The average amount of time spent by each group in all the base chambers for all ten trials is shown in Figure 3. Among the controls, the three Mus strains spent less than any of the other genera, and Microtus and Perognathus spent more time. The order from the least average amount to the greatest average amount of time spent in the base chambers is as follows: Saline -- M.m. CF-1, M.m. Mo., M.m. C57B1/6J, Onychomys, Peromyscus, Microtus and Perognathus; Pemoline 3 mg/kg -- Microtus, M.m. Mo., M.m. CF-1, Peromyscus, M.m. C57B1/6J, Onychomys and Perognathus; Pemoline 12 mg/kg -- M.m. Mo., Microtus, M.m. C57B1/6J, M.m. CF-1, Onychomys, Perognathus and Peromyscus. The least mean base times were those of M.m. Mo. and Microtus, each with Pemoline 12 mg/kg, while the longest mean base times were those of Perognathus both saline controls and Pemoline 3 mg/kg.

Regarding the change in performance of the animals when treated with Pemoline, one notes that five of the seven had poorer base times with the 3 mg/kg dose than with saline, while five had better base times with the 12 mg/kg dose than with saline. The two with better base times with the 3 mg/kg dose were Microtus and Peromyscus, and the two with
poorer base times with the 12 mg/kg dose were M.m. CF-1 and Peromyscus.

B. AVERAGE CLIMBING TIMES

The average amount of time spent by each group in all the climbing screens for all ten trials is shown in Figure 4. Among the controls, the three Mus strains had short climbing times and Microtus had the longest climbing time. The order from least to greatest mean amount of time spent climbing is as follows: Saline -- M.m. Mo., M.m. C57Bl/6J, Onychomys, M.m. CF-1, Perognathus, Peromyscus and Microtus; Pemoline 3 mg/kg -- Onychomys, M.m. Mo., M.m. C57Bl/6J, M.m. CF-1, Peromyscus, Microtus and Perognathus; Pemoline 12 mg/kg -- M.m. Mo., Perognathus, M.m. C57Bl/6J, M.m. CF-1, Onychomys, Microtus and Peromyscus.

The shortest mean climbing time was that of M.m. Mo. under the influence of Pemoline 12 mg/kg, while the longest mean climbing time was that of the Microtus ochrogaster saline control. Only three of the seven had poorer climbing times with the 3 mg/kg dose, these being M.m. C57Bl/6J, M.m. Mo. and Perognathus. For these three this worsened performance with the 3 mg/kg dose was also present in their base times. Only one of the seven had a poorer performance in climbing times with the 12 mg/kg dose, this being M.m. C57Bl/6J.

It is also noted that two of the seven, M.m. Mo. and Perognathus,
showed exactly the same pattern with both base and climbing times with both doses, i.e., decreased performance with the 3 mg/kg dose but increased performance with the 12 mg/kg dose. Microtus was unique in showing increasingly better performance with the 3 mg/kg and the 12 mg/kg dose for both base and climbing times. M. m. CF-1 was unique in showing increasing performance with dose levels in climbing times but decreasing performance with dose levels in base times.

C. ANALYSIS OF THE DATA BY LEVEL

1. Saline

In order to gauge the possible interaction of the animals with the machine, one must be aware that instead of 50 consecutive identical avoidance escape situations, we may be dealing with only ten, each of which is composed of five parts (the levels of the base compartments of the climbing screen). An analysis of base chamber and climbing screen times for each of the five chambers and screens for each genus or strain with saline is shown in Fig. 11. As can be readily seen M. m. CF-1 varied little from base chamber to base chamber, while M. m. Mo. and Perognathus had longer times in chambers 1, 3, and 5 than in 2 and 4. Onychomys, on the other hand, seemed to be better in 1, 3 and 5 than in 2 and 4. Microtus had much better scores with 4 and 5 than with 1, 2 and 3. Climbing times did not show this variable effect, since all except Peromyscus climbed better with experience.
Peromyscus showed much the same approach to the climbing screen portion that M. m. Mo. and Perognathus showed in the base chambers. This analysis demonstrates intergeneric variance which appears independent of climbing screen levels.

2. Pemoline 3/mg/kg

Figure 12 presents a similar analysis for Pemoline 3 mg/kg. M. m. CF-1, Microtus and Peromyscus show relatively constant reaction to each base chamber, while Perognathus and M. m. Mo. show better performance with succeeding levels. Onychomys does poorly in base chamber 2, but better in chamber 5, and M. m. C57Bl/6J shows poorer performance in 1 and 3 than in 2, 4 and 5. In climbing times, only Microtus shows difficulty after the first screen. Perognathus does better in 2, 3 and 4 than it does in 5, although it performs worst in chamber 1.

3. Pemoline 12 mg/kg

Figure 13 represents a similar analysis for Pemoline 12 mg/kg. Onychomys, Perognathus and Peromyscus have long base times in chamber 2, and M. m. CF-1 has longer base times for 2, 3, 4 and 5 than for base chamber 1. M. m. C57Bl/6J, M. m. Mo. and Microtus have rather similar base times in each of the chambers. Climbing times show more variance at this dose level than with either Pemoline 3 mg/kg or saline. Peromyscus' climbing times are long at level 2 while
Microtus' climbing times are long at level 3.

D. LEARNING CURVES -- BASE TIMES

1. Saline

The average total base times is perhaps the clearest measure of escape shown by the data but this evaluation gives no indication of the modulation of escape due to learning. This quality is reflected from the learning curves (Figs. 5 - 10). The average base times spent by each genus or strain in all five base chambers for each of the ten trials through the machine with saline is shown in Fig. 5. There are three basic patterns discernable from this figure: (1) Convex, there is an initial decrease in performance until a point is reached after which performance steadily increases; (2) Concave, i.e., there is an initial increase in performance with later flattening out; and (3) Erratic behavior, i.e., gaps of improving performance interspersed with poor performance (forgetting?). Convexity is shown by M.m. C57Bl/6J, M.m. CF -1 and Microtus. Concavity is shown by M.m. Mo. and Onychomys. Erratic behavior is shown by Perognathus and Peromyscus. Concavity is the only pattern shared by mice of similar background, i.e. both M.m. Mo. and Onychomys are feral strains. However, Microtus had a higher average in trial ten than in trial one, and only the Mus strains managed to achieve an average base time than was less than five seconds, and thus avoided shock. Erratic behavior (forgetting) was shown by Peromyscus in the mid-range (Trials 5 - 7).
2. Pemoline 3 mg/kg

A similar plot for the 3 mg/kg dose of Pemoline is shown in Fig. 6. In this instance, four patterns were seen: Concave, convex, erratic and a new straight-lined pattern. The concave pattern was shown by Onychomys and Peromyscus, the convex by M.m. Mo., Microtus and Perognathus, while erratic behavior was seen with M.m. CF-1. The M.m. C57Bl/6J animals showed a more or less straight-lined pattern of improvement which has a slight sigmoid shape. Late forgetting was seen, however, with Perognathus, Onychomys and Microtus.

3. Pemoline 12 mg/kg

A similar treatment of the data for the 12 mg/kg dose of Pemoline is shown in Fig. 7. In this instance, the same four patterns are present. Convexity is shown with M.m. CF-1, Onychomys and M.m. C57Bl/6J, concavity with Perognathus and erratic behavior with Peromyscus. Straight-line behavior was shown by M.m. Mo. and Microtus, although in opposite directions. Late forgetting did not appear to occur with this dose level.

E. LEARNING CURVES--CLIMBING TIMES

1. Saline

By applying the same analysis to the climbing times, the results are presented in Figures 8, 9 and 10. (8 - Saline, 9 - Pemoline 3 mg/kg, and 10 - Pemoline 12 mg/kg) Among the saline controls
(Fig. 8), concavity was shown by M.m. Mo., Onychomys and Perognathus, convexity by Microtus and M.m. CF-1, erratic behavior by Peromyscus and straight-line behavior by M.m. C57Bl/6J.

2. Pemoline 3 mg/kg

Pemoline 3 mg/kg results (Fig. 9) showed concavity for M.m. CF-1, Onychomys and Perognathus, convexity for M.m. C57Bl/6J, M.m. Mo. and Microtus, and erratic behavior for Peromyscus.

3. Pemoline 12/mg/kg

Pemoline 12 mg/kg results (Fig. 10) showed concavity for M.m. Mo., Microtus, Perognathus and Peromyscus, convexity for M.m. CF-1 and Onychomys, and straight-lined behavior by M.m. C57Bl/6J. No straight-line behavior was apparent with Pemoline 3 mg/kg, and no erratic behavior noted with Pemoline 12 mg/kg.

F. LEARNING CURVES AS A REFLECTION OF CONSISTENCY OF BEHAVIOR

The average base and climbing times for each trial at each dose level with saline controls is presented for each genus or strain in Figures 14 - 20. Differences between the base and climbing times are less clear due to overlapping; but a composite picture is convenient for the learning curves. Each genus except Peromyscus shows a final base time with Pemoline 12 mg/kg well below that of the saline control. Peromyscus was impaired by the dose. Its behavior was erratic in the first trials. However, its climbing behavior was still much less erratic.
with the higher dose than with saline or with the lower dose of the drug. This characteristic of erratic behavior may be used to differentiate the base chamber performance of the genus or strain in question.

Particularly M. m. Mo., M. m. CF-1, and Microtus, and also Onychomys, showed little change from trial to trial with saline (Figures 15, 16, 17 and 18). On the other hand, M. m. C57Bl/6J showed marked change from trial to trial (Figures 16 and 14), while Peromyscus and Perognathus exhibiting a similar tendency to a lesser extent (Figures 19 and 20). With Pemoline 3 mg/kg, Peromyscus, M. m. C57Bl/6J, and M. m. CF-1, and especially Microtus, showed little change from trial to trial. M. m. Mo., Perognathus, and to a lesser degree Onychomys, showed much variance. With Pemoline 12 mg/kg, all Mus strains and Microtus showed little variance, while Onychomys showed a large amount, and Perognathus and Peromyscus exhibited a moderate amount.

These statements are based on the fact that when variance for the base times is calculated as a pooled standard error for the base trials, (cf. Figures 14 - 20), the size of the error term is in these sequences.

G. LEARNING CURVES AS A MEASURE OF AVOIDANCE

The criteria for avoidance conditioning of a given group in this experiment were as follows: (1) average avoidance of shock by attaining average base times equal to or less than 5.0 seconds for any given trial through the machine; or (2) partial avoidance of shock by attaining average
base times between 5.0 and 5.5 seconds. Partial avoidance means that while some of the mice of a group avoided, the majority did not. The majority of the mice in the group, however, had base times very close to avoidance.

Onychomys, Perognathus, and Peromyscus (Figures 18, 19 and 20) were poor avoiders. They did not have any base times less than 5.5 seconds with saline, 3 mg/kg, or 12 mg/kg Pemoline. Onychomys had average base times for the last five trials between 5.6 and 6.8 seconds with 12 mg/kg Pemoline and 6.1 and 6.8 seconds with saline. Perognathus had no average base times less than 9.0 seconds, but with 12 mg/kg Pemoline had base times for the last five trials lower than either those with 3 mg/kg Pemoline or Saline. Peromyscus had no base times less than 6.0 seconds but most nearly approached avoidance criteria with 3 mg/kg Pemoline, with base times less than 7.0 seconds for the last seven trials at this dose level.

Microtus (Fig. 17) had no base times less than 9.0 seconds with saline and only partial avoidance for trials 8, 9 and 10 with 3 mg/kg Pemoline. Microtus with 12 mg/kg Pemoline had partial avoidance for trials 4, 5, and 7, and average avoidance for trials 1, 2, 3, 6 and 9.

The Mus strains were generally good avoiders. M.m. C57B1/6J had partial avoidance for trials 6, 7 and 8 and average avoidance for trials 9 and 10.
M. m. CF-1 (Fig. 15) had partial avoidance for trials 1, 2, 6, 7, 8, and 9, and average avoidance for trial 10 with saline. With 3 mg/kg Pemoline, however, it had partial avoidance only on trial 10 and no average base times which met criteria for average avoidance.

M. m. CF-1 had average avoidance for trials 8, 9 and 10 with 12 mg/kg Pemoline. M. m. Mo. (Fig. 16) had partial avoidance on trial 9 and average avoidance on trial 10 with saline, but only partial avoidance on trial 10 with 3 mg/kg Pemoline. The M. m. Mo. animals treated with 12 mg/kg Pemoline had partial avoidance on trials 3, 4 and 5, and average avoidance for the last five trials.
CHAPTER IV

DISCUSSION

A. AVERAGE BASE TIMES

The average total base times is perhaps the clearest measure of escape shown by the data but it does not show modulation of escape by learning. Average total base times may be used in comparing strains and genera for genetic tendencies toward escape and consideration of other aspects of an animal's behavioral profile should permit correlation with these tendencies. The mouse with the least mean base times is a highly escaping animal. This escape tendency may be a reflection of learned avoidance or of hereditary predispositions.

Animals such as *Microtus*, *Peromyscus*, and *Perognathus* are ecologically found in sparsely covered areas whereas *M.m. C57Bl/6J* has been shown in the laboratory to prefer corners and walls. One might expect space-seeking animals to exhibit low mean base times and corner-seeking animals to maintain long mean base times. Inspection of the data, however, does not support these assumptions. The apparent paradox may be due in part to a modification of natural tendencies which resulted from inbreeding in the laboratory of *Microtus* and *Peromyscus*. *Perognathus*, on the other hand, was always feral. It is a timid and jumpy creature of relatively large size. These characteristics conceivably could have inhibited natural escape tendencies. In this case
long mean base times would reflect equally an inhibition of natural behavioral responses (i.e., space-seeking) and timidity for entrance into a new situation. *Perognathus* may also have had difficulty in leaving the base chamber, and when confronted with a shock it may have adopted freezing behavior. The animal with the lowest mean base times in general was *M. m. Missouri*. This animal seemed best adapted naturally to the characteristics and dimensions of the climbing screen. *Onychomys* has been described as capable of both aggressive and/or freezing (catatonia) responses. This mouse exhibited escape tendencies intermediate between the highly escaping animals and the inhibited animals.

Performance varied among the drugged and undrugged animals of a genus or strain irrespective of general escape tendencies. Four groups of animals had average base times near the five-second avoidance criterion. Three of these groups were animals which had received a dose of 12 mg/kg Pemoline (*M. m. C57Bl/6J* -- 5.57 ± 0.097 sec.; *M. m. Mo.* -- 5.01 ± 0.075 sec.; and *Microtus* -- 5.19 ± 0.112 sec.). The fourth group was the *M. m. CF-1* control (5.59 ± 0.153 sec.). Also these four groups had little variance from trial to trial as reflected by the standard errors and reduction of variance was a reflection of behavior in a drugged state. The high dose level of Pemoline reduced trial to trial variance for a majority of the genera.
and in general also lowered average base times. The intermediate
dose level lowered average base times for only two genera, *Microtus*
and *Peromyscus*, but these were the two space-seeking genera whose
escape behavior was inhibited in the control animals. This inter­
mediate dose level also reduced the variance from trial to trial for
these genera; but was without effect on two other genera and increased
variance for the remaining three genera.

This inconsistent drug effect is perhaps explained by correlating
average base times at the intermediate dose level with average base
times at the high dose level and in the undrugged state. At least two
genera had average base times which were equal for the untreated and
the 3 mg/kg Pemoline-treated animals, implying a lack of drug effect
at this dosage level, but had lower average base times with the high
dose. The 3 mg/kg dose of Pemoline inhibited the escape behavior of
at least three genera, and the 12 mg/kg dose level inhibited the escape
behavior of at least two genera. These comparisons are admittedly
complex but in general Magnesium Pemoline was effective at both
dose levels in lowering average base times. The 12 mg/kg dosage
level was more effective than the 3 mg/kg dose level. Inhibition of
escape tendencies was manifest at the 3 mg/kg dose level but much
less evident at the 12 mg/kg dose level. Enhancement of escape ten­
dencies was manifest at the 12 mg/kg dose level but much less evident
at the 3 mg/kg dose level. Variance of drug effect appeared to be dependent on both the dosage level and intergeneric differences in drug sensitivity and natural escape tendencies.

B. AVERAGE CLIMBING TIMES

The average climbing times of these animals reflect neuromuscular coordination, innate geotropism, and escape tendencies. The variance term (standard error) is larger for the climbing times than for the base times since the grid shock occurred at ten-second intervals ascending the grid floor in four segments following the shock in the base chamber floor. This gave a forty second delay before a mouse was forced into the subsequent chamber. However, none of the average climbing times were more than ten seconds and all but one were less than six seconds. Sometimes mice entered the subsequent chamber at once, at other times they stopped along the runway and waited for the shock to ascend. The animals also tried to run down the runway, and to climb out through the top. Many of the mice from all genera were rarely shocked on the runway. Pemoline facilitated climbing behavior at the 12 mg/kg dose level for almost all genera and facilitated climbing behavior for a majority of the genera at the 3 mg/kg dose level. Pemoline at both dose levels had most effect on Microtus and the 12 mg/kg dose level of Pemoline markedly enhanced the average climbing times of Perognathus. Pemoline had less effect on the Mus strains which are
generally good climbers.

C. ANALYSIS OF THE DATA BY LEVEL

There was no single level of the climbing screen apparatus which had a differential effect on either the base or climbing times of the animals (Fig. 11, 12 and 13). From this we may conclude that each animal was presented with fifty essentially identical avoidance escape situations. The results of the experiment represent, then, valid inferences of performance. Response variability at any level of the climbing screen is better correlated with genetic differences among the genera than with mechanical differences in the environment presented to the mice. There is also no correlation between dose levels and performance in a given segment of the machine.

D. LEARNING CURVES -- BASE TIMES

The learning curves for base times (Figures 5 - 7, 14 - 20) reflect the interactions of drug effects, generic reactivity, and experience. The interactions of these three influences are manifest in the overall patterning of the learning curves. Convexity refers to the initial decrease in performance after which performance steadily increases. The point on the curve after which performance progressively improves has been termed the inspiration point (IP) by Scudder et al.\textsuperscript{150} If, as was suggested, the genera are regarded as probabilistic homeostatic machines, this initial decrease in performance may be considered a clue
to the complexity of the decision pathways. Sutherland would describe these decrements as reflections of learning which analysers and outputs to attach to the incoming stimuli. If the assumption of homeostasis includes a goal directed behavior of avoiding shock and this behavior if arrived at by a choice of possible paths in the nervous system, the animals with the more complex nervous system networks with many initially equally probable decisions will make more errors in trying out one major strategy after another than a comparatively simple system. Concavity refers to an initial increase in performance with later flattening-out of the curve. Concavity represents early discovery of the correct solution and rapid attainment of the maximum in performance which is not further improved upon. The animals exhibiting concave learning curves would then be homeostatic machines capable of relatively few decisions or capable of more rapid and accurate analysis of initial strategies. Erratic behavior intersperses gaps of improving performance with poor performance. This poor performance may represent forgetting or may be further attempts at more correct solutions to the problem. It seems unlikely, however, that a system would attempt new strategies which exceed present homeostasis. If such were the case, it would not be functioning as a probabilistic machine since its behavior would represent unlearning of a correct response. Straight-line behavior refers to progressive improvement in performance. The mouse system does not seem to
completely discover the correct solution but it does behave more homeostatic. The neural pathway for the correct solution only slowly exercises a predominant influence. Of these four learning curve patterns, only the erratic pattern is poorly adapting. That each of the other three is exhibited preferentially by certain genera may well be a reflection that genetic differences predispose a mouse to choose among initially equally probable decisions in a particular sequence. The sequence may be altered by drug influences, as demonstrated in Table II.

Two mice, Microtus and Onychomys, had the same patterns in both base and climbing times with saline that they had with Pemoline 3 mg/kg. Two strains had the same patterns in both base and climbing times with saline that they did with 12 mg/kg Pemoline. Peromyscus was the most persistently erratic performer, having four erratic patterns and two concave patterns. It is interesting to note that Peromyscus had erratic patterns in base and climbing times for saline, was erratic for climbing times with 3 mg/kg Pemoline but erratic for base times with 12 mg/kg Pemoline. Perognathus had consistently concave patterns for climbing times, but was erratic with saline, convex with 3 mg/kg Pemoline and concave with 12 mg/kg Pemoline. Concavity is the only pattern shared by mice of similar background since both Onychomys and M. m. Mo. are feral.
Erratic behavior is characteristic of Peromyscus and straight-line behavior is characteristic of M. m. C57Bl/6J, both of which are highly inbred strains. The other patterns are almost equally exhibited by the animals. The convex pattern is as frequently seen in the control as in the treated animals. The concave and erratic patterns are seen less, and the straight-line pattern more, in Pemoline treated animals than in control animals, in a dose-response relationship. Pemoline thus seems to enhance homeostatic behavior.

The quantity of learning may be assessed by comparing the average of the base times for the first five trials with the average of the last five trials for each genus or strain. This results in a gradient reflecting improvement with experience. The order, from greatest to least amount of improvement among the genera and strains with saline is as follows: Onychomys, M. m. Mo., Peromyscus, M. m. C57Bl/6J, M. m. CF-1; Microtus and Perognathus had negative values, and thus cannot be included in this gradient. With 3 mg/kg Pemoline, the order is: Perognathus, Onychomys, M. m. C57Bl/6J, Peromyscus, M. m. CF-1, M. m. Mo., and Microtus. With 12 mg/kg Pemoline, the order is: Perognathus, Peromyscus, M. m. Mo., M. m. CF-1, Onychomys, M. m. C57Bl/6J; Microtus is again not included in the gradient. Quantitative improvement in learning is influenced by initial latency, final performance, and overall learning.
curves. Animals with convex learning curves tend to have more quantitative improvement than is present with any of the other patterns. And the relative quantity of learning does not reflect successful avoidance (e.g., M.m. Mo. in Fig. 16).

E. LEARNING CURVES -- CLIMBING TIMES

The learning curves for climbing times can be discussed as the base times have been discussed in reference to drug effects, generic reactivity, and experience. There was little change in relative stability of response to saline and to the two drug doses in M.m. CF-1, Onychomys and Perognathus, but Peromyscus performed better with Pemoline than with saline in a dose-response relationship. M.m. C57Bl/6J, on the other hand, had a poorer value with the 3 mg/kg dose than with either saline or the 12 mg/kg dose. The performance of M.m. Mo. became more stable with the 3 mg/kg dose but less stable with the 12 mg/kg dose, as did that of Microtus. Pemoline facilitated improvement in the climbing times of almost all genera but there was a tendency to slightly slower running on the last trial. This probably represents a fatigue decrement.

Innate geotropism rather than learning is expressed by the general superiority of some animals in basic climbing ability. Climbing performance was relatively stable for all genera. Only two animals showed marked changes in their ranking regarding escape tendency.
Onychomys was the most escaping animal at the 3 mg/kg level, but was fifth at the 12 mg/kg level; and Perognathus was much more escaping with the 12 mg/kg dose than with saline. Pemoline does not affect climbing performance as much as base time performance.

Quantitative improvement in climbing performance was also less affected by Pemoline than was base performance. Certain animals, e.g., Peromyscus untreated, exhibited both quantitatively more improvement and change in climbing time learning curves to more homeostatic solutions. M. m. C57Bl/6J was improved, and M. m. Mo. and Peromyscus much improved quantitatively with the Pemoline doses, while Microtus, Onychomys and Perognathus were quantitatively less improved relative to the other animals with the drug in either dose. M. m. CF-1 showed some relative improvement with the 3 mg/kg dose but was the poorest quantitatively with the Pemoline 12 mg/kg dose.

F. LEARNING CURVES AS A REFLECTION OF CONSISTENCY OF BEHAVIOR

Consistency of behavior is reflected by both variance of base and/or climbing times from trial to trial and adherence to a learning curve pattern of performance closely described by a mathematical expression. This continuity of the learning curves refers to how closely subsequent points lie to the closest continuous curve or quadratic equation possibly represented by them. Hypothetically divergence from a continuous curve represents forgetting and indicates high variance from...
performance to performance among mice of a genus. Scudder et al. have previously demonstrated that in the climbing screen the wild animals, the least inbred, generally show the most constant behavior. Survival probably depends on large measure on a good uniform escape behavior or a well-regulated processing of information leading to superior escape; and the wild forms, although they may not be highly inbred or homozygous for other traits show a strong tendency for uniform escape behavior. The most interesting results were with the animals treated with 3 mg/kg Pemoline. In this instance the inbred strains showed little variance in performance while the feral strains showed more variance. Onychomys and M. m. Mo. are feral mice and had little variance in performance when given saline. In the drugged state, both of these feral mice became less consistent in their learning performances. The highly inbred strains, on the other hand, showed uniform responsiveness in the drugged state. Pemoline presumably acted to facilitate selection of behavioral responses which are subordinated in the untreated inbred animals and to disrupt normal behavioral responses in the feral mice.

G. LEARNING CURVES AS A MEASURE OF AVOIDANCE

The quality of learning is important for this is basically an avoidance situation. Criteria were established to determine the qualitative learning (i.e., avoidance) exhibited by the animals. Quality of learning is
influenced by neuromuscular coordination, innate geotropism, and drug-mouse and mouse-machine interactions. We have little evidence with which to evaluate neuromuscular coordination in this experiment and must therefore consider it an unknown variable. Innate geotropism is reflected in the consistency with which various genera react to the avoidance-escape situation. The Mus strains are generally good avoiders but Perognathus, Peromyscus, and Onychomys are generally poor avoiders. Microtus, which showed no avoidance with saline, performed very well with either dose of Pemoline.

If one analyses the learning curves for quality of learning, quantity of learning, convexity (IP), stability and continuity, and escape tendencies, the results appear as in Tables III for base times and Table IV for climbing times. These tables represent means for evaluating the intelligence of each genus as reflected in the performances in drugged and undrugged states. The complete lack of qualitative improvement with the 3 mg/kg dose of Pemoline is significant from the standpoint of a possible biphasic action of Magnesium Pemoline. Thus quality ratings for all genuses and strains with Pemoline doses of 3 mg/kg were entered as zero. If, however, the maximum poor rating, i.e., 7, is given, the final intelligence totals change. This adjusted score does not alter inter-generic and inter-strain relative differences in performance, but it does change the evaluation of the drug effects. Three
mice, *M. m. Mo.*, Onychomys and *Peromyscus* have the same relative performance with and without adjustment. This analysis is shown in Table V.

*Perognathus* and *M. m. C57Bl/6J* change from most intelligent performance to least. *M. m. CF-I* still has more intelligence with Pemoline, but its adjusted performance is worse at the lower dose than at the higher. *Microtus*, on the other hand, does best with the higher dose. Two animals are profoundly influenced by the drug: *M. m. Mo.* and Onychomys. The *M. m. Mo.* animals treated with 3 mg/kg Pemoline do very poorly, while their cohorts do extremely well. The Onychomys controls are second in intelligence only to *M. m. Mo.*, but drugged Onychomys does very poorly. Both *M. m. Mo.* and Onychomys are feral. When performance is adjusted, two patterns develop. The highly inbred *Mus* strains are affected similarly by the dosages and have comparable total scores. They are best without the drug, and are inhibited by the lower dose. Some of the feral animals, *M. m. Mo.*, *Microtus* and *Perognathus*, do best with the higher dose and poorest with the lower dose. *Peromyscus* in general does poorly but less poorly with 3 mg/kg Pemoline.

There was a divergence of dose effects in the relative intelligence rankings for base and climbing times. These animals which in general showed increases in relative rank for climbing times showed decreases
for base times. Microtus was unique in exhibiting progressive improvement in base times with increasing drug dose levels but a decline in climbing performance with the 12 mg/kg dose of Pemoline.

Microtus is a very interesting animal in its reaction to both the climbing screen apparatus and to Pemoline. The controls show absolutely no avoidance, and are quantitatively poorer performers in the last five trials than in the first five. Microtus was shown to be a poor learner and a poor avoider in our earlier undrugged studies. With the lower dose of Pemoline, the animals approached the machine more adaptively, and some of them were avoiding in the last six trials, having reached maximum performance (and learning) by the fourth trial through the machine with 3 mg/kg Pemoline. With the higher dose, they seemed adjusted to the machine from the start, having an average of less than 5.00 seconds for the first three trials, then hovering around the avoidance level for the next four trials, then apparently waiting for the shock before leaving the chambers. It is noteworthy that even in the last three trials, some of the animals are still avoiding. Microtus also shows much less variance in its climbing behavior under the influence of the drug than with saline, although it learns running performance progressively better with saline after an early marked convexity, which is less pronounced with the lower dose of Pemoline.

An analysis of Microtus for all 50 avoidance-escape situations for
all ten mice of each group for base times is shown in Fig. 21. The very erratic behavior of the saline controls is obvious, as is the relatively stable performance of the higher dose animals. The animals treated with the lower dose of Pemoline showed erratic behavior for much of the first half of the experiment, but became more stable than the 12 mg/kg animals in the second half. The dotted line represents the 5.0 second avoidance area, and it can be seen that the drugged animals have learned to associate their motor responses better to their sensory input than the saline control animals. The question of whether Pemoline influenced the sensory recognition of the shock is beyond the scope or feasibility of this paper.

An analysis of Microtus for the 50 avoidance-escape situations for all ten mice of each group for their climbing times is shown in Fig. 22. Again the control animals are more erratic, and the lower dose animals show erratic behavior early with better stability than the higher dose animals at the end.

H. SIGNIFICANCE OF THE RESULTS

The results may be correlated with the theories of learning and memory. Straight-line patterns of learning curves correspond well with the trial-and-error patterns proposed by Thorndike and, in a sense, with those of all SR theorists. Guthrie's shifting of associations theory is demonstrated by the concave learning curve patterns. The convex
patterns are obvious detour patterns and may result from the complexities of neural pathways. That no one psychological theory explains the results is to be expected from the state of controversy about learning and memory processes.

It is likely that molecular modulation and repression are the bases of learning and memory. Nucleic acid is the most probable site of memory coding and an increase in RNA would enhance memory and learning. Neurophysiological postulations have been proposed by Plotnikoff and by Glasky to explain the effects of Pemoline on learning and memory. According to the postulated mechanism of action of Pemoline, true-RNA polymerase is stimulated in a linear dose-response relationship. This presumably facilitates the development of preferential neural pathways and synaptic connections by increasing available RNA. 

We have demonstrated variable and complex behavioral effects of Pemoline on learning and memory in mice. Our results failed to substantiate a dose-response relationship between Pemoline and performance. Performance was enhanced by Pemoline at both dose levels, the higher dose being generally more effective, but there was a divergence of dose effects in the relative rankings of the genera for performance in base and climbing times. The enhancement of learning produced by Pemoline is probably due to a non-specific action as a CNS stimulant.
SUMMARY AND CONCLUSIONS

Employing an automated avoidance conditioning climbing screen, of a study was made of the comparative effects saline controls, and 3 mg/kg and 12 mg/kg dose levels of Magnesium Pemoline on learning behavior in several genera of mice. Results were recorded in terms of the amount of time the animals spent in the base chambers of the machine and the climbing time from leaving the base chamber until entering the next chamber. The mice were Microtus ochrogaster, Onychomys leucogaster, Perognathus penicillatus, Peromyscus maniculatus Bairdii, and three strains of Mus musculus, M.m. CF-1, M.m. C57Bl/6J, and M.m. Missouri.

The learning curves of the mice revealed marked variability in avoidance conditioning among the strains and genera in both drugged and undrugged states.

The shapes of the learning curves were analyzed for (1) quality of learning, (2) quantity of learning, (3) stability and continuity, (4) escape tendency and (5) inspiration point or convexity. Tables were constructed quantitating the relative behavior of the strains and genera for both base and climb times, and an intelligence - performance scale derived.

The feral animals, except Perognathus penicillatus,
were the most adaptive animals as saline controls, and the feral Mus, M. m. Missouri, was the most intelligent animal whether drugged or not. Microtus ochrogaster, which showed no avoidance learning with saline, performed very well with either dose level of Magnesium Pemoline.

There was a divergence of dose effects in the relative intelligence rankings for base and climb times. Those animals which in general showed increases in relative rank for climb times exhibited decreases for base times. Microtus ochrogaster was unique in showing progressive improvement in base times with increasing drug doses but a decline in performance with the 12 mg/kg dose of Magnesium Pemoline.

The results failed to show enhanced performance for all mice in a dose-response relationship. Rather, the data suggested that the 3 mg/kg dose of Magnesium Pemoline adversely influenced feral mice more than inbred mice, but produced learning increments in a poorly adapting, highly inbred strain, Peromyscus maniculatus Bairdii. Some of the feral animals, M. m. Missouri, Microtus ochrogaster, and Perognathus penicillatus, performed, in comparison to the saline control group, better with the 12 mg/kg dose but poorer with the 3 mg/kg dose. No qualitative learning was evident in any of the mice with the 3 mg/kg dose level.

That our results did not show the dose-response relationships
previously demonstrated by others may be due to our employment of
different dose levels than those employed by Lienert, Schmidt or
Plotnikoff. Our data indicates facilitation at higher doses contrary to
what was reported by Lienert. And the linear dose-response relation-
ship noted by Plotnikoff was absent in our results. Our data indicate
a doubly biphasic action on avoidance conditioning and learning in low-
moderate and high dose levels of the drug, which affects feral mice more
than highly inbred strains. Since puromycin is known to inhibit
learning in mice and interferes with protein synthesis, the conclusion
has been drawn that puromycin adversely affects the learning and
memory of mice by inhibiting the synthesis of protein via the
ribosomes. It may be of interest in the future to contrast the effects of
puromycin and those of Pemoline on the learning and memory of mice and
to correlate these results with molecular studies of the drugged animals.
In our opinion, the enhancement of learned avoidance conditioning in the
mice in our study was due to a nonspecific central stimulating effect of
this drug.
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      2  Wiring diagram of Avoidance Conditioning Machine
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      5  Average Base Times for each Genera (or Strain) with Saline
      6  Average Base Times for each Genera (or Strain) 3 mg/kg Pemoline
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      8  Average Climb Time for each Genera (or Strain) with Saline
      9  Average Climb Time for each Genera (or Strain) 3 mg/kg Pemoline
     10  Average Climb Time for each Genera (or Strain) 12 mg/kg Pemoline
     11  Avg. Base & Climb Times for ea. Genera (or Strain) with Saline
     12  Avg. Base & Climb Times for ea. Genera (or Strain) 3 mg/kg Pem.
     13  Avg. Base & Climb Times for ea. Genera (or Strain) 12 mg/kg Pem.
     14  Avg. Base & Climb Times for Mus musculus C57B1/6J
     15  Avg. Base & Climb Times for Mus musculus CF-1
     16  Avg. Base & Climb Times for Mus musculus Missouri
     17  Avg. Base & Climb Times for Microtus ochrogaster
     18  Avg. Base & Climb Times for Onychomys leucogaster
     19  Avg. Base & Climb Times for Perognathus penicillatus
     20  Avg. Base & Climb Times for Peromyscus maniculatus Bairdii
     21  Average Base Times for Microtus
     22  Average Climb Times for Microtus
<table>
<thead>
<tr>
<th><strong>TABLE I</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug Effects on Learning and Memory</td>
</tr>
</tbody>
</table>

**A. DRUG IMPAIRMENT OF LEARNING**
1. Barbiturates
2. Tranquilizers
3. Anticholinergics
4. Cholinomimetics
5. Inhibitors of RNA synthesis (8-azaguanine)
6. Cations (CaCl₂)

**B. DRUG IMPAIRMENT OF MEMORY**
1. Anesthetics
2. Topicals
3. Tranquilizers
4. Scopolamine
5. Depressants
6. Inhibitors of RNA synthesis
7. Inhibitors of protein synthesis

**C. DRUG FACILITATION OF LEARNING**
1. Strychnine
2. Amphetamines
3. Nicotine
4. Chlordiazepoxide
5. Cholinomimetics
6. Thiamine
7. Diphenyldiazadamantanol (1757 I.S.)
8. Pemoline

**D. DRUG FACILITATION OF MEMORY**
1. Stimulants
2. Analeptics
3. Convulsants
4. Cholinomimetics
5. Ribonucleic acid
6. Diphenyldiazadamantanol (1757 I.S.)
7. 1,1,3-tricyano-2-amino-1-propene (U-9189)
8. Pemoline (7)
TABLE II

<table>
<thead>
<tr>
<th>Genus/Strain</th>
<th>Saline</th>
<th>3 mg/kg Pemoline</th>
<th>12 mg/kg Pemoline</th>
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<tr>
<td></td>
<td>Base</td>
<td>Climb</td>
<td>Base</td>
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<tr>
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<td>convex/straight</td>
<td>straight/convex</td>
<td>convex/straight</td>
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<tr>
<td>M. m. CF-I</td>
<td>convex/convex</td>
<td>erratic/concave</td>
<td>convex/convex</td>
</tr>
<tr>
<td>M. m. Missouri</td>
<td>concave/concave</td>
<td>convex/convex</td>
<td>straight/concave</td>
</tr>
<tr>
<td>Microtus</td>
<td>convex/convex</td>
<td>convex/convex</td>
<td>straight/concave</td>
</tr>
<tr>
<td>Onychomys</td>
<td>concave/concave</td>
<td>concave/concave</td>
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</tr>
<tr>
<td>Perognathus</td>
<td>erratic/concave</td>
<td>convex/concave</td>
<td>concave/concave</td>
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<tr>
<td>Peromyscus</td>
<td>erratic/erratic</td>
<td>concave/erratic</td>
<td>erratic/concave</td>
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This represents a tabulation of the various learning curves exhibited by the genera. For explanation see text (Chapter IV--Section D) and Figures 5 - 10.
### TABLE III
Evaluation of Base Times

<table>
<thead>
<tr>
<th>Genus</th>
<th>Stability and Continuity</th>
<th>Inspiration Point or Convexity</th>
<th>Quantity</th>
<th>Quality</th>
<th>Escape</th>
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<tr>
<td></td>
<td>S 3 12</td>
<td>S 3 12</td>
<td>S 3 12</td>
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<td>S 3 12</td>
</tr>
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<td>M. m. C57 B1/6J</td>
<td>3 1 3</td>
<td>3 3 2</td>
<td>4 3 5</td>
<td>2 0 3</td>
<td>3 5 3</td>
<td>15 12 16</td>
</tr>
<tr>
<td>M. m. CF-I</td>
<td>4 6 6</td>
<td>4 4 3</td>
<td>5 5 4</td>
<td>1 0 2</td>
<td>1 3 4</td>
<td>15 21 19</td>
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<tr>
<td>M. m. Mo.</td>
<td>2 2 1</td>
<td>1 3 1</td>
<td>2 6 3</td>
<td>1 0 1</td>
<td>2 2 1</td>
<td>8 16 7</td>
</tr>
<tr>
<td>Microtus</td>
<td>5 5 2</td>
<td>5 3 7</td>
<td>0* 6 0*</td>
<td>2 0 6</td>
<td>1 2 15 18 12</td>
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<tr>
<td>Onychomys</td>
<td>1 3 4</td>
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<td>1 2 4</td>
<td>2 0 4</td>
<td>4 6 5</td>
<td>9 12 21</td>
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<td>Perognathus</td>
<td>6 7 5</td>
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<td>0* 1 1</td>
<td>2 0 4</td>
<td>7 7 17 21</td>
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<td>Peromyscus</td>
<td>7 4 7</td>
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<td>3 4 2</td>
<td>2 0 5</td>
<td>4 7 17 24</td>
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</tbody>
</table>

This represents a five-factor analysis of the average base times and the learning curves. For explanation see text (Chapter IV) and Figures 3, 5-7, and 14-20. S = Saline, 3 = 3 mg/kg pemoline, and 12 = 2 mg/kg pemoline. The asterisks are explained in the text.
This represents a three-factor analysis of the average climbing times and the learning curves. For explanation see text (Chapter IV) and Figures 4, 8–10, and 14–20. S = Saline, 3 = 3 mg/kg penicillin, and 12 = 12 mg/kg penicillin.

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<tbody>
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TABLE IV
Evaluation of Climbing Times
### TABLE V

#### Intelligence - Performance

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<th>Drug</th>
<th>SCORE</th>
<th>PERFORMANCE</th>
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<td>Saline</td>
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<td>Pem. 3 mg/kg</td>
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<td>Pem 12 mg/kg</td>
<td>16</td>
<td>3</td>
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<td>M.m. CF-I</td>
<td>Saline</td>
<td>15</td>
<td>1</td>
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<td></td>
<td>Pem 3 mg/kg</td>
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<td>Pem 12 mg/kg</td>
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<td>M.m. Missouri</td>
<td>Saline</td>
<td>18</td>
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<td>Pem 3 mg/kg</td>
<td>13</td>
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<tr>
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<td>Pem 12 mg/kg</td>
<td>7</td>
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<td>Microtus</td>
<td>Saline</td>
<td>18</td>
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<tr>
<td></td>
<td>Pem 3 mg/kg</td>
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<tr>
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<td>Pem 12 mg/kg</td>
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<td>Saline</td>
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<td>Saline</td>
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<td>Pem 3 mg/kg</td>
<td>13</td>
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<tr>
<td></td>
<td>Pem 12 mg/kg</td>
<td>24</td>
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This represents an adjustment for values in Tables III and IV. In place of the zero entered in the qualitative learning columns of all the genera the maximum poor rating is entered here since there was no qualitative improvement of learning with 3 mg/kg pemoline. As seen there is an effect on the relative effects of pemoline on the intelligence rating given the various genera.
FIGURE 1
Block Diagram of Avoidance Conditioning Machine
FIGURE 2

Wiring Diagram of Avoidance Conditioning Machine
BASE TIME FOR EACH GENUS (OR STRAIN)

SALINE CONTROL
3 mg/kg MAGNESIUM PEMOLINE
12 mg/kg MAGNESIUM PEMOLINE

- MUS M. C57/BL6
- MUS CFI
- MUS "MO"
- MICROSTUS OCHRO-GASTER
- ONYCHOMIS LEUCO-GASTER
- PEROGNA-THUS SP.
- PEROMYSCUS M. BAIRDII
FIGURE 4

Climb Time for Each Genus (or Strain)

Saline Control
3 mg/kg Mg. Pemoline
12 mg/kg Mg. Pemoline

Mus H. C57 BL/6j
Mus H. CF-1
Mus M. Missouri
Microtus ochrogaster
Onychomys leucopterus
Perognathus paniculatus
Peromyscus man机ulatus
Figure 5 -- Base times, Saline Control

M. m. C57Bl/6J

M. m. CF-1

M. m. "Mo."
FIGURE 5

Base times, Saline control

TIME IN SECONDS

Microtus

Onychomys

Perognathus

Peromyscus

TRIALS
Figure 6 -- Base times, 3 mg/kg Pemoline

M. m. C57Bl/6J

M. m. CF-1

M. m. "Mo."
FIGURE 6

Base times, 3 mg/kg Pemoline

TIME IN SECONDS

Microtus

Onychomys

Perognathus

Peromyscus

TRIALS
Figure 7 -- Base times, 12 mg/kg Pemoline

M. m. C57Bl/6J

M. m. CF-1

M. m. "Mo."
FIGURE 7

Base times, 12 mg/kg Pemoline

TIME IN SECONDS

TRIALS

Microtus

Onychomys

Perognathus

Peromyscus
FIGURE 8 -- Climbing Times, Saline control

M. m. C57Bl/6J

M. m. CF-1

M. m. "Mo."

Microtus

TRIALS
Figure 8 -- Climbing times, Saline control

Onychomys

Perognathus

Peromyscus

TIME IN SECONDS

TRIALS
Figure 9 — Climbing times, 3 mg/kg Pemoline

M. m. C57Bl/6J

M. m. CF-1

M. m. "Mo."

Microtus
Figure 9 -- Climbing times, 3 mg/kg Pemoline

Onychomys

Perognathus

Peromyscus
Figure 10 -- Climbing times, 12 mg/kg Pemoline

M. m. C57B1/6J

M. m. CF-1

M. m. "Mo."

Microtus

TIME IN SECONDS

TRIALS
Figure 10 -- Climbing times, 12 mg/kg Pemoline

Onychomys

Perognathus

Peromyscus

TIME IN SECONDS

TRIALS
Figure 11 -- Analysis by level, Saline control

**BASE**
- M. m. C57BL/6J

**CLIMB**
- M. m. C57BL/6J

**Microtus**

**TIME IN SECONDS**

Chambers

Screens
Analysis by level, Saline control
Figure 12 -- Analysis by level, 3 mg/kg Pemoline

Chambers

Screens
**FIGURE 2.2**

- **Onychomys**
- **Perognathus**
- **Peromyscus**

**Chambers**

**Screens**
Figure 13 -- Analysis by Level, 12 mg/kg Pemoline

- M. m. C57B1/6J
- M. m. CF-1
- M. m. "Mo."
- Microtus
- Onychomys

Chambers

Screens
FIGURE 13

Analysis by level, 12 mg/kg Pemoline
FIGURE 15
Mus Musculus CF-1

- Base time with saline
- Base time with 3 mg/kg pemoline
- Base time with 12 mg/kg pemoline
- Climb time with saline
- Climb time with 3 mg/kg pemoline
- Climb time with 12 mg/kg pemoline
FIGURE 16

Mus Musculus Missouri

- Base time with saline
- Base time with 3 mg/kg pemoline
- Base time with 12 mg/kg pemoline
- Climb time with saline
- Climb time with 3 mg/kg pemoline
- Climb time with 12 mg/kg pemoline

TIME IN SECONDS

TRIALS
FIGURE 17

Microtus ochrogaster

- - - - Base time with saline
△△△△ Base time with 3 mg/kg pemoline
■■■■ Base time with 12 mg/kg pemoline
○○○○ Climb time with saline
△△△△ Climb time with 3 mg/kg pemoline
■■■■ Climb time with 12 mg/kg pemoline

TIME IN SECONDS

0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32

TRIALS
FIGURE 18

Onychomys leucogaster

- Base time with saline
- Base time with 3 mg/kg pemoline
- Base time with 12 mg/kg pemoline
- Climb time with saline
- Climb time with 3 mg/kg pemoline
- Climb time with 12 mg/kg pemoline
FIGURE 19

Perognathus penicillatus

- Base time with saline
- Base time with 3 mg/kg pemoline
- Base time with 12 mg/kg pemoline
- Climb time with saline
- Climb time with 3 mg/kg pemoline
- Climb time with 12 mg/kg pemoline
FIGURE 20

Peromyscus maniculatus Bairdii

- Base time with saline
- Base time with 3 mg/kg pemoline
- Base time with 12 mg/kg pemoline
- Climb time with saline
- Climb time with 3 mg/kg pemoline
- Climb time with 12 mg/kg pemoline
FIGURE 21

BASE CHAMBER TIMES

MICROTUS OCHROGAST

- SALINE
- PEMOLINE, 3mg/kg
- PEMOLINE, 12mg/kg

TIME IN SECONDS

BASE CHAMBERS
Fig. 22 - MICROTUS OCHROGASTER

CLIMB SCREEN TIMES

- SALINE
- PEMOLINE, 3 mg/kg
- PEMOLINE, 12 mg/kg

TIME IN SECONDS

CLIMB SCREENS


115. Flexner, Boris B., Flexner, Josefa B., and Stellar, Eliot; Memory and Cerebral Protein Synthesis in Mice as Affected by Graded Amounts of Paarmycin.


118. Scudder, C. L. Personal Communication.


The dissertation submitted by Denis S. Avery has been read and approved by two members of the faculty of the Graduate School and a guest member from Abbott Laboratories.

The final copies have been examined by the Director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the dissertation is now given final approval with reference to content, form, and mechanical accuracy.

The dissertation is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

May 29, 1967  
Signature of Adviser