THE EFFECTS OF MAGNESIUM PEMOLINE ON DELAYED INTERVAL RESPONDING

by

Richard D. Millar

A Thesis Submitted to the Faculty of the School of Graduate Studies in partial fulfillment of the Degree of Master of Arts

Western Michigan University Kalamazoo, Michigan August 1967
ACKNOWLEDGEMENTS

The experimenter is indebted to Dr. David O. Lyon for the invaluable opportunities and academic stimulation which guided the development of the experimental repertoire necessary for this endeavor. Similar appreciation is extended to Dr. Richard W. Malott and Dr. Paul T. Mountjoy for their assistance and academic contributions.

Special mention is given to the author's wife, Linda, without whom this academic accomplishment could not have materialized.

Richard D. Millar
MASTER'S THESIS M-1299

MILLAR, Richard Dykes
THE EFFECTS OF MAGNESIUM PEMOLINE ON DELAYED INTERVAL RESPONDING.

Western Michigan University, M.A., 1967
Psychology, experimental

University Microfilms, Inc., Ann Arbor, Michigan
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>METHOD</td>
<td>8</td>
</tr>
<tr>
<td>Subjects</td>
<td>8</td>
</tr>
<tr>
<td>Apparatus</td>
<td>8</td>
</tr>
<tr>
<td>Procedure</td>
<td>9</td>
</tr>
<tr>
<td>RESULTS</td>
<td>12</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>23</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>27</td>
</tr>
</tbody>
</table>

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
INTRODUCTION

Learning is the subject of frequent speculation and considerable scientific investigation, but the learning process itself is not directly observable. Learning is an intervening variable which may be inferred only from changes in overt response probabilities (Gollub & Brady, 1965). Although there are no concrete organic learning processes which have been observed, different theories have attempted to postulate its properties. Some theories have concerned neurophysiological models and cybernetic interpretations while many models are based solely on the behavioral data. The primary obstacles to such research are due to the limitations in technology in producing an observable phenomena of the organic processes of learning.

In the last two decades a new member has been added to the list of learning theories which can be examined with certain degrees of credibility. This approach has attempted to explain learning and retention biochemically in terms of ribonucleic acid (RNA). The RNA theory was significantly strengthened through the research of Hyden (1962; 1963; 1965), in which he was able to detect changes in the chemical composition of RNA after subjects were exposed to a learning situation. By using a microanalytic technique Hyden removed glial and Deiters' nerve cells from rats trained to balance on a wire in order to obtain food reinforcement. Control subjects were stimulated in a manner which activated these nerve cells but did not result
in a conditioned response. Increases in RNA concentration were noted for both groups, however, only in the trained subjects were chemical compositions of the RNA notably altered. It was stated by Hyden since DNA is known to contain the "genetic code" which stores the "ancestral memories" then RNA might encode or "remember" an organism's personal memories. Although Hyden's work was neither proven nor disproven it served to stimulate interest in the biochemical approach.

Further interest in RNA was generated by a group of researchers working with planaria, or common fresh water flatworms. The planaria have many features which readily qualify them for learning research. It has a rudimentary brain which can be regenerated when removed, and the whole organism can regenerate itself when cut into sections (Best, 1963). A number of investigators (McConnell, 1962; McConnell, Jacobson, & Kimble, 1959; Thompson & McConnell, 1955) that when planaria are cut in half both the regenerated head and tail sections of the planaria retained a previously conditioned response. Once more when a naive planaria would ingest a trained planaria (cannibalism) the naive subjects could acquire the same conditioned response with less training. McConnell suggested perhaps the RNA molecules shown to be modified by other research (Hyden, 1961) were transferred to the naive worms. Corning and John (1961) using the planaria attempted to verify the speculations of McConnell. Here the regenerative planaria were cut in half transversely and some head and tail sections were regenerated in ordinary pond water
while others were placed in a ribonuclease solution, an enzyme which destroys RNA. The original head and tail sections regenerated in the pond water both showed retention of the learned response. A similar result was obtained with the head sections regenerated in the ribonuclease solution, however, there was no retention of the learned response in the tail sections regenerated in the ribonuclease solution. The authors suggest retention of the conditioned response was stored by the RNA and was subsequently erased for the experimental group by the RNA destructive chemical.

Even though much of the research with planaria has been replicated in various experimental laboratories, there is still evidence which contradicts many of the findings. Hartry, Keith-Lee, and Morton (1964) found it was also possible to get more rapid conditioning when unconditioned planaria would ingest other unconditioned planaria. As a result of these findings, Hartry, et al. suggest the improvement in training was related to metabolic or nutritional factors, or general activation and sensitization rather than RNA transfer.

The effects of RNA upon learning in higher organisms has been reported in three studies (Babich, Jacobson, Bubash, & Jacobson, 1965; Jacobson, Babich, Bubash, & Jacobson, 1965; Babich, Jacobson, & Bubash, 1965). The design of these studies involved extracting RNA from the brains of rats or hamsters which had been trained to press a lever for food and subsequently injecting this RNA into naive subjects. It was shown that the RNA injected subjects learned
the lever pressing response faster than the control subjects. However, these data could not be replicated by other investigators (Byrne, et al., 1966; Gross & Carey, 1965; Luttges, Johnson, Buck, Holland, & McGaugh, 1966). The study by Luttges, et al. (1966) included a number of independent experiments. The most significant study involved labeling RNA with radioactive tracers and injecting the RNA into mice. The results showed little, if any, RNA passing through the blood brain barrier after the intraperitoneal injections. Other research has attempted to explain any facilitative effect of RNA injections as being caused by the ability of the chemical to increase the general activity of the organism (Brown, 1966a; Brown, 1966b; Wagner, Carter, & Beatty, 1966). However, other studies have failed to find this stimulation effect (Corson & Enesco, 1966), while others contend RNA enhances only the learning of complex discriminations as opposed to simpler situations (Ison & Taplin, 1966).

Recently Glasky and Simon (1966) demonstrated after in vivo and in vitro analysis that magnesium pemoline activates the nuclear aggregate enzymes responsible for RNA synthesis. These authors also suggested that this drug, classified as a moderate central nervous system stimulant, might be useful in establishing a relationship between RNA and learning. Plotnikoff (1966) was the first to attempt an experimental study in which the effects of pemoline were tested in a learning situation. The resultant information of this study seemed to support the earlier contentions of Glasky and Simon (1966). The group which was injected with pemoline had significantly shorter
reaction times in the discriminated jump-out avoidance situation. Later replications of the Plotnikoff study indicated the findings could be explained by an increase in general performance (Beach & Kimble, 1967), a disruption of emotional freezing, or an increase in the sensitivity to sound or shock (Frey & Polidora, 1967). Furthermore, whether learning actually occurred is questioned since the pemoline group was shown to have shorter response latencies but there were no differences between groups in the frequency of shock avoidance. Additional research presented by Howard and Doty (1967) found magnesium pemoline to have facilitative effects on the learning of a simple avoidance response. These authors showed that pemoline injections occurring 1 minute after a daily session enhanced avoidance performance, although there were no changes with RNA injections 1 or 4 hours after the trials. Similar injections with a discriminated avoidance problem did not result in significant differences in performance. These data were interpreted as indicating "pemoline...somehow alters post-trial neural processes associated with the acquisition of new responses."

Human learning studies (Burns, House, Fensch, & Miller, 1967; Smith, 1967) failed to report any facilitative effect due to the influence of pemoline. The results of these studies indicated the effects of pemoline on human behavior are similar to the effects obtained by a general stimulant of the central nervous system (CNS).

In accordance with the many contradictions involved with RNA and magnesium pemoline research, it has been shown recently (Morris,
Aghajanian, & Bloom, 1967) that the results obtained by Glasky and Simon (1966) relating magnesium pemoline to RNA synthesis were not replicable when studied in vivo. However, no apparent attempts have been made to replicate the in vitro process.

In light of the preceding research this experimental endeavor will attempt to present further information concerning the possible facilitative effects of magnesium pemoline. The disciplines of this experimental design should make it possible to differentiate between learning and general performance stimulation. This is deemed necessary since one of the main contentions against the facilitative effects of RNA, as stated by a number of authors (Brown, 1966a; Brown, 1966b; Wagner, Carder, & Beatty, 1966) is the general performance stimulant effects caused by RNA which can be erroneously construed as learning enhancement. Similar evidence has been shown by Beach and Kimble (1967), Frey and Polidora (1967), and Millar and Thor (1966) concerning the possible stimulant effects of magnesium pemoline.

In order to establish a behavior which would show learning but would not be facilitated by a stimulant, a schedule of differential reinforcement of low rates (DRL) described by Wilson and Keller (1954) was selected. This schedule requires the animal to space his responses, those responses which occur only after a minimum duration since the previous responses are reinforced. For example, on a DRL 18 second schedule only those responses occurring 18 seconds after the previous response are reinforced. All responses which occur before
the 18 second criterion serve to reset the delay interval timer.
METHOD

Subjects

Ten experimentally naive male albino rats were selected from the Western Michigan University colony. The animals were approximately 120 to 140 days old and weighed 320 to 440 grams at the beginning of the experiment. Subjects were food deprived within approximately 15 grams of 80% free feeding weights. Noyes 45 milligram food pellets served as reinforcers in the experimental chamber, while Wayne Lab-Blox were used as a supplement after each session to maintain deprivation levels. Water was always available in the home cages.

Apparatus

A Scientific Prototype rodent test chamber #A-100 was used. The chamber was modified by removing the response lever and inserting a Switchcraft Lev-R switch #3002 (Verhave, 1958). The response lever consisted of a stainless steel rod 2 inches in length and 3/8 inches in diameter mounted 2 inches above the floor. A pressure of 6-8 grams was required to operate the lever. There was no auditory stimulus when the animal pressed the lever before completion of the interval. However, there was a click when the pellet dispenser was operated for a correct response at the end of an interval. Both the test chamber and pellet dispenser were situated in a sound attenuated, ventilated enclosure. Experimental contingencies were programmed by.
electro-mechanical timers and relay circuitry located in an adjoining room. Data was recorded from electro-mechanical counters and a Gerbrands cumulative recorder.

Procedure

The experimental environment was programmed to deliver one food pellet for each response which had an interresponse time of 18 seconds or more. Responses occurring at intervals less than 18 seconds postponed the availability of reinforcement for an additional 18 seconds (DRL 18). There was no shaping of the bar response or continuous reinforcement at any time during the experiment. The naive subjects were placed into the experimental chamber and through their own behaviors located the response lever as well as the food dish.

Oral injections were made by means of a No. 8 French catheter and were administered 30 minutes prior to each experimental session. All volumes were determined by the body weight of the subjects and fluctuated less than .1 of a milliliter for all subjects in both groups.

Drug Injections: Control Group

The 5 control subjects were initially maintained on the DRL schedule for 21 consecutive daily one hour sessions receiving prior injections of water. Following the initial 21 sessions subjects were injected for 3 sessions with 5 mg/kg of magnesium pemoline suspended
in a vehicle of 0.3 percent tragacanth. After this block of 3 ses-
sions with magnesium pemoline, the control subjects received another
block of 3 sessions with injections of tragacanth. This block alter-
nation procedure continued for 24 sessions with successive pemoline
dosages of 5 mg/kg, 10 mg/kg, 20 mg/kg, and 40 mg/kg. The final
five sessions consisted of water injections. The control group was
exposed to a total of 50 experimental sessions which were periodi-
cally interrupted for various intervals. One day intervals occurred
between sessions 32-33, 39-40, 42-43, 43-44; two day intervals be-
tween sessions 45-46; and a four day interval between sessions 36-37.

Drug Injections: Experimental Group

Of the 5 members of the experimental group subjects SE 6, SE 8,
and SE 10 were injected for 20 consecutive sessions with a 20 mg/kg
concentration of pemoline suspended in 0.3 percent tragacanth. How-
ever, due to the total ineffectiveness of the performance of subjects
SE 7 and SE 9 their experimental procedure included additional man-
ipulation.

For SE 7 magnesium pemoline injections were administered for
the initial 7 sessions, followed by a complete absence of injections
or exposure to the experimental environment for 4 days. This cess-
ation was succeeded by 3 consecutive experimental sessions with in-
jections of the tragacanth vehicle. Immediately following was one
session with a pemoline injection, a three day absence of injections
or exposure to the chamber, and three final trials with the pemoline.
The experimental subject SE 9 was initially injected with the 20 mg/kg pemoline suspension for 4 consecutive days followed by 1 experimental session with a tragacanth injection. The next day consisted of neither an injection nor placement into the chamber. This was succeeded by a tragacanth session, a 4 day suspension of injections and testing, 3 sessions on tragacanth, one session on pemoline, a three day cessation, and three final sessions on the pemoline.
RESULTS

These data were initially recorded on the basis of total responses for a session divided by the total number of reinforcements obtained. The mean number of responses per reinforcement for each session was calculated. These data are presented in Figure 1. Although these calculations were made on the basis of the entire 5 subjects of the control group, the experimental group data include only subjects SE 6, SE 8, and SE 10. The performance of the pemoline group seems to be superior to the control group during the initial stages of acquisition. The pemoline group made fewer responses per reinforcement and only after the first 19 sessions were the performances of the two groups relatively equivalent. However, after session 14 the pemoline group had a sharp increase in responses per reinforcement with continued high and low fluctuations in the final 5 sessions. The group receiving the water injections began to reach a stable performance level of 5 or less responses per reinforcement after session 16. This condition was never reached with the pemoline group during the 20 sessions. It was decided after looking at these data a more descriptive procedure should be used to illustrate the response and reinforcement patterns. The procedure decided upon was a response ratio and the percentage of the total possible reinforcements obtained.

The response ratio was calculated by subtracting the total number of responses for each session from 200, the optimum level of
Figure 1. Mean number of responses per reinforcement as a function of sessions.
responding, and dividing the remainder by 200. This ratio reveals the number of responses relative to the optimum level, where .00 represents the optimum level of 200 responses. A ratio of 1.0 indicates 100% more responses than required and -1.0 is a complete absence of responding. The mean response ratio for each group is presented in Figure 2 as a function of sessions. The difference between the response rates for the two groups is rather extensive. The mean percentage of reinforcements for each group are presented in Figure 3 as a function of sessions. These data show no corresponding difference in the percentage of reinforcements received by the two groups. In the latter sessions, however, the control group received a slightly higher percentage of reinforcements.

The alternation procedure (Figure 4) used on the control group after the first 21 sessions did not show any consistent effects on the response rate. There might have been a slight tendency at the higher dosages for the response rate to increase and decrease with successive tragacanth injections but the performances are too unstable to reach any general conclusions. As shown in Figure 5 the number of reinforcements received during these sessions was not changed by the variations in the agents or dosages.

The performance of 2 of the pemoline subjects (SE 7 and SE 9) can be seen in Figures 6 and 7 to be considerably different from the other subjects of the experiment. Both of these subjects showed a general decrease in response rate. Although the first session for SE 7 was not unlike those of SE 6, SE 8, and SE 10, the performance
Figure 2. The response inflection ratio as a function of sessions.
Figure 3. The percentage of reinforcements as a function of sessions.
Figure 4. The response inflection ratio during the alternation procedure as a function of sessions.
Figure 5. The percentage of reinforcements during the alternation procedure as a function of sessions.
Figure 6. The response inflection ratio for subjects SE 7 and SE 9 as a function of days.
Figure 7. The percentage of reinforcements for subjects SE 7 and SE 9 as a function of days.
of SE 9 consisted of 15 responses during the entire session. The second session produced an extensive drop in the response rate of SE 7, while SE 9 continued the suppressed performance. After the first session, SE 9 made less than 4 responses during any subsequent session and frequently none at all. SE 7, however, made between 26 to 87 responses and received from 21 to 42 reinforcements after the initial session. Upon observation of these subjects it was learned that the bar press response was occurring accidentally since the subjects were backing into the bar while on their hind legs. In a majority of the sessions half or more of the food pellets were left in the food dish. The subjects were usually situated in the back of the chamber and initiated little behavior directly toward the food dish or response lever. However, after the session these subjects would hastily grab for the supplemental food blocks placed in the home cages, although it was not observed whether these were ingested. After these initial sessions with the pemoline injections and the subsequent poor performance, the subjects were removed from the pemoline injection schedule. SE 9 was taken off pemoline after session 4 and placed on the tragacanth vehicle for session 5 during which no responding occurred. After a day without injection or exposure to the chamber, SE 9 was injected with tragacanth and again there was complete suppression. Since the performance of SE 7 was slightly better he was left on the pemoline until after session 7. After session 6 for SE 9 and session 7 for SE 7 there was a 4 day interval in which no injections or exposure to the chamber were
encountered by the subjects. Following this interim tragacanth injections were made and SE 7 and SE 9 were placed in the experimental chamber. During these sessions both subjects responded at rates more typical of the other subjects and ingested all available reinforcements. Session 15 was preceded by a pemoline injection and resulted in a decrease in the response rates of both SE 7 and SE 9. A second interval lasting 3 days was introduced after session 15. Following this second interim the subjects were injected with pemoline resulting in a sharp increase in responding similar to the tragacanth injections following the first interim. Continued injections with the pemoline produced a considerable drop in the response level of the subjects.
DISCUSSION

The distinct difference in the number of responses between the control and the pemoline group, with the later response rate being considerably lower, does not concur with the findings of other researchers (Beach & Kimble, 1967; Frey & Polidora, 1967). There does not seem to be any general stimulant effect of the drug on the bar press response. Whether or not the animals injected with pemoline were generally more active in the chamber, as shown by Beach and Kimble (1967) could not be determined with this experimental procedure. The only instances where the pemoline group (SE 6, SE 8, and SE 10) showed a higher response rate than the control group was after 15 consecutive injections of the pemoline. The effects of other central nervous system stimulants on this schedule (Schuster & Zimmerman, 1961) show a significant increase in responding with injections of dl-amphetamine (CNS-stimulant) on a DRL 17.5 second schedule. Therefore, it seems that if a stimulant effect was occurring it could be detected on this type of schedule.

However, just as there were no predominant stimulant effects neither were there any indications of a facilitative effect on acquisition as was suggested by Plotnikoff (1966). Although the rate of responding was nearer the optimum level for the pemoline group, there was no significant increase in the number of reinforcements obtained by the pemoline group and indeed at times this number was lower. Similarly, Beach and Kimble (1967) report response latencies

23
were shorter for the pemoline group, in a jump-out avoidance task, but there were no differences in the frequency of shock avoidance between their groups.

Perhaps the most salient data with respect to the lack of stimulant effects comes from subjects SE 7 and SE 9 of the pemoline group. For these subjects the response rate was severely suppressed by the pemoline. Although it is known that some central nervous system stimulants (dl-amphetamine) depress certain food reinforced operants (Dews & Morse, 1961); the present study showed no indication of suppression for all subjects, even though some subjects received dosages as high as 40 mg/kg.

Another possible explanation for the suppressed rates of SE 7 and SE 9, and for the low rates of the pemoline group might be suggested from the research of Beaulieu (1966). Here it was demonstrated that injections of RNA, with water deprived subjects produced an extensive increase in water consumption of these subjects as compared to the saline controls. If a thirst inducing effect is incurred from RNA injections as is indicated by Beaulieu's data, perhaps the same effect occurs with pemoline injections. If this could be assumed as true the response patterns of the entire pemoline group might not be atypical of what would be expected of animals on a food schedule while on water deprivation, since animals who have been water deprived show a decrease in rate for food reinforcement. If such an effect were generated by RNA and pemoline much of the high response rates with liquid reinforcements might be
explained (Brown, 1966a), as well as other research showing high activity. It seems possible that under previous testing procedures using avoidance techniques, increases in general activity could be related to induced "thirst" effects on the subjects. Most of the behavior in the present study could be explained by this "thirst" principle. However, explanations are not available for the high increases in response rates toward the later sessions for the pemoline group and the variations in time required for the drugs to take effect. Since there have been few studies using food or water reinforcement with RNA or pemoline further research is necessary to determine possible deprivational effects.

Further information obtained from subjects SE 7 and SE 9 indicates the time required for the drug to lose its effects on the organism may vary but could conceivably require 4 or more days, although this does not concur with other findings using an avoidance schedule (Millar & Thor, 1966). This contradiction could be added evidence of the effects of pemoline on "thirst" since avoidance schedules are not dependent on water deprivational levels.

From this research it seems possible to question two of the primary generalizations concerning pemoline. First, the drug does not have stimulative effects when used on a food reinforced DRL schedule. Whether this decrease in response rate as compared to the control was caused by a thirst inducing effect of the pemoline cannot at this time be completely established. If this is true such an effect might off set the stimulant qualities of the drug,
if indeed they are present. Second, if thirst was not affecting the performance of the subjects of the pemoline group it does not appear that acquisition was facilitated, since there were no substantial differences between the groups in reinforcements obtained. These results can add a number of new variables to the question of RNA, magnesium pemoline, and "learning enhancement." However, these questions are left for future research, which eventually will determine the fate of the biochemical theory of learning.
REFERENCES


Morris, R.N., Aghajanian, G.K., & Bloom, F.E. Magnesium pemoline: Failure to affect in vivo synthesis of brain RNA. Science,
1967, 155, 1125-1126.


