AN EXPERIMENTAL INVESTIGATION OF THE EFFECT OF MONOSODIUM GLUTAMATE ON THE LEARNING ABILITY OF BRIGHT AND DULL RATS

A Thesis

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by

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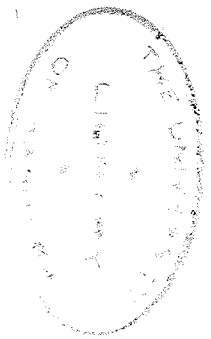

ABSTRACT OF THESIS

Early reports of the successful use of glutamic acid as a method of improving learning ability were followed by contradictory findings based on both clinical and animal experimentation. The need for the present study arose from the fact that there was an uncontrolled variable in the animal experiments which might explain why some investigators obtained an improvement in learning ability in their subjects while others reported no such effect. This variable was the possibility of strain differences in the learning ability of the animal subjects. The present study was designed to test the hypothesis that glutamate supplementation might have a beneficial effect on dull animals but fail to influence those of normal or superior learning ability.

Two separate experiments were carried out using rats of a bright and dull strain as subjects. Each strain of animals was divided into an experimental and control group at twenty-five days of age. The dull experimentals and bright experimentals received a daily supplement of 200 mg. of monosodium glutamate (in five grams of wet mash) for forty days, while the dull and bright controls received a

placebo supplement. Following a series of adaptation sessions and preliminary trials, the animals were tested on the twelve problems of the Hebb-Williams maze.

On the basis of the animals' performance it was concluded that dietary supplementation of monosodium glutamate from the age of twenty-five to sixty-five days significantly increased the learning ability of the dull rats but had no significant effect on the learning ability of the bright animals.

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CHAPTER I

THE PROBLEM AND INTRODUCTION

I. STATEMENT OF THE PROBLEM

In 1944 two reports appeared in the literature (4, 69) which suggested that dietary supplementation of glutamic acid could improve the learning ability of rats. Subsequently, however, a number of experiments were carried out which contradicted these earlier findings. As a result of these negative studies, the use of glutamic acid as a method of improving learning ability fell into disrepute.

The need for the present study arose from the fact that there was an uncontrolled variable in the animal experiments which might explain why some investigators obtained an improvement in learning ability in their subjects while others reported no such effect. This variable was the possibility of strain differences in the learning ability of the animal subjects. In two of the most important studies (50, 69), the animals used in the positive study (69) were actually much duller than those used in the negative study. In view of this it was deemed possible

that glutamic acid might have an effect on dull animals but fail to influence those of normal or superior learning ability. The object of the present investigation, therefore, was to test the effect of glutamic acid on strains of bright and dull rats.

II. INTRODUCTION

Among the distressing social problems of our time, mental deficiency stands out as one of the most difficult to cope with and one of the most costly to society. The cost is measurable not only in terms of the amount of money required to care for each institutionalized patient, but also in terms of the loss of the potential contribution of these persons to the commercial, educational and scientific enterprizes of the nation. Commonly, the mentally defective individual becomes a ward of the state and is given little hope of ever leading a normal life.

Occasionally, however, investigators report the successful use of some new method of treatment in certain types of mental deficiency. Such a discovery usually has widespread repercussions. Research teams around the world are alerted and tests of the new treatment are carried out, often with disappointing results.

Perhaps the most promising of the techniques of treatment to be announced in recent years has been the use of dietary supplementation of glutamic acid. In some cases such supplementation was reported to bring about not only an increase in the level of intelligence but also striking changes in emotional stability and personality. Several negative studies, however, appeared in both the clinical and the animal literature, reporting no beneficial effects following glutamic acid feeding. The present investigation is restricted to the animal field and seeks to explain the conflicting findings in this area by means of an experimental determination of the effect of glutamic acid on learning ability in bright and dull rats.

The thesis begins with a discussion of the historical background of the problem and goes on to present some of the more important research findings in this area. Included in this section is a discussion of the part played by glutamic acid in nerve metabolism. Following these introductory sections, the subjects, apparatus and procedures used in the experiments are described. The results are then presented together with a discussion and explanation of their implications. Finally, the concluding section

summarizes the results and discussion of previous sections.

III. HISTORICAL BACKGROUND

The origins of research problems in Psychology can frequently be traced to the experimental findings of some earlier investigator working in a related discipline. To give an adequate description of the line of thought leading ultimately to the present problem, one has to go back two decades to the experiments of the neurologist, L. A. Andreyev (5). This investigator was interested in those permanent functional changes in the brain which could be produced by altering some facet of cerebral metabolism during infancy. He found that tying off certain cerebral blood vessels in a very young dog caused the behavior of that dog two years later to be indistinguishable from that of a puppy. Metabolic deficiencies in early life were found to produce profound changes in later behavior.

Taking this work of Andreyev as their starting point, Himwich and Fazekas (24) went on to explore further the relationship between mental functions and cerebral metabolism. Their careful study of tissue preparations made from the brains of mentally defective persons showed that such tissue was incapable of utilizing normal amounts

of oxygen and carbohydrate. In cases of mongolism and phenylpyruvic oligophrenia, the brain removed much less than the normal amounts of oxygen and glucose from a given volume of blood passing through it. Emphasis in this research was placed upon the relationship between cerebral metabolism and mental deficiency.

In the course of further research on factors influencing cerebral metabolism, it was found that glutamic acid alone among the amino acids could serve as the respiratory substrate of the brain in lieu of glucose (9). Nachmansohn, John and Waelsch (37) further demonstrated that glutamic acid could speed up the rate of formation of acetylcholine, an important substance involved in neural conduction. In this case, glutamic acid was reported to achieve its effect by activating the enzyme choline acetylase. This enzyme is responsible for the synthesis of choline and acetic acid to form acetylcholine. Further studies showed that glutamic acid appeared in relatively high concentration in the brain where it was related via ketoglutaric acid with the tricarboxylic and citric acid cycles (11, 57).

Clinical Evidence

These advances in the knowledge of glutamic acid

and the part it played in cerebral metabolism prompted Waelsch (43) to suggest its use in the treatment of certain types of epilepsy. It was known at that time that a slightly acidic change in the blood of epileptics could relieve some of their symptoms but methods of producing this change had been quite unsatisfactory. By using glutamic acid, a normal constituent of all protein food, Waelsch and his colleagues hoped to relieve some of the epileptic symptoms without engendering any additional ill effects. The outcome of the treatment far exceeded their expectations. The patients evidenced not only a reduction of epileptic symptoms, but also striking improvements in "mental alertness" and other beneficial changes.

The unprecedented but portentous findings of these early investigations led naturally to further research. A preliminary study by Albert, Hoch and Waelsch (2) reported that the administration of glutamic acid to mental defectives increased the intelligence of seven of the eight patients so treated. F. T. Zimmerman and his colleagues at Columbia University published several reports from 1944 to 1951 (63, 64, 65, 66, 67, 68), concluding that glutamic acid supplementation could result in improvements in mental functioning as measured by a variety of test situations.

The news of these positive findings spread rapidly and additional work on the problem soon commenced in many countries. Reports coming from Britain, France, Spain and Belgium described findings similar to those of the Columbia University research group and gave impetus to further research. In Germany, Schwöbel (48) obtained improvements in personality and intelligence in both adults and children following controlled administration of glutamic acid. At the same time, a group of Mexican investigators (13) reported an average increase of 8.5 points in the intelligence of 28 children fed glutamic acid supplements.

Although the evidence for the beneficial effects of glutamic acid seemed well established, it was not long before contradictory findings were also being reported. Loeb and Tuddenham (31) found no improvement in a group of 33 adolescents, following glutamate supplementation. A similar conclusion was reported by McCulloch (34) after experimenting on institutionalized mental defectives. Several other investigators also reported negative findings and a review of the clinical literature by Arbitman (6) in 1952 concluded that glutamic acid had yet to be proven of definite value in treating mental deficiency.

Evidence from Animal Studies.

The positive findings of the early clinical studies were substantiated by two independent animal experiments published in 1944. The relationship between glutamic acid and learning ability was made the subject of a carefully controlled experiment carried out at Columbia University by Zimmerman and Ross (69) using albino rats as subjects. The animals were taken off their regular laboratory diet at six weeks of age and placed on a 24-hour feeding schedule in which the 13 rats of the experimental group received a daily supplement of 200 mg. of glutamic acid. The control group of nine rats received no glutamic acid supplement. The supplements were given for two weeks prior to testing and were continued during the 21 days of testing on a Warner-Warden single alternation maze. On this measure of learning ability, the glutamic-fed group required significantly fewer trials to reach criterion, made fewer errors and required less time to learn the maze than did the control group. Zimmerman and Ross concluded that the glutamic acid had a definite beneficial influence on the learning ability of the experimental animals.

The experimental results obtained by Albert and Warden (4), another team of Columbia workers, supported a

similar conclusion. These investigators were interested in the effects of glutamic acid on the rats' performance in a more complex reasoning problem. In this experiment, glutamic acid supplementation commenced when the animals were 30 days of age and was maintained for a period of 40 weeks. The nine experimental animals received 150 mg. of glutamic acid daily for the first 14 weeks and 250 mg. daily for the next 26 weeks. The performance of the animals was measured on the "Jenkins triple-plate problem box," an apparatus in which the rat is required to press one or more of three pedals in a certain defined sequence in order to open the door of the food enclosure and obtain its reward. In this problem situation the control animals had difficulty reaching the second problem, whereas the glutamic-fed rats mastered the first three problems with one animal going on to press four and five pedals in the correct sequence. The efficacy of glutamic acid seemed well established in the light of these findings.

In the animal studies as well as in the clinical work, however, reports of a negative nature soon began to appear. Hamilton and Maher (20) reported no improvement in the learning ability of rats tested on the Maier three-table test of reasoning after a period of glutamic acid

supplementation. The experimental animals ran faster and visited more tables than did the controls but there was little difference between the two groups in the number of errors made or the number of perfect trials completed. Marx (32) also failed to obtain any significant difference in performance of control and glutamic-fed rats on a "Stone multiple-T" water maze. Both Hamilton and Maher, and Marx suggested that the earlier positive results could be explained in terms of "heightened activity" rather than by improved learning ability.

In 1948, Stellar and McElroy (50) duplicated the original Zimmerman and Ross study following their experimental procedure in every detail except for the strain of rats used. The results of this study were completely opposed to the earlier positive findings. Braider (12) in 1949 also obtained negative results when her experimental animals were tested in both a water maze and a conditioning apparatus. Subsequent reports appearing from 1949 to 1951 (33, 41, 42, 61) all concluded, in effect, that excess glutamic acid was without influence on the learning ability of the rat. By 1952, the weight of experimental evidence in both clinical and animal research was not in favor of a specific relationship

between glutamic acid and intelligence.

Critique of Negative Animal Studies.

Among the negative studies, the experiment carried out by Stellar and McElroy (50) was perhaps the most crucial. This research project paralleled the procedures of Zimmerman and Ross in every possible detail, using the same maze, the same diet, the same feeding schedule and rats of the same age. In spite of these careful precautions, completely negative results were obtained. To test the possibility that the single alternation maze might be too easy to differentiate between their glutamic-fed and control animals, Stellar and McElroy continued testing the animals on two additional mazes of increasing difficulty. They used first a Warner-Warden, 8-cul, double alternation maze and then an elevated, 4-cul, double alternation maze. These more difficult tests still failed to reveal any significant differences between the experimental and control animals in terms of error scores, time scores or the number of trials required to reach criterion. The conclusive findings of this experiment appeared to put an end to the controversy regarding the effects of glutamic acid on the learning ability of rats and positive findings have subsequently failed to

appear.

The absence of positive experimental findings since 1948, however, does not necessarily indicate that Stellar and McElroy have made a final statement on the subject. The experimental replication of the Zimmerman and Ross study remains vulnerable in one important area. A major variable, that of possible strain differences in learning ability of the animals, had been overlooked. Table I provides a comparison of the results obtained by Zimmerman and Ross and those reported by Stellar and McElroy.

TABLE I

MEAN PERFORMANCE SCORES OF RATS ON A WARNER-WARDEN 8-CUL, SINGLE ALTERNATION MAZE OBTAINED IN TWO SEPARATE EXPERIMENTS CONDUCTED UNDER IDENTICAL CONDITIONS

GROUPS	Trials to Meet Criterion			Total Errors		Total Time (secs.)	
	N	M	σ Mean	M	σ Mean	M	σ Mean
Zimmerman and Ross:							
Glutamic-fed	17	3.9	2.7	8.5	5.2	287.7	196.7
Controls	9	12.1	3.7	36.5	15.5	666.1	382.0
Stellar and McElroy:							
Glutamic-fed	14	5.1	2.3	14.2	4.8	597.0	287.0
Controls	14	5.1	4.0	11.4	4.5	663.0	568.0

The figures of this table indicate that the control animals used by Zimmerman and Ross required twice as many trials to reach criterion and made more than three times as many errors as did the control animals of Stellar and McElroy even though the latter animals ran at approximately the same speed, on the same maze, and under similar conditions. From this it would appear that one group of investigators (Zimmerman and Ross) had used a much duller strain of animals than had the other. If this is the case, and it appears to be so, then strain differences in learning ability may be the cause of the conflicting data, for it is possible that glutamic acid may facilitate the learning ability of dull animals but have no effect on those of normal or superior ability. An obvious test of this hypothesis would be to obtain strains of bright and dull rats and then determine whether glutamic acid has a differential effect on the two groups of animals, that is, improving the learning ability of the dull animals but having no effect on the bright ones. Since such animals were readily available in the bright and dull strains maintained by the University of Manitoba Psychology Laboratory, it was decided to use them in an experimental test of the above hypothesis. The aim of this thesis,

therefore, was to study the effect of glutamic acid on the learning ability of bright and dull rats.

CHAPTER II

EXPERIMENTAL TECHNIQUES AND RESULTS

I. EXPERIMENT I

The Problem.

The discussion of Chapter I revealed the conflicting findings reported in earlier studies of the relationship between glutamic acid and learning ability. Previous investigators, it was pointed out, used normal animals as subjects and overlooked the important variable of possible strain differences in the learning ability of their subjects. The present experiment was designed to control this factor by measuring the effect of glutamic acid on a strain of rats selectively bred for subnormal learning ability as measured by their performance on the Hebb-Williams maze. More specifically, the problem of the present experiment was to determine the effect of dietary supplements of monosolium glutamate on the learning ability of dull rats.

Subjects

The animals used as subjects in this experiment were dull rats from a strain of animals developed in a

selective breeding project by C. F. Wrigley at McGill University. This work has since been continued by W. R. Thompson of Queen's University, and by the Department of Psychology, University of Manitoba.

The technique employed in this project and in previous selective breeding experiments (23, 29, 55) was to separate from a large original population of animals, those which were high or low on a particular behavioral continuum, and selectively inter-mate them. Wrigley and Thompson made their selection on the basis of operationally defined rat "intelligence," or learning ability as measured by performance on the Hebb-Williams maze.

A large number of hooded rats of the McGill strain were tested on this maze and a record was kept of the error scores of each animal. These scores indicated that the animals were not of uniform ability. Some rats made a great many errors on the maze while others made very low scores. The experimenters picked out the brightest and dullest rats in the group and began a program of selective breeding. The rats of poorest ability were mated with each other, using brother-sister pairs whenever possible, and the rats of superior ability were similarly intermated. When the offspring of these animals were

mature, they too were tested on the Hebb-Williams maze. Again, the brightest and dullest animals were selectively mated and their offspring were later tested on the maze. Six generations of the animals were subjected to this same regimen of testing, selection and breeding, with the result that a significant difference in the learning ability of the two groups soon appeared. The mean error scores of the first six generations of bright and dull rats are shown in Figure 1. These results indicate that

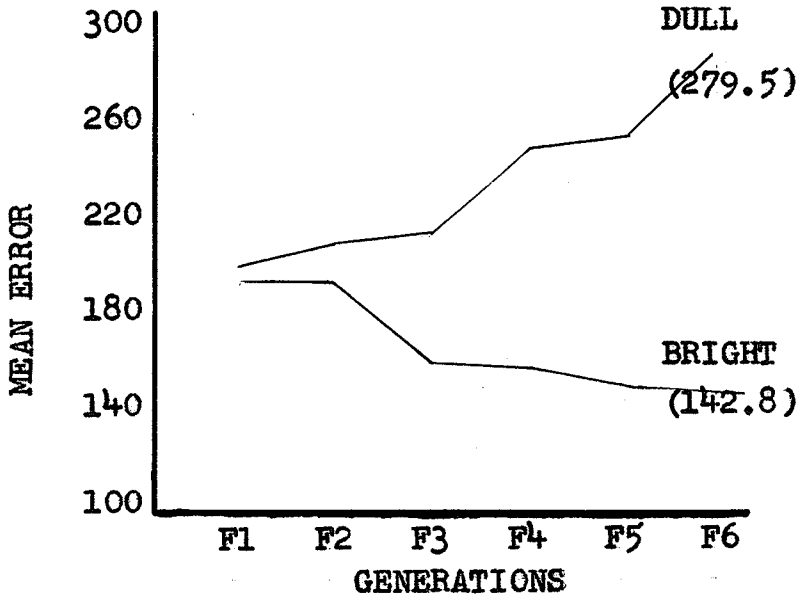


Figure 1. Mean error scores of six generations of bright and dull rats on 24 problems of the Hebb-Williams maze. (Thompson, 1954.)

the two groups differ markedly in their learning ability.

Further studies have shown that the strains do not differ significantly in weight, emotionality, motivation (53) or exploratory behavior (51). These results support the contention that the difference between the bright and dull rats is one of learning ability as measured, unconfounded by other factors.

The thirty-one animals used in the present experiment are direct descendants of Thompson's F7 dull group and represent the tenth generation of this strain. The young rats were separated from their mothers at twenty-five days of age and the litters were then split to form an experimental group of eighteen and a control group of thirteen animals. During the experiment the young rats lived, six or seven per cage, in steel mesh cages, 18" x 18" x 5".

Apparatus

The selection of bright and dull rats in Thompson's selective breeding project was made on the basis of their scores on the Hebb-Williams maze. This maze, known also as a "close field test of intelligence," was suggested by D. O. Hebb and Kenneth Williams in 1946 (22). Their aim was to devise a measure of animal intelligence similar to

measures of human intelligence, being based not on a single measure but on a number of problems of varying complexity. They argued that the ordinary fixed maze patterns commonly used in animal experimentation could be a measure of timidity, of need for food, or a complex of these with intellectual factors but not necessarily a measure of learning ability. The maze designed by Hebb and Williams and standardized by Rabinovitch and Rosvold (46) differs from all other mazes in that the animal has to solve a number of problems rather than just a single problem, which is the usual procedure.

The present form of the maze consists of a box four inches high and thirty inches square, having an entrance box at one corner and a food compartment in the corner diagonally opposite. Fourteen separate barriers of lengths varying from five to twenty-five inches make it possible to set up any one of the six practice or twelve test problems used with the maze (Figure 3.) The walls and barriers of the maze are made from ½" x 4" dressed lumber, painted black to contrast with the white floor. Thirty-six five-inch squares are outlined in black on the floor of the maze to facilitate the placing of barriers and to define error zones during the test situation, (see Figure 2.) Drop

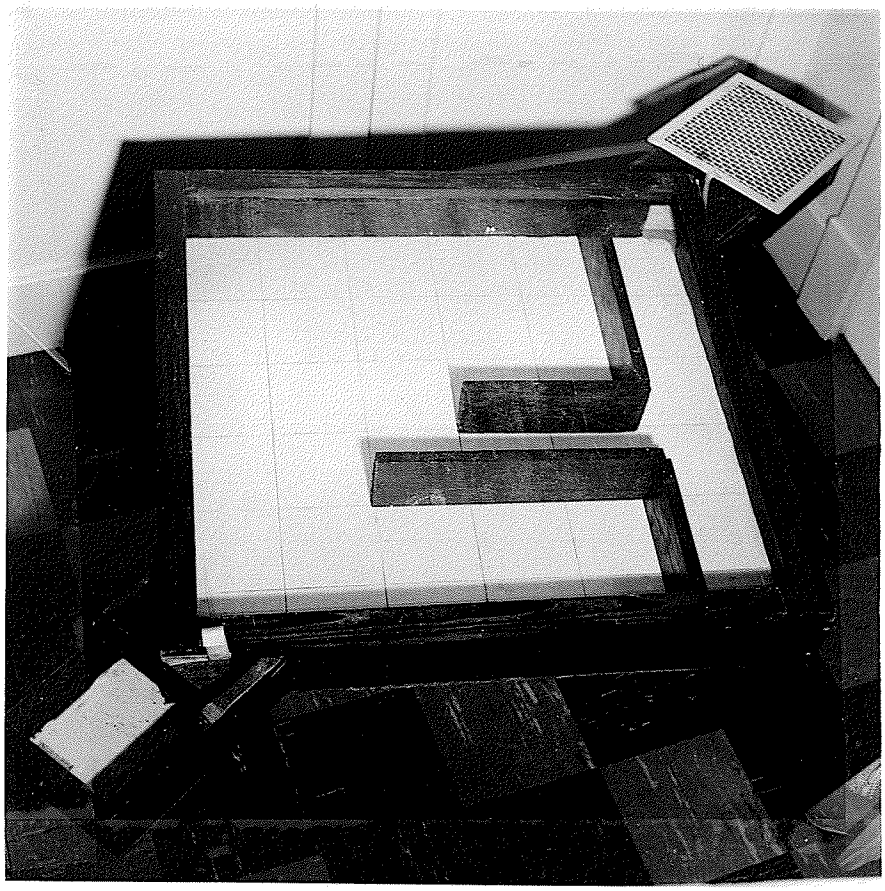


Figure 2. Photograph of Hebb-Williams Maze.

doors at the entrance box and food box were employed to facilitate timing and handling and to prevent the animal from re-entering the maze once he had reached the food dish.

Experimental Procedure

Supplementary feeding. At the age of twenty-five days the young rats were divided into an experimental and control group. They were then placed on a 24-hour feeding schedule in which they had access to a 25 per cent protein laboratory mash for one hour each day. At the beginning of each feeding period, the animals were placed in the individual compartments of a feeding rack where the experimental animals were given a five-gram dish of the basic diet containing 200 mg. monosodium glutamate. The control group received the extra five grams of food but did not receive the glutamate supplement. The animals were kept on this feeding schedule from the age of twenty-five days until they reached sixty-five days of age.

At the age of sixty-five days, the supplementation of monosodium glutamate was discontinued. Experimental and control animals were then started on the training regimen of the Hebb-Williams maze.

Maze adaptation sessions. Having been deprived of food for nine hours, the rats were placed four at a time in the entrance box of the maze and allowed to find their way around the barriers of the first adaptation problem (Figure 3, Problem A) to the food compartment. Two one-hour adaptation periods were given each day with practice problem A set up in the first period, problem B in the second, and so on, until the animals appeared well adapted to the apparatus.

Preliminary trials. Completion of the adaptation sessions was followed by a series of timed runs on the six practice problems. The rat was placed in the entrance box and time was recorded from the moment it passed through the entrance door until it reached the food in the food compartment. The animal was then allowed to eat for five to six seconds and then he was replaced in the entrance box and again allowed to go to the food, being timed as previously. This was repeated nine times on each practice problem, twice a day, until all the animals were able to make their nine runs on the practice problems in sixty seconds on two consecutive occasions. Rats slow to reach this criterion were given less food and more trials

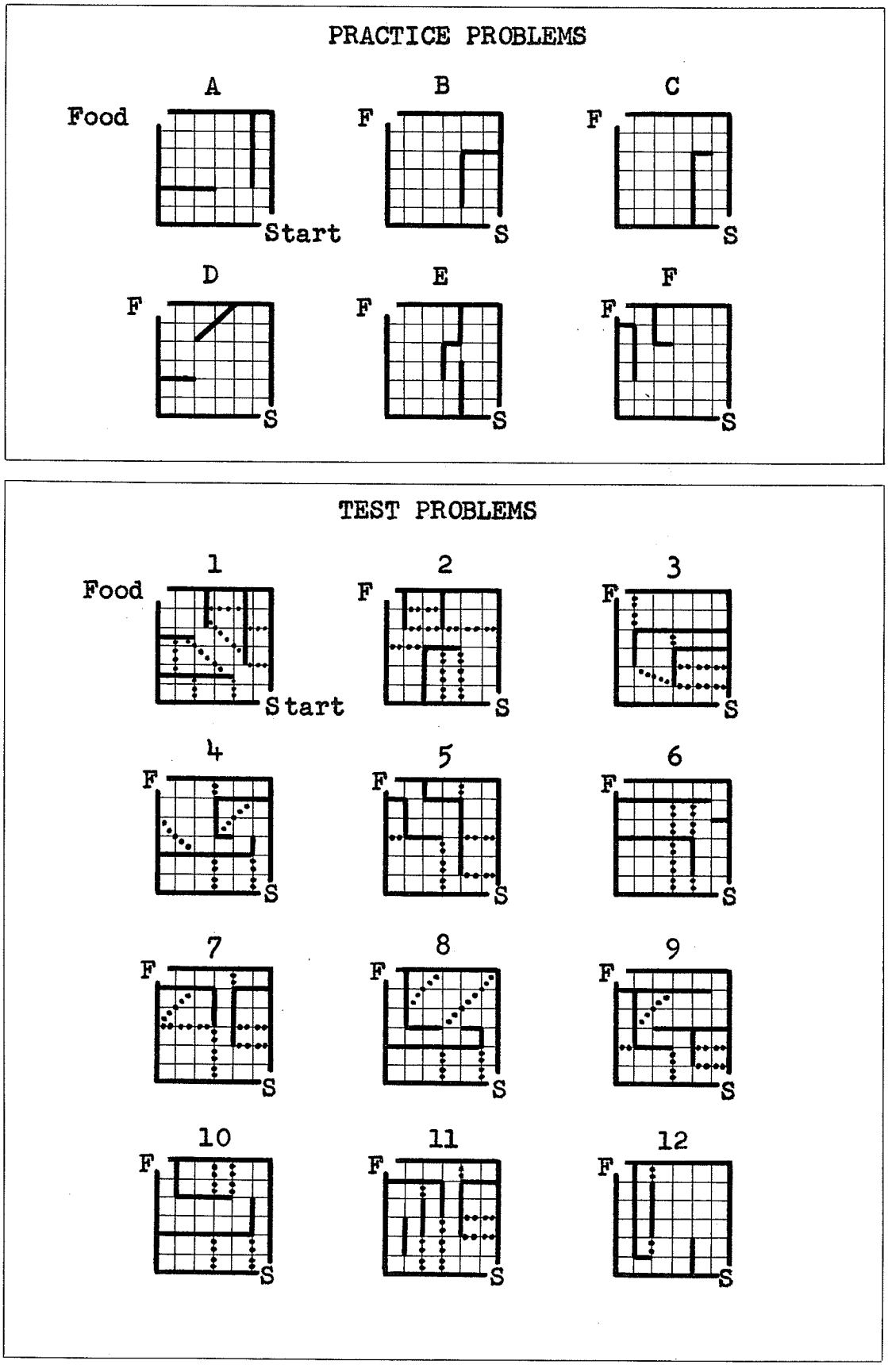


Figure 3. Floor Plan of Training and Test Problems.

each day, while those reaching the criterion early were given fewer trials in each session. This preliminary training serves to reduce emotional and motivational differences between animals. Only those animals which become completely adapted to the maze and to handling are used in the test problems.

Test problems. Upon completion of the preliminary training, the twelve test problems shown in Figure 2 were then administered two per day for six days. In the test sessions an animal was given eight runs on the first problem and was then permitted to eat moist mash for fifteen minutes before being returned to its home cage. After a delay of several hours, the same procedure was repeated for problem two. This was continued in morning and evening sessions until the twelve test problems had been completed. Time and error scores for each trial were recorded for every animal.

Scoring procedure. Time for each trial was measured from the moment the rat passed through the entrance door until it reached the food in the food compartment. An error was scored each time the rat's two forefeet crossed one of the error zones indicated by broken lines in Figure 3.

Where a blind alley contained two error zones (two broken lines), two errors were scored if the animal crossed the second error line, but no error was scored when he emerged from the alley through the first error zone. If an animal emerged from an error zone with both forefeet, but then turned and went back, a further error was scored. The total number of error zones entered by an animal in the twelve test items was that animal's score on the test.

Results.

Individual error and time scores for each rat are given in Table 1 and Table 2 of the appendix. The mean error and time scores for the experimental and control groups are indicated below in Table II. A study of the

TABLE II

MEAN ERROR AND TIME SCORES FOR EXPERIMENTAL AND CONTROL GROUPS OF DULL RATS IN EXPERIMENT I

	Dull Experimentals	Dull Controls
Mean Errors	107.6	144.9
Mean Time (secs.)	407.2	541.2

figures contained in this table reveals that the average number of errors made by the rats fed the glutamate supplement was much lower than that of the control group. There is a difference of 37.31 between the mean error scores of the glutamate-fed and control group. When the error scores of both groups were subjected to statistical analysis according to the method of Dixon and Massey (15, p. 103), a "t" value of 3.016 was obtained. This value is statistically significant beyond the one per cent level of confidence ($p < .01$). That is to say, there is a probability of less than one in a hundred that the difference between the two groups was due to the operation of chance factors.

Table II also shows that the dull experimentals made better time scores than the controls. The difference of 13¼ seconds in favor of the experimentals is also significant at the one per cent level of confidence ($t = 3.172$, $p < .01$). From all this data it is clear that the glutamate supplement had a beneficial effect on the learning scores of the dull animals.

II. EXPERIMENT II

The Problem

Since the results of Experiment I were the first positive ones to be obtained since the early Columbia studies, and because they will undoubtedly reopen the whole problem, it was decided to repeat the entire procedure with another group of dull animals in order to be absolutely certain that monosodium glutamate really does have a beneficial effect on maze learning ability. In addition, a strain of bright rats was also used to test the hypothesis that glutamate supplementation could improve the learning ability of dull animals but have no influence on those of superior ability.

Experimental Procedure

Bright and dull rats from the strains maintained by the University of Manitoba Psychology Laboratory served as subjects in the experiment. The young rats were eleventh generation descendants of the original Thompson-Wrigley strain.

The animals were separated from their mothers at twenty-five days of age and the litters were then split

to form experimental and control groups. The dull group consisted of thirteen experimentals and eleven controls. The bright group contained eight experimental and eleven control animals.

At the age of twenty-five days the animals were placed on a 24-hour feeding schedule in which they had access to a 25 per cent protein laboratory mash for one hour daily. The animals were placed in individual compartments of a feeding rack where the experimental animals received a five-gram dish of the basic diet containing 200 mg. of monosodium glutamate. Control animals received the daily five-gram supplement of mash but received no glutamate supplement. During this time the animals lived six or seven per cage in 12" x 10" x 8" cages.

When the animals reached sixty-five days of age, the supplementary feeding was discontinued. The animals were then started on the preliminary training sessions prior to being tested on the problems of the Hebb-Williams maze.

Following six days of preliminary training, the animals commenced the twelve test problems. Eight trials were given on each problem and there were two problems a day, spaced approximately nine hours apart. A period of

two weeks was required to complete the preliminary training and test problems.

Results

Individual error and time scores for the dull rats are given in Tables 3 and 4 of the appendix. Tables 5 and 6 in the appendix give the individual scores for the bright rats. The mean error and time scores for experimental and control groups of bright and dull rats are shown in Table III below.

TABLE III

MEAN ERROR AND TIME SCORES OF DULL AND BRIGHT, EXPERIMENTAL AND CONTROL RATS IN EXPERIMENT II

	Dull		Bright	
	Experimental	Control	Experimental	Control
Mean Errors	127.5	164.0	116.5	117.0
Mean Time (secs.)	601.8	850.0	372.0	391.5

From this table we again see that the dull experimentals make fewer errors ($t = 2.156$, $p > .01 < .05$) and take less time ($t = 3.048$, $p < .01$) in solving the maze

problems than do the dull control animals. These results are again statistically significant.

However, as far as the learning ability of the bright animals is concerned, Table III shows that monosodium glutamate had no effect. The error scores made by the bright experimental and control groups are almost identical. Statistically, the slight difference of 0.5 errors in favor of the experimentals is not significant ($t = .045$, $p > .10$). The time scores are slightly in favor of the bright experimentals but the difference is not statistically significant ($t = .99$, $p > .10$).

Thus, from these results it is clear that monosodium glutamate significantly improved the learning ability of dull animals but had no effect on the bright.

III. DISCUSSION

In discussing the results of the present experiment it is important to remember that the present study is not simply a replication of the earlier Zimmerman-Ross and Stellar-McElroy experiments. Although the study was suggested by the differences in learning ability of the animals used in these previous experiments, it differs from them in certain important respects. The distinguishing

features of the present experiment are the use of bright and dull rats, the use of a more complex maze and the feeding of monosodium glutamate to much younger rats than those used in the previous studies. The early feeding procedure was employed so that the effects of the supplement could operate at a time when the nervous system of the young animals was still developing and amenable to change. Some studies, however, started the feeding at almost the same time and continued it for a comparable period with negative results (12, 32). The effect on dull rats of glutamic acid fed at different periods of the life-span will form the subject of future experimentation.

The results of Experiment I showed that the learning ability of dull rats as measured by the Hebb-Williams maze could be significantly improved by a period of monosodium glutamate supplementation started at an early age. A repetition of this study using a second group of dull rats was carried out as part of Experiment II and the results supported a similar conclusion. When the supplement was administered to bright rats, however, no similar beneficial effects were obtained.

These results support the original hypothesis that glutamate supplementation might improve the learning ability

of dull subjects but have little or no influence on those already possessing superior ability. The findings serve also to clarify the reasons for the conflicting reports of earlier investigators. The animals used by Stellar and McElroy actually started off at a higher level of ability than did those used by the Columbia investigators. If the Zimmerman and Ross animals can therefore be regarded as a somewhat duller group, then the improvement in their performance is understandable in the light of the findings of the present experiment. Both Stellar and McElroy and Porter and Griffin (41) in reporting their negative studies, suggested that the effects of glutamic acid might be specific to the Sherman strain kept at Columbia University, by virtue of some unknown metabolic deficiency in these animals. This suggestion, however, was never followed up and the question of "strain specific effects" remained unanswered.

Zimmerman (64), on the other hand, disregarded this possible explanation and explained away the negative findings as due to "lack of technical precision" and experimental inaccuracy. He had reason to defend his findings but the defence lay elsewhere than in an attack on the integrity of his critics. Porter and Griffin (41, p. 13) were closer

to the truth when they observed that, "The clinicians select their subjects from a population of defectives, [whereas] the analogous step is lacking in the animal experiments."

When the variety of studies on glutamic acid are viewed with regard to the experimental diets employed, it becomes apparent that the acid does not mediate its effects simply by alleviating some preëxisting dietary deficiency. All the investigations have employed seemingly adequate diets and in addition it is known that normal animals can synthesize glutamic acid in quantities sufficient to meet their bodily requirements (57.) Furthermore, Porter and Griffin (42) have demonstrated that animals raised on diets deficient in glutamic acid still show no improvement in learning ability following excess glutamate supplementation. The evidence does not point to the amount of glutamic acid in the normal diet as the crucial factor in explaining the positive and negative experimental findings.

An alternative explanation proposed by Hamilton and Maher (20) and by Marx (32), is that the reported beneficial effects of the supplement might be due to heightened activity level rather than to any increase in learning ability.

This possibility of a rise in general activity level

is in line with Weil-Malherbe's proposal (58, 60) that the effects of glutamic acid are more likely to be due to an adrenergic mechanism than to any direct stimulation of brain cells. He considers it unlikely that glutamic acid could pass through the blood-brain barrier in sufficient quantities to influence the brain directly and in view of this he suggests that the effects of the substance result from increased output of adrenalin brought about by excess glutamic acid in the blood. Sauri (47), however, has shown that glutamic acid can influence the brain, particularly the excitability of the sensory cortex, and in addition, Weil-Malherbe has overlooked the possibility that the permeability of the brain to glutamic acid might be altered in the pathological conditions of mental deficiency.

Heightened activity certainly differs from improved learned ability and if the effects of glutamic acid were explainable in terms of activity alone, this increase in speed should be apparent in all groups receiving the supplement. Since differences in learning ability would not be a point in question in this case, both bright and dull experimental animals should show similar increases in activity. This, however, was not found to be the case. The supplement had no appreciable influence on the time

scores of bright rats. An interpretation of the effects of glutamic acid in terms of heightened activity level or increased adrenalin output thus fails to account for the experimentally observed facts.

The literature on glutamic acid suggests more than one tenable explanation for the basis of this improvement in learning ability. There is a possibility that its action may be to alter some basic metabolic process only indirectly concerned with the nervous system. Its connection via ketoglutaric acid with the tricarboxylic and citric acid cycle supports this alternative (11, 57). Ginsburg and his colleagues (19) accept this explanation to account for the protection afforded by glutamic acid against sound induced seizures in mice.

Another encouraging hypothesis is that the beneficial effects of glutamic acid are due to the direct influence it exerts on the nervous system by facilitating acetylcholine formation. This substance has long been known to be essential for the production of various electrical changes occurring during neural transmission. Nachmansohn, John and Waelsch (37) have shown that the rate of acetylcholine formation could be increased four to five times by adding glutamic acid to dialyzed extracts

of rat brain. Zimmerman (68) suggests that this mechanism is sufficient to account for the intellectual gains in his subjects following glutamic acid supplementation. He argues that since learning ability and intelligence depend on sensations from the environment being conducted, through nerve impulses, to the integrating centres of the brain, then glutamic acid can achieve its effect simply by facilitating this process of conduction. This connection between glutamic acid and acetylcholine synthesis, its high concentration in the brain, and its involvement in cerebral metabolism (10, 56, 57, 59), all point to an important role for this substance in neural activity.

Whatever the theoretical basis for the effectiveness of glutamic acid may be, its importance in normal nerve metabolism seems well established. What is perhaps more important, especially for the present discussion, is the part this substance plays in the metabolic reactions of an abnormal or defective nervous system.

Knowledge in this area is far from complete but many studies have demonstrated the importance of adequate cerebral metabolism for proper mental functioning. It has long been known, for example, that oxygen lack can seriously impair the functions of the brain. Furthermore,

Himwich and Fazekas (24) have shown that in certain types of mental deficiency the brain is incapable of utilizing normal amounts of oxygen and carbohydrate. Thus there appears to be a definite relationship between mental deficiency and cerebral metabolism.

It is possible that further research could reveal a difference in the cerebral metabolism of bright and dull rats similar to that reported for humans. It would also be interesting to determine whether the concentration of glutamic acid in the brains of bright and dull glutamate-fed rats differs from that of the control animals in each strain. The results of the present study are thus important not only in reconciling earlier conflicting findings, but also in suggesting new possibilities for research which may further facilitate the study of mental deficiency.

CHAPTER III

SUMMARY AND CONCLUSIONS

I. SUMMARY OF THE STUDY

Interest in the use of glutamic acid as a method of treating mental deficiency was first stimulated by experimental studies reporting that this substance could reduce certain epileptic symptoms and bring about striking changes in personality and mental ability. Early reports based on both clinical and animal research indicated that supplementary feeding of glutamic acid could result in definite improvements in mental functioning.

The hopeful reports of the early investigators, however, were soon followed by contradictory findings. A number of clinical studies failed to obtain improvements in their subjects following controlled administration of glutamic acid. In the animal field, a careful replication of one of the original positive studies failed to substantiate the earlier findings. No improvement in the learning ability of the subjects followed glutamic acid supplementation. This crucial study was followed by several additional experiments all concluding that the

supplement failed to improve the learning ability of rats.

In the clinical studies, however, the effects of glutamic acid had been measured on mentally defective subjects. When subjects of normal or above normal ability were used, the results were negative. The animal studies, on the other hand, have invariably employed normal laboratory rats, carefully raised, and free from observable deficiencies. Although this has been the case, certain differences were found to exist in the initial learning ability of the strains of rats used by different experimenters. The animals used in one of the original positive studies were actually much duller than those used in a replication of that study which obtained negative results. These facts suggested the possibility that glutamic acid might exert its effects on subjects of below average mental ability and yet have no influence on those of normal or superior ability.

A research project was therefore designed to test the hypothesis that glutamic acid exerts a differential effect on subjects of below normal and superior mental ability. The subjects in this project were bright and dull rats, selectively bred on the basis of their ability to learn the test problems of the Hebb-Williams maze. Two

separate experiments were carried out.

In the first study, thirty-one rats of a dull strain served as subjects. An experimental group of eighteen animals, and a control group of thirteen were separated from their mothers at twenty-five days of age and placed on a 24-hour feeding schedule. The experimental group received a daily supplement of 200 mg. of monosodium glutamate in a five gram dish of wet mash. The control group received the extra mash but received no glutamate supplement. At the age of sixty-five days the supplements were discontinued, and the animals were introduced to the training and testing regimen of the Hebb-Williams maze. The learning ability of the glutamate-fed group as measured by this instrument, was significantly superior to that of the littermate controls.

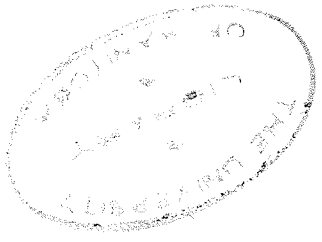
In the second experiment a similar procedure was followed with another group of twenty-four dull rats and a group of nineteen bright animals. The bright and dull experimental and control animals were placed on a 24-hour feeding schedule from the age of twenty-five days until they reached sixty-five days of age. During this time the experimental animals received a daily supplement of 200 mg. monosodium glutamate. When the animals were tested on the

problems of the Hebb-Williams maze after the age of sixty-five days, the thirteen dull experimentals were clearly superior to their eleven litter-mate controls. No such significant difference was observed between the bright experimental and control animals.

II. CONCLUSIONS

Within the limitations set by the design of the present research project, the following conclusion is warranted: Dietary supplementation of monosodium glutamate from the age of twenty-five to sixty-five days significantly increases the learning ability of dull rats but has no significant effect on the learning ability of bright animals.

The results of the present experiments aid in clarifying the reasons for the conflicting findings reported in previous animal investigations. They serve also to suggest topics for further study. The effect of feeding glutamic acid during different periods of the life-span and during the prenatal period is a worthy problem for research. The most efficient dosage and the proper duration of supplementation also deserve attention. A measure of the glutamic acid concentration in the brains of bright and dull rats



would be of particular value since metabolic differences in these animals would make possible a number of studies capable of illustrating further the role of glutamic acid in normal and defective cerebral metabolism.

The relationship between glutamic acid and mental ability can be investigated from many viewpoints. It is hoped that the techniques and results embodied in the present investigation will suggest new avenues of approach and facilitate further research in this area.

A P P E N D I X

TABLE 1.

ERROR AND TIME SCORES OF DULL EXPERIMENTAL ANIMALS IN EXPERIMENT I

Rat No.	Error Score	Time Score (secs.)
1	89	642
2	102	372
3	130	541
4	137	434
7	150	391
8	63	342
12	104	388
13	75	328
15	133	604
17	47	390
20	108	336
21	124	359
22	120	382
23	91	365
24	100	268
25	96	412
26	112	369
28	156	407
Sum	1937	7330
Mean	107.61	407.22

TABLE 2

ERROR AND TIME SCORES OF DULL CONTROL ANIMALS IN EXPERIMENT I

Rat No.	Error Score	Time Score (secs.)
6	151	533
9	196	928
10	193	719
16	195	551
18	115	460
29	123	492
30	111	536
31	212	479
32	108	485
33	148	563
34	99	417
35	122	463
36	111	409
Sum	1884	7035
Mean	144.92	541.15

TABLE 3

ERROR AND TIME SCORES OF DULL EXPERIMENTAL ANIMALS IN EXPERIMENT II

Rat No.	Error Score	Time Score (secs.)
54	117	572
55	143	606
58	107	527
62	73	528
64	150	422
66	160	645
68	83	607
71	179	448
130	114	394
131	157	507
132	157	620
133	106	997
136	112	951
Sum	1658	7824
Mean	127.54	601.84

TABLE 4

ERROR AND TIME SCORES OF DULL CONTROL ANIMALS IN EXPERIMENT II

Rat. No.	Error Score	Time Score (secs.)
53	181	645
56	129	468
57	110	695
63	270	1012
65	192	753
102	225	822
134	100	815
135	160	1031
137	137	1069
138	148	816
139	152	1225
Sum	1804	9351
Mean	164.00	850.01

TABLE 5

ERROR AND TIME SCORES OF BRIGHT EXPERIMENTAL ANIMALS IN EXPERIMENT II

Rat No.	Error Score	Time Score (secs.)
77	142	325
79	115	353
81	111	321
83	128	412
85	91	368
86	146	321
121	122	429
123	77	447
	Sum 932	2976
	Mean 116.5	372.0

TABLE 6

ERROR AND TIME SCORES OF BRIGHT CONTROL ANIMALS IN EXPERIMENT II

Rat No.	Error Score	Time Score (secs.)
78	141	355
80	83	354
82	104	378
84	142	410
87	147	471
88	103	396
89	94	416
124	89	394
125	140	396
126	108	374
127	136	363
Sum	1287	4307
Mean	117.00	391.54

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