THE EFFECTS OF BILATERAL STIMULATION OF THE HIPPOCAMPUS ON PERSEVERATIVE NEURAL PROCESSES

by

John P. Harcourt, Jr.

Submitted in partial fulfillment of the requirements for graduation with honors in the Department of Psychology, Washington and Lee University

May 1, 1963

Approved
Milton M. Hunter
Leonard E. Jarrard

ACKNOWLEDGMENT

The writer wishes to express his appreciation to Dr. Leonard E. Jarrard, who served as director of the present research, and to Dr. William M. Hinton for his added assistance and encouragement.

TABLE OF CONTENTS

List of Tables

LIST OF ILLUSTRATIONS

INTRODUCTION

In recent years there has been a significant amount of work done in the field of memory and the physiological processes that are involved. One main area of research has been connected with perseveration theory. The first clear statement of the perseveration - consolidation theory was made by Muller and Pilzecker (1900). They stated that previous to consolidation of memory traces the traces perseverate over neural pathways. They further stated that these neural perseverative processes were requisite to the consolidation of the memory trace for recently acquired memory; and that these processes may be subject to external interference.

Although several methods have been used in research on perseveration theory (Glickman, 1961), the most successful, to date, appears to be the employing of electroconvulsive shock (ECS). The initial study on perseveration theory using ECS was done by Duncan (1949). In this study, rats in an avoidance conditioning problem were given one trail per day for 18 days. The animals were divided into eight groups, which were administered ECS 20 sec., 40 sec., 60 sec., 4 min., 15 min., 1 hr., 4 hrs., and 14hrs., respectively, following the termination of each trial. Duncan found that if an hour or more elapsed between the learning trial and administration of

ECS, there was no effect upon memory. Electroconvulsive shock within 15 min., however, resulted in a depressed learning rate of the avoidance response. The learning deficit was found to be inversly related to the length of time intervening between the learning trial completion and onset of the ECS convulsion. In a study patterned after Duncan, Gerard (1955) used hampsters, and found that the perseverative processes could be found to last for an hour.

In a more recent study, Thompson and Dean (1955) using a discrimination problem and five groups of 12 subjects each administered ECS 10 sec., 2 min., 1 hr., and 4 hr. after learning trials. The fifth group served as a control. Significant differences were found for the 10 sec., 2 min., and 1 hr. groups. The memory deficit was inversely related to the length of time intervening between learning trials and administration of ECS. There was no significant difference between the 4 hr. and the control groups.

The results of these studies, and others with similar results (Thompson and Pennington 1957, Thompson 1958), point to the acceptance, by many investigators, of the perseveration theory (Glickman, 1961). The next step in studying perseveration theory was to try to localize the specific area of the brain which controls perseveration. In an excellent review

of the area, Glickman (1961) reported very little work along these lines. In one study by Mahut (1958), chronically implanted electrodes were used in the thalamic neuclei. Glickman (1958) implanted electrodes in the midbrain tegmentum. In both studies some interference with the normal processes of memory was reported.

The present study is a further attempt to localize the specific area of the brain which controls perseveration. Attempted localization of perseveration in the hippocampus is suggested in Glickman's review (1961). Glickman, referring to Milner and Penfield (1955) and Scoville and Milner (1957), points out that experiments using hippocampal lisions indicate that subjects are unable to learn postoperative material, but retain material learned preoperatively. Although the specific areas controlling such phenomena are not definitely known, Glickman points to a need for research in the hippocampus.

The present experiment is designed to measure the effects of bilateral stimulation of the hippocampal area of the white rat on the acquisition of a Lashley Maze Type III. With stimulation at a sufficiently low level to eliminate spreading, and administered at an interval long enough to eliminate association, it was assumed that any memory deficit could be attributed to a disturbance of the perseverative neural processes.

Method

Subjects: The Ss were 24 male albino rats of the Sprague-Dawley Disease Resistant strain obtained from the Dublin Laboratories. The Ss were approximately 110 days old at the beginning of the experiment. They were deprived of food until they reached approximately 85% of their normal body weight. At that time the 24 Ss were divided into three groups of eight animals each which were equal in weight.

Sugery: Bilateral bipolar electrodes embedded in a common nylon base were chronically implanted in the Ss brains. The electrodes consisted of 0.010 inch triple insulated nilstain wires wound together. A diagram of the electrodes can be found in Figure 1. Cross sections of the tips of the electrodes were bared and separated. The Ss under Nembutal anesthesia (40 mg./kg.), were placed in a C. H. Stoelting Stereotoxic instrument. Two holes were drilled in the skull at locations as defined in the de Groot atlas (1959). Eight operated experimental animals had electrodes implanted in the hippocampus at the following coordinates: 3.7 mm. posterior to Bregma;; 3 mm. laterally on each side of the midline; 3 mm. down from the top of the skull. Eight operated control animals had electrodes implanted at the following coordinates in the neocortex: 3.7 mm. posterior to Bregma;; 3 mm. laterally on either side of the midline; 1.5 mm. down from

the top of the skull. The eight remaining Ss served as unoperated controls.

Apparatus: A Lashley Maze Type III (Lashley 1929) was used in the learning task. The maze is pictured in Figure 2. The maze was constructed of 3/4" plywood with an inside length of 46" and alleys of 4" in width. The maze was 6" high and covered with hardware cloth. A $\frac{1}{4}$ teaspoon in an aluminum holder served as the food cup in the goal box. A microswitch connected to the start box door started a Standard Electric timer when the door was raised. When the door was lowered the first clock was stopped and another of the same type started. When the goal box door was lowered the second clock was stopped by means of a microswitch. Thus, it was possible to record latency and running time for each trial.

Procedure: Two days following surgery the Ss were placed on a deprivation schedule. Animals were deprived for 10 days before pretraining began. During this period, each was handled approximately 15 min. a day. Training consisted of a pretraining period of four days and a test period of 16 days.

Day 1 of pretraining consisted of 10 feeding trials in the goal box. The goal box was modified to prevent com-

plete familiarization with the test environment. During these trials S remained in the goal box until it had eaten, and was then removed while more food was put in the food cup. Throughout preliminary training and the test period two Noyes Co. food pellets in several drops of water were used as reward. The second day of pretraining consisted of 10 more trials in the modified goal box. Day 3 of the pretraining period consisted of 10 trials in a straightway. The straightway was of the same dimensions as an alley of the test maze. A guillotine door of the same type as those used in the test situation was placed half down the straightway. This was to familiarize the Ss with the operation and noise of the door. Day 4 consisted of 10 more trials in the straightway.

During the test period each animal was given three massed trials per day for 16 days in the Lashley Maze Type III. Following each block of three trials the animal was returned to a holding cage for 10 min. The animal was then transferred to the stimulating box. The stimulating box and equipment are pictured in Figure 3. Ten min. was chosen as the interval between learning trials and stimulation. This interval was employed to assure no association (Kimble 1961); while getting the greatest possible effect from the stimulation. Following the procedure of Mahut (1958), each

experimental and operated control animal was given a 15 sec. burst of 60 cycle sine wave at .25 volts. Control animals were placed in the stimulation box without stimulation for a period equivalent to that of the two stimulation groups. Latency, running time and number of errors were recorded for each trial.

Results

The following measures were used in evaluating the data: (1) latency, (2) running time, (3) trials to criterion, and (4) total number of errors for the test period. In evaluating the data, four Ss were eliminated. Subjects 1, 2, and 18 would not run in the test apparatus, and S 20 died on the fourteenth day of testing. Subjects 1 and 2 were cortical control animals. Subject 18 was a control animal; and S 20 was a hippocampal animal.

A between-within analysis of variance (Edwards, 1950) was used in evaluating the data. In cases where the F value was significant, the test for the difference between the means required for significance (Lindquist, 1953) was employed. Table I summarizes the data and results of the analysis of variance for latency. The F of 4.944 was significant at the .05 level of confidence. This indicates a significant difference between the three main groups. The test for the difference between means required for significance revealed

that the cortical control and hippocampal groups had significantly faster average latencies than the control group. The differences between the means were 1.598 and 1.678, respectively. There was no significant difference between the hippocampal and cortical control groups, whose means differed by 0.80.

The data and analysis of variance results for average running time are summarized in Table II. The F value of 2.118 obtained was not significant. A second analysis was run, eliminating the data for S 10 because of the radical deviation of these scores from the group mean. The data and results of the second analysis of variance are summarized in Table III. Figure 4 presents the trials to criterion data for each animal in each group. The F value of 3.761 was significant at the .05 level of confidence. The difference between the means of the hippocampal and control groups was 5.643. This difference was not sufficiently large enough for significance. The difference between the means for the hippocampal and cortical control groups was 8.476. It revealed that the hippocampal group required significantly more trials to reach the criterion than the cortical control group. There was no significant difference between the cortical group and the control group, whose means differed by 2.833.

Table IV summarizes the results of the analysis of variance and data for total errors. Figure 5 shows the cumulative number of errors per group as a function of days. The F value of 4.141 was significant at the .05 level of confidence. The difference between the means of the hippocampal and control groups of 2.15 is not large enough for significance. The differences between the means of the cortical control group and hippocampal and control groups were significant, revealing that the cortical control group had significantly fewer errors than the other two groups. The differences between the means were 22.14 and 24.29.

An analysis of variance was also run on the average weight of the animals during the experiment. The data and results of analysis of variance are summarized in Table V. The F value of 7.844 was significant at the .05 level of confidence. The test for the difference between the means required for significance revealed that the cortical control and hippocampal groups had significantly lower average weights than the control groups. Mean differences of 18.76 and 25.29 were obtained. The difference between the means of the cortical control and hippocampal groups was 6.53. It was not large enough for significance.

Discussion

The purpose of this study was to determine the effects of bilateral hippocampal stimulation on perseverative neural processes as indicated in a learning task. The evaluation of the data indicates that the hippocampal and cortical control groups had significantly lower latencies and running times than the control group, but did not differ significantly themselves. The Ss in the hippocampal group required significantly more trials to criterion than the cortical control group. Further, the cortical control group made significantly fewer errors than either of the other two groups.

The fact that the hippocampal and cortical control groups had significantly faster latencies and running times than the control groups can probably be explained in terms of differences in motivation. The results of the analysis of the data for body weight revealed that the cortical control and hippocampal groups weighed significantly less than the control group. The lower body weight would indicate greater motivation, and, thus, may account for the differences obtained.

Although there was no significant difference in trials to criterion between the hippocampal animals and the control animals, there is a definite trend indicated. The similarity of the data for the control and cortical control groups and

the obvious dissimilarity of the hippocampal group is evident. That the hippocampal group differed significantly from the cortical control group indicates that chance factors may have played an important part because of the small number of Ss. With more Ss to eliminate the strong influence of chance factors, it may be that significant differences between the hippocampal and control groups would be found.

The significant differences between the cortical control group and the control and hippocampal groups for total errors can be explained in two ways. The first possibility is that the difference was merely chance; and, thus, a replication of the study would not reveal the same results. A second possibility is that stimulation of the neocortex has a facilitating effect on learning. The first explanation--chance--is the more likely for several reasons. First, is the fact that in the other measures, the cortical control group showed no trend suggesting a facilitating effect. In the most important measure of learning, trials to criterion, the cortical control group was very similar to the control group and did not approach a significant difference. The differences between the cortical control and control groups for average latency and running time have already been explained in terms of possible differences in motivation. The final argument against the possibility of a facilitating effect is neo-

cortical ablation data. In a study by Jarrard, Isaacson and Wicklegren (1963) it was reported that ablation of the neocortex had no effect on the acquisition or extinction of a learning task.

A major part of any experiment of this type is the data that is obtained from histological analysis of the brains. Such data is necessary to assure proper placement of the electrodes. It is entirely possible that the coordinates in the present experiment were, for some reason, wrong. The only method to assure that the electrodes in this study were in the desired areas of the brain is through examination of the brain by histology. Since the histology is a long process, histological data was not available for inclusion in this thesis. Histological data will, however, be available in the near future.

Another possible explanation of the negative results obtained in this study, other than improper placement of electrodes, is that the amount of stimulation used was not sufficient. The major problem in this area is to avoid spreading of the stimulation to other areas of the brain. It is, therefore, necessary to use only a small amount of stimulation. It seems unlikely that there was any spreading effect in the present study. A spreading effect would be

almost analagous to ECS, in that it would effect several areas of the brain, and probably give results similar to the ECS results. Another problem in the present study was control of motivational factors. The difference in motivation between the control animals and cortical control and hippocampal animals was evident. It certainly effected latency and running time data, and may well have effected the other measures as well. Finally, there is an apparent need to run a larger number of Ss in order to eliminate the influence of chance factors due to the small number of Ss employed in the present study.

In Müller and Pilzecker's presentation of the perseveration theory it was noted that perseverative neural processes may be subject to external interference. As pointed out in the introduction, there is some evidence that the hippocampus might play an important role in controlling these perseverative neural processes (Glickman, 1961). The present study was designed to determine the effects of bilateral stimulation of the hippocampus on perseverative neural processes. The results obtained in this study indicate that there is no significant effect on such processes from hippocampal stimulation. This does not point to the rejection of the theory, nor of the hypothesis that the hippocampus may play an important role in controlling perseverative processes. There does, in fact, appear to be a definite trend suggesting some possible effect of stimulation. With

a larger number of Ss and more closely controlled motivational factors significant differences could possible be obtained.

Summary

The purpose of the present study was to determine the effects of bilateral stimulation of the hippocampus on perseverative neural processes as indicated by acquisition of a learning problem.

Twenty-four male albino rats were divided into three equal groups. An experimental group of 8 Ss had electrodes implanted in the hippocampus. An operated control group of 8 Ss had electrodes implanted in the neocortex. A third group of 8 Ss served as unoperated controls. Each animal was given three trials per day for 16 days in a Lashley Maze Type III. Ten min. after each block of three trials the hippocampal and neocortical animals were each stimulated by a 15 sec. burst of 60 cycle sine wave at .25 volts.

Analysis of variance and tests for the difference between means required for significance indicated, that under the conditions, of the present study, bilateral stimulation of the hippocampus has no apparent effect on the perseverative neural processes. Several suggestions were made for the improvement of the present study.

BIBLIOGRAPHY

1. deGroot, J. The rat forebrain in stereotoxic coordinates. Amsterdam: Noord-Hollandsche Uitgivers Maatschoppij, 1951.

2. Duncan, C. P. The retroactive effects of 3 electroshock on learning. J. comp. physiol. Psychol., 1949, 42, 32-44.

3. Edwards, Alden L. Experimental design in psychological research. New Yrok: Rinehart and Company, Inc. 1950.

4. Gerard, R. W. Biological roots of psychiatry, Science. 1955, 122, 225-230.

5. Glickman, S. E. Perseverative neural processes and consolidation of the memory trace, Psychol. Bull. 1961, 58 218-233.

6. _____ Deficits in avoidance learning produced by stimulation of the ascending reticular formation. Canad. J. Psychol., 1958, 12, 97-102.

7. Kimble, Gregory A. Hilgard and Marquis conditioning and learning second edition. New York: Appleton-Century-Crafts, Inc., 1960.

8. Lashley, K. S. Brain Mechanisms and intelligence. Chicage: University of Chicage Press, 1929.

9. Lindquist. Design and Analysis of Experiments in Psychology and Education. Cambridge. Houghton and Mifflin Co., 1953.

10. Mahut, H. Discussion in H. H. Jasper (Ed.), Reticular formation of the brain. Boston: Little, Brown, 1958. p. 528.

11. Milner, B. and Penfield W. The effect of hippocampal lesions on recent memory. Trans Amer. Neural Assn. 1955, 42-48.

12. Muller, G. E. and Pilzecker, A. Experimentelle britrage zur lehre vom gedachtnis. Z. Psychol. 1900 Suppl. No. 1.

13. Scoville W. B. and Milner, B. Loss of recent memory after bilateral hippocampal lesions. J. Neural. Neurosurg. Psychiat. 1957, 20, 11-21.

14. Thompson, R. The effects of degree of learning and problem difficulty on perseveration. J. exp. Psychol. 1958, 55, 496-500.

15. Thompson, R. and Dean, W. A further study of the retroactive effects of ECS. J. comp. physiol. Psychol. 1955, 48, 488-491.

16. Thompson, R. and Pennington, D. F. Memory decrement produced by ECS as a function of distribution in original learning J. comp. physiol. Psychol.

APPENDIX

Tables I to V

and

Illustrations 1 to 5

TABLE I

Summary of Data and Results of Analysis of Variance for Average Latency Per Trial

GROUP:	HIPPOCAMPAL	CORTICAL CONTROL	CONTROL
Rat. No.:	7. 2.01	5. 2.63	3. 2.97
	8. 2.29	6. 1.79	4. 4.51
	11. 1.93	9. 2.47	10. 4.06
	12. 1.18	14. 1.76	13. 2.38
	17. 2.19	15. 2.27	16. 3.14
	22. 3.17	24. 2.37	19. 2.07
	23. 2.16		21. 7.56
	EX = 14.93	EX = 13.29	EX = 26.69
	$\bar{X}$ = 2.135	$\bar{X}$ = 2.215	$\bar{X}$ = 3.813

	Sum of Squares	df	Mean Square	F
Between groups	12.29	2	6.13	4.944 *
Within groups	23.56	.9	1.24	

*Significant at the .05 level of confidence.

differences between means:

cortical control - hippocampal = .080

control - hippocampal = 1.678

control - cortical control = 1.598

difference between means required for significance: 1.279

TABLE II

Summary of Data and Results of Analysis
of Variance for Average Running Time Per Trial

Group:	HIPPOCAMPAL	CORTICAL CONTROL	CONTROL
RAT NO: 7	10.58	5. 7.30	3. 9.12
8.	7.96	6. 6.21	4. 18.86
11.	8.98	9. 8.79	10. 24.44
12.	6.89	14. 5.75	13. 7.80
17.	8.24	15. 10.11	16. 9.34
22.	8.17	24. 7.21	19. 7.50
23.	7.84		21. 37.00
ΣX =	58.66	ΣX = 45.37	ΣX = 114.06
M =	8.38	M = 7.48	M = 16.29

	Sum of Squares	df	Mean Square	F
Between groups	315.01	2	157.51	3.876 *
Within Groups	772.24	19	40.64	

*Significant at the .05 level of confidence.

differences between means:

hippocampal - cortical control = 0.90
control - cortical control = 8.81
control - hippocampal = 7.91

difference between means required for significance: 7.328

TABLE III

Summary of Data and Results of Analysis of Variance for Trials to Criterion

GROUP:	HIPPOCAMPAL	CORTICAL CONTROL	CONTROL
RAT NO.:	7. 17	5. 12	3. 13
	8. 17	6. 9	4. 26
	11. 15	9. 13	10. 42 (Eliminated)
	12. 14	14. 12	13. 12
	17. 22	15. 9	16. 11
	22. 21	24. 15	19. 10
	23. 35		21. 15
	ΣX = 141	ΣX = 70	ΣX = 87
	$\bar{M}$ = 20.143	$\bar{M}$ = 11.667	$\bar{M}$ = 14.500

	Sum of Squares	df.	Mean Square	F*
Between groups	212.99	2	106.495	3.761
Within groups	509.68	18	28.316	

*Significant at the .05 level of confidence.

differences between means:

hippocampal - control = 5.643
hippocampal - cortical control = 8.476
control - cortical control = 2.833

difference between means required for significance: 6.045

TABLE IV

Summary of Data and Results of Analysis of Variance for Total Number of Errors

GROUP:	HIPPOCAMPAL	CORTICAL CONTROL	CONTROL
RAT NO. :	7. 51	5. 25	3. 20
	8. 27	6. 21	4. 44
	11. 38	9. 20	10. 64
	12. 31	14. 11	13. 28
	17. 46	15. 25	16. 22
	22. 38	24. 24	19. 28
	23. 65		21. 75
	EX = 296	EX = 126	EX = 281
	$\bar{M}$ = 42.29	$\bar{M}$ = 18.00	$\bar{M}$ = 40.14

	Sum of squares	df.	mean square	F
Between groups	1732.26	2	866.13	4.141*
Within groups	3974.29	19	209.17	

*Significant at the .05 level of confidence.

differences between means:

hippocampal - cortical control = 24.29
control - cortical control = 22.14
hippocampal - control = 2.15

difference between means required for significance: 16.62

TABLE V

Summary of Data and Results of Analysis of Variance for Average Weight

GROUP:	HIPPOCAMPAL	CORTICAL CONTROL	CONTROL
RAT NO.:	7. 126	5. 145	3. 169
	8. 161	6. 132	4. 184
	11. 145	9. 148	10. 153
	12. 127	14. 144	13. 155
	17. 133	15. 164	16. 152
	22. 134	24. 123	19. 163
	24. 127		21. 152
	ΣX = 856	ΣX = 953	ΣX = 1128
	$\bar{M}$ = 136.14	$\bar{M}$ = 142.67	$\bar{M}$ = 161.43

	Sum of squares	df	mean squre	F*
Between groups	2337.50	2	1168.75	7.844
Within groups	2831.05	19	149.00	

*Significant at the .05 level of confidence.

differences between means:

control-hippocampal = 25.29
control-cortical control = 18.76
cortical control-hippocampal = 6.53

difference between means required for significance: 14.032

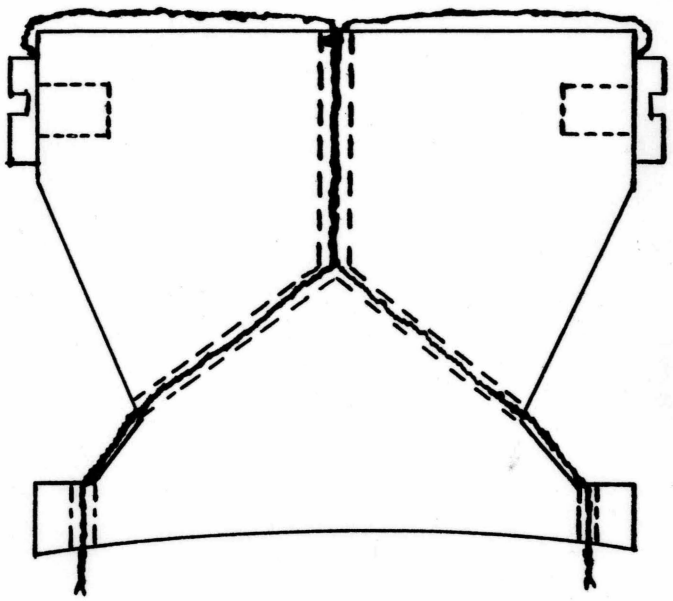

FIG. I. DIAGRAM OF ELECTRODE EMPLOYED

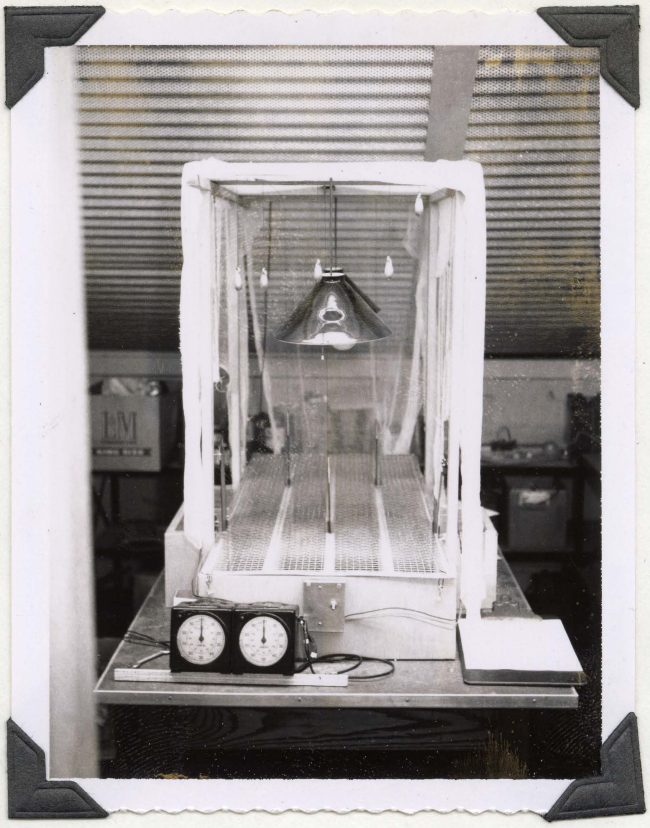

Fig. 2. Picture of Lashley Maze Type III

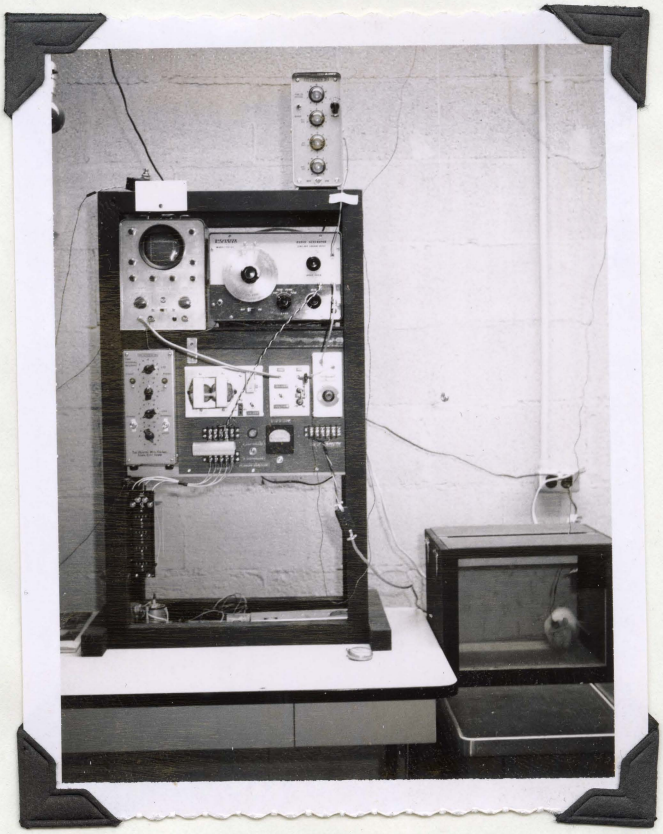

Fig. 3. Picture of stimulating box and equipment

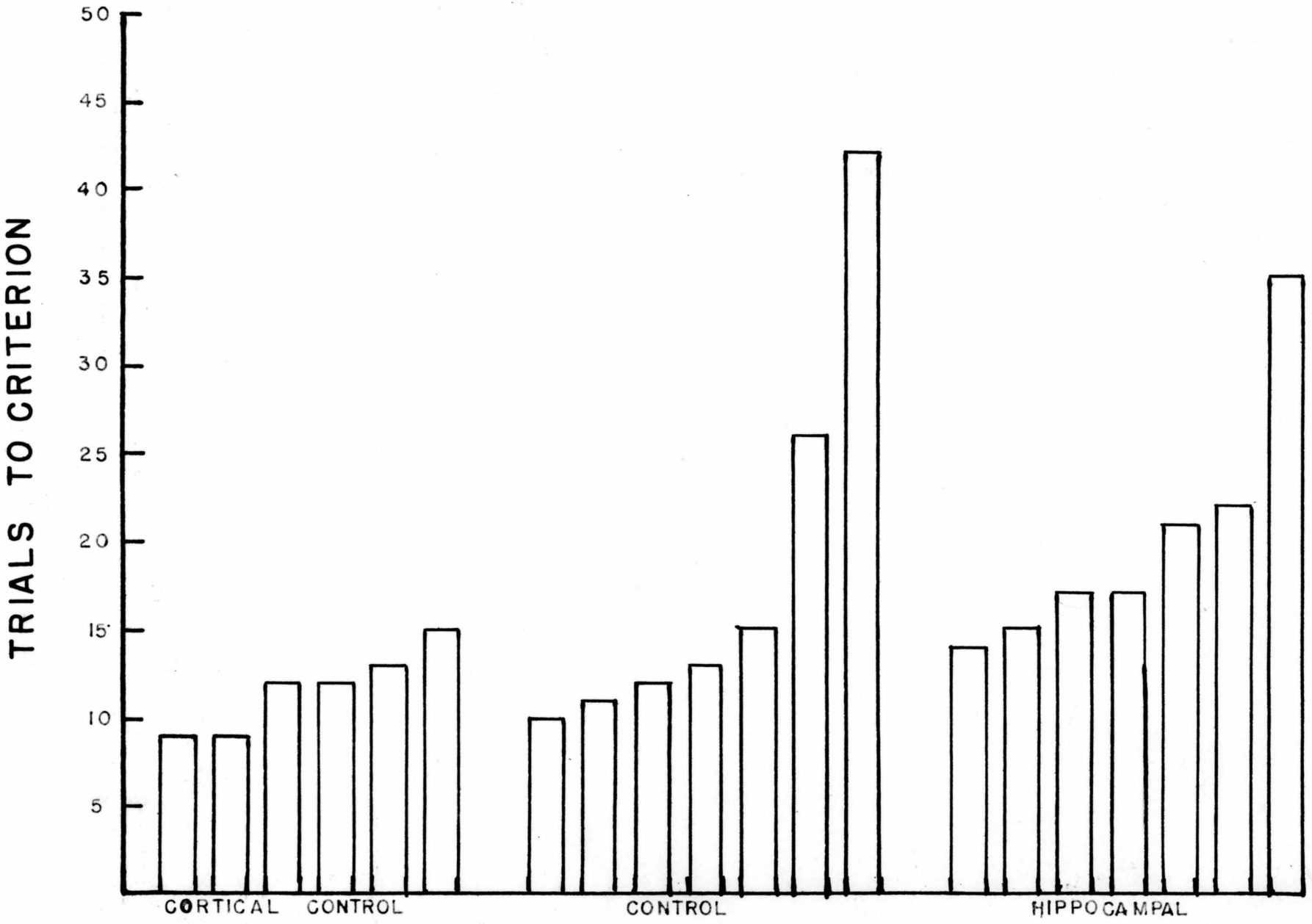

FIG. 4. TRIALS TO CRITEION PER ANIMAL BY GROUPS

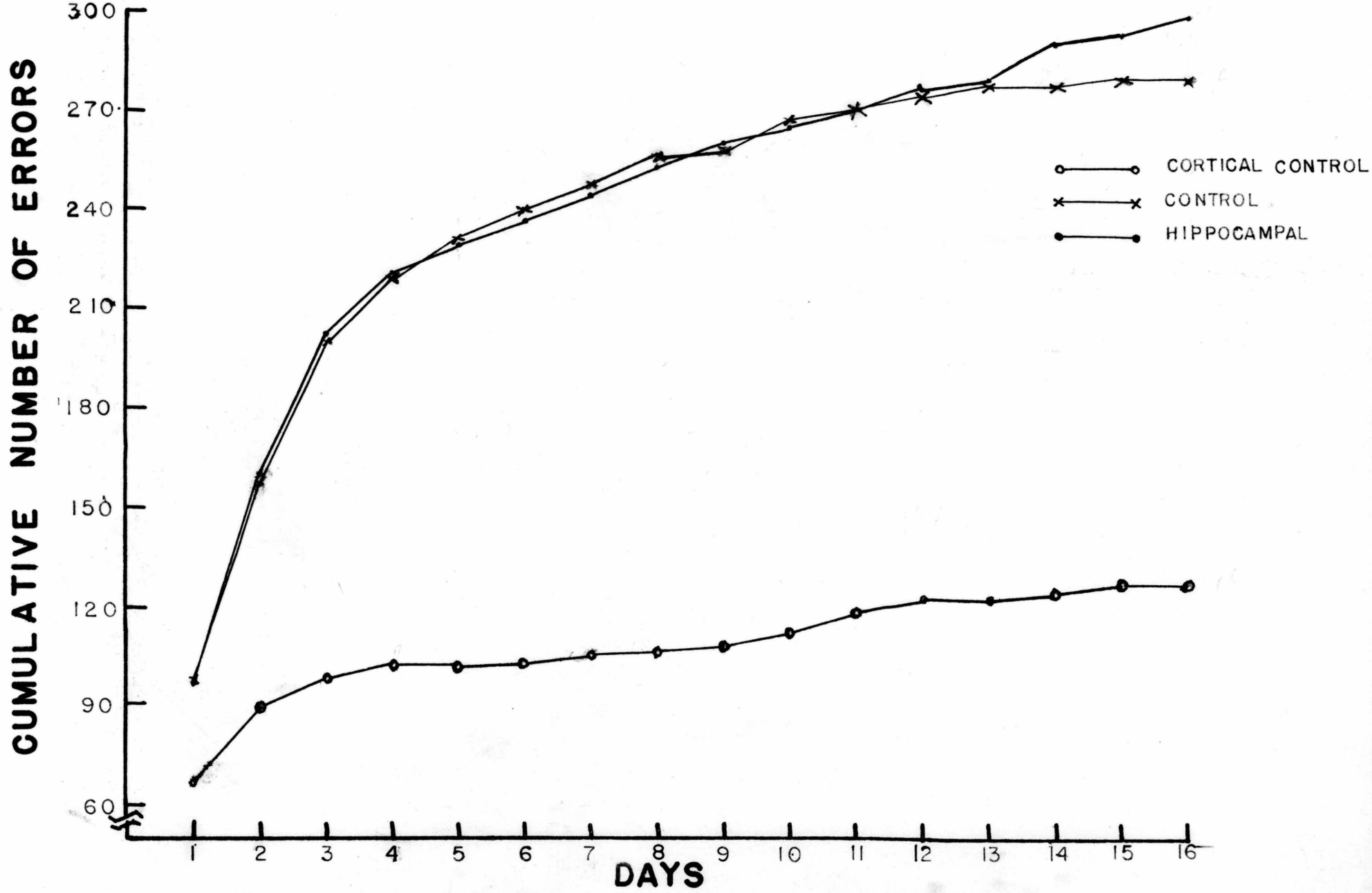

FIG. 5. CUMULATIVE NUMBER OF ERRORS AS A FUNCTION OF DAYS