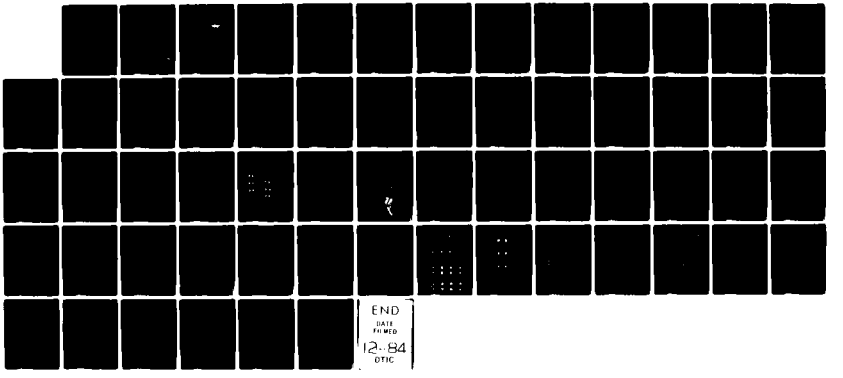
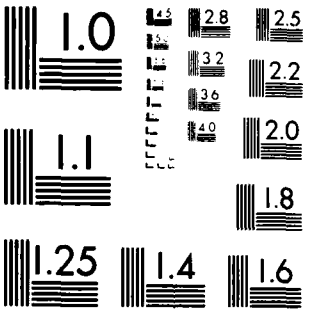
AD-A147 577 NEUROCHEMICAL MECHANISMS MEDIATING RECOVERY OF FUNCTION 1/1
(U) JOHNS HOPKINS UNIV BALTIMORE MD DEPT OF PSYCHOLOGY
G WENK ET AL. NOV 84 DAMD17-82-C-2225

UNCLASSIFIED F/G 6/15 NL



END
DATE FILMED
12-84
DTIC



AD_____

Neurochemical Mechanisms Mediating Recovery of Function

David Olton, Gary Wenk, Zoltan Annau

November, 1984

Supported by:

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD-17-82-C-2225

The Johns Hopkins University
Baltimore, Maryland 21218

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-A147577	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Neurochemical Mechanisms Mediating Recovery of Function		5. TYPE OF REPORT & PERIOD COVERED Annual 1 August'82-31 July '83
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Gary Wenk, David Olton, Zoltan Annau		8. CONTRACT OR GRANT NUMBER(s) DAMD17-82-C-2225
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Psychology The Johns Hopkins University Baltimore, Maryland 21218		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 61102A.3M161102BS11.EF.010
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick, Frederick, Maryland 21701-5012		12. REPORT DATE [redacted] 13. NUMBER OF PAGES 59
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Cholinergic neurotoxins, medial septal area, nucleus basalis magnocellularis, recovery of function, behavioral alterations following neurtoxic insult

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

See reverse side

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

Block 20: Abstract

We have completed studies on the changes in behavior which occur following damage to the basal forebrain cholinergic (Ch) system in the mammalian central nervous system.

Sodium dependent high affinity uptake of the Ch precursor choline was used as an indication of activity in the Ch neuronal system. This measure is specific for the Ch system and is increased following increased firing and decreased following decreased firing of these neurons. There was a significant increase in choline uptake in the hippocampus following performance on a T-maze and the radial arm maze. This laboratory has previously shown that the performance of these behaviors typically requires an intact hippocampal Ch system. In contrast, the Ch system afferent to the neocortex was not activated during the performance of these tasks.

Presently, experiments have been designed using various pharmacotherapies in an attempt to produce a significant recovery of both biochemical and behavioral indices.

In summary, we have accomplished our goals for the first year. We have established all the procedures and baseline conditions necessary to examine recovery of function; obtained significant amounts of data describing the biohcemical and behavioral changes that occur subsequent to destruction of the Ch system by a toxic agent, and begun the critical experiments necessary to determine whether we can accelerate the recovery processes with various pharmacotherapies or behavioral testing.

AD_____

Neurochemical Mechanisms Mediating Recovery of Function

David Olton, Gary Wenk, Zoltan Annau

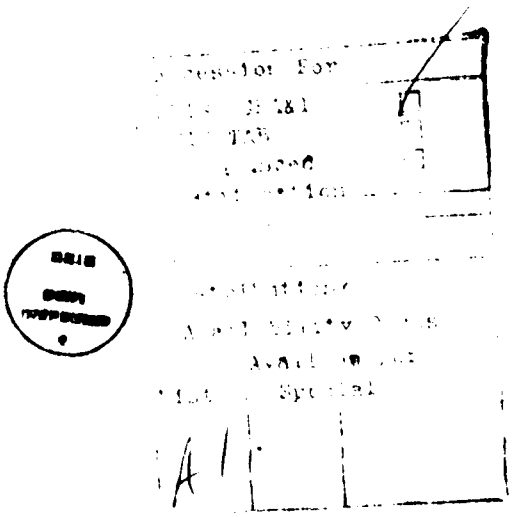
November, 1984

Supported by:

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD-17-82-C-2225

The Johns Hopkins University
Baltimore, Maryland 21218



SUMMARY

In this annual report we discuss our accomplishments during the previous year, outline our goals for the next contract period, and introduce briefly some of the experiments we are now undertaking.

We have completed studies on the changes in behavior which occur following damage to the basal forebrain cholinergic (Ch) system in the mammalian central nervous system. Changes in biochemical indices of Ch integrity in the neocortex and hippocampus following physical and chemical destruction of the Ch neurons in the nucleus basalis magnocellularis (NBM) and the medial septal area (MSA) have been correlated with changes in avoidance behavior, as well as radial arm and T-maze performance.

We used sodium-dependent high affinity choline uptake as an indication of activity of Ch neurons. This measure is specific for the Ch system and is increased following increased firing and decreased following decreased firing of Ch neurons. Choline uptake increased in the hippocampus following performance on the T-maze and radial arm maze. This laboratory has previously shown that the performance of these behaviors typically requires an intact hippocampal Ch system. In contrast, the Ch system afferent to the neocortex was not activated during the performance of these tasks.

Presently, experiments have been designed using various pharmacotherapies in an attempt to produce a significant recovery of both biochemical and behavioral indices.

In summary, we have accomplished our goals for the first year; established all the procedures and baseline conditions necessary to examine recovery of function; obtained significant amounts of data describing the biohcemical and behavioral changes that occur subsequent to destruction of the Ch system by a toxic agent, and begun the critical experiments necessary to determine whether we can accelerate the recovery processes with pharmacotherapy.

FOREWARD

In conducting of research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

Table of Contents

NEUROCHEMICAL INTERACTIONS MEDIATING RECOVERY OF FUNCTION

In this annual report we discuss our accomplishments during the previous year, outline our goals for the next contract period, and introduce briefly some of the experiments we are now undertaking.

We have completed studies on the changes in behavior which occur following damage to the forebrain cholinergic (Ch) system in the mammalian central nervous system. Changes in biochemical indices of Ch integrity in the neocortex and hippocampus following physical (electrocoagulation) and chemical (ibotenic acid) destruction of the Ch neurons in the nucleus basalis magnocellularis (NBM) and the medial septal area (MSA) have been correlated with changes in avoidance behavior, as well as impairments in radial arm and T-maze performance. Progess in this area has been difficult due to the problems in reliably and accurately producing the lesions, as well as providing effective post-operative care to ensure adequate survival rates. (see appendix 4)

Preliminary studies examined the value of quinolinic acid as a selective neurotoxin. Quinolinic's effects were too variable to be useful for our studies on recovery of function in the Ch system. It was very toxic in the NBM, but was only slightly effective in the MSA. Kainic acid has also been found to be unsuitable for our studies because it produces destruction at a distance from its site of injection. We have therefore discontinued the use of kainic or quinolinic acid in these studies.

During the past year experiments were designed to find certain behaviors that would activate the basal forebrain Ch system. It was reasoned that if these neurons are involved in the performance of a particular task, then one should be able to detect this activity and correlate it with behavior. Such a correlation would indicate which behaviors are likely to be impaired due to destruction by an excitotoxin. We used sodium-dependent high affinity choline uptake as an indication of activity in the Ch neuronal system. This measure is specific for the Ch system and is increased following increased firing and decreased following decreased firing of these neurons. Sodium-dependent high affinity choline uptake increased in the hippocampus following the performance of the T-maze and the radial arm maze. This laboratory has previously shown that the performance of these behaviors typically requires an intact hippocampal Ch system. In contrast, the Ch system afferent to the neocortex was not activated during the performance of these tasks. The data are outlined in detail in appendix 2.

We have studied the effects of time on recovery of neocortical Ch markers following unilateral destruction of Ch neurons in the NBM by ibotenic acid. There was a gradual increase towards normal ChAT levels in the neocortex; by three months, no detectable interhemispheric differences existed. (see appendix 5) The degree of recovery may depend upon the amount of tissue destroyed, and especially upon the

ability of the surviving neurons to reinnervate the vacant nervous tissue regions. Studies with specific inhibitors of ChAT enzyme suggested that the recovery may be due to ingrowth of Ch terminals, and not other neuronal cells, which might contain carnitine acetyltransferase. The recovery of ChAT activity may be related to ingrowth of terminals spared during the ibotenic acid injection. To test this hypothesis, we injected ibotenic acid into the NBM of rats who had received similar injections three months prior. ChAT activity decreased, but the decrease was not as great as with the initial ibotenic acid injection. If the innervation to the neocortex originated outside the NBM, the second injection would have had only a slight effect on neocortical ChAT activity levels. We are presently trying to determine the nature of this innervation.

We were also interested in whether behavioral testing could accelerate recovery of a biochemical parameter such as ChAT activity. Rats received ibotenic acid lesions in their MSA. Ch efferents were effectively destroyed as determined by a significant decrease in ChAT activity in the hippocampus. Following recovery from the operation, a group of rats underwent extensive behavioral training, while a second group sat in their cages. Behavioral testing continued at an intense pace for two weeks, and the rats were then sacrificed. Biochemical and histological examinations were performed to determine the extent of damage due to the lesion and the degree of recovery produced by the behavioral testing. Another study was designed similarly, except that the rats had a long (1 month) post-operative period prior to the initiation of extensive behavioral testing. These studies were designed to investigate the influence of time and behavioral testing on the recovery of a single biochemical indicator of the integrity of the Ch system. This study is outlined in detail in appendix 1. The results indicated that neither time nor behavioral testing significantly accelerated the recovery of ChAT levels in the hippocampus.

Presently, experiments have been designed using various pharmacotherapies in an attempt to produce a significant recovery of both biochemical and behavioral indices. We have produced rats with lesions in the MSA and will begin treating these rats with either pentoxifylline, a cerebral metabolic enhancer in the xanthine class of drugs, nerve growth factor, or the ganglioside GM1. Each of these drugs has been shown to produce significant recovery of function in studies on lesioned rats.

Recent reports on the influence of Ch agents on female sexual behavior suggested the following set of experiments. A small group of female rats received injections of ibotenic acid into their nucleus basalis magnocellularis and were allowed to recover before being placed with males of known reproductive potency. Two of the four females became pregnant and gave birth to small litters (3 to 7 pups). Nestling and suckling behaviors were apparently normal. Similar lesions were also produced in males and the experiment repeated to determine whether the performance of the males can be affected by loss of neurons in the nucleus basalis magnocellularis. Two of four males were incapable

of mating. The two litters that were sired were very small (4 pups).

In summary, we think that we have accomplished our goals for the first year. We have established all the procedures and baseline conditions necessary to examine recovery of function; obtained significant amounts of data describing the biochemical and behavioral changes that occur subsequent to destruction of the Ch by a toxic agent (see appendix 3), and begun the critical experiments necessary to determine whether we can accelerate the recovery processes with various pharmacotherapies or behavioral testing.

Given our progress during the first year, we are very excited about the prospects for continued success during the second year. We look forward to maintaining the very positive relationship that has developed between our laboratory and the Army Research Institute. Again, thank you for your support and enthusiasm.

Appendix:1

RECOVERY OF FUNCTION: INFLUENCE OF TIME AND BEHAVIORAL EXPERIENCE

BACKGROUND

The mechanisms underlying spontaneous recovery after toxic damage to the brain are of considerable importance, yet are not well defined. The peripheral nervous system is capable of collateral sprouting to achieve adequate reinnervation following partial denervation. However the central nervous system was thought to be incapable of such a response to injury. Recently, reports have described the ability of the central nervous system pathways to develop collateral and terminal sprouting in response to lesions, including such regions as the lateral geniculate body (1), septum (2), dentate gyrus (3), cerebellar cortex (4), red nucleus (5) and the thalamus (6). Two basic mechanisms that may be involved in this recovery (7): reactive synaptogenesis, which occurs with rapid onset, usually within one week, and has a fairly short duration (only a few weeks); and compensatory collateral sprouting, which provides far more accurate reinnervation, occurs much later than reactive synaptogenesis (up to a few weeks), and continues for a much longer time (up to 10 months).

The purpose of the present set of experiments was to accelerate the rate of functional recovery by intensively challenging rats with MSA lesions on various behavioral tasks. The degree of recovery was infered by hippocampal ChAT activity levels, as an indication of the integrity of the cholinergic (Ch) afferent system.

METHODS

Experimental Design

The subjects were thirty-five male albino rats. Ten rats did not receive a lesion and simply remained housed individually in their home cage. Twenty-five rats were radomly selected to receive lesions in the medial septal area with ibotenic acid (25 nanomoles/1.0 ul). Ten rats with lesions were randomly chosen to remain in their cages for two months before beginning testing. The remaining fifteen rats with lesions began training and testing immediately after recovery from the operation. All rats received water ad lib. Each rat in the experimental groups were maintained at 85% of their normal weight.

The rats were sacrificed at the completion of their behavioral testing. Choline acetyltransferase (ChAT) activity levels were determined (8) in the hippocampus to estimate the extent of recovery in the Ch system. ChAT is a reliable marker enzyme for Ch system integrity and reflects terminal density. This measure is an indirect indication of the presence of collateral sprouting. Protein in each sample was determined according to the method of Lowry et al., (9). Histological sections were prepared from the medial septal area of each rat to determine the effectiveness of the lesion.

Behavioral Testing

The behavioral testing involved three tasks: performance on the radial arm maze, T-maze, and activity in an open field. All of the rats were tested for a minimum of 9 days. The specific training and testing procedures have been published previously by this laboratory (10). All rats spent at least 30 minutes per day on each task.

RESULTS

Behavioral experience did not alter the time-related rate of recovery in the Ch system, as determined by levels of ChAT in the hippocampus. The results are summarized in the table I. Their were no changes in neocortical ChAT levels.

DISCUSSION

Clearly, the behavioral challenges had no effect on the level of ChAT in the hippocampus. This lack of evidence for collateralization of surviving neurons suggests that functional recovery would also not have occured. There are two possible reasons for this lack of an effect: 1) the testing was not sufficiently challenging to the rats or 2) the testing did not last long enough. However, recovery of ChAT levels to normal may not be required for recovery of lost functions. Because there is a natural surplus of endogenous ChAT, it is entirely possible that functional recovery in the Ch system can occur with less than normal levels of ChAT activity.

Future studies will explore these possible alternatives and pursue a combined approach of behavioral testing and specific pharmacotherapies.

Table I.

	BEHAVIORAL GROUP		
	MSA LESION		
	Tested	Not Tested	Unlesioned Controls
Post-Op Time			
Dorsal Hippocampus			
One Week	34.35 ± 4.93	31.37 ± 2.34	55.98 ± 1.94
Two Months	36.96 ± 0.66	24.50 ± 1.78	
Ventral Hippocampus			
One Week	47.47 ± 5.81	48.08 ± 4.57	65.56 ± 5.79
Two Months	36.52 ± 5.18	30.70 ± 3.76	

The Influence of Time and Behavioral Training on the Cholinergic System. ChAT activity is expressed as nmole/hr/mg protein. The mean (± S.E.M.) is shown for two measurements in each rat. Tested - Each rat in this group was lesioned and underwent extensive behavioral training; Not Tested - Each rat in this group was lesioned, but did not undergo any behavioral testing, but remained in their home cage; Controls Each rat received sham lesion and remained in the home cage for the duration of the experiment.

Appendix: 2

BEHAVIOR ALTERS THE UPTAKE OF (3H)-CHOLINE INTO ACETYLCHOLINERGIC NEURONS OF THE NUCLEUS BASALIS MAGNOCELLULARIS AND MEDIAL SEPTAL AREA.

BACKGROUND

The forebrain Ch system may be involved in many cognitive functions. The MSA forms the most rostral extent of a core of Ch neurons extending caudally along the basal forebrain into the substantia innominata. Within the substantia innominata lies a less circumscribed group of Ch neurons, the NBM. Ch afferents to the cerebral cortex originate in the NBM in the same general topographical manner that Ch afferents to the hippocampus originate in the MSA. SDHACU in vitro reflects Ch activity in vivo (11). Drugs that increase the activity of Ch neurons (e.g. scopolamine and pentylenetetrazol) and increase the release of (3H)-acetylcholine also increase in vitro SDHACU. Consequently, SDHACU in the hippocampus ought to change if behavior alters the firing of MSA Ch neurons, and SDHACU in the frontal cortex ought to change if behavior alters the firing of NBM Ch neurons.

SDHACU has the advantage of being able to reflect the activity of nerve cells that use a specific transmitter substance. Thus, unlike electrophysiological recording techniques, it provides information about a specific transmitter system in the brain.

Behavioral training and testing has been shown to alter SDHACU (12,13). SDHACU and incorporation of (3H)-choline into (3H)-acetylcholine was higher in the hippocampus of rats showing good retention in a brightness discrimination task, and in rats that were placed in a novel environment.

Experiment 1 was designed to determine: 1) the extent to which behavioral experience can influence the activity of cholinergic nerve cells in the brain, and the types of behavioral experience that are sufficient to produce this activation, and 2) the possible dissociations between two different components of the cholinergic system, the projections of the nucleus basalis to the frontal cortex and those from the medial septal area to the hippocampus. Experiment 2 was designed to examine the time course of the changes in SDHACU produced by behavioral experience, with special emphasis on the rate at which SDHACU would change in response to a behavioral challenge, and the length of time that changes in SDHACU persist following termination of the behavioral experience that produced those changes.

EXPERIMENT 1

SDHACU was recorded in the frontal cortex and the hippocampus of rats following experience in several different behavioral tests. The recording from two different areas allows a comparison of the extent to which these two different components of the cholinergic system are functionally distinct. If the entire basal forebrain cholinergic system is activated by a behavioral experience, then equivalent changes might be seen in both the frontal cortex and hippocampus.

If, on the other hand, behavioral experience selectively activates one or the other of these two components, then differential activation of the frontal cortex and the hippocampus should appear.

The behavioral tasks were chosen in such a way as to enable conclusions to be drawn about the types of experiences that could produce changes in SDHACU. Rats were taken from their home cages to provide an indication of SDHACU levels in the absence of training on any task or stimulation in any obvious way. Rats were also placed in a treadmill, in which they had to run for extended periods of time, to provide information about the extent to which SDHACU was affected by a sensory-motor task or by handling. Performance in a radial maze was chosen because lesions of the MSA or the hippocampus produce impairments of this behavior. Consequently, we expected that SDHACU would be increased during the performance of this task. Performance in a T-maze used two different types of memory discriminations: the comparison between the performance in these two tasks, and with that found in the radial arm maze, indicates the extent to which a particular memory component is important for changes in SDHACU. Thus, a comparison of the results obtained in the different behavioral tasks allows conclusions to be drawn about the interrelationship of basal forebrain Ch activity and behavior.

Rats were trained on their respective tasks and sacrificed usually within 2 minutes of the last experience. One group, the radial maze trained-untested, was sacrificed 24 hours after the last experience.

Caged

Twenty-six male Sprague-Dawley rats (250 - 300g), housed individually with ad lib food and water. The colony room was maintained on a 16:8 light-dark cycle. Two rats were taken daily from their home cages, sacrificed, and prepared for neurochemical analyses as described below. Preliminary studies on rats taken directly from the home cage, without any prior behavioral experience, found that cortical and hippocampal SDHACU levels were consistent across many days. Therefore, rats from this group were *always sacrificed and assayed when any other group* was being assayed in Experiments 1 and 2. Two Caged rats were sacrificed on each day of testing of the other groups of rats. All of the rats in these studies were obtained from the same supplier, were of the same age weight, and were sacrificed between 8 and 10 AM.

Treadmill

Subjects. The subjects were 16 male albino rats housed individually. Each rat was deprived to 85% of his ad-lib weight and maintained at this weight, plus 5 g a week for growth throughout the experiment. Once each day, the rat was fed the appropriate amount of Charles River Rat Formula and 12 pieces of cat food (Thrive, Purina). The food deprivation and the cat food was to equate their diets with that of the rats tested in the other tasks (see below).

Apparatus. An electric treadmill was constructed from a belt sander. The treadmill moved at 6 cm/sec. A Plexiglas box, 30 cm long and 9 cm wide, was placed over the treadmill.

Procedure. Each rat was placed in the treadmill apparatus for approximately 45 minutes; then sacrificed immediately and prepared for chemical analysis.

12 Arm Maze

Trained: Tested and Untested Groups

Subjects. The subjects were 18 male albino rats (250-325 g) obtained from Charles River. Each rat was housed in an individual cage, and food-deprived to 85% of its normal body weight.

Apparatus. The test apparatus was a radial maze. The maze had 12 arms, each 97 cm long and 9 cm wide, connected to a duodecagonal center platform, 30 cm across, elevated 135 cm above the floor.

Procedure. Rats were first shaped to go down to the ends of the arms of the maze to get food reward. At the start of each trial, one pellet of food was placed in the cup at the end of each arm. The rat was then placed in the center platform and allowed to choose among the arms. The rat was confined for one minute in the center platform after each choice by guillotine doors. If the rat entered an arm for the first time, a correct response was recorded. If the rat entered an arm previously visited in that trial, an incorrect response was recorded. A trial was terminated when the rat visited each arm once, visited 20 arms, or 10 min had passed, whichever came first. Each rat was trained to a criterion of at least 12 correct choices in the first 13 arms entered for three consecutive test sessions. The training period required about 4 weeks.

On the day of sacrifice, half of the rats were given consecutive trials for 20 minutes and then sacrificed within 2 minutes after the completion of testing. This group was called Trained-Tested. The other half of the rats were in the Trained-Untested group. They were taken directly from the colony room and sacrificed without any behavioral testing. Thus, the only difference in procedure for these two groups of rats was the length of time between the last testing on the maze and the sacrifice; 2 minutes or less for the Trained-Tested, 24 hours for the Trained-Untested.

T-Maze

Subjects. Twenty-four male albino rats (250-325g) were maintained and food-deprived as described above.

Apparatus. The apparatus was a wooden T-maze with a stem, 33.6 cm long and 9 cm wide, and two arms, 49 cm long and 9 cm wide. On each side of the stem and arms was an edge, 5 cm high. The maze was elevated 22 cm above the surface of a table. At the end of each arm was a food cup, 1.0 cm in diameter and 1.0 cm deep.

Procedure. All rats to be tested were shaped to go down the arms of the maze for a food reward. Each rat was then reinforced on only one arm of the maze. A trial began with the rat being placed on the start arm and ended when he ate the food. A correct response was recorded when the rat chose the arm with the reinforcement in the food cup. An incorrect response was recorded when the rat chose the unreinforced arm. Each rat was given 8 trials a day, 5 or 6 days

each week for 2 weeks, until it reached a criterion of 7 correct choices in the first 8 trials for 3 consecutive days.

On the day of sacrifice, all rats were given 5 trials with the same discrimination. Half of the rats continued with this discrimination throughout the day's testing while the other half were given a reversal. For rats in the Reversed group, the food was placed on the opposite side of the maze. Testing continued for each rat until 5 consecutive correct responses were made. Each of the rats that continued in the Same discrimination was a yoked control for a rat in the reversal condition, and was given the same number of trials with the same discrimination that had been learned previously. The rats in the reversal situation usually took about 18 trials (approx. 30 min.) to learn the task. The intertrial interval was approximately 2 min.

Cued Non-match-to-sample

Subjects. Five male rats (250 - 325g) were food-deprived and maintained as described above.

Apparatus. The apparatus was composed of a start platform, a runway, and two compartments which contained the discriminative stimuli. The starting platform was 14 cm long and 10 cm wide, separated from the runway by an opaque guillotine door. The runway was 15 cm long and 10 cm wide, at the end of which were placed the two compartments. Each compartment was 14 cm wide, 15 cm high, and 15 cm deep. In each compartment was a speaker, connected to a source of white noise, and three lights, which flashed at a rate of 3 times per second when turned on. The apparatus was placed in a dimly lit room, which minimized spatial cues.

Procedure. A standard procedure for a cued non-match-to-sample, working memory discrimination was used. Each trial was composed of two runs. For the forced run, a piece of clear plexiglass was placed in front of one of the compartments. The rat was placed on the starting platform, the guillotine door was raised, and the rat was allowed to run to that compartment to obtain sucrose. The rat was then picked up and returned to the starting platform. For the choice run, the barrier was removed so that the rat could enter either compartment. The guillotine door was raised, and the rat allowed to choose between the two compartments. Going to the compartment which did not have the same stimulus (white noise or flashing lights) as the one on the forced run was rewarded with sucrose. Entering the other compartment was not. In either case, the rat was removed from the apparatus and returned to his home cage.

For each run, one compartment had flashing lights while the other compartment had white noise. The compartment which contained each stimulus varied randomly from run-to-run with the constraint that all possible combinations occured equally often during a day's testing, and no single combination occurred for two consecutive trials. Consequently, in order to choose correctly for the choice run of any given trial, the rat had to remember the stimulus that was encountered during the forced run of that trial.

Eight trials were given each day, five days a week. During the intertrial interval, which was approximately 5 minutes, the rat remained

in his home cage in the same room with the apparatus.

The rats began the discrimination performing at the level expected by chance (4 correct responses on the 8 choice runs of each test session). After several weeks of testing, choice accuracy gradually improved. At the time of measurement of SDHACU, approximately 5 months after the beginning of testing, each rat was performing almost perfectly and rarely made errors. After the last 8 trials, each was immediately sacrificed.

Neurochemistry

Each rat was sacrificed by decapitation and the brain was rinsed with ice-cold 0.9% NaCl, and rapidly cooled on ice. The frontal dorsolateral cerebral cortex was removed from the surface of the brain to expose the hippocampus which was subsequently removed with fine forceps. The section of frontal cortex was lateral to cingulate and anterior to sensory-motor cortex. The selected brain regions were weighed (cortex: 30 mg, hippocampus: 75 mg) and homogenized in 20 vols of 0.32 M sucrose, using a Teflon-in-glass homogenizer (10 strokes; clearance 0.40 mm). The homogenate was centrifuged at 1,000 g for 10 minutes to obtain a crude nuclear pellet which was discarded. The resulting supernatant was centrifuged at 17,000 g for 15 minutes to obtain a crude mitochondrial pellet. The supernatant was discarded and the pellet was resuspended in the original volume of 0.32 M sucrose, homogenized and was then utilized in uptake studies. Aliquots of this suspension were added to both normal Krebs-Ringer phosphate media and Na-free media, pH 7.4. Uptake values obtained with the latter media were an indication of sodium independent uptake and were subtracted in all cases as a blank value from the values obtained utilizing the former media. The level of SDHACU was determined according to the method of Atweh et al., (11). The composition (mM) of the normal Krebs-Ringer phosphate media was as follows: NaCl, 126; KCl, 4.75; $CaCl_2$, 1.27; Na_2HPO_4, 15.8; $MgCl_2$, 1.42; dextrose 2 mg/ml. The composition of the sodium free media was the same, except that NaCl and the Na_2HPO_4 were replaced by sucrose (252 mM) and Tris-phosphate (15.8 mM).

Routine assays were performed as follows. Aliquots (0.1 ml) of the synaptosomal suspension were added to 0.8 ml of incubation medium and transferred to a water bath (37°C). After 4 minutes, 0.1 ml of (^{3}H)-choline solution (final concentration: 0.4 uM, 0.8 uCi) was added. Uptake was terminated after 4 minutes by addition of 2 ml of ice-cold, Na-free Krebs-Ringer solution, which was followed immediately by rapid filtration on Watman GF/C filters using a Millipore vacuum filtration manifold. The filters were washed 3 times with 2 ml of ice-cold Tris KrebsRinger and then placed in liquid scintillation vials. Ten milliliters of Aquasol II (New England Nuclear Corp., Boston, MA) were added, and the radioactivity counted in a Packard Tricarb liquid scintillation spectrometer. The total radioactivity, measured in counts per minute, was converted to picomoles of choline taken up for 4 minutes per milligram protein. (3H)-choline was obtained from New England Nuclear (80 Ci/mmol) and non-radioactive choline was obtained from Sigma. Protein was measured in every sample according to the method of Lowry et al.(9), with bovine serum albumin as external

standard.

RESULTS

Rats tested in a treadmill had SDHACU levels similar to those seen in the Cage group, but the variability was smaller (see Figure 1).

Experience in all of the other behavioral tasks elevated SDHACU in hippocampus ($p < 0.05$) but not in frontal cortex. In fact, decreases in SDHACU in the frontal cortex were commonly observed following behavioral testing.

DISCUSSION

These results show that behavioral experience can differentially influence SDHACU levels in the brain, increasing those in the hippocampus, but not in the frontal cortex. These data provide information about the types of behavioral experiences that involve cholinergic activity in the brain, and demonstrate a functional dissociation between the forebrain cholinergic systems of the NBM and the MSA.

Experience in all of the behavioral tests, except the treadmill, significantly elevated SDHACU in the hippocampus above levels found in rats taken from their home cage. A comparison of the components of these tasks, especially in conjunction with those involved in the treadmill, begins to identify the types of experiences that elicit activity of the Ch system. Both the discrimination in the radial arm maze and the reversal of the T-maze discrimination require a short-term, flexible memory that is impaired by damage to the hippocampal system. The straight left-right discrimination on the T-maze involves a different type of memory, and performance in this task is not impaired by lesions of the hippocampal system. Nonetheless, SDHACU was elevated by experience in this task, indicating that the specific type of memory involved was not the critical factor. Experience in the cued non-match--to-sample task also elevated SDHACU. Because this task involved non-spatial stimuli, experience with spatial discriminations is not necessary to elevate SDHACU. This pattern of results suggests that any kind of learning experience may be sufficient to elevate SDHACU levels in the hippocampus.

A comparison of the data from the discrimination tests with those from the treadmill, however, indicate that some learning or memory component must be an important factor involved in the elevation of SDHACU. Experience in the treadmill, even for extended periods of time, was not sufficient to increase the level of SDHACU. Consequently, the increase in SDHACU in the learning task cannot be attributed to any component in common with that in the treadmill; sensory-stimulation, handling, motor movement, food deprivation, etc. These data suggest that the process of learning was a very important component producing the increase in SDHACU in the hippocampus.

None of these experiences increased the SDHACU level in the frontal cortex. In fact, levels in frontal cortex were decreased. The dissociation in the pattern of results seen for the NBM and MSA indicate

that they may be differentially activated by behavioral experience. Consequently, although the NBM and MSA are components of the same basal forebrain cholinergic system, nonetheless, they clearly are functionally distinct.

When rats were taken from the colony room without any training, SDHACU levels were more variable than seen in any other group of rats. This variability may have been due to the different behaviors of the rats prior to sacrifice. The group of rats trained on the treadmill may have provided a more consistent behavioral environment prior to sacrifice, and controlled for many of the sensory, motor, and motivational components of the rats' behavior in the maze. Although the variability was smaller in the Treadmill group, the absolute level of the mean did not change.

EXPERIMENT 2

The results of the first experiment demonstrated that SDHACU in the hippocampus increased with behavioral experience involving learning and memory, and that in the case of the radial arm maze task, SDHACU remained elevated, although not significantly above Cage Controls, for at least 24 hours. The second experiment was designed to investigate the rate at which behavioral testing increases SDHACU. The acquisition of an active avoidance response to an aversive stiumlus was examined. Three groups of rats spent different amounts of time in the shuttle--box apparatus.

The second experiment also examined the possible long-term effects of training on SDHACU. If the increase in SDHACU is an acute phenomenon only, reflecting the immediately preceding experience of the animal, then SDHACU levels should fall rapidly following the cessation of testing. If, however, the experience produces a relatively long-term change in brain activity, then increases in SDHACU should be seen for much longer following the cessation of testing.

Active Avoidance

Subjects. Twenty-one male albino rats (250-325 g) were housed individually as described before, but with ab lib. food and water.

Apparatus. The apparatus was a box, 13.0 X 43.0 X 27.0 cm, with the sides and top constructed of clear Plexiglas. The floor consisted of stainless-steel rods, 0.9 cm in diameter, spaced 1.7 cm apart. The box was divided into two compartments by an opaque guillotine door which slid down to the top of a barrier, 4.5 cm high, in the middle. The CS was 50-decibel white noise and the illumination of a six-watt light which were presented in the compartment occupied by the rat. The UCS consisted of a 0.5 ma shock delivered in 0.2 sec pulses by a shock generator (GSC model E1064GS). The CS-US interval was 10 sec.

Procedure. All rats were placed two at a time in the shuttle-box for 30 minutes on the day prior to active avoidance testing. The guillotine door was raised and the rats were allowed to explore freely; neither the CS nor UCS were present.

The next day, each rat was trained in a standard two-way shuttle

task. Each rat was given unavoidable shock on the first two trials; the guillotine door remained shut and was not raised until the onset of the shock. At the start of each subsequent trial, the CS was presented and the guillotine door was raised. If the rat did not cross over the barrier to the other compartment within 10 sec after the onset of the CS, the UCS was presented until the response was made. After the rat moved to the other compartment, the gullotine door was lowered and the CS and UCS were terminated. The rat remained in the compartment which it had just entered for a 30 sec intertrial interval.

On the day of sacrifice, one rat was randomly assigned to each of 3 groups: Group 1 received 8 trials, which was not a sufficient opportunity to learn the avoidance response; Group 2 were trained to a criterion of at least 7 correct avoidance responses in 8 consecutive trials; Group 3 were trained to criterion and then given 20 additional trials. Each rat was sacrificed immediately following the completion of its behavioral testing. SDHACU was determined as described above.

Radial Maze: Post-Training Changes in SDHACU

Subjects. Eighteen male rats (250-325 g) were housed individually and food-deprived to 85% of their ad-lib weight, as described above.

Procedure. Each rat was trained on the radial maze (approx. 5 weeks) until it reached a criterion performance of at least 12 correct choices in the first 13 arms entered for three consecutive days. Training was then stopped and the rats remained in their home cages, individually housed, with ad lib food and water. One day after the last training session, 4 rats were sacrificed to confirm the changes in hippocampal and frontal cortical SDHACU levels observed in Experiment 1. On selected days thereafter (see Figure 2), 2-4 rats were removed from their cages and quickly sacrificed by decapitation. SDHACU was determined as described in Experiment 1.

RESULTS

Hippocampal SDHACU levels gradually increased with the amount of time and the number of trials, and was significantly ($p < 0.05$) increased only in those rats that surpassed a criterion level of performance (see Figure 1, Criterion + 20). Frontal cortical SDHACU decreased slightly, but not significantly. The rats in Groups 2 and 3 required a mean of 42 trials to reach criterion performance (approx. 30 min.).

Hippocampal SDHACU levels were still elevated one day after the cessation of training on the radial maze (see Figure 2), confirming the results of Experiment 1. The elevation was statistically significant ($p < 0.05$) only when compared to rats ran in the treadmill task. It was maintained for 20 days, but returned to near control levels 40 days later. By day 20, neocortical SDHACU returned to near control levels; then gradually decreased for 20 days, paralleling the changes seen in the hippocampus.

DISCUSSION

In the active avoidance experiments, rats tested long enough to reach criterion plus 20 trials were in the task for a mean of 50 minutes, which was sufficient to produce a significant increase in SDHACU. Thus increases in SDHACU can occur in less than 50 minutes from the beginning of testing (rats tested for only 8 trials, and especially in rats tested to criterion performance, already showed a tendency for increasing hippocampal SDHACU levels), and persist for 20 days. Further testing, of course, is required to identify more precisely the parameters of this time course. The increase in hippocampal SDHACU and decrease in cortical SDHACU may be partially related to stress associated with the aversive stimuli and not just restricted to appetitive tasks. Appropriate controls need to be examined to rule out this important factor.

The time course of continued elevation of SDHACU activity in the hippocampus was described by the results. The elevation induced by behavioral training on the radial arm maze was maintained for at least a few weeks after the end of training. The prolonged elevation in the activity of cholinergic neurons afferent to the hippocampus may indicate increased processing in this system. The hippocampus may be more capable during this period of encoding information related to learning and memory, processes that are more typically related to the cholinergic system in this region of the brain.

GENERAL DISCUSSION

The present experiments measured SDHACU in the hippocampus and frontal cortex to gain an indication of the extent to which behavioral experience can alter the activity of cholinergic nerve cells in the MSA and NBM, respectively. The results of these experiments show that this approach to recording brain activity can be very valuable. Like other recording techniques, it is able to examine in a non-manipulative manner the types of variables that alter the activity of the system being examined. It has the advantage of being able to reflect specific changes of an individual neurotransmitter system, and can measure the activity of given sets of nerve cells at distances far removed from those nerve cell bodies. For groups of nerve cells that are tightly packed together but have widespread projections, this spatial distribution allows a discrimination among the activity of the nerve cells at the terminals that can be much finer than any obtained at the nerve cells themselves.

The pattern of results obtained from this experiment indicate that experience in many different kinds of discrimination tasks is sufficient to elevate SDHACU in the hippocampus, while simple sensory-motor experience (as in the treadmill) is not. Clearly, some aspect of learning and memory triggers activity of these nerve cells. The exact component remains to be identified.

The dissociation between the results obtained from the hippocampus and frontal cortex indicate a marked functional distinction between the MSA and the NBM. The experiences investigated in the present study that elevated SDHACU in the hippocampus were either not intense

enough to produce changes in the frontal cortex (suggesting a quantitative distinction between these two areas), or they may have been inappropriate to activate the NBM (suggesting a qualitative distinction between these two areas). Of particular importance for future research is defining the types of stimuli that can activate the cholinergic system from the NBM to the frontal cortex. Also, given that cholinergic interneurons exist in the cortex, the changes in cortical SDHACU will need to be interpreted accordingly.

The present study demonstrates that SDHACU can be a valuable technique to investigate the functional organization of the cholinergic system, and that the hippocampal and frontal frontal cortical components of this system are functionally distinct. Many questions remain to be answered, and these can be approached in the same way that other studies using 2-deoxyglucose have examined the functional organization of the brain. In essence, the SDHACU technique uses the same rationale and general approach as that already established for 2-deoxyglucose, and has the advantage of providing specific information about a single neurotransmitter system.

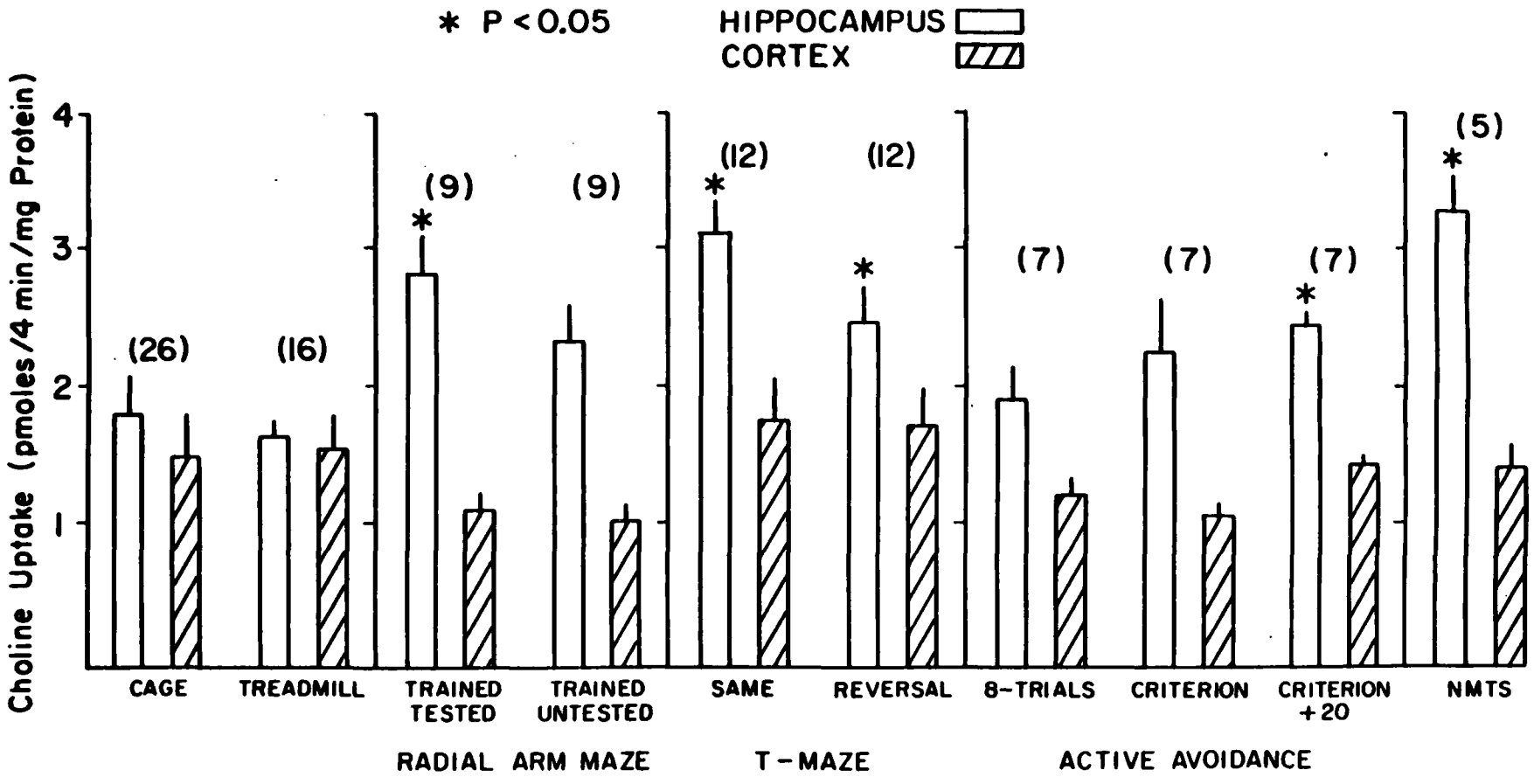

* P < 0.05
HIPPOCAMPUS
CORTEX
Choline Uptake (pmoles/4 min/mg Protein)
4
3
2
1
(26)
(16)
(9)
(9)
(12)
(12)
(7)
(7)
(7)
(5)
*
CAGE
TREADMILL
TRAINED TESTED
TRAINED UNTESTED
SAME
REVERSAL
8-TRIALS
CRITERION
CRITERION +20
NMTS
RADIAL ARM MAZE
T-MAZE
ACTIVE AVOIDANCE

Figure 2

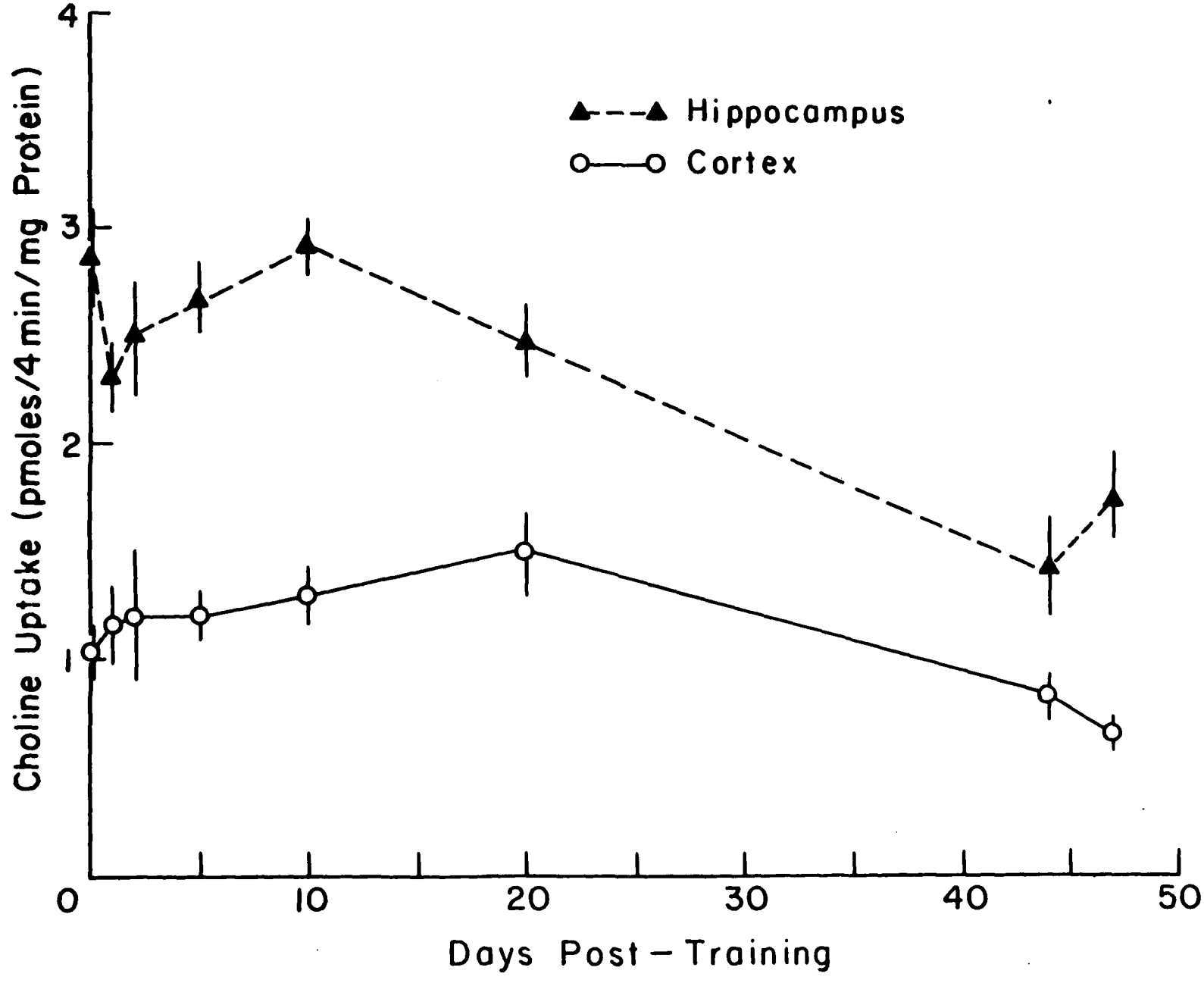

Choline Uptake (pmoles/4 min/mg Protein)
4
3
2
1
0
10
20
30
40
50
Days Post – Training
Hippocampus
Cortex

FIGURE CAPTIONS

Figure 1. Sodium-dependent high affinity choline uptake following experience in various behavioral tasks. SDHACU is expressed as picomoles of choline taken up in 4 minutes per mg of protein in the synaptosomal suspension. X $\pm$ SEM. Numbers in parentheses indicate the number of animals in each group. Statistical comparisons were made by t-tests to the Cage group. NMTS indicates the cued non-match-to-sample task.

Figure 2. Time-dependent changes in neocortical and hippocampal SDHACU following the last day of radial maze training. Day 0 represents SDHACU levels on the last day of training. This data point is reproduced from Figure 1 (radial maze, trainedtested). SDHACU is expressed as picomoles of choline taken up in 4 minutes per mg of protein in the synaptosomal suspension. Data points represent X $\pm$ SEM for 4 rats on day 1 and 2-4 rats on days 2 through 47.

Appendix: 3

BASAL FOREBRAIN: OPTIMAL COORDINATES FOR LESIONS ANALYSIS

BACKGROUND

Three groups of Ch nuclei lie in the basal forebrain of mammals and project to the hippocampus and entire neocortex (14). The most ventrolateral and caudal group, the NBM, provides the major Ch projection to neocortex (15). The middle group is the diagonal band of Broca (DBB), which projects to cingulate and occipital cortex and may provide up to 60% of the Ch input to the hippocampus. The most dorsal and anterior group, the MSA, sends a Ch projection to the hippocampus. Thus, the cell bodies in these Ch nuclei have a regular topological relationship that is reflected in the termination of the axons in the neocortex and the hippocampus. Animal models are now examining the changes that occur subsequent to destruction of the NBM (16). Ibotenic acid (IBO) is often used to produce these lesions because it destroys cell bodies and dendrites, while sparing axonal processes. However, its use poses two problems. First, the NBM is a relatively diverse, widely spread group of cell bodies in the rodent. Second, the cell bodies in the NBM are close to other Ch cell bodies in the basal forebrain. Consequently, ibotenic acid must be spread through a large enough area in order to destroy the cell bodies in the NBM, while not diffusing to the adjacent Ch nerve cell bodies and producing unwanted damage. Furthermore, destruction of the MSA-DBB Ch neurons which innervate the hippocampus produces substantial behavioral changes (10). These changes are very similar to many of those reported following destruction of the NBM (16). An important question is whether or not the behavioral effects following lesions of the NBM are due to selective destruction of the NBM or to spread of that destruction into the MSA-DBB complex, and impairment of hippocampal Ch function.

The present experiment was designed to determine the optimal placement of ibotenic acid in the NBM in order to get a substantial destruction of Ch input to the frontolateral neocortex, while sparing that to the hippocampus. Two different volumes of ibotenic acid were injected at four different stereotaxic placements in the basal forebrain. ChAT levels were measured in three different regions of neocortex and two regions of hippocampus. The study was designed to indicate the most effective placement of lesions in the NBM to produce maximal decreases of ChAT in the frontal cortex and minimal decreases of ChAT in the hippocampus.

METHODS

Subjects:

The subjects were 32 male Sprague-Dawley rats obtained from Charles River breeders, weighing from 250 to 300 grams. After surgery, the rats were housed individually and maintained on a 12-hour light/dark cycle. The rats were allowed *ad lib* access to Purina lab chow.

Surgery:

The rats were injected with atropine methyl bromide (1.25 mg; Sigma), anesthetized with Chloropent (Fort Dodge), 0.3 ml/kg IP, and placed in a sterotaxic instrument. The incisor bar of the stereotaxic instrument was set 2 mm below the interaural line. Ibotenic acid (25 nanomoles) in phosphate-buffered saline (pH 7.4), was infused via a 1.0 uL Hamilton microsyringe. Four different sets of coordinates were used to produce the NBM lesions: 0.9 mm or 0.4 mm posterior to bregma; ± 2.0 mm or ± 2.6 mm lateral to the midline. The needle tip was always lowered 6.8 mm below the surface of the brain and was left in place 2 minutes before the infusion began. The infusion volume was either 1.0 or 0.6 microliters and was delivered at a rate of 0.1 ul/minute. The needle was then left in place 5 minutes. All placements were bilateral. Lesions were also produced in the MSA by placing the tip of the needle 0.8 mm anterior to bregma and 5.8 mm ventral to the brain surface at the midline. In sham operated control rats, the needle was lowered into the basal forebrain region and left there for five minutes without any infusion.

Neurochemistry:

One week after surgery, the rats were sacrificed by decapitation and their brains were removed and rapidly dissected at 5° C. Three bilateral sections of frontolateral neocortex (approx. 25 mg each) and two of hippocampus were isolated. Sections of neocortex included a) frontal cortex including the frontal pole (lateral to cingulate cortex), b) dorsal cingulate and parietal cortex, and c) lateral parietal cortex. The tissues were stored at -40° C until assayed. The tissues were then homogenized in 40 volumes of 0.5% Triton X-100:10 mM EDTA, pH 7.4; ChAT activity was determined according to the method of Fonnum (8). The protein content of the homogenates was assayed according to the method of Lowry et al. (9), with bovine serum albumin as standard. Statistical comparisons were made to sham-operated controls by multiple t-tests.

Histology:

The remaining brain tissue was fixed in 10% buffered formalin with 30% sucrose for cryoprotection. Frozen sections were mounted on glass slides and stained with cresyl violet. Lesion size was defined as the area in which magnocellular neurons were absent or had no nucleoli, or gliosis was present.

RESULTS

Histology:

Examination of coronal sections through the lesion sites revealed that the lesions were located in the ventromedial region of the globus pallidus. With coordinates that were more anterior and/or medial (0.4 mm P; ± 2.0 mm L), the lesion included sections of the horizontal limb of DBB (See Figure 1). Lesion coordinates which were more posterior (0.9 mm P) and medial (± 2.0 mm L) involved less of the diagonal band and often extended into the lateral hypothalamus and ventral thalamus along the lesion's anterior to posterior axis. The shapes of the lesions tended to follow natural hydrophobic myelinated borders which may have channeled the flow of the ibotenic acid.

Lesions that were posterior and lateral extended into the posterior globus pallidus, as it becomes oriented more dorsoventrally near the CA1 region of the hippocampus. These lesions also included large regions of the body of the globus pallidus, including the Ch magnocellular neurons which border the caudate nucleus.

Generally, lesions produced by 1.0 ul injections were about twenty percent larger, yet they tended to destroy a smaller percentage of cells within the region.

NBM lesions which were anterior (0.4 mm P) and medial ($\pm$ 2.0 mm L), and MSA lesions, and which were made with 1.0 ul ibotenic acid, produced 100% mortality. None of the other lesions coordinates were lethal for any of the other groups of rats.

Neurochemistry:

All lesions in the NBM produced decreases in neocortical ChAT (up to 54%), with the greatest and most extensive effects being produced by lesions that were more lateral and caudal (0.9 mm P; $\pm$ 2.6 mm L; See Figure 2). All lesions that were placed at 0.4 mm posterior to bregma, or placed 2.0 mm lateral to the midline, produced significant ($p < 0.05$) decreases in ChAT activity levels in the ventral hippocampus. ChAT activity levels in the dorsal hippocampus were rarely affected.

The concentration of the toxin appeared to have some affect on the extent and degree of the neocortical ChAT decreases. Smaller injection volumes were more potent, even though equimolar amounts of the toxin were infused.

DISCUSSION

ChAT activity in the frontal cortex and hippocampus was reduced by microinfusion of ibotenic acid into the NBM. With the most posterolateral placement, and the smaller volume of acid, ChAT in the frontal cortex was reduced substantially while ChAT in the hippocampus was unaffected. With all the other placements, ChAT levels in the ventral hippocampus were reduced, often significantly. Furthermore, these other placements did not produce a greater depletion of ChAT levels in the frontal cortex. These results show that the specific coordinates and volumes of ibotenic acid are critical to produce selective and substantial destruction of cells in the NBM.

The relative amount of the neocortical decrease was seen to be a function of the region of cortex sampled and the placement of the lesion within the NBM. The decrease in ChAT activity in the ventral hippocampus following infusion of ibotenic acid into the NBM may be due to the the spread of destruction into the DBB or the presence of hippocampal-projecting Ch neurons in the NBM region. It is important therefore to have optimal placement of ibotenic acid infusions in the NBM to get the maximal widespread cortical decrease in ChAT levels without altering hippocampal ChAT levels.

Far greater decreases in neocortical ChAT activity were seen when equimolar amounts of ibotenic acid were infused in smaller volumes than in larger volumes. A critical concentration of the toxin in the neurons' immediate environment may be required to kill a cell; more dilute concentrations may not be sufficient to kill these neurons.

Cellular density in the NBM region may be an important factor in this consideration.

The present study also provides data which describe the anatomy of this diffuse and extensive Ch system. According to the differential decreases in ChAT levels, far more neocortical neurons projecting to the neocortex are in the more lateral aspects of the NBM region than are in the more medial aspects. A similar dorsal-ventral organization of Ch neuronal projections from the MSA-DBB complex exist along the dorsal-ventral axis of the hippocampus (17).

The high mortality rate seen when 1.0 ul of ibotenic acid was injected may have been due to the unintended destruction of lateral hypothalamic centers, or to the presence in the ventricles of the excitotoxin which may have destroyed vital vegetative centers.

In conclusion, the results of this experiment show that small changes in the placements and volumes of ibotenic acid injected in the basal forebrain area have profound effects on the type of destruction produced in those areas, and the reduction of Ch activity in the projection areas of those neurons. These results provide information about the neuroanatomical organization of the projections of these cells to the rest of the brain, and also have substantial implications for the interpretation of results of experiments examining the behavioral changes that occur following injections of ibotenic acid into the basal forebrain.

Figure 1.

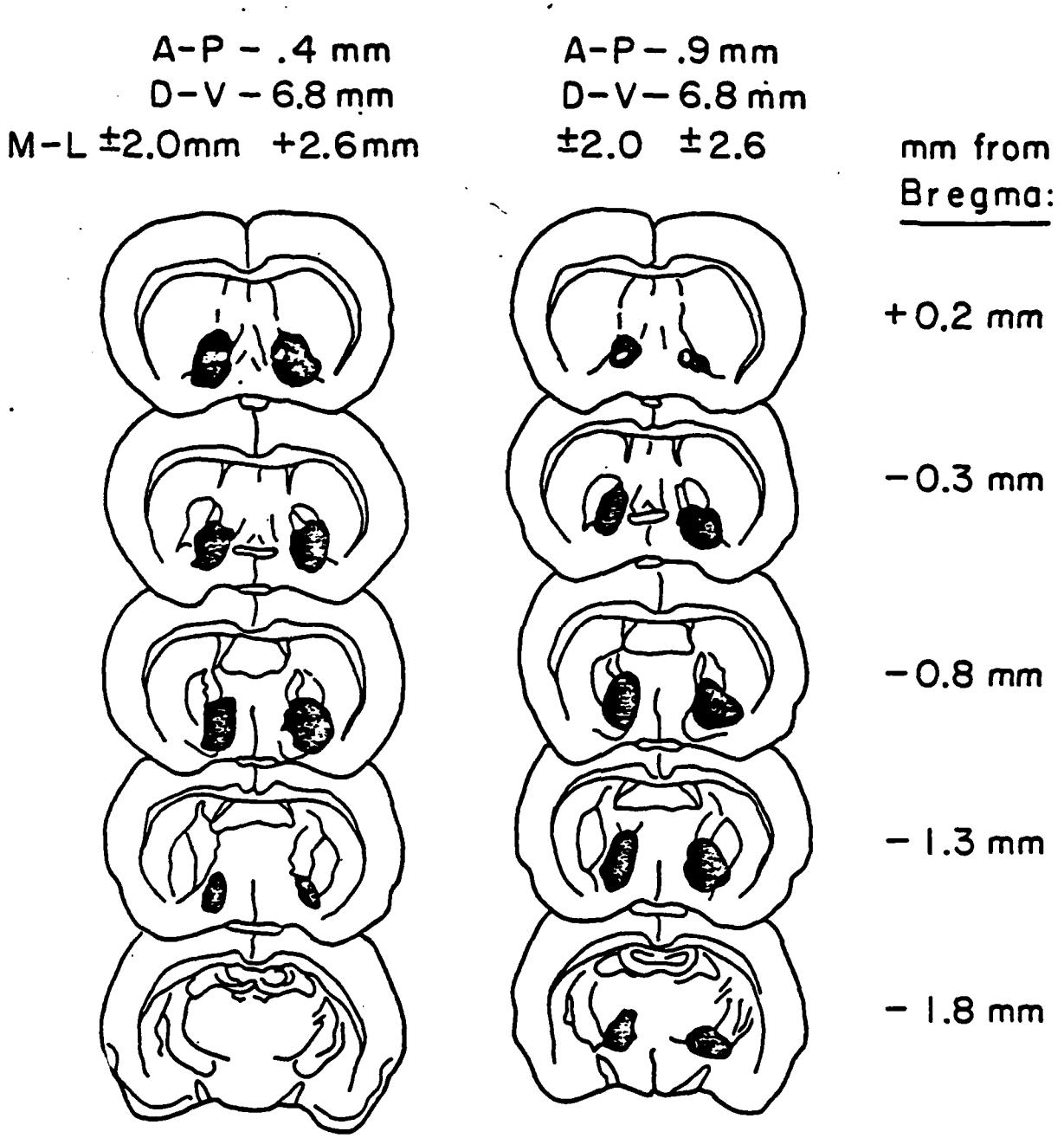

A-P - .4 mm
D-V - 6.8 mm
M-L ±2.0mm +2.6mm
A-P - .9 mm
D-V - 6.8 mm
±2.0 ±2.6
mm from Bregma:
+0.2 mm
-0.3 mm
-0.8 mm
- 1.3 mm
- 1.8 mm

Figure 2.

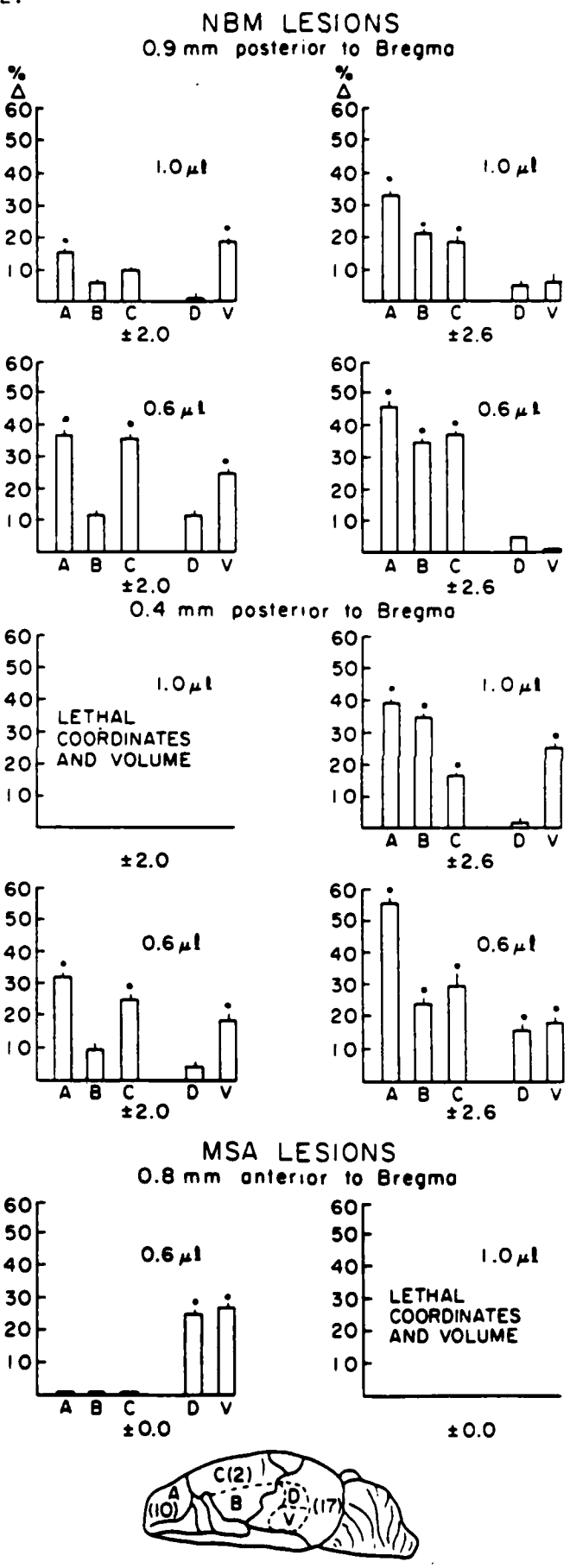

NBM LESIONS
0.9 mm posterior to Bregma
% Δ
1.0 µl
0.6 µl
A B C D V
±2.0
±2.6
0.4 mm posterior to Bregma
LETHAL COORDINATES AND VOLUME
MSA LESIONS
0.8 mm anterior to Bregma
±0.0
C(2)
A
(10)
B
D
V
(17)

Figure 3

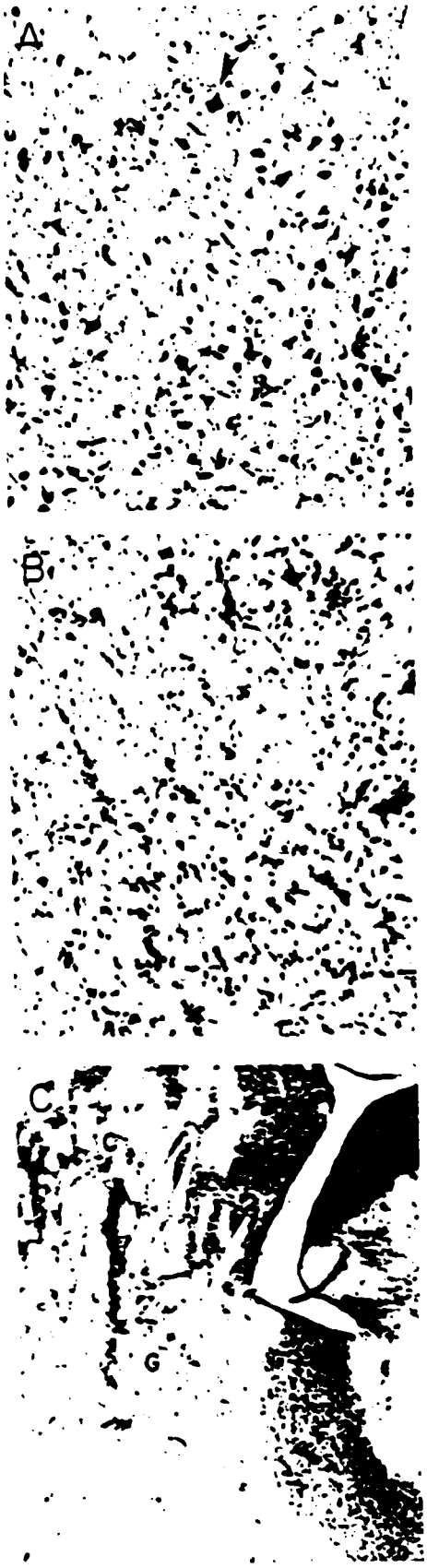


FIGURE CAPTIONS

Figure 1. Schematic representations of lesion size and placement. Cresyl-violet stained preparations were examined and composites of the lesioned areas were obtained. The lesions represented are composites from the brains of rats that had been injected with 0.6 ul of ibotenic acid.

Figure 2A-C. Effects of ibotenate lesion on ventral globus pallidus and substantia innominata. A. Low power photomicrograph of cresyl violet stained section through the globus pallidus (G) and caudate (C) of a rat that received an injection of 25 nmoles of ibotenic acid seven days prior to sacrifice. B. Large (40-60 um) neuronal perikarya located in ventral globus pallidus are indicated by arrows in this higher power photomicrograph of control rat. C. Higher power photomicrograph of cresyl violet stained section of the nucleus basalis region. Note the absence of neuronal perikarya and the intense gliotic reation.

Figure 3. Percent decrease in ChAT activity in the cortex and hippocampus following the production of lesions at various stereotaxic coordinates. * = $p < 0.05$ *vs.* control. Coordinates for the NBM lesions were -6.8 mm ventral to the brain surface. Coordinates for the MSA lesions was -5.8 mm ventral to the brain surface. The posterior coordinates are with respect to bregma, and the lateral coordinates are with respect to the midline. The regions of neocortex and hippocampi that were sampled are indicated on the drawing of a lateral view of the rat brain.

Appendix: 4

LESIONS IN NUCLEUS BASALIS MAGNOCELLULARIS AND MEDIAL SEPTAL AREA OF RATS PRODUCE QUALITATIVELY SIMILAR MEMORY IMPAIRMENTS

BACKGROUND

Within the basal forebrain of the rat is a core of Ch neurons that has been divided into several regions which include the NBM, diagonal band of Broca, and the MSA (14). The NBM projects Ch afferents primarily to neocortex, and the MSA and vertical limb of the diagonal band, project Ch afferents primarily to the hippocampus.

The frontal neocortex and hippocampus, and the cholinergic systems afferent to these areas, have an important role in memory (18). Recently, the behavioral effects of lesions produced in either the NBM (16) or MSA (19) have been evaluated separately in individual tasks, but the behavioral effects of these two lesions have not been compared on the same tasks.

In the present study, both IBO and radiofrequency (RF) current were used to produce lesions. Ibotenic acid causes degeneration of neuronal perikarya at the site of injection, without damaging axons of passage or neurons distant from the injection site. RF lesions destroy both neuronal perikarya and fibers of passage. The comparison of RF and IBO lesions allows for the assessment of the relative importance of damage to the cell bodies, as compared to the axons passing through the lesioned area, for these behaviors.

IBO lesions were also made in the DGP to control for the possibility that the behavioral changes produced by IBO in the NBM and MSA were due to generalized effects of the IBO rather than specific damage in the NBM and MSA. The DGP is anatomically close to the NBM and MSA and contains cholinergic neurons (14). These neurons do not project to the frontal cortex or hippocampus, and the DGP has not been linked to memory functions. If the behavioral changes following IBO lesions in the NMB and MSA are due to diffuse effects of IBO, then IBO lesions of the DGP should produce behavioral effects similar to those following IBO lesions in the NBM and MSA. If these behavioral changes following IBO injections in the NBM and MSA are due to specific damage to these particular structures, then IBO injections into the DGP should have no effect on performance in these tasks.

METHODS

Subjects

The subjects were 94 male albino rats (350-400 gm) obtained from Charles River. Each rat was housed individually throughout testing with free access to water, and was maintained on a 16:8 light/dark cycle with lights on at 7:00 A.M. Each rat was deprived to 85% of its ad-lib weight prior to shaping and was maintained at this weight, plus 5 grams per week for growth, throughout the experiment. At the completion of the day's testing, each rat was fed the appropriate

amount of Charles River Rat Formula.

Apparatus

Two identical wooden T-mazes (Figure 1) were used. On either side of the starting platform, stem, and arms were wooden edges, 2.0 cm high. The starting platform was separated from the stem by a guillotine door, 11.5 cm high and 6.2 cm wide, mounted in a frame, 22.5 cm high and 15.0 cm wide. The stem was divided into two halves by a hardware cloth partition, 12.0 cm high and 31.5 cm long, which started 24.0 cm from the guillotine door and was 24 cm long. At the end of the partition proximal to the arms, on both sides of the stem, was a white opaque curtain, 14.0 cm high and 7.0 cm wide, suspended from a frame, 14.0 cm high and 15.5 cm wide, which was perpendicular to the stem. A clear Plexiglas barrier, 14.0 cm high and 7.8 cm wide, was placed behind one curtain to block access to the arms on that side of the stem. A food cup, 1.0 cm wide and 1.5 cm deep, was located 1.0 cm from the distal end of each arm. The two mazes were located in different locations to provide unique extra-maze cues for each maze.

Procedure

Shaping

Each rat was trained for five days to run to the ends of the arms for a food reward (Thrive Cat Food, Purina). During the first day, food was liberally spread thoughout the maze; each rat was allowed 20 min to explore. During each succesive day, the amount of food was progressively restricted so that by the fifth day food was present only in the food cups. Each rat received an equal amount of shaping at each of the two maze locations. The rats were given no food in their home cages during the shaping procedure and received only what food they ate during the daily shaping sessions.

Preoperative Testing

The trial-independent memory discrimination (reference memory) consisted of a simultaneous left/right discrimination on the stem of each T-maze. While one side of the hardware cloth partition was blocked by the Plexiglas barrier in one maze location, the opposite side was blocked in the other maze location. In order to choose the unobstructed side of the stem, the rat had to determine the maze location in which it was being tested. A response was recorded for the stem discrimination when the rat placed its head more than 10 cm beyond the start of the hardware cloth barrier. If the rat chose the open side of the stem, a correct stem response was recorded and the rat continued to the arms. If the rat chose the blocked side of the stem, an incorrect stem response was recorded and the rat was allowed to turn around and choose the open side. The side that was blocked remained constant for any particular rat in all trials, but varied among rats.

The trial-dependent memory discrimination (working memory) was a discrete trial rewarded alternation in the arms of the T-maze.

Each trial consisted of a forced run, in which the rat was directed to one arm, and a choice run, in which the rat was able to choose either arm. At the start of each trial, one piece of food was placed in the cup on the end of each of the two arms. During the forced run, a large piece of wood was used to block the entrance to one of the arms. The rat was placed on the starting platform and the guillotine door was raised, allowing the rat to run down the stem and enter the unblocked arm to get the reward. For the choice run, the block was removed and the rat was again placed on the starting platform. After a 5 sec delay, the guillotine door was raised and the rat was allowed to run down the stem and choose one of the arms. A response was recorded when the rat placed its head more than 10 cm down an arm. If the rat chose the arm which had been blocked during the forced run, a correct arm response was recorded. If the rat chose the arm to which it had been previously forced, an incorrect arm response was recorded. The rat was then returned to its home cage.

Once per day, 5 days each week, the rat was given a test session consisting of 8 trials separated by an inter-trial interval of approximately 2 min. The particular maze on which the rat was tested and the arm to which the rat was forced was varied pseudo-randomly between trials so that in each test session 4 trials were given on each maze and each arm on each maze was blocked for the forced run of 2 trials. Also, the two T-mazes were interchanged approximately once each week to be certain that the rats were not using particular cues on the mazes to solve the task.

Preoperatively, each rat received at least 10 days of testing. Testing was continued until the rat reached a criterion of at least 7 correct responses in the 8 trials of the arm discrimination and at least 15 correct responses in the 16 trials of the stem discrimination each day for five consecutive days. Each rat was then assigned to one of three experimental groups for surgery.

Experimental Design

Lesions were placed in the NBM and in the MSA by IBO and RF. These four groups are identified by a compound abbreviation indicating the lesion site and the type of lesion: NBM-IBO, NBM-RF, MSA-IBO, MSA-RF. Lesions produced by IBO were also placed in both the NBM and MSA (NBM+MSA), and in the dorsal globus pallidus (DGP). One group of rats received operations but no lesion, and is identified by the abbreviation CON.

The experiment proceeded in 4 sections, each of which included a group of CON rats. The lesion groups in each section were: (1) NBM-RF, MSA-RF, (2) NBM-IBO, MSA-IBO, (3) NBM+MSA, (4) DGP. An additional group of rats with IBO lesions in the NBM and their controls received no behavioral testing and were sacrificed 10 days after surgery for biochemical analysis.

Surgery

Prior to surgery, each rat received 0.3 cc of 0.5 mg/ml atropine methyl bromide (Sigma, St. Louis, Mo.) intraperitoneally. Thirty

minutes later, each rat was anesthetized with 0.3 ml/kg Chloropent (Fort Dodge Laboratories; Fort Dodge, Iowa). The rat was placed in a David Kopf stereotaxic instrument with the incisor bar set so that Bregma and Lambda were in the same horizontal plane. The scalp was incised and retracted. Holes were drilled through the skull in the appropriate locations, and the lesion was made. The scalp was sutured, and the rat received 0.1 cc Bicillin (Wyeth, Phila., Pa.) intramuscularly in both hind legs. The rat was then removed from the stereotaxic instrument and placed under a heat lamp until it awakened.

CON rats (n=39) received the same surgical treatment as the lesioned rats except that no electrode or syringe needle was lowered into the brain.

NBM lesions (n=28) were made at the following coordinates for both RF and IBO lesions: 0.3 mm posterior to Bregma, 2.8 mm lateral to the central sinus, and 6.9 mm ventral from dura. The rats received RF lesions in two stages: a unilateral lesion was produced, the rats were allowed seven days to recover, and then the contralateral lesion was produced. RF lesions (n=17) were produced by passing 16 milliamps of current (Grass Lesion Maker Model LM 4, Grass Instruments, Quincy, Mass.) for 18 sec through an electrode 0.2 mm in diameter, insulated except for 0.6 mm at the tip. Bilateral IBO lesions (n=11) were placed by injecting 25 nanomoles of IBO (Sigma, St. Louis, Mo.) in 1.0 ul of phosphate-buffered saline with a 1.0 ul Hamilton syringe during 10 min. The syringe was left in place for 5 minutes after the completion of the infusion. The contralateral NBM then received a similar injection of IBO.

MSA lesions (n=20) were made at the following coordinates for both the RF and IBO groups: 0.8 mm anterior to Bregma, at the midline, and 5.5 mm ventral from dura. RF lesions (n=10) were produced by passing 12 milliamps of current for 14 sec through an electrode 0.2 mm in diameter, insulated except for 0.6 mm at the tip. IBO lesions (n=9) were produced by injecting 18 nanomoles of IBO in 0.7 ul of phosphate-buffered saline during a period of 5 min.

NBM+MSA lesions (n=9) were made at the same coordinates with IBO as described above. All injections were made in one surgical procedure.

DGP lesions (n=8) were placed 0.4 mm posterior to Bregma, 2.6 mm lateral to the central sinus, and 3.0 mm ventral from dura by injecting 25 nanomoles of ibotenic acid in 1.0 ul of phosphate-buffered saline during a period of 5 min.

Postoperative testing

Retention. Postoperatively, the test procedure was the same as that at the end of preoperative testing. Each rat was tested until it reached criterion.

Stem Reversal. The stem discrimination was reversed. The Plexiglas barrier was moved to the opposite side of the stem of each maze. Thus, to gain access to the arms, the rat had to go down the side of each stem opposite that to which it had been trained preoperatively. With the exception of the reversal on the stem, the task was unchanged. Each rat was tested until it reached criterion.

Extended Delay. Nine rats with NBM+MSA lesions and 10 CON rats continued testing after reaching criterion performance. For 6 test sessions, the delay between the forced and choice runs was 5 sec. For the next 18 test sessions, the delay was 10 min. For the next 6 test sessions, the delay was 5 sec. The rats were then sacrificed.

Biochemical Analyses

Four untrained rats with NBM-IBO lesions and 4 rats without lesions were sacrificed 10 days after surgery to determine the effectiveness and specificity of the IBO lesions. Each rat was decapitated and the brain rapidly removed. Tissue samples (50-75 mg) were taken from of the frontolateral cortex and combined, and separately from the dorsal and ventral hippocampus.

All other rats were sacrificed at the completion of behavioral testing, approximately 8 weeks after surgery. Tissue samples (50-75 mg) were taken from frontolateral cortex and the dorsal hippocampus. ChAT levels were measured by the method of Fonnum (8). Protein was measured according to Lowry et al. (9). For both the IBO and RF sections of the experiment, all rats within each group were sacrificed within three days of each other.

Histology

After the removal of samples for biochemical analysis, the remaining brain tissue was fixed in a 10% formalin: 30% sucrose solution. The brain was frozen and sectioned coronally at 30 um with a frozen stage microtome. Every fifth section throughout the lesion site was mounted on a glass slide and stained with cresyl violet. The size and location of the lesions were determined by microscopic examination for loss of magnocellular neurons and the presence of gliosis.

Statistics

The data were analzyed using an analysis of variance with post hoc Sheffe contrasts.

RESULTS

ChAT Levels. The levels of ChAT activity in the rats with lesions were compared to the values in rats with CON operations to determine the percentage decrease in activity caused by the lesion. The levels of ChAT activity for the rats with NBM-IBO lesions sacrificed 10 days after surgery are shown in Table 1. IBO lesions decreased the levels of ChAT activity in the frontal cortex, ($p < .01$), but did not affect levels of ChAT activity in either the dorsal or ventral hippocampus, ($p < .05$).

The levels of ChAT activity in the frontal cortex and hippocampus of rats that received behavioral testing are summarized in Table 2. For CON rats, the mean levels of ChAT activity of both the frontal cortex and hippocampus were significantly lower for the IBO section of the experiment than for the RF section, ($p < .01$). Therefore, the

data for the rats with lesions were compared to that of the CON rats in their own section. NBM lesions, produced by both IBO and RF, significantly decreased levels of ChAT activity in the frontal cortex but not in the dorsal hippocampus, ($p < .01$). MSA lesions, produced by both IBO and RF, significantly decreased levels of ChAT activity in the hippocampus but not in the frontal cortex, ($p < .01$). NBM+MSA lesions significantly decreased levels of ChAT activity in both the frontal cortex and hippocampus, ($p < .01$). DGP lesions did not significantly alter levels of ChAT activity in either the frontal cortex or hippocampus (99 and 100% of control regions, respectively).

Histology.

Lesions in the NBM were centered in the substantia innominata for both the NBM-IBO and NBM-RF groups and extended 2.0 mm caudally from the anterior commissure. A typical set of lesions is shown schematically in Figure 2. IBO lesions in the MSA destroyed most of the cells of the medial septum and the dorsal part of the vertical limb of the diagonal band. RF lesions in the MSA destroyed only the medial septal area. Lesions in the DGP involved the most dorsal extent of the globus pallidus and striatum just below the corpus callosum (see Figure 3).

Behavior

Preoperative

Choice accuracy in both discriminations was near the level expected by chance for all rats at the start of preoperative testing. Within ten pre-operative test sessions, however, every rat performed at or above criterion levels in both the stem and arm discriminations.

Postoperative

After the second lesion, many of the rats in the NBM-IBO and NBM-RF groups showed normal posture but did not eat, drink, or groom. These rats were intubated and fed intragastrically (SMA, Wyeth Lab.Inc., Philadelphia, PA: 10 cc every 6 hr). Four rats with IBO lesions, and 5 rats with RF lesions never recovered and subsequently died. Rats who did recover usually began eating about one week after the contralateral lesion. Rats in the MSA-IBO and MSA-RF groups were generally as healthy as the CON rats immediately after surgery. Two rats in the MSA-IBO group died several days later. All rats in the NBM+MSA group showed normal posture, eating, and drinking by the third day after surgery.

Because the behavioral data for the CON groups from both the IBO and RF sections of the experiment were not significantly different, they were combined, and all the behavioral data were analyzed together in a three-way ANOVA. The percent of total responses that were correct for each test session was averaged into 2-day blocks for the analysis.

Stem Discrimination.

CON rats continued criterion level choice accuracy (see Figure 4). All six groups of rats with lesions also continued criterion level choice accuracy. No main effect or interaction was significant, ($F < 1$).

Arm Discrimination.

CON and DGP rats had only a small transient decrease in choice accuracy (see Figure 5). They returned to criterion performance in a mean of 3.8 test sessions. At the start of postoperative testing, NBM, MSA, and NBM+MSA groups had significant decreases in choice accuracy. These rats relearned the task and reached criterion. The mean number of trials to criterion for each group of rats was: NBM-IBO, 9.6; NBM-RF, 9.8; MSA-IBO, 13.4; MSA-RF, 15.8; NBM+MSA, 13.8 (see Figure 4).

Analysis of variance showed that choice accuracy for all groups improved during testing, [$F(8,764) = 354.33$, $p < .01$]. The choice accuracy for rats with lesions in the NBM, MSA, and NBM+MSA was impaired relative to that of CON rats [$F(6,764) = 798.54$, $p < .01$]. The choice accuracy of rats with MSA lesions was impaired relative to that of rats with NBM lesions, ($p < .05$). A Group by Test Session effect, [$F(48,764) = 71.01$, $p < .01$], and Sheffe contrasts revealed that the choice accuracy of rats with NBM, MSA, and NBM+MSA lesions was significantly impaired relative to that of CON rats for blocks 1 through 7 inclusive, ($p < .05$). The choice accuracy of rats with MSA and NBM+MSA lesions was impaired relative to that of rats with NBM lesions for blocks 3 through 7, inclusive, ($p < .05$). No significant difference in choice accuracy was found between the MSA and NBM+MSA groups.

Rats with RF lesions in the NBM and MSA had impaired choice accuracy relative to rats with IBO lesions in these areas [$F(1,622) = 5.13$, $p < .05$]. A Lesion by Test Session effect, [$F(8,622) = 14.16$, $p < 0.01$], and Sheffe contrasts revealed that the rats in the NBM-RF and MSA-RF groups had impaired choice accuracy relative to rats in the NBM-IBO and MSA-IBO groups for blocks 3 through 7 inclusive, ($p < .05$).

Finally, a Lesion by Group by Test Session effect was found, [$F(16, 622) = 4.19$, $p < .01$]. This interaction was due to the MSA-RF lesion group which had impaired choice accuracy relative to the MSA-IBO lesion group for blocks 4 through 7 inclusive, ($p < .05$).

Reversal of Stem Discrimination.

For the first few trials of the reversed stem discrimination, every rat made errors in the stem. All rats quickly learned the reversal, and reached criterion. The CON group reached the criterion level in a mean of 4.2 test sessions. The mean number of trials to criterion for each lesion group was: DGP, 4.1; NBM-IBO, 4.6; NBM-RF, 4.4; MSA-IBO, 4.9; MSA-RF, 5.0; NBM+MSA, 10.5. Choice accuracy of the rats in the NBM+MSA group differed significantly from that of CON group [$F(6,511) = 36.00$, $p < .01$]. With the exception of Test Sessions, [$F(5,511) = 44.49$, $p < .01$], no other main effect or interaction was significant ($F < 1$).

Extended Delay

Stem Discrimination. With the initial delay of 5 sec., choice accuracy of rats in both the CON and NBM+MSA groups was at or above the criterion level (see Figure 5). When the delay was increased to 10 min., however, the choice accuracy of rats in the NBM+MSA group was slightly, but significantly, impaired relative to the performance of rats in the CON group [$F(1,52) = 4.29$, $p < .05$], and did not improve during the 18 test sessions with the 10 minute delay. When the delay was decreased to 5 sec, the mean number of trials for choice accuracy to return to criterion for rats with CON lesions (1.6) was not significantly different from the number required for rats with lesions (1.9).

Arm Discrimination. With the initial delay of 5 sec., the choice accuracy of rats in the CON and NBM+MSA groups was also at or above the criterion level. With the 10 min delay, however, the choice accuracy of rats in both groups was substantially decreased relative to their performance with the 5 sec delay [$F(1, 52) = 21.2$, $p < .01$]. Of greater importance, the choice accuracy of rats in the NBM+MSA group was markedly impaired relative to the performance of rats in the CON group [$F(1,52) = 33.28$, $p < .01$]. The choice accuracy of rats in both these groups did not improve during the 18 test sessions. When the delay was decreased to 5 sec, the mean number of trials for choice accuracy to return to criterion for rats with CON lesions (1.7) was not significantly different from the number required for rats with lesions (2.1).

DISCUSSION

The behavioral effects of lesions in either the NBM or the MSA were similar in all three behavioral tasks. Choice accuracy was 1) impaired in the retention of the trial-dependent discrimination on the arms of the T-maze, 2) unimpaired in the retention of the trial-independent discrimination on the stem of the T-maze, and 3) unimpaired in the reversal of this discimination (except for the NBM+MSA group which was slightly impaired). The impairment in the ability to choose the correct arm indicates that both of these brain structures normally mediate choice accuracy in this type of task. The frontal cortex and hippocampus may form a closely interrelated system for processing of different types of information about memory.

The pattern of behavioral changes observed here suggests that the rats suffered a specific impairment in memory. In order to perform the stem discrimination accurately, the rat had to identify the location in the maze where it had been placed, determine right from left, and respond correctly. Therefore, perceptual, motivational, and motoric processes were intact. What was not intact was the rat's ability to remember short term trial-specific information. This trial-dependent memory deficit can not be attributed to a general retrograde amnesia because the lesioned rats were unimpaired in the re-acquisition of the stem discrimination. Further, the dissociation of performance between the arm and stem discriminations can not be attributed to a difference in the relative difficulty of the two tasks because the

rats learned both discriminations at approximately the same rate. Thus, the pattern of dissociations suggests that rats with NBM, MSA, or NBM+MSA lesions had an amnesic syndrome with a specific impairment in trial-dependent (working) memory.

During the initial preoperative testing with the 5 second delay between the forced run and the choice run in the arm discrimination, all rats with lesions in either the NBM or MSA had impaired choice accuracy. However, all rats eventually reached criterion levels of accuracy. This improvement in performance did not reflect a complete recovery of function, however. When the memory requirement of the task was emphasized by increasing the delay from 5 seconds to 10 minutes, the impairment in the lesioned rats returned. This sensitivity of choice accuracy to the delay interval is additional support for the interpretation that these lesions produced a specific impairment in memory. All the components of the task were the same at the long delay as at the short delay except for the length of time the forced run had to be remembered. Consequently, all those processes necessary for normal performance at the short delay must have been intact, and the most likely explanation for the impairment at the longer delay involves the psychological processes that were related to the delay interval, and these are generally interpreted as memory. This interpretation is compromised slightly by the impairment on the stem discrimination during the extended delay. However, the magnitude of the deficit on the arm discrimination was much greater than that in the stem discrimination. The slight impairment in the stem discrimination may have been due to interference from the reversal of the stem discrimination that occurred just prior to the testing with the extended delay.

The recovery of behavioral performance with the short delay interval between the forced run and choice run may have been due to compensatory biochemical changes made by the brain in an attempt to adjust to the localized damage. Immediatlely after lesions in the basal forebrain, sodium-dependent high affinity choline uptake (20) and 2-deoxyglucose uptake were both decreased in the frontal cortex and hippocampus. Six weeks after the lesions both measures returned to control levels. Post-synaptic muscarinic receptors in the neocortex become supersensitive following basal forebrain lesions. Low affinity sites decreased immediately after the lesion and returned to normal in 3 weeks, while high affinity sites were increased chronically after the lesion. Therefore, the behaviorial recovery observed with the short delay may have been due to increased activity of the cholinergic neurons that survived the lesion, restored glucose utilization in the neocortex, or compensatory changes in postsynaptic muscarinic receptors. However, these biochemical changes were not sufficient to reestablish normal behavior because an impairment persisted with the long delay.

The impairment of choice accuracy in the arms was probably due to changes in the cholinergic system. The majority of the cells within the NBM are cholinergic (14). Furthermore, injections of IBO in the NBM with techniques similar to those used here reduced cholinergic markers in the neocortex but did not affect other neurochemical systems, including GABAergic neurons intrinsic to cortex as well as noradrenergic, serotonergic and histaminergic afferents. Similar neurochemical data are not available following injections of IBO into the MSA. However,

anticholinergic drugs produced an impairment of choice accuracy in memory tasks that required trial-dependent memory. Individual assessment of all other transmitter systems is necessary before any final conclusions can be drawn. In any case, the similar behavioral effects produced by the RF and IBO lesions show that the cell bodies themselves (whatever their transmitter) in the NBM and MSA are functionally important for normal choice accuracy in the arm discrimination; damage to the fibers of passage in these areas is not necessary to obtain memory impairments.

Lesions in either the NBM and MSA produced similar behavioral deficits. However, this result was not due to similar effects on ChAT levels. Lesions in the NBM significantly decreased ChAT levels in the frontal cortex but not the dorsal or ventral hippocampus, while lesions in the MSA produced the opposite effect. Consequently, the lesions themselves were clearly distinct both in terms of their placement and their effect on cholinergic projections.

The large difference in ChAT levels between the control groups of the various sections of the present study was probably due to the different times that the rats were obtained from the breeders. ChAT levels and sodium-dependent high affinity choline uptake have varied between groups of rats obtained *at different times from the same breeder* (unpublished observations). Such variability does not affect the interpretation of the ChAT data within each section, because appropriate control rats were included from each shipment.

DGP lesions had no significant effect on choice accuracy in any of the discriminations, nor did they significantly reduce ChAT levels in the frontal cortex or hippocampus. These results are important because they demonstrate that the behavioral changes following injections of IBO into the NBM and MSA were due to selective damage of neuronal cell bodies located in those areas, rather than to IBO damage to the brain or destruction of contiguous portion of the GP.

Table I. Choline acetyltransferase levels (mean ± S.E.M) in frontal cortex, dorsal hippocampus, and ventral hippocampus for rats with lesions produced by ibotenic acid lesions.

Group	N	Choline acetyltransferase (nmol/mg protein/h) frontal cortex	hippocampus dorsal	hippocampus ventral
Control	4	22.70 ± 0.61	27.00 ± 1.49	30.85 ± 1.04
NBM	4	11.78 ± 2.06**	26.27 ± 1.31	30.20 ± 1.47

**$p < 0.01$ compared to corresponding region of control rats using Student's two-tailed test. These rats received no behavioral testing and were sacrificed 10 days after the lesions were placed.

Table 2. Choline acetyltransferase levels (mean ± S.E.M) in frontal cortex and dorsal hippocampus for rats with lesions produced by ibotenic acid (top) and radio-frequency current (bottom).

Ibotenic acid

Group	N	Choline acetyltransferase (nmol/mg protein/h) Frontal Cortex	Hippocampus
Control	26	22.85 ± 1.49	29.53 ± 1.99
NBM	7	9.91 ± 2.36**	27.72 ± 2.36
MSA	7	24.47 ± 2.10	14.86 ± 3.03**
NBM+MSA	9	14.10 ± 1.16**	19.32 ± 0.96**

**$P < 0.01$ compared to corresponding region of control rats using Student's two-tailed test.

Table 2 (cont.)

		Radio-frequency	
		Choline acetyltransferase (nmol/mg protein/h)	
Group	N	Frontal Cortex	Hippocampus
Control	13	31.94 ± 1.07	36.20 ± 1.36
NBM	12	24.03 ± 2.10**	37.41 ± 3.12
MSA	10	26.91 ± 2.01	22.90 ± 2.97**

**P < 0.01 compared to corresponding region of control rats using Student's two-tailed test.

Figure 1.

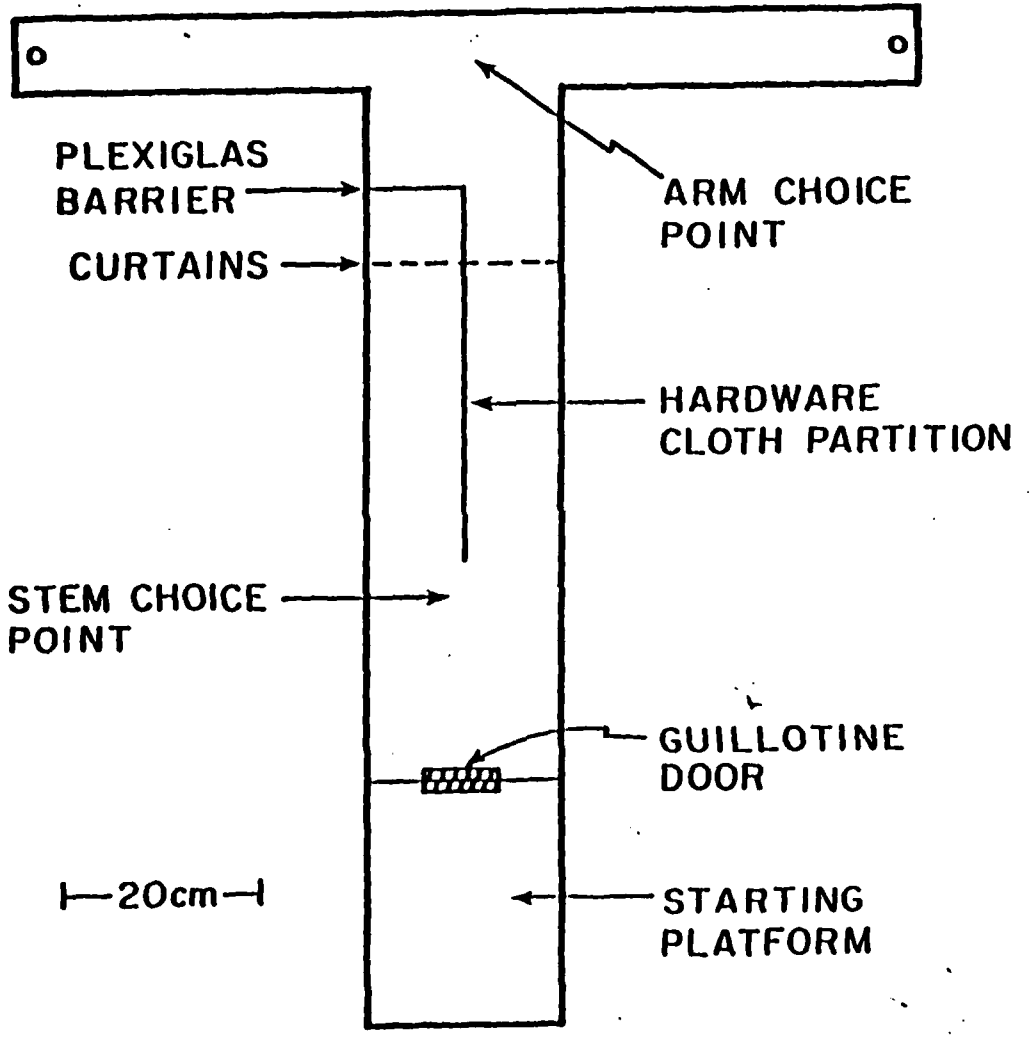

PLEXIGLAS
BARRIER
CURTAINS
ARM CHOICE
POINT
HARDWARE
CLOTH PARTITION
STEM CHOICE
POINT
GUILLOTINE
DOOR
20cm
STARTING
PLATFORM

Figure 2

NBM
IBOTENIC ACID
RADIOFREQUENCY CURRENT
MSA
IBOTENIC ACID
RADIOFREQUENCY CURRENT

Figure 3

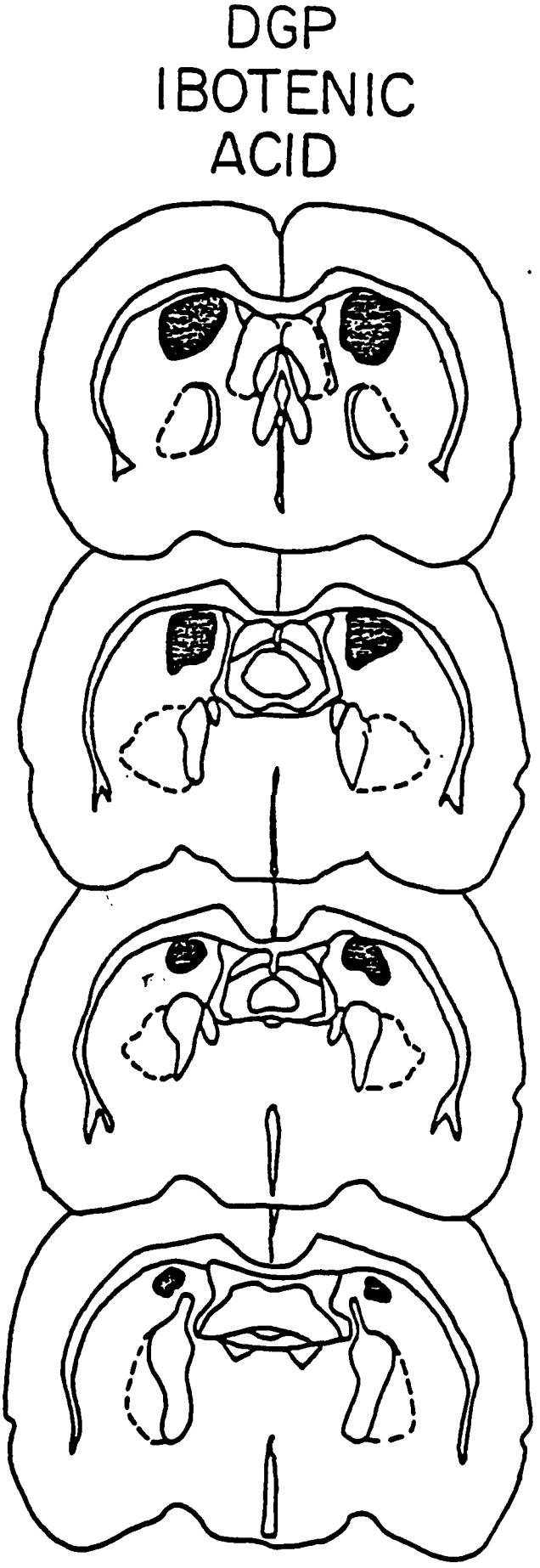


Figure 4

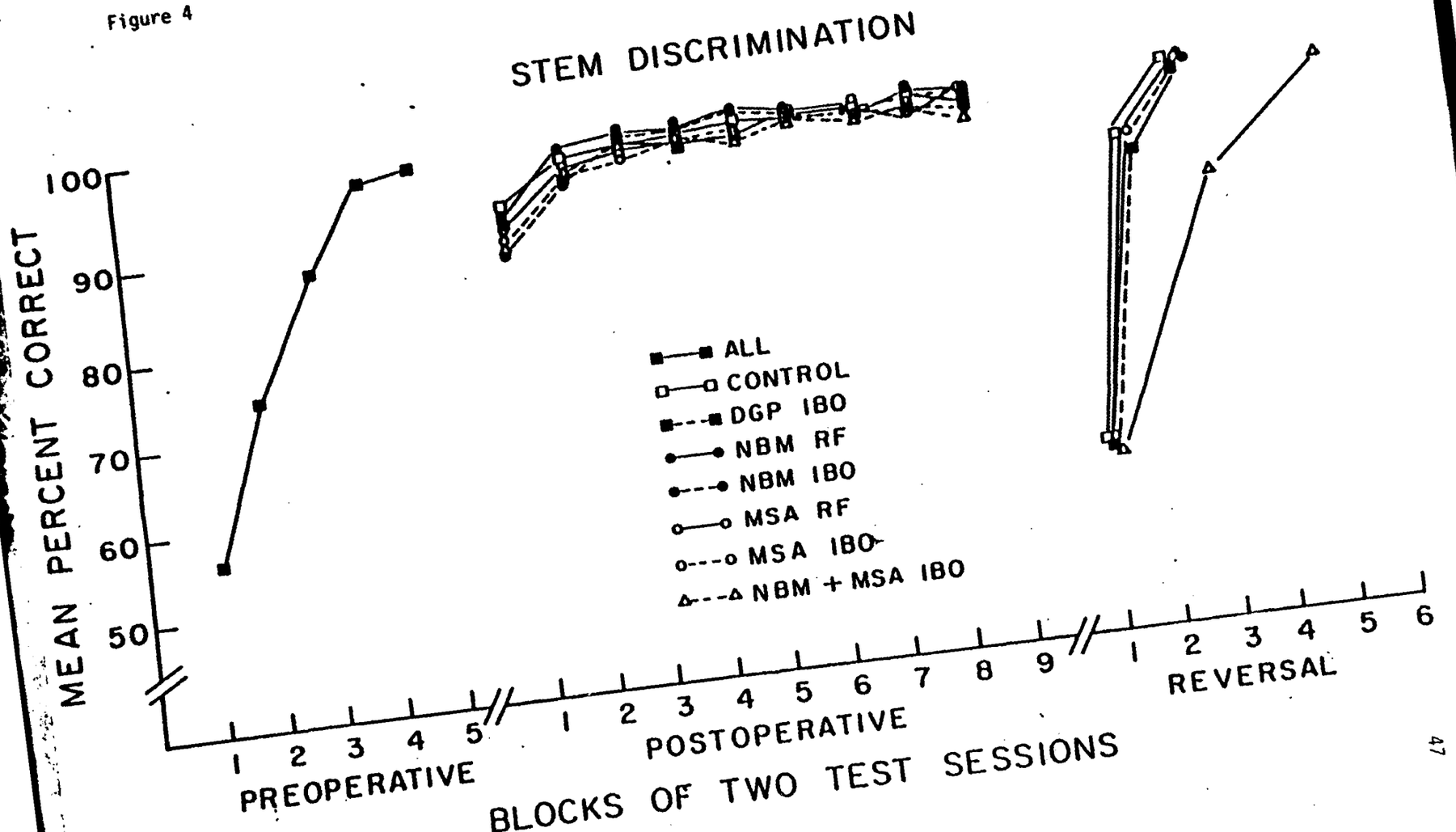

STEM DISCRIMINATION
MEAN PERCENT CORRECT
100
90
80
70
60
50
ALL
CONTROL
DGP IBO
NBM RF
NBM IBO
MSA RF
MSA IBO
NBM + MSA IBO
1 2 3 4 5
PREOPERATIVE
1 2 3 4 5 6 7 8 9
POSTOPERATIVE
1 2 3 4 5 6
REVERSAL
BLOCKS OF TWO TEST SESSIONS

Figure 5

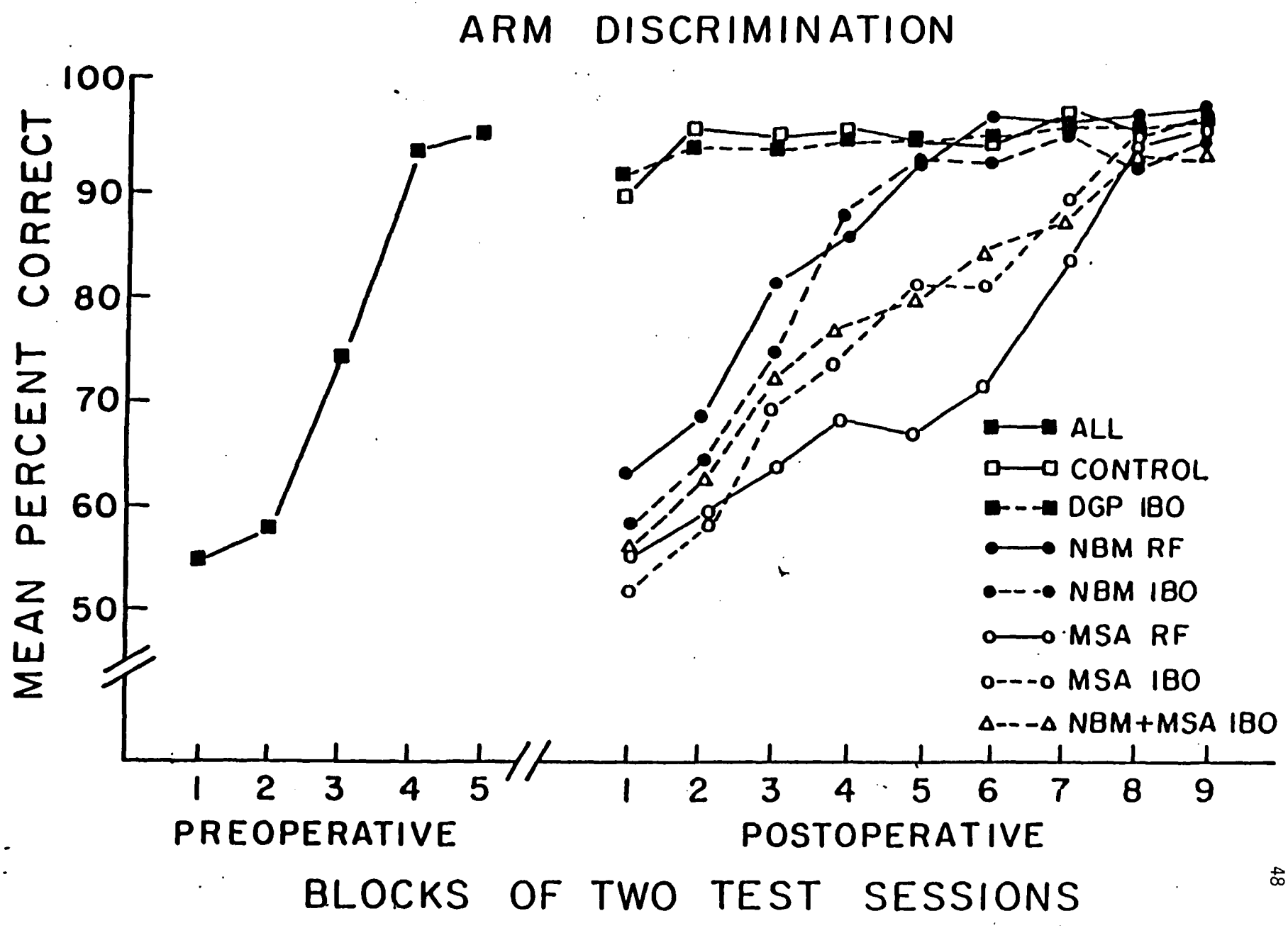

ARM DISCRIMINATION
MEAN PERCENT CORRECT
100
90
80
70
60
50
ALL
CONTROL
DGP IBO
NBM RF
NBM IBO
MSA RF
MSA IBO
NBM+MSA IBO
1 2 3 4 5
1 2 3 4 5 6 7 8 9
PREOPERATIVE
POSTOPERATIVE
BLOCKS OF TWO TEST SESSIONS

Figure 6

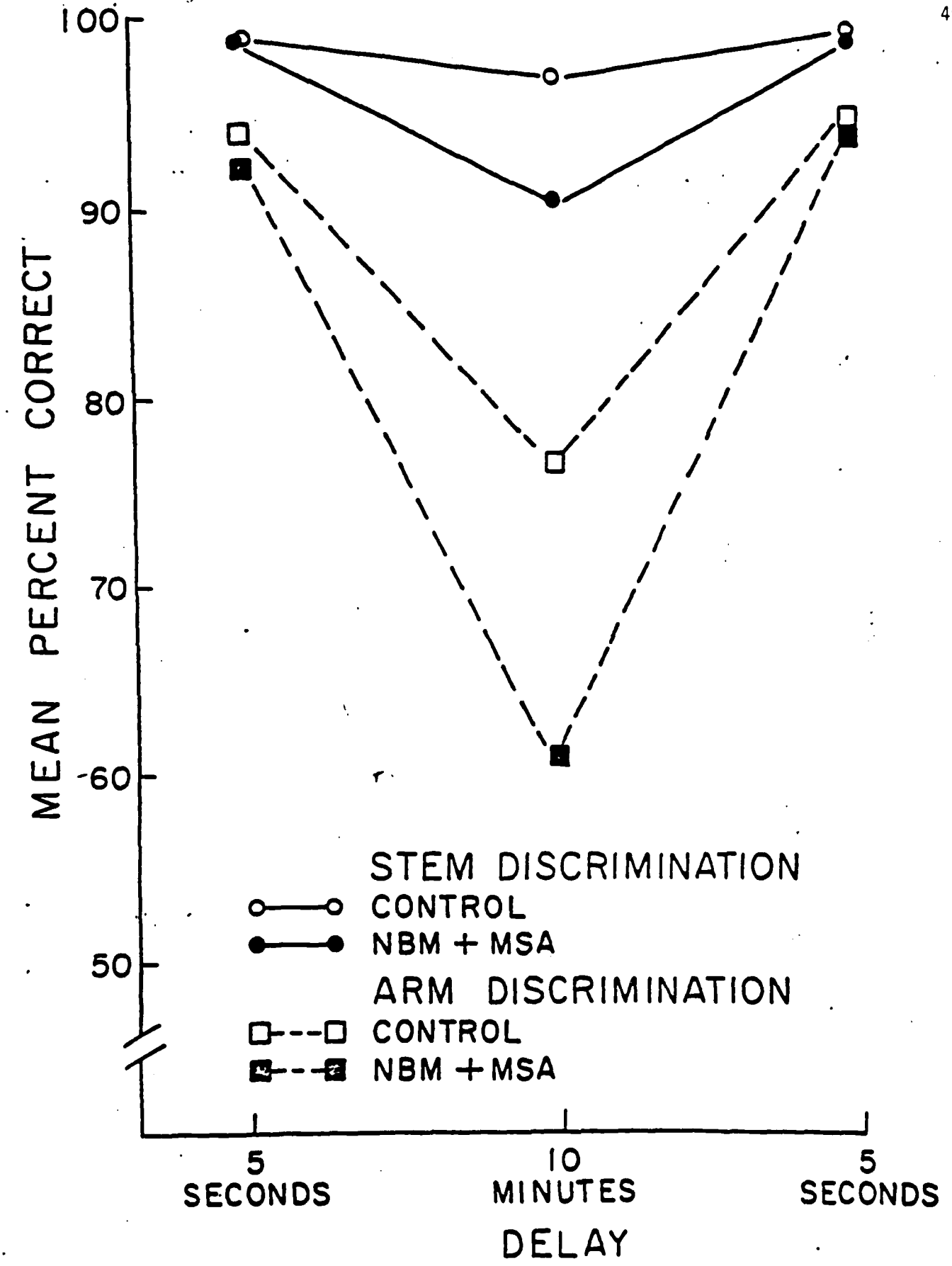

100
90
80
70
60
50
MEAN PERCENT CORRECT
STEM DISCRIMINATION
CONTROL
NBM + MSA
ARM DISCRIMINATION
CONTROL
NBM + MSA
5
SECONDS
10
MINUTES
5
SECONDS
DELAY

FIGURE CAPTIONS

Figure 1. Scale drawing of the apparatus. See text for detailed description.

Figure 2. Drawings of coronal sections showing typical IBO and RF lesions in the NBM (left), MSA (right). Shaded areas indicate area of cell loss.

Figure 3. Drawings of coronal sections showing a typical IBO lesions of the DGP. Shaded areas indicate area of cell loss.

Figure 4. Summary of the behavioral data from the stem discrimination. The vertical axis presents the mean percent correct choices. The horizontal axis presents blocks of two test sessions. Preoperatively, all rats learned the stem discrimination within ten test sessions. Postoperatively, the choice accuracy on the stem was unimpaired for all groups of rats. All groups of rats acquired the stem reversal at the same rate, reaching criterion by the fifth test session (except for the NBM+MSA group).

Figure 5. Summary of behavioral data from the arm discrimination. The vertical axis presents the mean percent correct choices. The horizontal axis presents blocks of two test sessions. Preoperatively, all rats learned the arm discrimination within ten test sessions. Postoperatively, the choice accuracy on the arms was impaired during the first several blocks of test sessions following NBM and MSA lesions. Choice accuracy rapidly improved to criterion levels.

Figure 6. Summary of behavioral data from the extended delay task. The vertical axis presents the mean percent correct choices. The horizontal axis presents the different delays (6 test sessions for each of the 5 sec delays, 18 test sessions for the 10 min delay). At the 5 sec delay, all rats performed at or above criterion level of performance on both the stem and arm discriminations. At the 10 min delay, the choice accuracy of the NBM+MSA group was silghtly impaired relative to that of the CON group in the stem discrimination, and greatly impaired in the arm discrimination. All rats quickly returned to the criterion level of performance when the delay was decreased to 5 sec.

Appendix: 5

RECOVERY OF NEOCORTICAL CHOLINE ACETYLTRANSFERASE ACTIVITY FOLLOWING IBOTENIC ACID INJECTION INTO THE NUCLEUS BASALIS OF MEYNERT IN RATS

BACKGROUND

The NBM contains magnocellular Ch neurons which project primarily to ipsilateral neocortex (14). The destruction of these Ch neurons by electrocoagulation (16) or by injection of ibotenic acid produces a significant decrease in acetylcholine production and ChAT activity in the ipsilateral neocortex.

The present report shows that the decrease in ChAT in the neocortex following a lesion of the NBM is transitory in rats. Following the lesion, ChAT levels gradually increased until after three months they had returned to normal. These results indicate that the NBM Ch projections to the frontal cortex exhibit considerable plasticity.

METHODS

Male, Sprague-Dawley rats (120 days old, 250 g) were anaesthetized with Chloropent (Fort Dodge Labs, Fort Dodge, IA) and placed in a stereotaxic apparatus with the incisor bar set at 2 mm below the intra-auricular line. Ibotenic acid (0.5 ul in 50 mM sodium phosphate, pH 7.4; 50 nanomoles) was injected into the left NBM using a Hamilton syringe. Coordinates for the injection were A: 6.4 mm, L: 2.5 mm, V: 6.5 mm, from the intra-auricular line, midline and dura, respectively. At selected times after the operation, rats were sacrificed by decapitation. The neocortex and hippocampus were dissected on ice and assayed for ChAT activity according to the method of Fonnum (8). Protein was measured according to the method of Lowry et al., (9). The neocortical sample (4 mm sq.; approx. 50 to 75 mg) was taken from a region immediately lateral to cingulate cortex and anterior to motor cortex. Preliminary studies had shown that the decrease in ChAT activity was greatest in this area of the cortex following and IBO injection at the coordinates described above. A selective inhibitor of ChAT enzyme (hydroxyethyl-4-naphthyvinyl pyridium; NVP, Calbiochem) was included in certain assays to determine that the acetyltransferase activity measured was due to the presence of ChAT.

The placement and extent of the lesions was confirmed by placing the remainder of the brain in 10% formalin:30% sucrose. The area through the NBM was sectioned into 30 micron slices and stained with cresyl violet.

RESULTS

One week after the operation, ChAT activity decreased 60 percent in the frontolateral neocortex, ipsilateral to the lesion, as compared to the contralateral side. ChAT activity in the neocortex increased gradually during three months until the levels in the lesioned and unlesioned hemispheres were the same (see figure I).

Sodium-dependent high affinity choline uptake (11) in the fronto-

lateral neocortex initially decreased 45 percent. It then increased to control levels during the next four weeks.

Histological examination of the brain shoed extensive cell loss in the NBM. The lesion did not affect the Ch neurons in the MSA or diagonal band of Broca, as confirmed by the fact that hippocampal ChAT levels did not change from normal (59 .68 $\pm$ 7.62 nanomoles/mg protein/hour). At the light-microscopic level, the post-operative appearance of the lesion was similar at one week and twelve weeks. The lesion extended 1.5 mm in all direction from the injection site at the level of the ventral globus pallidus.

Addition os NVP decreased in vitro acetyltransferase activity to 4 percent of control levels. The ChAT levels in the cortex were determined to be due to the presence of Ch neuronal terminals.

DISCUSSION

Surviving NBM Ch neurons may contribute to the ChAT recovery by arborizing at their cortical terminals in a manner similar to the long-term compensatory collateral sprouting described in the hippocampus following partial dennervation (7). Alternatively, ChAT enzyme levels within surviving cortical neuronal terminals may have had a compensatory increase. Research is in progress to determine the precise nature of the processes underlying, and the behavioral implications of, this recovery.

Figure 1.

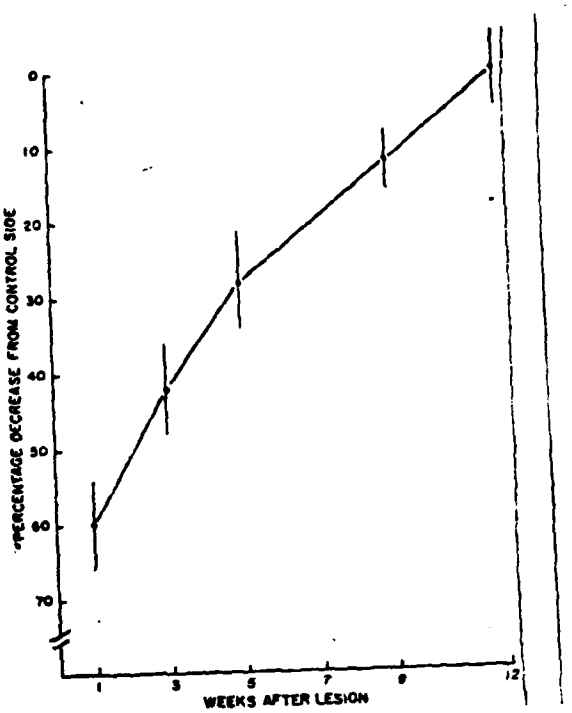

0
10
20
30
40
50
60
70
PERCENTAGE DECREASE FROM CONTROL SIDE
1
3
5
7
9
12
WEEKS AFTER LESION

FIGURE CAPTION

Figure I. Gradual increase in neocortical ChAT levels with increasing post-operative recovery time. The left side of the brain had a lesion in the NBM produced by IBO. The right side was intact. The vertical axis show the percentage difference between the level of ChAT activity in the left and right frontolateral neocortex. The horizontal axis show the number of weeks between the lesion and date of sacrifice. Data points indicate mean $\pm$ SEM for at least four rats. The first and last data points include data from at least 10 rats.

REFERENCES

1. Stenevi, U., A. Bjorklund, R.Y. Moore (1972) Growth of intact central adrenergic axons in the denervated lateral geniculate body. Exp. Neurol. 35:290-299.
2. Moore, R.Y., A. Bjorklund, U. Stenevi (1971) Plastic changes in the adrenergic innervation of the septal area in response to denervation. Brain Res. 33:13-35.
3. Lynch, G. D.A. Matthews, S. Mosko, T. Parks, C.W. Cotman (1972) Induced acetylcholine-esterase-rich layer in rat dentate gyrus following entorhinal lesions. Brain Res. 42:311-318.
4. Pickel, V., H. Krebs, F.E. Bloom (1973) Proliferation of nor epinephrine--containing axons in rat cerebellar cortex after peduncle lesions. Brain Res. 59:169-179.
5. Nakamura, Y., N. Mizuno, A. Konishi, M. Sato (1974) Synaptic reorganization of the red nucleus after chronic deafferentation from cerebellorubral fibers: an electron microscope study in the cat. Brain Res. 82:298-301.
6. Wall, P.D., M.D. Egger (1971) Formation of new connections in adult rat brains after partial deafferentation. Nature 232:542-545.
7. Gage, F.H., A. Bjorklund, U. Stenevi (1983) Reinnervation of the patially deafferented hippocampus by compensatory collateral sprouting from spared cholinergic and noradrendergic afferents. Brain Res. 268:27-37.
8. Fonnum, F. (1969) Radiochemical micro assays for the determination of choline acetyltransferase and acetylcholinesterase activities. Biochem. J., 115:465-472.
9. Lowry, O.H., N.J. Rosebrough, A.C. Farr, R.S. Randall (1951) Protein measurement with the Folin phenil reagent. J. Biol. Chem. 193:265-275.
10. Olton, D.S., J.T. Becker, G.E. Handelmann (1979) Hippocampus, space, and memory. Behav. Brain Sci. 2:313-365.
11. Atweh, S., Simon, J.R. and Kuhar, M.J., Utilization of sodium-dependent high affinity choline uptake in vitro as a measure of the activity of cholinergic neurons in vivo, Life Sci., 17:(1976)1535-1544.
12. Raaijmakers, W.G.M., Active avoidance learning and choline uptake in rat hippocampal synaptosomes, Neurosci. Lett., (suppl) 1:(1978)86.
13. Burgel, P. and Rommelspacher, H., Changes in high affinity choline uptake in behavioral experiments, Life Sci., 23:(1978)2423-2428.
14. Saper, C.B. (1984) Organization of cerebral cortical afferent systems in the rat. I. Magnocellular basal nucleus. J. comp. Neurol., 222:313-342.
15. Lehmann J, Nagy JI, Atmadja S, Fibiger HC (1980) The nucleus basalis magnocellularis: The origin of a cholinergic projection to the neocortex of the rat. Neurosci. 5:1161-1174.
16. Lo Conte G, Bartolini L, Casamenti F, Marconcini-Pepeu I, Pepeu G (1982) Lesions of cholinergic forebrain nuclei: changes in avoidance behavior and scopolamine actions. Pharmacol. Biochem. Behav. 17:933-937.
17. Lewis PR, Shute CCD (1967b) The cholinergic limbic system: brain. J. Neurol. 90:521-540.
18. Bartus, R.T., Dean, R.L., Beer, B. and Lippa A. S. (1982) The

cholinergic hypothesis of geriatric memory dysfunction: A critical review. Science, 47: 408-417.
19. Mitchell, S. J., Rawlins, J. N. P., Steward, O. and Olton, D. S. (1982) Medial septal area lesions disrupt theta rhythm and cholinergic staining in medial entorhinal cortex and produce impaired radial arm maze behavior in rats. J. Neurosci., 2: 292-302.
20. Wenk, G and Olton, D. (1984) Recovery of neocortical choline acetyltransferase activity following ibotenic acid injection into the nucleus basalis of Meynert in rats. Brain Res., 293:184-186.

DISTRIBUTION LIST

4 copies	Commander US Army Medical Research and Development Command ATTN: SGRD-RMS Fort Detrick, Frederick, Maryland 21701-5012
5 copies	Commander US Army Medical Research and Development Command ATTN: SGRD-PLE Fort Detrick, Frederick, Maryland 21701-5012
12 copies	Defense Technical Information Center (DTIC) ATTN: DTIC-DDAC Cameron Station Alexandria, VA 22304-6145
1 copy	Dean School of Medicine Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, MD 20814-4799
1 copy	Commandant Academy of Health Sciences, US Army ATTN: AHS-CDM Fort Sam Houston, TX 78234-6100