



1965

The Effect of Bilateral Fornix Lesions upon the Learning and Retention of an Olfactory and Auditory Discrimination in the Albino Rat

John F. Snyder
Loyola University Chicago

Recommended Citation

Snyder, John F., "The Effect of Bilateral Fornix Lesions upon the Learning and Retention of an Olfactory and Auditory Discrimination in the Albino Rat" (1965). *Dissertations*. Paper 805.
http://ecommons.luc.edu/luc_diss/805

This Dissertation is brought to you for free and open access by the Theses and Dissertations at Loyola eCommons. It has been accepted for inclusion in Dissertations by an authorized administrator of Loyola eCommons. For more information, please contact ecommons@luc.edu.



This work is licensed under a [Creative Commons Attribution-Noncommercial-No Derivative Works 3.0 License](https://creativecommons.org/licenses/by-nc-nd/3.0/).
Copyright © 1965 John F. Snyder

**THE EFFECT OF BILATERAL FORNIX LESIONS UPON THE
LEARNING AND RETENTION OF AN OLFACTORY
AND AUDITORY DISCRIMINATION
IN THE ALBINO RAT**

BY

John P. Snyder

**A Dissertation Submitted to the Faculty of the Graduate
School of Loyola University in Partial Fulfill-
ment of the Requirements for the Degree
of Doctor of Philosophy**

June

1965

LIFE

John F. Snyder was born in Chicago, Illinois, on March 29, 1933.

He was graduated from De La Salle High School, Chicago, Illinois, in June 1951.

In June 1954, he received a Bachelor of Science degree from St. Mary's College, Winona, Minnesota.

From February 1959 until June 1963, he was a full-time student in the Department of Psychology at Loyola University, Chicago, Illinois. He was awarded a National Defense and Education Act (1955) Fellowship for the years 1960-1962.

He was on the staff of the Behavior Laboratory during these years and was Assistant Director from June 1963 until September 1964.

He held the rank of Instructor on the Loyola University Faculty from September 1963 until June 1965.

ACKNOWLEDGEMENTS

The writer wishes to express his sincere appreciation

To Magda B. Arnold, Professor of Psychology, Loyola University,
for her suggestions and direction of this study.

To Reverend V. V. Herr, S.J., Chairman of the Department of
Psychology, especially for his guidance and assistance.

To Reverend Michael J. O'Brien, C.S.V., for his challenge and
kindness.

To his colleagues in the Loyola Behavior Laboratory, Gerald Driessen,
Thomas Planek, Margaret Condon, Victor Dufour, William Pizzi, John Coneely,
and Jose Lamas whose friendship and assistance were invaluable.

To the faculty of Loyola University for their stimulation and
encouragement.

To his wife Mary Kay, for her typing and psychometric assistance,
and most especially, for her total involvement in the completion of the
dissertation.

This study was supported by the Illinois Psychiatric Training and
Research Authority as a part of project 1791, The Role of the Hippocampal
System in Learning; grantee: Dr. Magda B. Arnold.

TABLE OF CONTENTS

Chapter		Page
1	Introduction	1
2	Review of Related Literature	10
3	Procedure	19
4	Results	33
5	Discussion and Summary	55
6	References	64

LIST OF TABLES

Tables		Page
1	Mean preoperative learning and postoperative retention scores for all groups on auditory-vibratory discrimination	35
2	Postoperative retention scores for animals run alternately on auditory-vibratory (speaker-in) discrimination and auditory only (speaker-out) discrimination	37
3	Mean preoperative learning and postoperative retention scores for all groups on olfactory discrimination	39
4	Mean postoperative learning scores for all groups on auditory-vibratory discrimination	40
5	Postoperative learning scores for animals run alternately on auditory-vibratory (speaker-in) discrimination and auditory only (speaker-out) discrimination	41
6	Mean postoperative learning scores for all groups on olfactory discrimination	42
7	Mean postoperative learning scores for all groups on olfactory-somesthetic discrimination	43
8	Mean postoperative learning scores for all groups on motor discrimination	44
9	Mean postoperative learning scores for all groups on visual discrimination	45
10	Mean postoperative learning scores for all groups on tactual discrimination	46
11	Raw scores achieved by each experimental animal for all discriminations	48
12	Behavior deficits on each discrimination for each animal .	49

LIST OF FIGURES

Figures		Page
1	Schematic diagram of rat brain showing lesion sites . . .	7
2	Lateral and dorsal views of hippocampal formation in several species	9
3	Two views of the apparatus used for olfactory discrimination	21
4	Apparatus used for olfactory-somesthetic discrimination	23
5	Four test chambers (Skinner boxes) used for both the auditory and visual discriminations	23
6	Apparatus used for the motor discrimination	26
7	Apparatus used for the tactual discrimination	26
8	Three views of the brain of experimental animal 0-9 . . .	51
9	Photographs of stained brain sections of experimental animals with anterior-posterior designations . . .	52

CHAPTER I

INTRODUCTION

Memory, as a process and primary function, is involved in the learning and retention of both humans and animals. In recent years, there has been an abundance of interdisciplinary investigations which have emphasized the intention to uncover the electro-chemical mechanisms of memory registration, and the sites of this registration. The general trend has been the extirpation of more and more brain tissue without obtaining a clear deficit. Even the studies that have concentrated on one memory modality reported significant discrimination deficits only when the larger part of the association cortex serving this modality had been removed. As a result, research of this kind has gone out of vogue. The 1961 American Psychological Association Convention listed eight major papers concerning this subject, 1962 listed one paper, and 1964 none. There are, however, patients with severe memory defects in clinics today who, upon neurological examination, are suspected of having brain lesions much smaller than the 99% of association cortex which many studies indicate would have to be gone in order to explain such a deficit. What seems to have been generally overlooked is an inquiry regarding the re-activation of the memory trace. This is precisely why the present study is concerned with how the brain mediates recall and what circuit is necessary for it. Moreover, its intent is to demonstrate that a very small lesion interrupting this circuit can prevent recall which is necessary for both

learning and retention.

Another indication of the Zeitgeist is that many contemporary investigators of memory have been overly concerned with blindly damaging or stimulating nervous mechanisms just to see what would happen. While many have hunches as to some expected behavior changes, few have testable hypotheses and even fewer have a coherently organized and substantial theoretical framework. The present writer feels that in order to embark upon an efficient and meaningful investigation, a preparation comparable to the following would be necessary.

1. A compilation and integration of the data from relevant studies separated partially from their discrete interpretations which have been nearly as varied as the studies themselves. A consolidation of the abstracted essentials, characteristic of trends and communalities.

2. Construction of a theory which:

- a. attempts to synthesize and relate known memory function with anatomically identifiable nervous structures.
- b. is consistent with all the available data.
- c. is experimentally verifiable both behaviorally and neurophysiologically by its production of testable hypotheses.

3. Testing of the theory in all its ramifications including replication, and incorporation of data with a view to eventual theory modification and sharpening.

Arnold's theory of brain function (1960) meets the above criteria. It provides a phenomenological analysis of human experience which relates specific behavior to proposed definitive neurophysiologic circuits. According to Arnold, a complete sequence from perception to overt action is accomplished in the following manner. Something experienced is appraised as "good to know"; this spontaneously initiates the recall of past experiences relevant to the present circumstances. The result or effect of these past experiences in regard to action taken previously is in turn appraised. This initiates imagination of what could be done here and now together with what could be expected from this possible action. When these recalled past actions and presently imagined possibilities are appraised, there is the initiation of an action impulse which can then lead to overt response. Arnold states that the neurophysiologic mediation of the above sequence involves rhinencephalic structures.

Each sense impression is received in primary sensory cortex and registered as a neurophysiologic event in the nearest association cortex. The next time this sense impression is experienced, sensory projections arrive at primary cortex and concurrently signal reactivation of the formerly registered impression in association cortex. By this process, recognition of the past experience occurs. The recall of past sensations, according to Arnold, however, involves the hippocampal system in the following manner.

Impulses from sensory cortical areas are appraised in adjacent limbic cortex. This appraisal initiates impulses to the nearest point in the hippocampal circuit (hippocampal rudiment or hippocampus proper). These impulses are then transmitted via fornix, brainstem, and sensory relay nuclei in the thalamus back to cortical association areas, and so mediate recall.

Thus the hippocampal system (hippocampus, hippocampal rudiment, and fornix) mediates the initiation of memory recall while the limbic system (subcallosal, cingulate, retrosplenial, and hippocampal gyri and the island of Reil) mediates the appraisal of both past and imagined experiences as well as the registration of affective memory.

Memory then, is modality-specific. It depends on the areas of registration (association cortex) and on the circuit that mediates recall. While large cortical areas would have to be obliterated to eliminate registered memories of a given modality, recall of such memories can be disturbed by the comparatively small lesions necessary to transact this circuit at a given point. Impulses from olfactory, motor, somesthetic and gustatory areas can flow via the subcallosal and cingulate gyri into the hippocampal rudiment as it moves posteriorly over the dorsal surface of the corpus callosum. The auditory and visual impulses are thought to pass via the hippocampal and retrosplenial gyri into the hippocampus and through the fimbria of the hippocampus to its anterior extension, the fornix. When a lesion of the hippocampal rudiment or the hippocampus produces a memory loss,

it should be modality-specific, depending on the precise locus of the lesion.

PURPOSE

The purpose of the present dissertation is to investigate that aspect of Arnold's theory which states that the hippocampal system (i.e., hippocampal rudiment, hippocampus, and fornix) is necessary for recall in the various sense modalities. Specifically, this study will attempt to demonstrate the differential effects on the behavior of the albino rat of a bilateral lesion severing the post-commissural fornices at their most anterior aspect and the immediately dorsal pre-commissural fornices. According to Arnold's theory, this lesion will prevent the recall of sensations in all sense modalities.

HYPOTHESIS:

As a first step in testing the above, it is hypothesized that the aforementioned lesion will prevent recall based on olfactory, motor, tactual, visual and auditory cues. If this hypothesis is correct, appropriately lesioned rats should neither be able to learn nor retain responses necessary to the performance of sensory discriminations in any of the sense modalities.

This study is primarily concerned with the two sense modalities of olfaction and audition. These were chosen in order to represent both projections of the hippocampal system (e.g., hippocampal rudiment for olfactory discrimination and the hippocampus for auditory discrimination) into the fornix. While it will remain for a future investigator to test more fully

the hypothesis with regard to motor, tactual and visual discriminations, a few animals were tested on all discriminations. This was done with the intent of procuring information which will act as a guide to future research.

The present study is the fifth in an initial series of five designed to test separate aspects of Arnold's theory regarding the relay of memory by the hippocampal system. Figure 1 shows the lesion sites for each of the five experiments. In experiment one, the hippocampal rudiment was bilaterally interrupted at the genu of the corpus callosum; in experiment two, the same structure was cut caudal to the motor cortex; in experiment three, the same structure was cut at the splenium of the corpus callosum; in experiment four, the hippocampus was bilaterally transected approximately half way between its lateral tip and its junction with the fornix; in experiment five, the fornix was bilaterally transected.

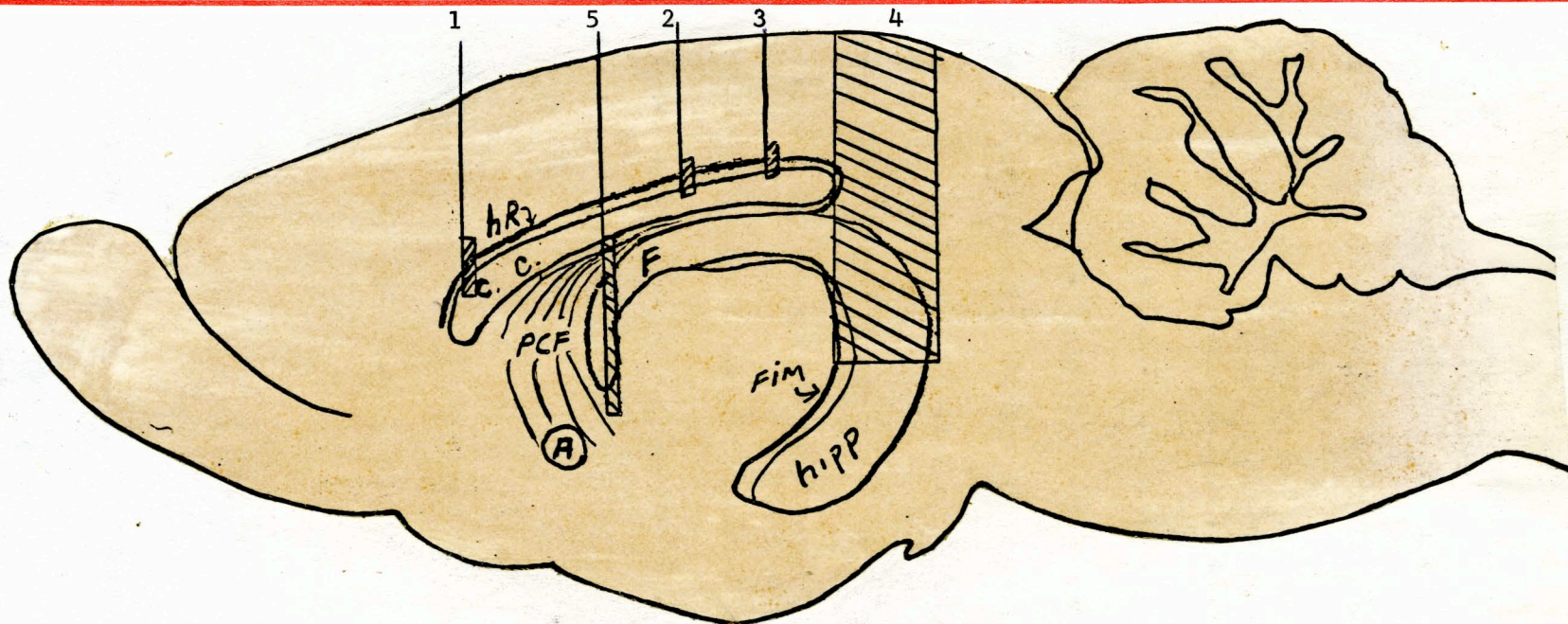


Fig. 1. Schematic diagram of rat brain showing lesion sites.*

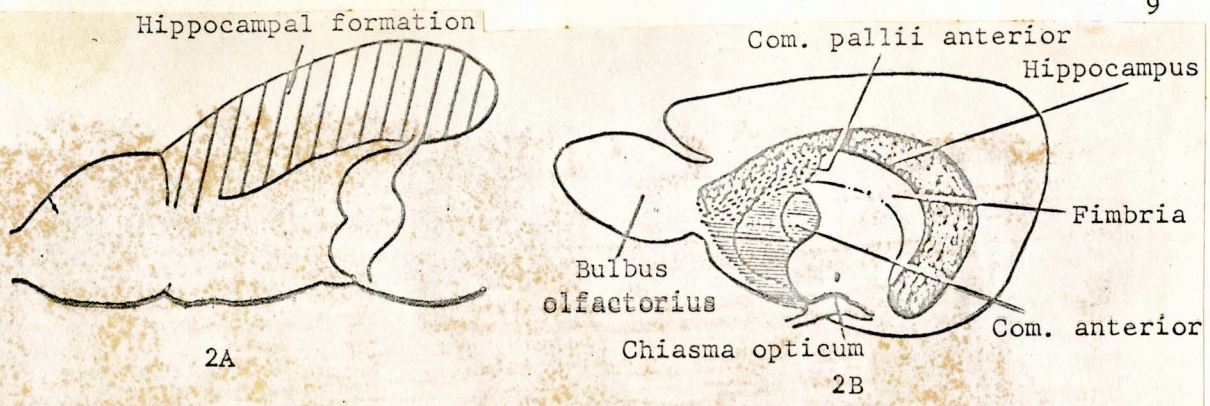
***Key to symbols and abbreviations:**

hR - hippocampal rudiment
 cc - corpus callosum
 PFC - precommissural fornix
 F - postcommissural fornix
 hipp - hippocampus
 fim - fimbria of hippocampus
 A - anterior commissure

1. bilateral lesion of hippocampal rudiment at genu of corpus callosum (Fagot, 1962)
2. bilateral lesion of hippocampal rudiment at trunkus of corpus callosum caudal to motor area (Gavin, 1963)
3. bilateral lesion of hippocampal rudiment at splenium of corpus callosum; actual lesions were further caudal (Planek, 1965)
4. bilateral lesion transecting hippocampus (Driessen, 1965)
5. bilateral lesion transecting fornix (Snyder, 1965)

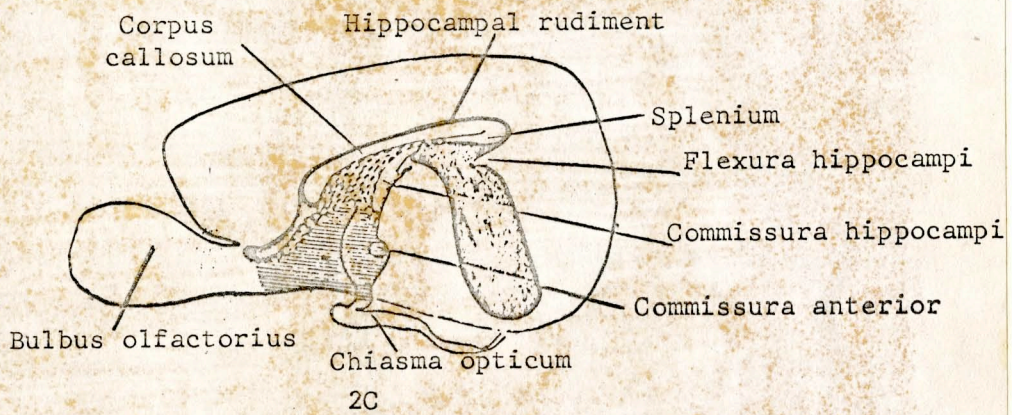
In order to provide a better understanding of the hippocampal positioning in the brain, Figures 2A, 2B and 2C show lateral views of the hippocampal formation as it appears in the frog (2A), marsuipal (2B), and rat (2C). Figure 2D shows the same structure from a dorsal view in the salamander (2d,1), the rat (2D,2) and man (2D,3). Embryologically, the hippocampus derives from the medial wall of the cerebral hemisphere. Together with the pyriform cortex, it serves as the cerebrum of primitive vertebrates. As the neocortex grows longitudinally as well as transversely in the higher species, the posterior parts of the hemisphere are pushed downward. Consequently, the originally straight hippocampal formation is bent down, curving around until its posterior end points anteroventrally in the temporal region of the hemisphere. The corpus callosum also influences the positioning of the hippocampal formation by growing through it in such a way that while the major portion of the hippocampal structure retreats into the temporal lobe, an elongated band of fibers remains superior to the corpus callosum and arches forward around its genu. This smaller, arching portion of the hippocampal system is the hippocampal rudiment or indusium griseum (Green, 1960). Though the term "indusium griseum" is more widely used, hippocampal rudiment is more appropriate from both a functional and an embryological point of view.

The hippocampus proper receives afferent projections from the hippocampal gyrus, cingulum and hippocampal rudiment (Brodal, 1947). The efferent pathway from the hippocampus begins in the fimbria (part of the hippocampus) and flows into the fornix.

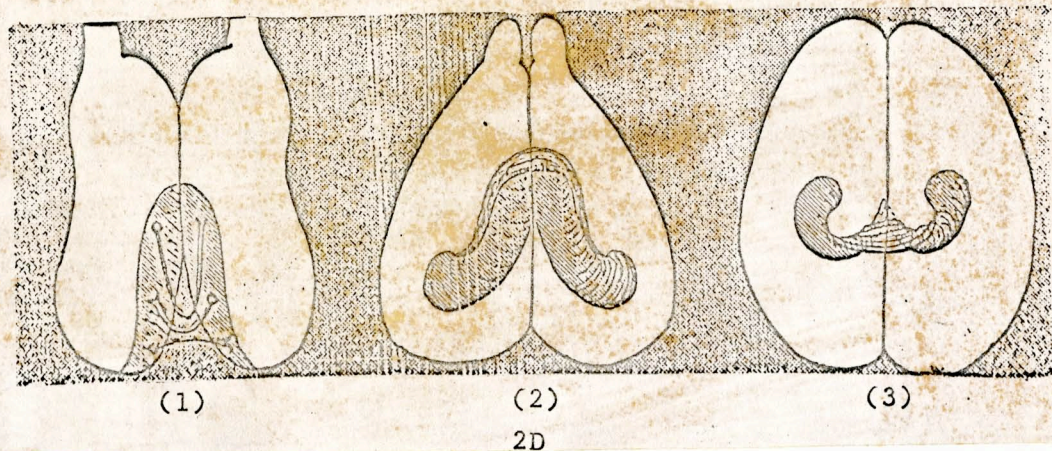


2A

2B



2C



(1)

(2)

(3)

2D

Fig. 2. Lateral view of hippocampal formation in frog (2A), marsupial (2B), and rat (2C). (Adapted from Zeman and Innes, 1963.) Dorsal view of hippocampal formation in salamander (2D, 1), rat (2D, 2), and man (2D, 3). (Adapted from Krieg, 1950.)

CHAPTER 2

REVIEW OF RELATED LITERATURE

The research that is most relevant to this experiment involves the fornix as it is related to memory and the effect of fornix lesions on olfactory and auditory discriminations. However, since the fornix is the main efferent of the hippocampus, a brief overview of hippocampal function is provided for the reader. More detailed reviews of the early theories of hippocampal functioning as well as recent research dealing with sensory discriminations have been presented in the following dissertations and shall not be repeated here: Fagot (1962), olfactory discrimination and the hippocampal rudiment; Gavin (1963), motor learning and the hippocampal rudiment; Planek (1965), somesthetic discrimination and visual discrimination and the hippocampal rudiment; Driessen (1965), visual and auditory discrimination and the hippocampus. The following discussion will attempt to describe particular investigations, report results, and, when appropriate, provide a possible interpretation in terms of the theory being tested in this experiment.

The Hippocampus

Olfactory functions were first attributed to Ammon's formation or the hippocampus by Broca in 1878. Campbell (1905) and Brodmann (1909) indicated that there was considerable morphological correspondence between the olfact-

ory bulbs, the olfactory tubercle and the hippocampus. The implication that the hippocampus was the cortical receiver of olfactory impulses gained widespread support. Herrick (1933), however, saw the hippocampus as being involved in activity having a broader spectrum than merely olfaction. He suggested that functions such as learning, memory and emotion were activated by the hippocampus, yet he tendered no circuit to show how this might be accomplished. Papez (1937) and later, MacLean (1949), have suggested that the hippocampus is primarily concerned with the mediation of emotion, Specifically, Papez (1939) suggested a path from the anterior thalamus, to the cingulate gyrus, to the cingulum, to the hippocampus, to the fimbria hippocampi, to the fornix, returning via the mammillary bodies back to the anterior thalamus. MacLean (1949) extended Papez's notions to include the probability that the hippocampus acted as integrator and distributor for all sensory information. Thus memory in relation to hippocampal activity came under scrutiny. During this same period Brodal (1947), in a review of the literature concerning the relation between the hippocampus and the sense of smell, presented evidence to show that the hippocampus could not be an olfactory structure. Many contemporary investigators have followed MacLean's general analysis that the hippocampus serves memory functions by relaying neocortical impulses to subcortical structures. Opinion regarding the specific function and role of the hippocampus are, however, widely divergent. Milner (1954) and Penfield and Milner (1958) have distinguished be-

tween short-term and long-term memories concerning the role of the hippocampus. They suggest that short-term memories go through a process of "consolidation" in the hippocampus until such time as a cortical neural change takes place; once this occurs, memory is thought of as long-term and independent of any further hippocampal mediation. If, however, hippocampal ablation occurs, new experiences could never be consolidated into permanent memory traces. In a review of these findings, Pribram (1961) reports that when a plan of action is written out on paper by patients with hippocampectomies, they overcome their short-term memory deficit effectively. He proposes that the inability to execute complex sequences of action is the common factor in short-term memory deficits and suggests that the hippocampus is part of the individual's planning mechanism. Nielson (1958) has suggested that the sequential remembering of one event as preceding another in time depends on hippocampal storage while "retentive memory of acquired knowledge" is stored in cortical association areas. According to Arnold (1960), however, the memories in their temporal sequence go to cortical association areas to remain there until they are reactivated by impulses transmitted through hippocampal relays.

Most of the above studies support the contention that the hippocampus is directly involved with memory. However, it is apparent that they have not furnished an unequivocal clarification of its precise function and role.

Memory and the Fornix

Dott (1938) and Garcia Bengochea, de la Torre, Esquivel, Vieta, and Fernandez (1954) reported no memory loss or any other deficit due to bilateral postcommissural fornix ablation. However, since the precommissural fornix connections were left intact in both of these studies, their results are not surprising. According to Arnold (1960), intact precommissural fornices would allow recall via septal nuclei to the midbrain and then to cortical association areas. Brady and Nauta (1953, 1955) found that rats with lesions of the septal area, which included damage to pre-and postcommissural fornix fibers, exhibited behavior that could be interpreted as memory defects. The animals acted as if it was impossible for them to remember the effect of past experience. Jasper, Gloor and Milner (1956), commenting on the work of Brady and Nauta, noted that deficits produced by septal lesions were greater when more of the fornix was involved. Furthermore, in evaluation of lesions on the floor of the third ventricle in humans where there was selective impairment of memory, they, "...the parallels with hippocampal lesions ... is immediate and striking" (Jasper et al, 1956, p. 375).

Sweet, Talland, and Ervin (1959) report sectioning bilaterally the anterior columns of the fornices in a man which resulted in a permanent loss of memory for recent events. They suggest that the hippocampal-fornix-mammillary system is necessary in order to make past experience accessible to recall. In the same article, Milner related a bilateral fornix

section with only some memory disturbance in contrast to severe memory loss in hippocampal cases, and suggested that the fornix section only interferes with a part of the system.

Both the fornix section of Sweet and that of Milner were prompted by colloid cysts of the third ventricle. It is not unlikely that since the operations were performed independently by different surgeons that different amounts of tissue might have been removed. Moreover, Sweet described the cyst of his patient as "large" while Milner used no quantitative description. Should there have been a difference, a possible interpretation could be that Sweet's patient sustained removal of both pre- and postcommissural fornices and Milner's patient only postcommissural fornices due to less tissue removal. This anatomical difference, if interpreted in light of Arnold's theory, would explain the apparent behavioral differences between the two patients.

Isaacson, Douglas and Moore (1961) reported no auditory retention impairment following hippocampal damage in rats but a portion of the dorso-rostral hippocampus remained, allowing intact connections from hippocampus to fornix. McCleary (1961) found that cats with precommissural lesions could perform active avoidance after the presentation of an auditory signal, suggesting that intact postcommissural fornices mediated recall.

Moore (1964) found that four cats with 50-100% bilateral fornix destruction showed permanent deficits in retention of an auditory conditioned

reaction (CAR). These animals could not be retrained. His groups consisted of (1) cortical control lesions, (2) cingulate lesions, (3) septal lesions, and (4) septal-hippocampal lesions. While cortical controls showed perfect retention, there were deficits in nine of eleven animals in the septal group, retention deficits in five of six animals in the cingulate group, and deficits in seven of seven in the septal-hippocampal group. Of these, three animals with septal lesions and two with septal-hippocampal lesions were completely unable to relearn. Three of these five animals had maximal bilateral fornix destruction, and one had only moderate damage. According to Arnold's theory this should eliminate memory in all sense modalities since the fornix is the main efferent of the hippocampal system. However, in Moore's study, other memory modalities were not tested. The fifth animal had bilateral damage to the stria terminalis, which is the primary efferent of the amygdaloid complex. Arnold holds that the amygdaloid complex mediates imagination; thus, interruption of this circuit prevented the animal from being able to imagine what to do in response to the auditory stimulus.

Other studies in the literature concerning the fornix deal with only physiology or such gross tests of behavior that their deficits explain only general impairment rather than a modality-specific behavior deficit. It would seem relevant now to indicate that the present experiment is a necessary follow-up to the four which preceded it from the Loyola Behavior Laboratory. This will be done by briefly discussing each experiment in

light of its findings concerning lesions of the hippocampal rudiment and hippocampus proper.

The first study (Fagot, 1962) investigated the role of the hippocampal rudiment in the learning and retention of an olfactory discrimination. Using a barpress olfactometer, rats were trained to discriminate between the odor of extract of pine (a barpress in its presence led to water reinforcement) and oil of hyacinth (a barpress here was ineffectual in obtaining water). Animals sustaining bilateral transections of the hippocampal rudiment (at the genu of the corpus callosum) were unable to retain or to relearn the discrimination. In comparison, intact animals, as well as those having lesions in neighboring structures, relearned swiftly. Incomplete hippocampal rudiment lesions, while producing retention deficits, did not prevent eventual relearning. The same results were found in animals who were trained only postoperatively. Learning, in this case, was impossible for animals with bilateral transections, very slow for animals with incomplete transections, and very quick for animals with lesions in neighboring structures as well as intact animals.

Gavin (1963) tested the hypothesis that motor memory was mediated via the hippocampal rudiment posterior to Fagot's lesion. Rats were trained on a T-maze alternation problem. In order to make a correct response, the animal had to remember which way it had turned on the preceding trial. Those rats with bilateral transections of the hippocampal rudiment at a point post-

erior to the motor area were unable to learn, retain or relearn the problem. These results indicate that the hippocampal rudiment does play a role in the recall of olfactory and motor cues.

Planek (1965) found no deficit in somesthetic recall due to bilateral lesions of the hippocampal rudiment at the splenium of the corpus callosum in rats. Another circuit, however, was suggested by Arnold (personal communication) which would explain these results. It seems that recall of tactual foreleg and head sensations may be mediated via the lateral parietal cortex, insula and claustrum to the hippocampus, while hindleg and trunk sensations go via the posterior cingulate gyrus and hippocampal rudiment. It is important to note, however, that impulses from both circuits must, according to Arnold, flow into the fornix regardless of whether it is via the hippocampal rudiment or the hippocampus proper.

Driessen (1965) indicated that bilateral transection of the hippocampus approximately half-way between the lateral tip of the structure and its entrance into the fornix produced significant deficits in auditory and visual retention but failed to prevent relearning altogether. Since fibers mediating both visual and auditory recall flow into the hippocampus, this lesion would interfere with both memory modalities. Two animals in the auditory group were unable to relearn the discrimination but were thought to be deaf on the basis of startle tests. However, this failure to respond to startle tests is not surprising if, in fact, they could hear but sounds had lost all

meaning for them (e.g., due to the lesion preventing recall of past experience with sounds). Driessen concludes that the hippocampus does play a role in auditory and visual memory.

It is appropriate then, that this experiment follows those investigating the function of the hippocampus and hippocampal rudiment in an attempt to relate the fornix to memory as the efferent transmitter of the hippocampal system.

CHAPTER 3

PROCEDURE

Subjects

Seventy-one male albino rats of the Sprague-Dawley strain were used. They were approximately 100 days old at the beginning of training. Each animal was gentled for several days before training commenced.

Two animals died, one preoperatively, the other postoperatively, and three animals were excluded due to lack of histological evidence indicating a lesion. All operated animals were allowed an eight-day recovery period following the operation. Unoperated control animals were given an eight-day rest period prior to retesting. Both operated and unoperated animals were allowed free access to food and water during the eight days.

Prior to training and/or operation, animals were randomly assigned to groups. Final grouping, however, depended on histological results, since the stereotaxic placement of lesions according to Krieg's Atlas (1946) did not prove as reliable as had been hoped. An attempt was made to produce bilateral fornix transections in all animals of the operated groups. Those animals which upon post-mortem examination were found to have lesions in other structures were used as controls.

Subject Groupings

1. Experimental group for the study of retention. These animals were trained to criterion on auditory and olfactory discriminations, operated on

and then retrained. Some of these animals were also postoperatively trained on motor, visual, and tactual discriminations. Those animals which did not show retention were tested for at least a period of time equal to or greater than the length of time required for relearning by the slowest of the subjects in the control groups. In most cases, however, testing continued much longer.

2. Experimental group for the study of learning. These animals were operated on before training commenced.

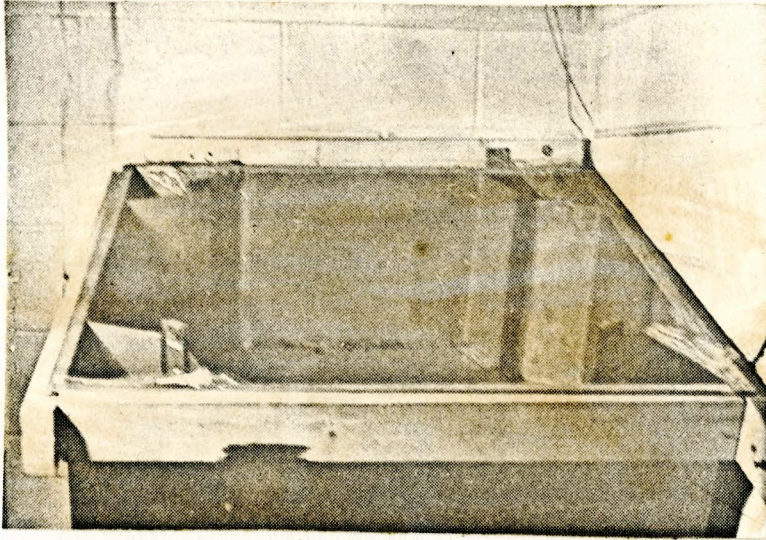
3. Operated control group. These animals were found to have lesions in neighboring structures upon histological verification. They provide controls for the trauma of the operation and bio-chemical cerebral changes due to a lesion.

4. Intact control group. These animals were trained, rested for eight days and retrained. They were not subjected to any surgery.

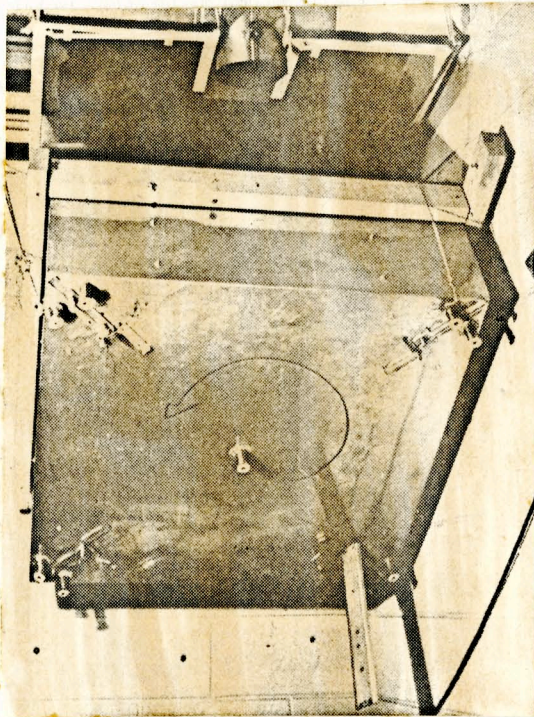
Apparatus

Olfactory

(See Figures 3A and 3B) The manually operated triple choice olfactometer was constructed of bacteriostatic stainless steel and measures 35x36x16 inches. The animal was placed in the center of the apparatus to allow random investigation of the four identical choice points. A vertical sliding door allowing the animal access to each corner is operated from underneath the apparatus. In each corner is a small compartment 9x15x10 inches (triangular). On the



View (A)



View (B)

Fig. 3. Two views of the apparatus used for olfactory discrimination. (A) shows the apparatus in position for training and testing. In (B) the apparatus is lifted up, showing the underside with runners and cups.

floor of each compartment are two holes, separated by a barrier. Fixed to the underside of the apparatus are runners carrying two cups which appear under these holes. The cup toward the interior of the apparatus contained the discriminative stimuli, while the cup toward the exterior contained reinforcement. The discriminative stimulus of powdered orange was paired with the reinforcement of water while the discriminative stimulus of rosemary leaves was paired with the reinforcement of an empty cup. Visual cues were controlled for by placing gauze over both cups.

An overhead exhaust system continually exchanged the air in order to eliminate irrelevant olfactory cues.

The apparatus was suspended from the ceiling, five feet above the floor, with a mirror above, which allowed observation of the animals without disturbing them.

Olfactory-Somesthetic (See Figure 4): The apparatus to be described was developed to provide faster and more efficient training. While the mean number of trials to criterion for Olfactory 1 was 120 (12 days), the mean number of trials to criterion using this apparatus was 30 (3 days). A rectangular box 16x12x6 inches with a glass wall in front and wire mesh ceiling served as the test chamber. A tray with ten small cups slid in a groove behind the glass and was moved by hand from right to left as the experimenter faced the glassed-in end. Clear water was in some cups and a saturated quinine solution in others. It was suspected, however, that the

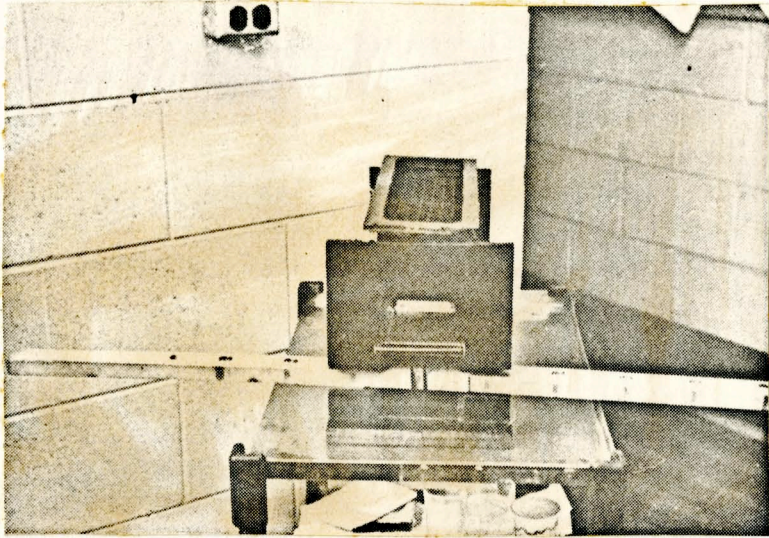


Fig. 4. Apparatus used for olfactory-somesthetic discrimination. The horizontal bar holds the discriminative stimuli and is moved by the experimenter.

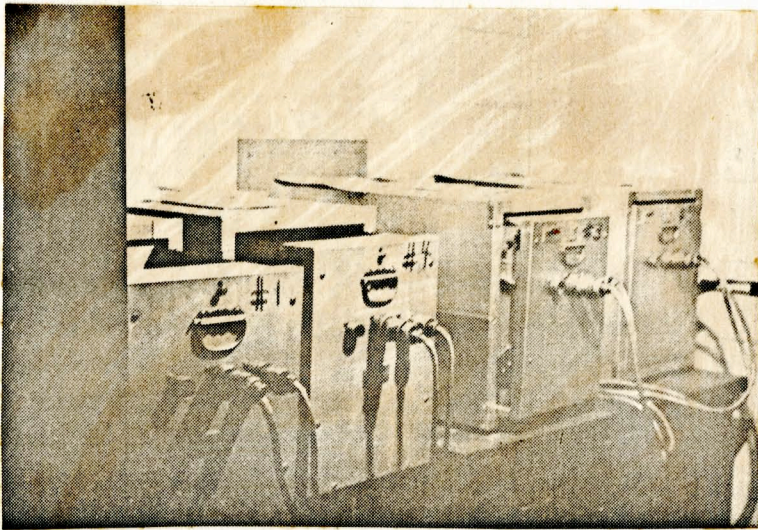


Fig. 5. Four test chambers (Skinner boxes) used for both the auditory and visual discriminations.

discriminative stimulus (saturated quinine solution using ethyl alcohol as solvent) might have been providing tactile cues to the animal. The alcohol may have produced an effect parallel to that of a person smelling ammonia or amyl acetate. Thus, the irritation of the mucous membranes, may have stimulated the trigeminal nerve and provided somesthetic cues. This discrimination, then, was probably an olfactory-somesthetic one, which could be learned on the basis of either kind of sensation.

Auditory (See Figure 5) Small animal test chambers (Skinner boxes) manufactured by Foringer Company (Model 1102-M1) were used in conjunction with the necessary programming accessories. The discriminative stimulus consisted of a clicking produced by a Grason-Stadler sound generator (#455B) and channeled into the test chamber via a Quam 2½ inch speaker located in the upper rear wall of the box or via a Quam 6 inch speaker suspended from the ceiling in the middle of the experimental room. The "speaker-in" arrangement, it was discovered late in the research, provided background vibrations that might serve as somesthetic cues to help the animal discriminate. Putting the speaker outside the box eliminated these undesirable cues. In the latter arrangement, "speaker-out," the small speakers inside the test chambers were disconnected and only the large speaker outside the test chambers served as stimulus source. The onset of the sound was controlled electronically by general purpose timers. These timers in combination with an alternator panel present the sound-on, sound-off phases for randomized intervals of time. None

of these intervals were shorter than seventeen seconds; none were longer than one minute. When the sound was on, positive reinforcement was available; a barpress, when the sound was off, resulted in a mild shock, produced by a standard Foringer shock generator (#1154) and grid scrambler (#1155), through the grid floor of the test chamber. Responses were recorded immediately and analyzed for accuracy at the end of each session. The length of each daily session was twenty minutes.

Visual (See Figure 5) The apparatus used for the visual discrimination was similar to that used for the auditory problem. Instead of speakers, however, the house-light within the test chamber was on (indicating that a barpress would deliver water) and off (indicating a barpress would not deliver water). Other than the change in the stimulus, and the introduction of constant "white noise" into the chamber to mask sounds and that shock was not used a negative reinforcer, the experimental program for the visual discrimination was parallel to that of the auditory.

Motor (See Figure 6) For the motor discrimination, a single-alternation T-maze was used. After one trial on which the animal found water whether he went right or left, he was run for fifteen trials during which he had to run alternately right and left (i.e., a subject had to recall what he had done on the previous trial) to obtain water. Water was put in a small dipper on the correct side during the trial. A saturated quinine solution was put on the incorrect side. Doors were operated by a system of strings and pulleys.

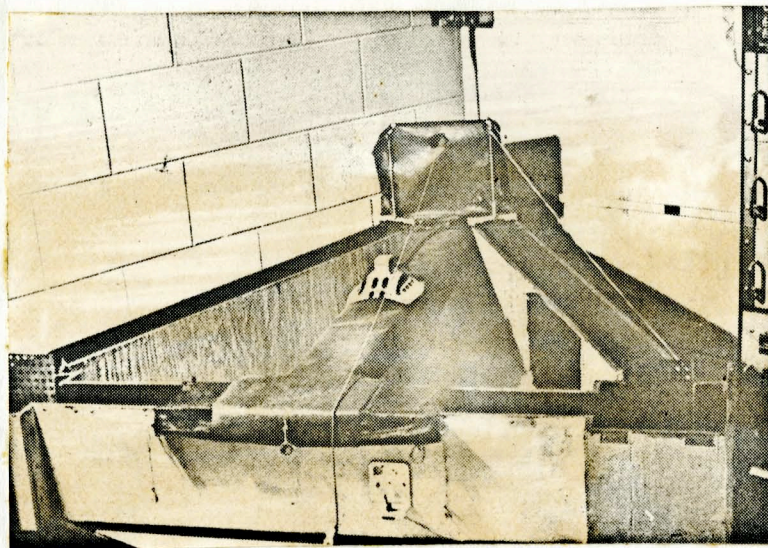


Fig. 6. Apparatus used for the motor discrimination. The starting box is located in the upper middle of the picture.

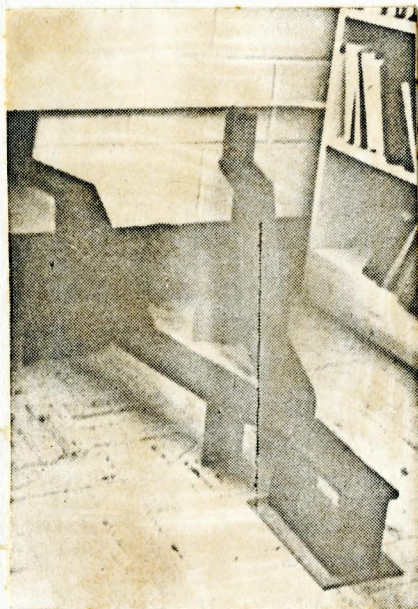


Fig. 7. Apparatus used for the tactual discrimination.

Oblique alleys returned from the goal boxes on the ends of the cross bar to the start box forming a triangular arrangement of pathways. This allowed the experimenter to run a series of trials without handling the animals.

White noise was introduced through a Quam 6 inch speaker placed over the middle of the central alley. A switch at the experimenter's position allowed him to control a small light in the starting box. This kept the animals light adapted and, thus, reduced any bias from visual cues. This light was turned off before opening the starting gate. The animals were run in a room totally darkened except for a small photographic red light that allowed the experimenter to record, etc. Retracing was prevented by the use of hinged doors located past the choice point.

Tactual (See Figure 7) A Y-shaped, elevated-path apparatus similar to that of Smith (1939) was used. The starting platform, twelve inches in length, led to a forked path, the arms of which presented the surfaces to be discriminated. The correct and incorrect pathways were constructed as separate units that could be assembled on a table to form the complete apparatus. The first eighteen inches of each path ran horizontally and led to a fourteen inch long incline of forty-five degrees. At the end of each incline was a platform ten inches in length, on which the animal received reinforcement. The correct pathway was covered with corrugated rubber. The coverings were removable from the main structure and were alternated randomly. The floor-boards of both runways, as well as the supports on which they were laid, were tapered

for a distance of $4\frac{1}{2}$ inches. In assembling the apparatus, both runways were placed in contact with the starting platform. Positive reinforcement was water; negative reinforcement was quinine. A response was not scored until the animal climbed the incline and reached a higher platform.

Training

Two days before the initiation of training, the subjects were placed on a schedule of water deprivation. The animals were given three ounces of water per day other than what they received during training and testing. They were given ad lib access to food. These patterns of maintenance prevailed throughout the experiment. On the third day, the shaping process began. Here, through selective reinforcement of responses that successively approximated the desired terminal behavior, the experimenter taught the rat what to do to get water, i.e., press the bar, run down the alley, etc. After the rats achieved a stable rate of response, the discrimination schedule was introduced.

Olfactory In order to respond to the test situation, sliding doors in all four corners are pulled down through the apparatus until they are flush with the floor of the chamber, thus allowing the animal to enter any corner. The animal may only put his head through the doorway to sniff at the stimulus cup immediately beyond it. If the scent is orange, the positive reinforcement of water awaits the animal should it choose to make the overt response of going around the barrier to the second cup. In order to accomplish this, the animals' body will have been fully introduced into the enclosed corner.

At this time, the experimenter pushes the sliding door up, thus barring exit. The corner entered was the new starting corner for the next trial. Stimuli were placed in a predetermined random order. During initial training, all four discriminative stimuli and reinforcement cups contained orange and water respectively. After shaping the animals to enter corners, rosemary and no water were paired in one corner. This progressed until after proficiency was established. Two corners contained the rosemary-empty configuration whereas one contained the orange-water arrangement. Animals performed ten trials daily to a criterion of 90% correct for three consecutive days.

Olfactory-Somesthetic With this apparatus, clear water was in some cups and a saturated quinine solution in others. The rat had to smell a single cup as it became accessible to him and drink or not drink. Subjects were given ten trials per day. A correct response was scored if the rats drank the water or did not drink the quinine. Incorrect responses were scored if the rats failed to drink water or did drink the quinine. Cups were refilled, as necessary, by means of hypodermic syringes. A criterion of 90% correct for three consecutive days was used.

Auditory Through the selective reinforcement of responses that successively approximate the desired terminal response, the experimenter taught the rat what to do to get water, i.e., press the bar. After the rats achieved a stable rate of response, the discrimination schedule was introduced. The length of daily training periods was twenty minutes. "Sound-on" signals

indicated that each bar press would produce reinforcement (continuous reinforcement schedule) while "sound-off" signaled that a barpress would not produce reinforcement (extinction schedule). Responses were recorded and analyzed immediately for accuracy. A criterion of 90% accuracy for three consecutive days were used.

Visual, Motor, and Tactual After appropriate shaping (press the bar, traverse the T maze, climb the elevated Y maze, respectively) the discrimination training, per se, began. This training in regard to reinforcement contingency was as follows. For the visual problem: light-on and light-off; for the motor problem: left turn versus right turn; for the tactual problem: smooth versus corrugated, served the same function as sound-on, sound-off for the auditory discrimination. The criterion used to indicate that an animal had learned was 90% accuracy or better for three consecutive days.

Operative Procedures

All operations were performed in one stage, using clean surgical technique. The animal was anesthetized with a mixture of ether and air. The average time for the anesthetic to take effect was approximately fifteen minutes. To begin surgery, the scalp on the dorsal surface was shaved and incised at the midline. The skull was then cleared of galea and periosteum to expose the bregma (the point at which the skull bone sutures meet, i.e., the reference point from which measurements are made). After suitable openings were drilled in the skull, both the pre- and postcommissural fornix were bi-

laterally transected at the point where the columns of the postcommissural fornix begin to turn ventral (Krieg coordinates 58.5). The lesion was accomplished in the following manner: A monopolar electrode (or knife in some lesions) was introduced at Right 84.5 and left 82 and aimed obliquely 30 degrees downward toward the opposite side and in each case crossing the midline of the brain. The depth of the lesion was from 3.0 to 5.5 mm in the case of the electrode which was insulated for the first 3 mm. Knife lesions, however, extended from 0 to 5.5 mm. The oblique method was used primarily in the interest of preserving the hippocampal rudiment and secondarily the mid-sagittal venous sinus where possible and still achieve a sub-callosal lesion in the middle of the brain. After surgery, the wound was covered with gelfoam and the scalp sutured. During the one week recovery period, the animal was given ad lib access to food and water.

Processing for Histology

In the Behavior Laboratory, the rats were perfused with formalin solution by the use of a twenty gauge needle and syringe. The brain was wholly excised and placed in buffered (saturated CaCl_2) formalin solution and fixed for six weeks.

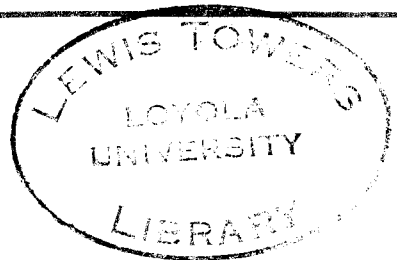
In the research laboratory of Dr. Patrick Toto of the Loyola Dental School (where the slides were made), the brain was trimmed, washed, dehydrated in ascending concentration of ethyl alcohol, transferred to three changes of xylene, and embedded in paraffin. The specimen was cut at ten

microns with a rotary microtome. The sections were stained with hematoxylin and eosin for general morphologic study. Luxol fast blue and Cresyl violet stain were used for combination nerve fibers and cells.

The slides were read by Dr. David Jones of the Anatomy Department of Loyola University Medical School (Division of Neurology) and Dr. Robert Yates Moore, Department of Anatomy, University of Chicago, who reported on the extent of the lesions.

CHAPTER 4

RESULTS



The final grouping of the animals, based upon histological verification, will now be reported. A total of seventy-one animals was used.

1. Experimental group for the study of retention (train-operate-retrain). Of a group of nineteen animals, two were deleted from analysis due to the absence of dependable histological information.
2. Experimental group for the study of learning (operate-train). Of a group of five animals, one was deleted due to the absence of dependable histological information. Fourteen animals from Group 1 are also included in this category because ten were trained postoperatively on motor discrimination and four of them on visual and olfactory discrimination.
3. Operated control group. Fifteen animals were used for these comparisons.
4. Intact control group. Thirty-six animals comprised this group. Many were trained on several discriminations.

Table 12, a summary table indicating the extent of behavioral deficits for every experimental animal, may be consulted to ascertain the discriminations on which each animal was trained. All listed anatomical sites indicate bilateral lesions unless specifically noted as unilateral. This was done for the sake of uniformity as well as the relative importance of bilateral over unilateral lesions. Table 12 also provides a complete analysis

of each animal in accordance with those structures deemed of primary importance to this experiment.

Since comparison of behavior based on lesion site is as important as comparison with control animals, the primary groupings for analysis will be according to the site of the lesion:

1. Precommissural fornix only
2. Hippocampal rudiment only
3. Precommissural fornix and hippocampal rudiment
4. Pre-and postcommissural fornix
5. Pre-and postcommissural fornix and hippocampal rudiment
6. Unilateral precommissural fornix
7. Unilateral hippocampal rudiment

There are two animals reported as having unilateral damage only, 0-2 (unilateral hippocampal rudiment) and 0-15 (precommissural fornix). They are, however, suspected of having bilateral damage. During histology, some tissue was lost and slides could not be made of the central aspect of the lesion site. Both of these animals have discrimination deficit scores in three sense modalities and differ significantly from both operated and intact controls. Furthermore, the magnitude of their discrimination deficits alone suggests more than unilateral damage. Since, however, this contention is histologically unverifiable, they must be designated as unilateral.

RetentionAuditory-Vibratory

Table 1 presents the mean number of sessions to achieve criterion preoperatively and postoperatively for the auditory discrimination which had

Table 1

Mean Preoperative Learning and Postoperative Retention Scores

For All Groups on Auditory-Vibratory Discrimination

Group	N	Preoper. Learning			Postoper. Learning <i>Retention</i>		
		Mean	S.D.	Range	Mean	S.D.	Range
<u>Controls</u>							
Intact	20	8.75	1.92	6-13	4.85	1.90	3-8
Hippocampal Lesion (Complete)	4	10.50	2.06	8-13	6.00	3.08	3-10
<u>Experimental Lesions</u>							
Precommissural Fornix	11	8.73	2.70	5-14	24.73*	19.19	7-74
Pre- and Postcommissural Fornix	2	8.00	-	6-10	63.00	-	57-69
Hippocampal Rudiment	1	14.00	-	-	16.00	-	-
Unilateral:							
Precommissural Fornix	1	10.00	-	-	69.00	-	-
Hippocampal Rudiment	1	7.00	-	-	15.00	-	-

*Significantly different from Mean of Learning scores and from Retention Controls (Intact and Hippocampal Lesion): $p < .025$ in all cases.

the speaker in the test chamber, allowing for vibratory cues as well as auditory cues. It shows that the mean performance of animals with precommissural fornix lesions differs significantly from their own learning mean and from both intact and hippocampal controls ($p < .025$). Statistically, this indicates an auditory-vibratory discrimination deficit. In addition, inspection of the retention scores of the two animals with pre-and post-commissural fornix lesions indicates large auditory-vibratory discrimination deficits. The animal with a bilateral hippocampal rudiment lesion, when compared with the controls, at first glance appears to be a deficit animal, but his initial score in learning suggests rather that it is a slow learner. Of fifteen animals with auditory-vibratory deficits, fourteen had fornix lesions. Five of these animals never reached criterion; the minimum number of sessions was forty-four. This indicates that both auditory and vibratory cues were useless to these five animals.

Auditory Only

Due to the discovery of vibratory cues late in the research, nine animals from the Auditory-Vibratory group were selected for continued training. A new condition was added; namely, the speaker was removed from the test chamber and a bigger speaker was placed nearby so that the intensity level remained the same. Table 2 indicates that eight of the nine animals were unable to reach criterion under the speaker-out condition. In the absence of

Table 2

Postoperative Retention Scores for Animals Run Alternately on
Auditory-Vibratory (Speaker In) Discrimination
and Auditory Only (Speaker Out) Discrimination

Experimental Lesion	Preoperative Learning	Postoperative Retention			
	Speaker In	Speaker In 1	Speaker Out 1	Speaker In 2	Speaker Out 2
Precommissural Fornix					
0-3	5	74*	22*	---	---
0-10	10	44*	16*	---	---
0-12	9	21	15*	---	---
0-13	11	26	73*	3	7*
0-18	11	3	3	---	---
Pre- and Postcommissural Fornix					
0-9	6	57*	65*	5	8*
0-17	10	69*	61*	10	3*
Unilateral: Hippocampal Rudiment					
0-2	7	15	67*	6	4*
Precommissural Fornix					
0-15	10	69*	16*	---	---

*Criterion not reached.

vibratory cues, these animals were unable to perform the task, indicative of auditory discrimination deficits. Note that animal 0-13 and 0-2 finally reached criterion with the speaker in the chamber but promptly lost it when the speaker was again removed. That this was not an artifact of the change in testing conditions is shown by the performance of 0-18, who maintained criterion in the minimum number of sessions under both conditions. In every case, animals who did not reach criterion under the speaker-out condition and were subsequently retested under speaker-in, achieved criterion in three to ten days. Having been brought up to criterion it would be expected that the animal continue criterion performance, but upon retesting under the speaker-out condition, they immediately failed. Even though these animals relearned by vibratory cues, the large number of trials indicates that they had vibratory as well as auditory discrimination deficits, though the latter appear more severe. Of the eight auditory discrimination deficit animals, seven sustained fornix damage.

Olfactory

Table 3 shows that there is no complete loss of discriminative ability due to any one or combination of lesions. However, since one of the pre-commissural animals served as its own control over time (achieving a retention score of 30), its postoperative retention score of 80 would seem indicative of a small olfactory discrimination deficit.

Table 3

Mean Preoperative Learning and Postoperative Retention Scores
For All Groups on Olfactory Discrimination

Group	N	Preoper. Learning Trials to Criterion			Postoper. Retention Trials to Criterion		
		Mean	S.D.	Range	Mean	S.D.	Range
<u>Controls (Intact)</u>	5	142.00	17.20	120-170	30.00	0.0	-
<u>Experimental Lesions</u>							
Precommissural Fornix	6	134.83	18.26	97-150	40.00	10.00	30-60
Precommissural Fornix and Hippocampal Rudmt.	2	155.00	-	140-170	60.00	-	40-80
Pre- and Postcommissural Fornix	1	143	-	-	30	-	-
Unilateral:							
Precommissural Fornix	1	120	-	-	30	-	-
Hippocampal Rudiment	1	120	-	-	60	-	-

LearningAuditory-Vibratory

Table 4 shows that animals with precommissural lesions are significantly inferior in learning ability as compared to both intact animals and those with partial hippocampal lesions. The animal listed as "Other" is suspected of having damage to primary auditory cortex.

Table 4

Mean Postoperative Learning Scores For All Groups
on Auditory-Vibratory Discrimination

Group	N	Mean	Range
<u>Controls</u>			
Intact	28	8.86	4-13
Hippocampal Lesion (Partial)	3	11.67	9-17
Other (ventro-posterior abscess)	1	62	-
<u>Experimental Lesion</u>			
Precommissural Fornix	3	69.33*	39-86

*Significantly different from both control groups using the Mann-Whitney U test: $p = .05$.

Auditory Only

As in the retention Auditory-Vibratory group, three animals from those included in Table 4 were selected to continue training under conditions of both speaker-in and speaker-out. Table 5 shows learning data comparable

Table 5

Postoperative Learning Scores for Animals Run Alternately on
 Auditory-Vibratory (Speaker In) Discrimination and
 Auditory Only (Speaker Out) Discrimination

Group	Postoperative Learning				
	Speaker In 1	Speaker Out 1	Speaker In 2	Speaker Out 2	Speaker In 3
<u>Control</u>					
Other (ventro-posterior abscess)					
02-4	62	68*	3	3*	6
<u>Experimental Lesion</u>					
Postcommissural Fornix					
02-1	86*	17*	-	-	-
02-5	83*	66*	10	3*	10

*Criterion not reached.

to that presented under retention in Table 2. Two animals (02-5 and 02-4), while failing to reach criterion on speaker-out, achieved it in three to ten days when returned to the speaker-in condition. Again this was repeated so as to remove any doubt as to the auditory discrimination deficit. The discrimination deficit seen in 02-4 was probably due to auditory cortex damage. Again, as with retention animals, two of these gave evidence (large number of sessions to criterion) of having both auditory and vibratory discrimination

deficits. Animal 02-1 never reached criterion and also falls into the dual discrimination deficit category.

Olfactory

Table 6 shows that animals sustaining precommissural fornix lesions are inferior in learning compared to the intact control group ($p < .01$). Relevant to this is the comparison to the "Other" operated animal who sustained severe damage to the auditory cortex and neighboring structures. The extent of this

Table 6

Mean Postoperative Learning Scores For All Groups on

Olfactory Discrimination

Group	N	Mean	S.D.	Range
<u>Control</u>				
Intact	19	136.69	22.86	97-190
Other (ventro-posterior abscess)	1	110	-	-
<u>Experimental Lesion</u>				
Precommissural Fornix	3	316.33*	41.50	258-351

*Significantly different from Mean of Control Group: $p < .01$.

damage involved much more brain tissue than any of the other three operates. Yet, on the basis of its score, it would appear to be no different from the average score of intact animals. Of the three experimental animals, one had bilateral hippocampal damage, one had unilateral hippocampal damage, and the other had no hippocampal involvement. All three had precommissural damage.

Olfactory-Somesthetic

As was noted in the procedure section, the apparatus used for this discrimination probably involved somesthetic as well as olfactory cues.

Table 7 indicates only one unusual score, that of an animal with pre-and postcommissural fornix as well as hippocampal rudiment damage. Upon initial

Table 7

Mean Postoperative Learning Scores For All Groups
on Olfactory-Somesthetic Discrimination

Group	N	Mean	S.D.	Range
<u>Control</u>				
Intact	28	30.0	0.0	-
<u>Experimental Lesion</u>				
Precommissural Fornix	3	33.3	4.71	30-40
Precommissural Fornix and Hippocampal Rudiment	5	30.0	0.0	-
Hippocampal Rudiment	1	40	-	-
Pre-and Postcommissural Fornix and Hippocamp- al Rudiment	1	75	-	-

inspection this seems to be not very significant. However, of thirty-eight animals trained on this discrimination, only three did not instantly learn the task. Two animals had a score of 40 and only one had a score as high as 75. That the standard deviation of the intact control group is 0.0 sup-

ports the contention that the artifact of criterion (3 sessions at 90% or better) does not allow the observer to note that most animals perform 80 to 100% the first day (rarely lower) and the next days are perfect or nearly so.

Motor

Table 8 indicates that the mean score of the group with fornix damage differs significantly from the mean score of the intact control group ($p < .025$).

Table 8

Mean Postoperative Learning Scores For All

Groups on Motor Discrimination

Group	N	Mean	S.D.	Range
<u>Control</u>				
Intact	26	62.69	26.39	30-120
<u>Experimental Lesion</u>				
Precommissural Fornix	6	127.50*	51.05	75-210
Precommissural Fornix and Hippocampal Rudiment	2	127.50	-	60-195
Unilateral:				
Precommissural Fornix	1	75	-	-
Hippocampal Rudiment	1	135	-	-

*Significantly different from mean of control group: $p < .025$.

It is noteworthy that of the two animals with both precommissural fornix damage and hippocampal rudiment damage, one score is average but one appears rather high. It would seem that the motor discrimination deficits can be best explained by fornix damage alone except in the case of the unilateral hippocampal rudiment animal. Again, it is suspected that this animal has fornix involvement though the histological report does not warrant claiming it unequivocally.

Table 9

Mean Postoperative Learning Scores For All

Groups on Visual Discrimination

Group	N	Mean	S.D.	Range
<u>Control</u>				
Intact	6	14.00	2.38	11-17
Operated (Neighboring Structures)	7	14.57	4.37	9-22
<u>Experimental Lesion</u>				
Pre-and Postcommissural Fornix	3	44.67*	9.46	43-57
Unilateral (Precommissural Fornix)	1	57	-	-

*Significantly different from both control groups: $p < .025$.

Visual

It is apparent in Table 9 that animals with fornix damage show a visual discrimination deficit in comparison with both operated and intact controls. The one unilateral animal also seems to be a visual discrimination deficit. Again, it is suspected that it has bilateral fornix damage which is not histologically apparent. That there is no difference between control operated and intact animals indicates that the operation per se has little to do with a visual discrimination deficit.

Table 10

Mean Postoperative Learning Scores For All

Groups on Tactual Discrimination

Group	N	Mean	S.D.	Range
<u>Control</u>				
Intact	28	116.79	32.52	80-180
<u>Experimental Lesion</u>				
Precommissural Fornix	2	90.00	-	80-100
Pre- and Postcommissural Fornix	1	100	-	-
Unilateral (Precommissural Fornix)	1	100	-	-

Tactual

Table 10 shows no tactual discrimination deficits due to lesions of the fornix. Learning appears to be consistently faster for the experimental animals.

Table 11 contains a summary of the raw scores achieved by each experimental animal according to sensory discrimination categories. The site of the lesion and whether it is bi-or unilateral is noted for each animal.

Table 12 contains a summary of discrimination deficits for each animal according to sensory discrimination categories. The site of the lesion is also indicated. The severity designation of discrimination deficits (medium or large) was determined quantitatively. The criterion was how distant a given animal's score was from the mean of the control group in terms of standard deviation. For a designation of "medium", the minimum distance was two S.D.s from the mean and for a designation of "large", three S.D.s was the minimum.

Table 11

Raw Scores Achieved by Each Experimental Animal For All Discriminations

Rat	Lesion*	Postoperative Retention				Postoperative Learning						
		Auditory & Vibra.	Auditory Only	Olfact.	Visual	Auditory & Vibra.	Auditory Only	Olfact.	Olfact. Somes.	Motor	Visual	Tactual
0-1	1b, 3b	23	-	40	-	-	-	-	30	195	-	-
0-2	3u	15	71	60	-	-	-	-	135	-	-	
0-3	1b	74	22	30	-	-	-	-	165	-	-	
0-5	1b, 3u	11	-	40	-	-	-	-	30	75	-	
0-7	1b, 3u	14	-	40	-	-	-	-	30	75	-	
0-8	1b, 3u	33	-	80	-	-	-	-	30	60	-	
0-9	1b, 2b, 3b	62	73	-	-	-	-	-	75	-	-	
0-10	1b, 3b	44	16	-	-	-	-	-	30	-	-	
0-11	1b, 3b	7	-	-	-	-	-	-	30	-	-	
0-12	1b, 3u	21	15	30	-	-	-	-	-	210	43	
0-13	1b, 3u	26	80	40	-	-	-	-	-	90	34	
0-15	1u	69	16	30	-	-	-	-	-	75	47	
0-17	1b, 2b	79	64	30	-	-	-	-	-	150	57	
V-17		-	-	-	20	-	-	-	-	-	-	
0-18	1b, 3u	3	3	60	-	-	-	-	-	-	-	
0-19	1b, 3b	16	-	-	-	-	-	-	-	-	-	
0-20	1u, 3b	11	-	-	-	-	-	-	30	-	-	
02-1	1b, 3b	-	-	-	-	-	-	-	40	-	-	
02-2	1b, 3u	-	-	-	-	86	17	258	-	-	-	
02-5	1b	-	-	-	-	39	-	340	40	-	-	
						83	68	351	-	-	-	
Control Group**												
	Mean	6.00	5.00	30.00	6.17	11.67**	5.00	136.69	30.00	62.69	14.57**	116.79
	S.D.	3.08	3.75	0.0	2.48	11.31	3.75	22.86	0.0	26.39	4.37	32.52
	N	4	7	5	6	3	7	19	28	26	7	28

* Code for lesions (b-bilateral; u-unilateral) 1. Precommissural Fornix; 2. Postcommissural Fornix;

**Indicates operated control group; all others are intact controls.

3. Hippocampal Rudiment

Table 12

Behavior Deficits on Each Discrimination For Each Animal

Rat	Lesion*	Postoperative Retention				Postoperative Learning						
		Auditory & Vibra.	Auditory Only	Olfact.	Visual	Auditory & Vibra	Auditory Only	Olfact.	Olfact. Somes.	Motor	Visual	Tactual
0-1	1b, 3b	Large	-	None	-	-	-	-	None	Large	-	-
0-2	3u	Medium	Large	None	-	-	-	-	-	Medium	-	-
0-3	1b	Large	Large	None	-	-	-	-	-	Medium	-	-
0-5	1b, 3u	Medium	-	None	-	-	-	-	None	None	-	-
0-7	1b, 3u	Medium	-	None	-	-	-	-	None	None	-	-
0-8	1b, 3b	Large	-	Small	-	-	-	-	None	None	-	-
0-9	1b, 2b, 3b	Large	Large	-	-	-	-	-	Medium	-	-	-
0-10	1b, 3b	Large	Large	-	-	-	-	-	None	-	-	-
0-11	1b, 3b	None	-	-	-	-	-	-	None	-	-	-
0-12	1b, 3u	Large	Large	None	-	-	-	-	-	Large	Large	None
0-13	1b, 3u	Large	Large	None	-	-	-	-	-	None	Large	None
0-15	1u	Large	Large	None	-	-	-	-	-	None	Large	None
0-17	1b, 2b	Large	Large	None	-	-	-	-	-	Medium	Large	None
V-17	1b, 2b, 3b	-	-	-	Large	-	-	-	-	-	-	-
0-18	1b, 2u	None	None	None	-	-	-	-	-	-	-	-
0-19	1b, 3b	Large	-	-	-	-	-	-	None	-	-	-
0-20	1u, 3b	Medium	-	-	-	-	-	-	None	-	-	-
02-1	1b, 3b	-	-	-	-	Large	Large	Large	-	-	-	-
02-2	1b, 3u	-	-	-	-	Large	-	Large	None	-	-	-
02-5	1b	-	-	-	-	Large	Large	Large	-	-	-	-

*Code for lesions: (b - bilateral; u - unilateral) 1. Precommissural Fornix; 2. Postcommissural Fornix; 3. Hippocampal Rudiment

Histological Results

The references used for identification of structures and lesion sites were the rat brain atlas of Krieg (1946) as well as the atlas Craigie's Neuroanatomy of the rat (1963). The micron (μ) designations refer to the anterior-posterior position of each slide and are based on the Konig and Klippel atlas (1963). The micron (μ) range is from 480 , posterior pole (not including cerebellum), to 12760 , anterior pole.

Bilateral or unilateral designation for each animal is provided in both Table 11 and 12 according to the following structures: 1. precommissural fornix, 2. postcommissural fornix, and 3. hippocampal rudiment. Photographs of three animals sustaining both pre-and postcommissural damage are provided in Figures 8, 9A, and 9B.

Rat number 0-9 (Figure 8) died and was not able to be perfused before his brain was removed from the skull. As a result, the tissue did not harden in the formalin solution sufficiently to allow sectioning for slides. For this reason, photographs were taken of the gross specimen. The brain was found to contain a large abscess which when removed, left the apparent cavity. From the gross specimen it was determined that there was extensive damage to cortex, hippocampal rudiment, corpus callosum, pre-and postcommissural fornices and septum.

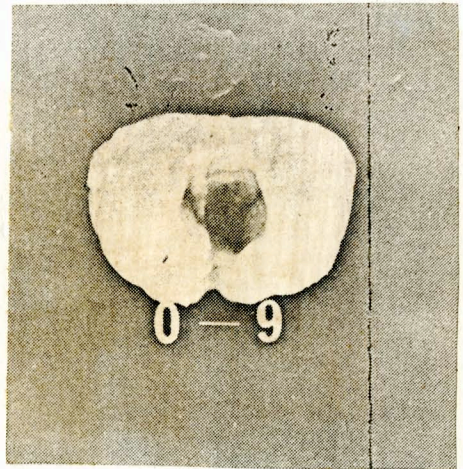
Figure 9A (animal number 0-17) shows extensive bilateral damage to the septum, pre-and postcommissural fornices, anterior commissure, unilateral

Posterior



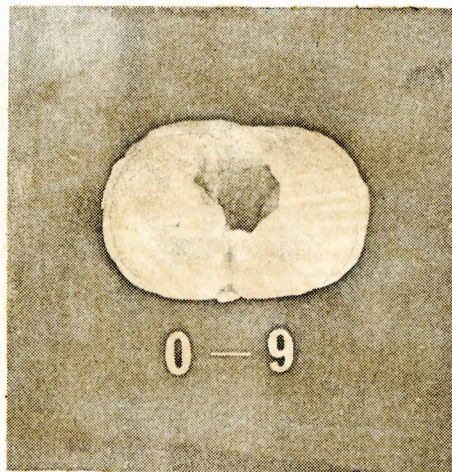
Anterior
 Dorsal view: looking down
 to the top of the brain

Posterior



Anterior
 Dorso-frontal view: brain is
 tilted to partially expose
 anterior aspect

Dorsal

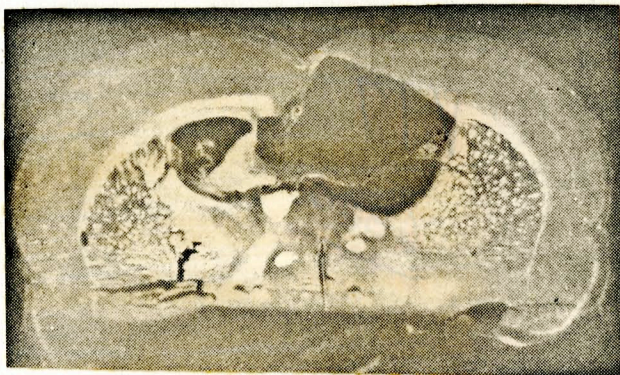


Ventral
 Anterior view: view from
 front to back

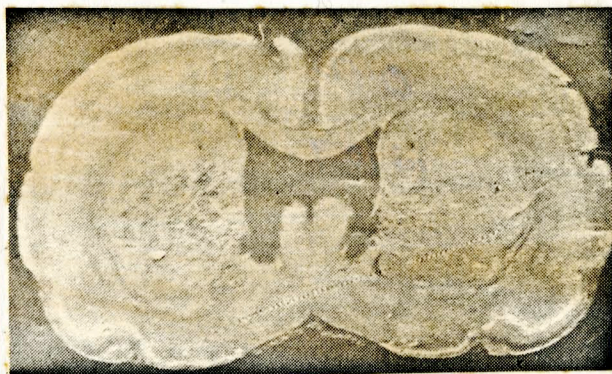
Fig. 8. Three views of the brain of experimental animal 0-9.



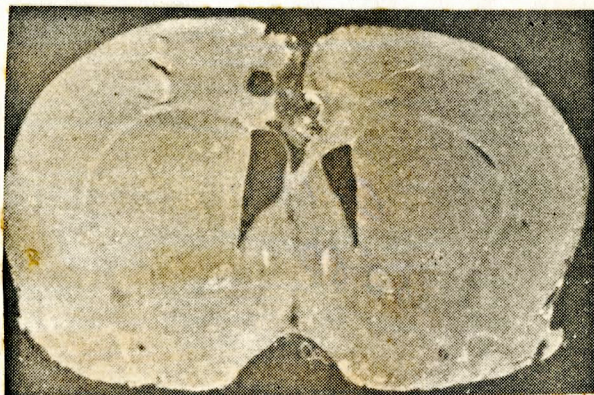
(A) Animal O-17
6890 μ



(B) Animal V-17
6320 μ



(C) Animal O-12
7470 μ



(D) Animal O-11
8380 μ

Fig. 9. Photographs of stained brain sections of experimental animals with anterior-posterior designations.

corpus callosum, minimal cortical and no hippocampal rudiment damage. Figure 9B (animal V-17) indicates extensive unilateral cortical damage and bilateral damage to the hippocampal rudiment, corpus callosum, pre-and post commissural fornices, hippocampal commissure and hippocampus.

Figure 9C (animal O-12) is a representative photograph of a bilateral precommissural fornix lesion with no damage to either the hippocampal rudiment or the corpus callosum. Note that the bottom tips of the lateral ventricles are enlarged and surrounded by jagged circular tissue indicating the most ventral aspect of the electrolytic lesion. Figure 9D (animal O-11) is a representative photograph of a hippocampal rudiment lesion involving damage to the corpus callosum with some precommissural fornix damage.

The following is a general summary of the bilateral lesions. Of the twenty experimental animals, three had bilateral pre-and postcommissural fornix lesions; two of these three also sustained bilateral hippocampal rudiment damage. Fourteen animals (not including the three already mentioned) had bilateral precommissural fornix lesions; six of these also sustained bilateral hippocampal rudiment damage.

One of the three remaining animals had only unilateral damage to the precommissural fornix and bilateral damage to the hippocampal rudiment. The last two animals sustained only unilateral damage; animal number O-2 (hippocampal rudiment) and O-15 (precommissural fornix). It has already been

noted that both of these animals are suspected of having significant bilateral pre-and postcommissural fornix damage but that this is not verifiable histologically.

CHAPTER 5

DISCUSSION

The results indicate that a bilateral precommissural fornix lesion prevented recall (inability to learn or retain) of an auditory discrimination and impaired recall (retarded learning) of olfactory, motor and visual discriminations. This lesion did not prevent learning olfactory, motor and visual discriminations, nor did it prevent relearning of an olfactory or visual discrimination.

The auditory results are consistent with those of Moore (1964). He found that four cats were unable to relearn an auditory conditioned reaction (CAR) due to septal and septal-hippocampal lesions; these lesions included the common factor of bilateral fornix damage. Moore's study did not specify whether fornix damage was pre-or postcommissural, but it implied damage to both. In the present study, there was precommissural fornix damage in all eight of the animals who indicated a permanent auditory discrimination deficit, while only two of the eight also had postcommissural fornix damage.

Schwartzbaum, Kellicutt, Spieth and Thompson (1964) report that rats were deficient in performing an auditory discrimination between two tones as a result of septal lesions (including medial and lateral septal nuclei, the nucleus accumbens, and precommissural fibers of the fornix). While only positive reinforcement was used (food) in their study, and both water and

shock were used in the present study, a comparison of results may still be made. Schwartzbaum et al (1964) found two animals who, within a set limit of 25 sessions, failed to regain criterion. These two animals, the four animals of Moore (1964), and the eight in this experiment all failed to reach auditory criterion and all animals sustained septal-precommissural fornix lesions.

Harvey, Lints, Jacobson and Hunt (1965) found that extensive bilateral septal lesions (including pre- and postcommissural fornices) interfered with the learning and retention of both an auditory and visual discrimination.

Of the four animals indicating retarded olfactory discrimination learning, in the present study, all sustained bilateral precommissural fornix damage and two also had bilateral hippocampal rudiment damage. Six other animals with both areas damaged were able to retain a learned olfactory discrimination. This latter finding is not in accord with Fagot (1962) who bilaterally lesioned the hippocampal rudiment in rats and found olfactory discrimination deficits. His lesion, however, was at the genu of the corpus callosum, anterior to the lesions reported in this experiment. Olsen and Magee (1961) give evidence showing that fibers from the hippocampal rudiment perforate the corpus callosum and join the distribution of subcallosal fornix fibers. Other fibers pass around the genu of the corpus callosum to the

septum where they then become part of the precommissural fornix (Ariens, Kappers, Huber, and Crosby, 1936). Consequently, it is possible that in recall, impulses are relayed to the hippocampal rudiment and go to the pre-commissural fornix, either around the corpus callosum or through it. Arnold's theory does not specify which fornix fibers mediate olfactory discrimination, only that they do. Animals who retained the olfactory discrimination undoubtedly had some precommissural fibers intact. Pribram and Kruger (1954) state that the precommissural fornix is several times larger by actual fiber count than the postcommissural fornix. Since fornix fibers penetrate the corpus callosum throughout its length, it would be difficult to destroy all fornix fibers, even with bilateral damage to pre-and postcommissural fornices. It would seem that future research must designate exactly where the precommissural fornix fibers are located which mediate olfactory discrimination.

The above explanation also applies to the recorded deficits in motor and visual discriminations. In regard to motor discrimination, Thompson and Langer (1963) investigated the effect of various lesions of the limbic system on alternation reversal performance in rats under conditions of escape from shock. They found that while precommissural fornix damage showed significant impairment in reversal learning, destruction of the postcommissural fornix failed to produce any significant deficits. They comment

that the reversal deficit noted in their experiment seemed a genuine impairment of some aspect of the memory process. They further remark that the animals "could not remember the side from which they received a shock 30 sec. previously..." (1963, p. 995). This seems to be very comparable to animals in this study. The latter could not remember which way they had gone on the previous trial, and therefore, could not choose the correct side.

Motor recall (what has been done before) is mediated by the hippocampal rudiment according to Arnold's theory; and recall, on the basis of visual cues, is mediated by the hippocampus, probably via the medial third of the hippocampal commissure. This implies that both memory modalities could be mediated via perforating fornix fibers and/or fibers running in the pre-and postcommissural fornix.

Concerning visual discrimination there is a suggestion in Planek's (1965) data that animals with bilateral hippocampal commissure (medial) damage were retarded in learning of a visual discrimination, when compared to animals having unilateral or no damage to this structure. Also, in the present experiment, animal V-17 showed significant impairment in the retention of a visual discrimination. The retention data of 20 sessions for V-17 is deceptive since this animal never reached criterion. V-17 sustained a large lesion which included the medial hippocampal commissure.

This visual retention deficit might be due to more complete destruction of either the fornix fibers perforating the corpus callosum, or to destruction of hippocampal projections via the pre-and postcommissural fornix. Since both pre-and postcommissural fornices were destroyed in V-17, it would be impossible to designate which fibers might have been instrumental in producing this deficit. It is important to note though, that of the other four animals in this study who had shown retardation in learning the visual discrimination, only one had postcommissural fornix damage; the other three had precommissural fornix damage. While this analysis is admittedly speculative, it does point to specific research in the future which might resolve the relationships in question.

The four animals trained on the tactual discrimination showed no retardation in their postoperative learning. Their scores indicate, if anything, consistently faster learning. The same animals, surprisingly enough, showed severe retardation on the auditory-vibratory discrimination, though all but one eventually came up to criterion. They were able to use only vibratory cues since none ever reached criterion under the speaker-out condition. Learning was impeded either because of the lesion or because the vibratory cues might be difficult to learn, even for an intact animal. Both the tactual and the vibratory cues are essentially somesthetic; yet the same animals performed well on the one task and were severely retarded on

the other. This may be difficult to interpret unless it is considered that sensory representation of the hind legs in the rat (if it is homologous with that of higher animals) could be located on the dorsal surface of the somesthetic cortex with relays to the posterior cingulate gyrus and hippocampal rudiment. The head (including the sensitive vibrissae) and forelegs may be represented on the lateral surface as it is in higher mammals. In this case, since the closest limbic area is the posterior insula, and the claustrum has monosynaptic connections with the insula and hippocampus (Rae, 1954), it would be possible for the hippocampus, rather than the hippocampal rudiment, to mediate recall on the basis of somesthetic sensations from head and forelegs (Planek, 1965). According to Arnold's theory, recall on the basis of auditory sensations is mediated via the hippocampus. Since the insula is near auditory cortex, it is possible that recall on the basis of both auditory and somesthetic sensations (from head and forelegs) may be mediated by the same area of the hippocampus. This is suggested by virtue of the fact that many animals in this study were completely unable to recall responses necessary for an auditory discrimination after pre-commissural fornix lesions. If both auditory and somesthetic recall relays begin in the same area of the hippocampus, it would not be surprising that their projection into the fornix system should be adjacent. This analysis too, is admittedly speculative and would have to be tested experimentally.

In conclusion, recall on the basis of auditory cues seems to be the only memory modality which can be eliminated by bilateral lesions of the precommissural fornix. Though retarded learning and retention in the other sense modalities seemed to occur, no definitive conclusions can be reached at this time. The precommissural fibers of the fornix seem to be implicated in the inability to learn or retain an auditory discrimination. However, the septal area (within which these fibers terminate) was always involved. Since there is a diversity of fiber systems and nuclei in the septal area, it would seem that other structures are also involved in a precommissural fornix lesion.

Many studies have connected the hippocampal system with auditory memory (Moore, 1964; Stepien et al, 1960; Karmos and Grastyán, 1962). It is not untenable that the main efferent of the hippocampus, the fornix, be involved as a necessary relay. Though it was not a specific hypothesis in this experiment to differentiate between the pre-and postcommissural fornix fibers, the precommissural fiber damage does seem to be the only common factor in eliminating auditory recall. It is important to state, however, that in this study there were no bilateral lesions of the postcommissural fornices only. Consequently, it is not possible to say whether such lesions would have produced the same defects as the precommissural lesions do in this study. The implications of the present experiment suggest the need

for a study of the effects of a postcommissural lesion on auditory memory.

Precise degeneration studies are needed not only to trace the fornix fibers but their direction as well. Furthermore, micro-lesion techniques may in future research be able to identify pathways indicating recall in specific modalities.

Summary

This study investigated one aspect of Arnold's theory regarding the mediation of recall by the hippocampal system. Specifically, the experiment was designed to determine the effect of a bilateral transection of the fornix on the learning and retention of auditory and olfactory discriminations. It was hypothesized that such a lesion would prevent recall based on olfactory, motor, tactual, visual and auditory cues.

Seventy-one albino rats were randomly assigned to experimental groups. Final groupings, based on histological results, showed three animals with bilateral pre-and postcommissural fornix lesions, fourteen with precommissural fornix damage, and two with unilateral damage. One other animal sustained supra-callosal damage only.

The results indicate that recall on the basis of auditory cues seems to be the only memory modality which can be eliminated by bilateral lesions of the precommissural fornix. Though retarded learning and retention

in other sense modalities seemed to occur, no definitive conclusion can be reached at this time. A lesion confined to the postcommissural fornix would be necessary to confirm that auditory discrimination is mediated only by the precommissural fornix. Results were discussed in light of Arnold's theory.

REFERENCES

- Ariens, M. F., Kappers, C. V., Huber, G. C., and Crosby, E. C. Comparative anatomy of the nervous system of vertebrates, including man. New York: MacMillan Co., 2 Vols., 1936.
- Arnold, M. B. Emotion and personality. New York: Columbia, 2 Vols, 1960
- Brady, J. V., & Nauta, W. J. H. Subcortical mechanisms in emotional behavior: affective changes following septal forebrain lesions in the albino rat. J. Comp. Physiol. Psychol., 1953, 46, 339-46
- Brady, J. C., & Nauta, W. J. H. Subcortical mechanisms in emotional behavior: the duration of affective changes following septal and habenular lesions in the albino rat. J. Comp. Physiol. Psychol., 1955, 48, 412-20
- Brodal, A. The hippocampus and the sense of smell: a review. Brain, 1947, 70, 179-222.
- Brodmann, K. Vergleichende lokalisationslehre der grosshirnrinde in ihren prinzipien dargestellt auf grund des zellenbaues. Leipzig: Borth Verlag, 324 pp., 1909.
- Campbell, W. A. Histological studies on the localization of cerebral function. New York: Cambridge, 1905.
- Dott, N. M. Surgical aspect of the hypothalamus. In: Clark, W. E. L., Beattie, J., Riddoch, G., and Dott, N. M. eds. The hypothalamus Edinburgh, Oliver and Boyd, 1938.
- Driessen, G. J. The effect of bilateral lesions of the hippocampus on the learning and retention of auditory and visual discriminations in the albion rat. Unpubl. doctoral dissert., Loyola Univ., 1965.
- Fagot, J. Effects of lesions in the hippocampal rudiment on conditioned olfactory discrimination in the albion rat. Unpubl. doctoral dissert., Loyola Univ., 1962.
- Fox, S. S., Kimble, D. P., & Lickey, M. E. Comparison of caudate nucleus and septal-area lesions on two types of avoidance behavior. J. Comp. Physiol. Psychol., 1964, 58, 380-386.
- Garcia Bengochea, F., de la Torre, O., Esquivel, O., Vieta, R., & Fernandez, D. The section of the fornix in the surgical treatment of certain

epilepsies. Trans. Am. Neurol. Assn., 1954, 176-78

Gavin, H. Effects of lesions in the hippocampal rudiment of T-maze single alternation in the albino rat. Unpubl. doctoral dissert., Loyola Univ., 1963.

Harvey, J. A., Lints, C. E., Jacobson, L. E. & Hunt, H. F. Effects of lesions in the septal area on conditioned fear and discriminated instrumental punishment in the albino rat. J. Comp. Physiol. Psychol., 1965, 59, 37-48.

Herrick, C. J. Morphogenesis of the brain. J. Morphol., 1933, 54, 233-258.

Isaacson, R., Douglas, R., and Moore, R. The effect of radical hippocampal ablation on acquisition of avoidance responses. J. Comp. Physiol. Psychol., 1961, 54, 625-28

Jasper, H., Gloor, P., & Milner, B. Higher functions of the nervous system. Ann. Rev. Physiol., 1956, 18, 359-384.

Karmos, G., & Grastyán, E. Influence of hippocampal lesions on simple and delayed conditioned reflexes. Acta Physiol. Acad. Sci. Hung., 1962, 21, 215-24.

Krieg, W. J. S. Accurate placement of minute lesions in the brain of the albino rat. Quart. Bull. Northwestern Univ. Med. School, 1946, 199-208.

---- Brain mechanisms in diachrome (2nd Ed.). Evanston: Basic Books, 1957.

MacLean, P. Psychosomatic disease and the "visceral brain:" recent developments bearing on the Papez theory of emotion. Psychosom. Med., 1949, 11, 338-353.

McCleary, R. A. Response specificity in the behavioral effects of limbic system lesions in the cat. J. Comp. Physiol. Psychol., 1961, 54, 605-613.

Moore, R. Y. Effects of some rhinencephalic lesions on retention of conditioned avoidance behavior in cats. J. Comp. Physiol. Psychol., 1964, 57, 65-71.

Nielson, J. Memory and amnesia. Los Angeles: San Lucas, 1958.

- Olson, R. N., & Magee, K. R. The effect of absence of the corpus callosum on the position of the hippocampus and on the formation of Probst's bundle. J. Comp. Neurol., 117, 3, 371-387.
- Papez, J. A proposed theory of emotion. Arch. Neur. Psychia., 1937, 38, 725-43.
- Penfield, W., & Jasper, H. H. Epilepsy and the functional anatomy of the human brain. Boston: Little, Brown, 1954.
- , & Milner, B. Memory deficit produced by bilateral lesions in the hippocampal zone. AMA Arch. Neur. Psychia., 1958, 79, 475-97.
- Planek, T. W. Effect of lesions in the hippocampal rudiment on tactual and visual learning and retention. Unpubl. doctoral dissert., Loyola Univ., 1965.
- Pribram, K. H., & Kuger, L. Functions of the "olfactory brain." Annals of N. Y. Acad. Sci., 1954, 58, 109-138.
- A further experimental analysis of the behavioral deficit that follows injury to the primate frontal cortex. Exper. Neurol., 1961, 3, 432-66.
- Rae, A. S. L. The connections of the claustrum. Confinia. Neurol., 1954, 14, 211-219.
- Schwartzbaum, J. S., Kellicutt, M. H., Spieth, T. M., & Thompson, J. B. Effects of septal lesions in rats on response inhibition associated with food-reinforced behavior. J. Comp. Physiol. Psychol., 1964, 58, 217-224.
- Stepien, L., Cordeou, J., & Rassmussen, T. The effect of temporal lobe hippocampal lesions on auditory and visual recent memory in monkeys. Brain, 1960, 83, 470-89.
- Sweet, W. H., Talland, G. A., & Ervin, F. R. Loss of recent memory following section of the fornix. Trans. Amer. Neurol. Assn., 1959, 76-82.
- Thompson, R. & Langer, S. K. Deficits in position reversal learning following lesions of the limbic system. J. Comp. Physiol. Psychol., 1963, 987-995.

APPROVAL SHEET

The dissertation submitted by John F. Snyder has been read and approved by five members of the Department of Psychology.

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

The dissertation is therefore accepted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

May 22, 1961
Date

Margda B. Arnold
Signature of Adviser