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Effects of Lesions in the Hippocampal Rudiment on Conditioned Olfactory Discrimination in the Albino Rat

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EFFECTS OF LESIONS IN THE HIPPOCAMPAL RUDIMENT
ON CONDITIONED OLFACTORY DISCRIMINATION
IN THE ALBINO RAT

Hacker J. Fagot

A Dissertation Submitted to the Faculty of the Graduate
School of Loyola University in Partial Fulfillment of
the Requirements for the Degree of
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Life

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Chapter 1

Introduction

One of the principal avenues of research open to contemporary psychology lies in the development of an adequate physiological psychology. The term is not to be understood in the sense that the branch of psychology known as physiological psychology constitutes a separate science distinct from both physiology and psychology. Rather, as its name implies, it is a link between two basically related disciplines, physiology and psychology.

More specifically, the content and nature of physiological psychology depend on a solid knowledge of the facts and methods of physiology and the related biological sciences. Such a knowledge must guide the psychologist's investigations of the processes underlying the sensory perceptual, and motor activities which lie at the roots of behavior.

The day is past when major progress in psychology can be expected from an approach that limits its study to input and output and relegates the reactions of the organism to a little black box beyond the pale of licit investigation. If psychology is to continue to live up to the promise of the past and to grow vigorous enough to meet the challenge of the future, the psychologist must lift the lid from this little black box and begin to investigate what goes on inside.

The conviction that more attention must be paid to the subject is growing today and is reaching an ever more numerous body of psychologists. At this point it will be sufficient to mention a few

works produced by authors of quite diverse backgrounds and orientations, but all bearing the stamp of such an idea: Allport (1947), May (1958), Arnold (1960), Turner (1960) and Mowrer (1960).

The methods and techniques of physiological psychology do not, to be sure, exhaust the wide gamut of possible avenues of research into the role played by the organism or subject. There are other methods of investigating and assessing this role in various experimental situations. But the fact remains that these methods constitute a means of predilection for the psychologist of today and that high hopes are cherished for considerable progress through their use.

Obstacles to this expected progress exist, however, and it is important that these obstacles be given due consideration at this point.

In the first place the neurophysiologist seems, with some justification, to distrust the work of the psychologist as being too exclusively behavioral or functional and too lacking in adequate controls of a biological nature, especially in the domain of histology. He tends to look askance at studies involving careful and precise testing of behavior or function without careful use of proper techniques for determining the precise location of brain damage or stimulation.

The psychologist, for his part, tends to consider that the precision and exactitude achieved in the determination of the locus of tissue damage or electrode implantation is wasted if the investigator is satisfied with rough observations of a naive kind without proper controls. This, he finds, is no proper way to determine the behavioral or functional effects of such neurophysiological intervention, no

matter how precisely localized.

Furthermore, the psychologist contends that careful psychological analysis of function is an essential prerequisite for intelligent understanding of the neurophysiological findings. See, for example, the comments of Scheerer (1954, p. 122) and Arnold (1960, Vol. I, p. 14).

Some psychologists tend to mistrust an approach which produces neurophysiological changes more or less at random and then speculates on what the results, (and those results only which happen to strike a particular observer), may mean. This procedure, they maintain, is putting the cart before the horse. What is needed, such psychologists claim, is a systematic effort at investigating functions previously established by approved psychological analysis. These functions should then be tested by approved psychological methods before, after, and (where possible) even during the physiological changes. Only in this way can we hope to make progress in understanding what results do actually follow upon neurophysiological changes.

It is notorious, of course, that workers in the borderline fields involving interdisciplinary approaches often employ inadequate methodology in one field or the other precisely because in our day and age it is an immense task to master even a single field adequately.

Perhaps the problem posed by the radical inability of a single individual to excel in more than one complex field can be solved only through the cooperative work of teams including specialists in each of the relevant branches of learning. Nevertheless, it does seem

in place to single out for emphasis that it would be well for both parties to turn their attention from the mote in their rival's eye long enough to consider honestly the beam in their own.

This suggestion would apply a fortiori to the physiological psychologist at work on the border between these two disciplines. He, more perhaps than any one else, should attempt to incorporate the valid claims of each group into his work. He should attempt to integrate into his methodology sufficient precision in each domain to achieve a result that satisfies the legitimate demands of both groups of investigators.

The present study represents an attempt to approach as nearly as possible to this ideal. To what extent or within what limits it has been successful only the future will reveal; but it appears valuable here to show briefly how the foregoing considerations directed the choice of the field of investigation, the delimitation of the problem, and the selection of the methods of research that were used. This sketch will constitute the remainder of this chapter.

Once it had been determined that this investigation should be directed toward the shedding of some light on the role of the organism in behavior, it was clear that the study must transcend an input and output approach to the subject. Input and output were not to be excluded from the study, of course, but the precise point of investigation was to be the processes within the organism that lie at the roots of behavior.

With this orientation in mind it is evident from what has already

been said that the study must proceed along the following lines in order to achieve its aims. In the first place, the study must begin with a systematic and consistent psychological analysis of functions. Secondly, it must continue by adopting an objective and dependable method of testing the functions or processes eventually selected for investigation, or, if none proved adequate as they stood, by devising possible adaptations of existing methods according to the approved standards of scientific work in psychology. Thirdly, the study must employ the available knowledge of structures and research methods available from the neurophysiological field. In short, it may be said that the study must begin in the theoretical field, proceed to employ the methods of the experimental field (once testable hypotheses had been formulated), and conclude with proper use of the physiological field.

In the theoretical field the psychological analysis of M. B. Arnold was adopted after careful consideration. The reasons which led to this choice may be very briefly outlined here.

In the first place the analysis of Arnold (1960) is based upon a clear and precise statement of the assumptions and embedded concepts used in the analysis and of the method of analysis employed. This clarity and precision made the identification of the various functions emerging from the analysis comparatively unambiguous. The lack of this clarity would have made the analysis extremely difficult to translate into an experimental situation.

In the second place, the analysis is amenable to translation into measurable operations. Unlike many of the hypothetico-deductive

systems in existence today, this analysis commits itself to testable propositions. For this reason it seemed an excellent basis for experimentally oriented work.

In the third place, this analysis clearly separates the data from which it is derived from any theoretical constructs or systematic assumptions involved in its presentation. This feature seemed apt to lead to greater precision in delimiting precisely what was being tested and in what measure this testing could be related to constructs or theoretical elaborations.

Finally, this analysis seemed ideal for research in the field of physiological psychology precisely because it is followed by concrete applications to the field of physiology and by concrete predictions and hypotheses within this field.

Careful study of these predictions and hypotheses led to a keen interest in the role and function of the olfactory and hippocampal systems of the brain. Since definite testable functions were suggested for these systems and concrete evidence for the hypotheses was presented, it seemed worthwhile to submit at least one of these perceptive hypotheses to the test of experimentation.

The next step, of course, involved elaborating an experimental method capable of testing the functions of these systems as inferred from the analysis. The method used was selected after due consideration of many possible techniques and methods of investigation. The final choice fell upon the methods of "operant conditioning." This method would employ as a fundamental variable the rate of operant response under different stimulus conditions. In this way it was hoped that

greater sensitivity of measurement might be attained by increasing the magnitude of the numbers employed. The method permitted precise determination of units of measurement without elaborate assumptions of a statistical nature. The fact that such methods are readily adaptable to the albino rat which was the chosen subject for the study, that commercially constructed experimental equipment designed for this technique was available, and that the simplicity of the data allowed greater objectivity were further decisive advantages.

Finally, the question of neurophysiological methods and techniques was critical. Although the experimenter spent more than a year and a half in acquiring the surgical and other techniques and methods that were to be employed in this study, it was clear that the necessary competence for the post-mortem histological studies could only be achieved by recourse to a professional technician. It may be considered that this step represented a move in the direction of the team approach suggested in the earlier part of this chapter.

This brief sketch of the approach used in this study and of the rationale behind the approach will serve to make clear the nature and scope of this research. The complexity of the problems encountered in even so simple an investigation and the more detailed realization of the bare outline given in this chapter should become clearer as the details of this study unfold in subsequent chapters.

Chapter 2

The Olfactory and Hippocampal Systems

One of the most fascinating enigmas in the field of physiological psychology and in the related areas of neurophysiology and neurology today is the question of the function of the part of the brain known as the rhinencephalon. Since this study bears directly on this area of the brain, it would be well at this point to review the literature on this subject in order to set the task undertaken here in a proper perspective.

The term, rhinencephalon, (meaning "nose brain") seems to have been first used by Kolliker and referred to those regions of the brain thought to be concerned with the sense of smell (Peele, 1961, p. 527). It was, of course, long known that the olfactory nerves arise in the epithelium of the upper nasal cavity, pierce the cribiform plate of the ethmoid bone, and enter the olfactory bulb of the brain. Cytoarchitectural studies by Campbell (1905) and Brodmann (1909) suggested that there is a basic structural similarity between the olfactory bulbs, the olfactory tubercle, and the hippocampus; thus it was natural to assume that these structures, which correspond to the rhinencephalon of Kolliker, are the brain structures that mediate the activities of the sense of smell.

On the basis of supposed connections between these areas it was simply assumed that the hippocampus was the cortical receiving area for smell corresponding to the cortical projection areas of the other senses. (See, for example, the successive editions of Ranson and

Clark, 1920, 1923, 1927, 1935, 1943, 1947, and even 1953; see also the successive editions of Morris and Schaeffer, 1914, 1921, 1942, 1947, and contrast Peele, 1954, with Peele, 1961, in the treatment of the hippocampus).

Eventually, however, Papez (1929, 1937, 1938) reviewed the evidence and concluded that much of the so-called "nose brain" was in reality not simply olfactory in function. Rather, certain of these structures seemed to be related to emotional moods and states. Prime among these structures was the hippocampus which he considered the discharging structure for the emotions.

At about the same time that Papez was writing these revolutionary suggestions, Kluver and Bucy began the experimental studies on the effects of temporal lobectomy which were to bring further attention to these areas of the brain. They reported that there were remarkable changes in the behavior of monkeys after ablation of the temporal lobes, including parts of the hippocampus. Their monkeys displayed remarkable activity, running from object to object and attempting to mouth and smell each, repeating this procedure no matter how often they encountered an object. Their eating habits changed drastically, leading them to devour all sorts of foods that normal monkeys would not eat. The animals did not chatter at each other or join in the normal noise of the colony at feeding time. (Kluver and Bucy, 1939).

It was at this point that Allen began his well known series of studies on olfactory discrimination in dogs. He applied the methods of classical conditioning and concluded that the correct performance of olfactory discrimination tasks did not depend on the integrity of

the hippocampus and fornix systems, but rather on the pyriform-amygdaloid areas (Allen, 1940, 1941). Later he found that even ablation of these areas did not prevent the learning of a conditioned response to clove vapor, nor the ability of the dogs to select a packet of meat from a collection of packets containing non-edible materials (Allen, 1944). Still later, he reported that bilateral frontal lobectomy prevented the acquisition of olfactory conditioned responses and the learning of a multiple choice olfactory discrimination between cloves and asafetida, although the dogs apparently could still smell since they could select the packets of meat as before. (Allen, 1948).

During this same period Brodal (1947) made a thorough review of the literature available at that date and came to the conclusion that the hippocampus was not directly involved in the sense of smell.

MacLean (1949) then took up Papez's suggestion that the hippocampus was involved in emotional reactions. He pointed out that the hippocampus is so placed that it can correlate every form of internal and external perception and that it also has relays to the hypothalamus and somatic motor system which make it capable of producing somatic and autonomic reactions.

MacLean's article brought widespread attention to this part of the brain and its possible functions. Experimental studies began to multiply. These studies ranged from the delicate histological work of Lorente de No (1949) on the cytoarchitecture of the hippocampus and related cortical areas, to the recording of electrical potentials in various parts of the rhinencephalon (Mayer and Allison, 1949;

Adrian, 1950; Sem-Jacobsen et al., 1953; Allison, 1953a, 1953b, 1954; Hernandez-Peon et al., 1960). The facts began to emerge more clearly as the effects of direct electrical stimulation of the hippocampus (Green and Adey, 1954, 1956; Green and Arduini, 1954) and of lesions in the rhinencephalon (Green, Clemente and DeGroot, 1957; Green, Steelman et al., 1958) were reported.

In the meantime, various investigators proposed hypothetical functions for the hippocampus. Thus Kaada (1951) suggested that the hippocampus might serve the role of a forebrain suppressor. Penfield (1955) suggested on the basis of neurological evidence that the hippocampus is a memory mechanism; later he presented further evidence to support this contention (Penfield and Milner, 1958). McLardy (1959), taking an analogy from computers, spoke of the hippocampus as a detector-coder of information from the temporal lobes.

The net result of this research and this collection of evidence was a conviction on the part of contemporary neurophysiologists and neurologists that, whatever might be the role of the hippocampus, it is certainly not the olfactory projection cortex. For one thing, as Peale (1954, 1961) points out, it is clear now, in the light of accumulated evidence, that there are no direct connections from the olfactory tracts to the hippocampus as had previously been supposed. For another, more and more evidence points to other areas as the regions which mediate these olfactory functions.

Thus, Pribram and MacLean (1953) have shown by neuronographic analysis that there are connections from the ventromedial neocortex of the frontal lobe (including the olfactory areas) to the subcallosal

and medial frontal orbital cortex. There are also other linkages to the prepyriform area. Allison and Meyer (Meyer and Allison, 1949 and Allison, 1954) have shown that the lateral olfactory striae terminate in the prepyriform area. Finally, Pribram, Lennox, and Dunsmore (1950) have shown that there are connections from the olfactory tubercle to the medial orbital cortex, subcallosal (septal) cortex, posterior orbital cortex, and the prepyriform area. In view of this evidence (as Papez (1959) and Peele (1961) point out), these areas must be considered the candidates for the function of projection and association cortices for the sense of smell.

On the basis of the analysis of functions mentioned in a previous chapter and on the basis of the evidence thus far accumulated, Arnold (1960) proposed a set of hypotheses on the functions of the rhinencephalon in its various parts. These hypotheses have formed the basis for the orientation of the research undertaken here and will be briefly outlined at this point.

Arnold suggests (1960, Vol. II, p. 48 and 1962) that the olfactory system contains structures that have in essence the same functions as those exercised by the brain stem and thalamus in other sense modalities. She hypothesizes that the olfactory bulb may be the olfactory equivalent of the thalamic sensory nuclei, that the anterior olfactory nuclei may correspond to the medial thalamic nuclei of the other senses, and that the olfactory tubercle may be the cortical receiving area for smell. She further suggests that by analogy with the other senses the medial orbital cortex may be the olfactory association area, while the subcallosal gyrus and

prepyriform area may be the limbic cortex concerned with the sense of smell.

Arnold's suggestion that the orbital cortex registers olfactory impressions is based on the experiments of Wenzel (1952) and Caldwell (1958) who found that after transorbital damage hospital patients could not recognize various odors, although the olfactory threshold was unaffected. (See Arnold, 1960, Vol. II, p. 60).

This set of structures, in Arnold's hypothesis, would constitute the olfactory system. The hippocampal system would serve another function which would not be directly olfactory.

In this hypothesis the hippocampal system would be a distinct system including the hippocampus proper including its anterior continuation over the corpus callosum (the hippocampal rudiment), together with the fascia dentata and subiculum and the fornix or efferent tract of the hippocampus. (Arnold, 1960, Vol. II, p. 32).

Arnold suggests (1960, Volume II, p. 55) that the hippocampus is the trigger for the function of recall and that it operates by collecting impulses from each sensory area (relayed to the hippocampus via the adjoining limbic regions) and then triggers the activity of the association cortex (locus of the memory traces) by a relay that goes via the fornix and midbrain to the sensory thalamic nuclei and association cortex, and via the medial thalamus to the whole cortex. Extensive evidence in favor of this suggestion is given and discussed by Arnold and need not be repeated here.

It is perhaps worth noting here that Arnold's theory of hippocampal functioning fits well with Penfield's suggestion, mentioned earlier,

that the hippocampus functions as a structure serving memory, though for Arnold this would not mean an exclusive memory "center." McLardy's suggestion that the hippocampus is a detector-coder of information would be consonant with such a hypothesis, as would be MacLean's concept of the hippocampus as a correlation center.

With this review of the relevant experimental studies and theoretical interpretations of the function of the olfactory and hippocampal systems, the way has been opened to consideration of the purpose, aim, and scope of this study. The precise implications of this review will be made clearer in a later chapter. Before this chapter can be presented, however, it seems important to consider the question of experimental methods in the field of olfactory discrimination. This will be treated in the chapter which immediately follows.

Chapter 3

Studies in Olfactory Discrimination

The rat has well developed olfactory brain structures (Krieg, 1955). This fact would seem to suggest that good sensitivity to odors might be expected in this species. Indeed, general observations and simple experiments were early reported in support of this contention by Small (1899), Watson (1907, 1914) and Strong (1911).

Nevertheless, it has only been in relatively recent years that carefully controlled experiments have given much support to this view. The technical difficulties involved in controlled presentation of odors have contributed, no doubt, to the late start of a scientific investigation of olfactory sensitivity in the rat. These technical difficulties stem largely from the serious lacunae in our knowledge of the significant dimensions of odor. Studies of the senses of vision and hearing have been greatly facilitated by the knowledge that pitch and hue depend on the frequency of vibration, while the amplitude of vibration is related to intensity. But in the study of the olfactory system, research is greatly handicapped by ignorance of the stimulus dimension which may be relevant to odor qualities.

That certain chemical factors play a role in the stimulus dimensions seems well established (Passy, 1892; Haycroft, 1889; Henning, 1924; Von Skramlik, 1925; Moncrieff, 1946). But it is equally clear that these factors are not a complete explanation (Zwaardemaker, 1922; MacDonald, 1922; Beck and Miles, 1947; Young,

Fletcher, and Wright, 1948; Beck, 1950; Pfaffmann, 1951; Jones and Jones, 1953; Wenzel, 1954). It has been found, for instance, that the volatility of the stimulus is very important (Elsberg, Brewer, and Levy, 1935-1936) and that the pressure of the applied stimulus is more important than the volume of stimulus used (Jerome, 1942; Wenzel, 1949). Another important factor is the subject's rate of inhaling (LeMagnen, 1942-1943; 1944-1945).

To complicate the picture, it has been found that olfactory adaptation (Allison and Katz, 1919; Komuro, 1921; Foster, Scofield, and Dallenbach, 1949) and cross adaptation (Ohma, 1922; LeMagnen, 1948a) play a large role in determining the sense qualities of an odor. This adaptation seems to vary with such factors as pregnancy (Hansen and Glass, 1936), menstruation cycle (Elsberg, Brewer, and Levy, 1935; LeMagnen, 1948b), the sex and age of the subject (LeMagnen, 1948b) and finally, the individual (Guillot, 1948a, 1948b; Krak, Amerine, Ough and Baker, 1959).

In any event, the first carefully systematic study of olfaction in the rat was done by Liggett (1928). He used four different methods of testing olfactory acuity.

In the first method he showed that normal rats had little difficulty in locating a piece of cheese hidden by sawdust under one of nine squares, while anosmic rats (rats in which the olfactory bulb had been ablated) failed to do so.

In the second method, the performance of normal and anosmic rats in learning a maze by following an odorous trail was compared. The results of this method were inconclusive.

The third method used a T-shaped maze in which the rat was to turn one way if an odor was present and the other way when it was absent. Apparently this task was unduly difficult, for only one rat learned to turn to the right for anise and to the left for amyl acetate, and this took 1000 trials.

The fourth method consisted in training rats in a Yerkes type discrimination box to discriminate between two odors presented simultaneously. Two animals showed some evidence of discrimination, but they did not retain the discrimination long enough for controls to be run. Only one rat gave clear evidence that he could discriminate between the presence and absence of amyl acetate, but even this result has been questioned because amyl acetate is known to have a tactile irritating quality which may have served as the basis for the discrimination.

When Liggett used human subjects for his odors he found that the discrimination was difficult though possible. Despite the elaborate precautions taken for the presentation and withdrawal of stimuli, it is possible that some blending of the odors made the task more difficult and it also seems probable that the odors were not sufficiently strong. In any event, Liggett's investigations failed in general to confirm the expected results of olfactory discrimination tests in the rat.

More recent investigators, however, have made careful efforts to simplify the experimental situation and to bring about a more direct connection between the odor and the behavior used as an indication of discrimination.

Swann (1933) tried unsuccessfully to bolster the evidence with a modification of the Lashley jumping stand arranged so that puffs of odor-bearing air came at the animal from the respective cards against which it was to jump.

After trying several other devices, also without success, Swann hit upon a method which proved more viable. A food box was constructed with two entrances, only one of which was accessible at any given time. These two entrances at opposite ends of the apparatus were each blocked with a pile of scented sawdust. Each pile of sawdust had a different odor and the box containing it was interchangeable so that the piles could be easily changed in position. When the animal succeeded in clearing away the "correct" pile of scented sawdust he could gain access to the food box through a trap-door. If the animal chose the "incorrect" pile he had to dig through the other pile of sawdust until he reached an open trap door before being admitted to the food box. The rats were given ten trials a day under mild food deprivation and after an average of 75 trials reached a criterion of 27 correct responses out of 30 trials. Totally anosmic rats (with removed olfactory bulbs) were completely unable to discriminate. A large number of controls were used and it seems clear that smell was the relevant factor in the discrimination.

Honzik (1936, p. 40) required rats to pull in one of three strings that was coated with the odor of anise. He reports that after string pulling was mastered, the olfactory discrimination was quickly formed. He gives no data on the number of trials required for learning.

Brown and Ghiselli (1938a) reported clear evidence of an olfactory discrimination in a multiple-unit maze requiring the animal to differentiate between the odor of anise and the odor of creosote by responding in a positive way to one of these odors. Twelve elevated maze units were used. They had small wells containing a drop of one odorant or the other, set in the choice points. Just beyond each well was an electrifiable grill. An incorrect choice was punished by electric shock, a correct response allowed the animal to escape punishment. The odors varied in a random order from left to right, and adequate precautions were taken against the use of outside cues. A criterion of 33 correct responses out of 36 was reached by 22 normal rats in an average of 62 trials (i.e. 744 choices).

Stone (1941) reported a deceptively simple and quite effective design that involved presenting rats with four dishes of their customary food, three of which contained enough quinine to produce emesis. A variety of inexpensive perfumes were used to serve as discriminative stimuli identifying the "correct" and "incorrect" dishes. Rotation of dishes and positions was employed in a random order, in five daily trials. Stone reports that the great majority of his rats made no incorrect responses after the first two days. He discovered that blind animals were not handicapped in learning the discrimination but that ten anosmic animals not only failed to learn the discrimination but gave no hint of beginning to learn it after thirty days of post-operative training. Stone's controls appear to have been adequate except for one defect he himself points

out: it was not possible with his apparatus to tell whether or not the animal licked the food slightly and so had access to taste cues.

French, a collaborator of Stone, devised a more adequate discrimination apparatus using five equally spaced food dishes, so constructed that the rat had to reach into a small hole in a wire mesh cover to obtain the food. This arrangement prevented the animal from reaching the dish with the tongue or tip of the nose. The controls appear to have been adequate for the series of experiments that were performed with this apparatus, which is essentially a modification of Stone's original idea. French reported that five rats were tested with eleven different odors and learned to discriminate easily. A control group of five anosmic rats failed to learn the discrimination (French, 1940).

Lashley and Sperry (1943) developed a simplified form of the French apparatus using a small perforated glass cover for the discriminative stimuli and only three food dishes. Using Stone's basic design with this further simplification rats reached a criterion of 28 out of 30 consecutive trials in as few as 15 learning sessions. Complete removal of the olfactory bulbs was followed by failure to discriminate. Adaptations of the French apparatus were used in most later experiments.

In the meantime, Foster and Dallenbach (1948) had made a great step forward in olfactometry by devising the olfactorium. This instrument was in essence a way of getting an odorless environment of limited space which permits a controlled flow of odor. The method was applied with some variations in design but no essential

changes by Wenzel (1955) to human subjects and by Kalmus (1958) to dogs. An ingenious application of the same principle of control by Cheesman and Kirkley (1959) permitted the use of the instrument for group threshold measurements.

Finally, Pfaffmann, Goff, and Bare (1958) adapted the technique for the rat. A bar-press apparatus and dipper mechanism for water reward were mounted in a cylindrical glass "wind tunnel." A stream of odorized air was made to flow through the cylinder at a known velocity. The odor was introduced in known volumes and concentrations. The animal was trained to face into the air stream when pressing the bar, so that all body odor or odorant absorbed on the animal's fur would be blown out to the rear of the apparatus. The method was used to study the effects of ablation of the olfactory bulbs, to measure olfactory thresholds, and to experiment on animals with altered glandular balance.

A similar apparatus was used by Michelson (1959) in establishing a discrimination based on olfactory stimuli in pigeons.

The apparatus employed in the present study is essentially a modification of Pfaffmann, Goff, and Bare's olfactometer for the rat. Of all the methods discussed, this method promises greatest sensitivity of measurement and greatest objectivity in recording the results.

This chapter concludes the study of the literature pertinent to this study. Now, the aims and scope of the study can be set forth against a background of information which will make it possible to grasp their import more clearly. The immediately following chapter will attempt to set down the scope and aims of the research clearly and simply while relating it to the literature reviewed.

Chapter 4

The Problem, Purpose, and Hypotheses

It is evident that learning of a conditioned olfactory discrimination requires not only the simple ability to smell but other functions as well. It is obvious, of course, that a completely anosmic animal, which cannot smell at all, cannot be expected to perform a task involving olfactory discrimination. It is perhaps less often noted, although obvious in itself, that even if an animal can smell, he may not be able to give evidence of this in a conditioning situation requiring him to perform some other response which may be impaired or prevented. For example, a mouse may be taught to jump at a certain auditory signal. When the mouse is thoroughly trained to jump whenever this signal is given, the experimenter then lames the mouse so that it has no use of its legs at all. It is obvious that the mouse will no longer jump when the signal is given, but no scientist worthy of the name would conclude that laming the legs of a mouse produces deafness!

Amusing as this example may seem, it points out an important lesson. We must be very careful to note that a discrimination may be prevented by any of several processes that intervene between the stimulation of the receptor cells of the nasal epithelium and the action that externally indicates that discrimination. Other deficits besides faulty sense of smell may result in a failure to learn or retain a conditioned olfactory discrimination. For this reason it is important at this point to look closely at the operant conditioning

procedure chosen as a means of testing olfactory discrimination.

The experimental method used for this test in the present study requires that a rat be trained to press a lever in an experimental chamber for a water reward. Next, the rat is trained to press the bar only when a certain odor is present in the chamber and to stop pressing the bar when another odor is introduced.

It is clear that, if a rat can learn to perform this task (assuming that other cues are excluded), he can discriminate between the two smells. But the failure of a rat to learn to perform this task does not necessarily imply that the animal cannot smell. Nor does it allow the experimenter to conclude that surgical or other procedures preceding testing necessarily eliminated the sense of smell.

For the rat to perform this task, it is true, he must be able to smell, but he must also be able to do several other things.

The rat must be able, for instance, to use his past experience with odors and bars and rewards. In the terminology of Arnold's analysis of functions (mentioned in a previous chapter) the rat must be able to recall on sniffing one odor that a bar is to be pressed and that this response will lead to water; and on sniffing another odor that pressing a bar will not be followed by the water. It is evident that, no matter how well the animal in question can smell, he will not be able to give evidence of a conditioned olfactory discrimination determined by this method unless he has this recall.

For such recall to be possible, according to the hypotheses of Arnold, not only must there be some storage area for the relevant

past olfactory impressions, but there must also be a trigger that touches off olfactory, visual, and motor recall, all of which would be necessary for the discriminatory response.

Arnold's suggestion, outlined in Chapter Two of this study, is precisely that the hippocampus serves as the trigger for recall and that the orbital cortex serves as the storage area for olfactory impressions. This means that the olfactory impressions would be registered in the orbital cortex and would be reactivated by the trigger action of the hippocampus. Visual and motor engrams would be registered in the occipital and prefrontal association areas and would also be reactivated via the hippocampal system.

For this triggering action to take place, again according to Arnold (1960, Vol. II, p. 55), it is necessary that "impulses from the sensory areas, relayed to the adjoining limbic region, can be sent to the hippocampus and from there via fornix and brainstem back to the sensory thalamic nuclei and the cortical association areas."

In the particular case of the sense of smell Arnold has suggested that the primary sensory area is the olfactory tubercle, and the limbic region the subcallosal gyrus. (Arnold, 1960, Vol. II, pp. 48-49), and that the connection to the hippocampus may go by way of the hippocampal rudiment or longitudinal striae that arch over the corpus callosum.

Now there are many hypotheses and an entire theory of brain function involved in Arnold's suggestions. These suggestions are in fact derived inferences from the general theory. To test the general theory adequately would take hundreds of studies. The present research,

therefore, does not even pretend to prove Arnold's general theory or to disprove it. What this study puts to the experimental test is only one aspect of one hypothesis derived from this theory.

The hypotheses of this study are two and they may be put concisely and precisely in the following form:

1) Rats which have undergone surgery resulting in lesions interrupting the hippocampal rudiment (including the indusium griseum and longitudinal striae) will not be able to learn a conditioned olfactory discrimination tested by operant techniques.

2) Rats which have undergone surgery resulting in lesions interrupting the hippocampal rudiment (including the indusium griseum and longitudinal striae) will not retain a preoperatively learned conditioned olfactory discrimination tested by operant techniques.

It is to be noted that these two hypotheses, though arrived at on purely theoretical grounds on the basis of Arnold's theory, are operationally definable and completely testable. They constitute the immediate aim of this research. A more distant aim may be said to be shedding some light on the functions of this part of the rhinencephalon and, ultimately, on the processes which intervene in the organism between stimulus and response.

Chapter 5

Experiments on Retention

The experiments on retention for this study may be conveniently discussed in terms of the five successive steps in which they were carried out: 1) training the animals to use the bar-pressing apparatus 2) selection of subjects for the experiments 3) training of the subjects in an olfactory discrimination 4) surgery aimed at placing lesions in the hippocampal rudiment 5) testing of the animals for retention of the preoperatively learned discrimination. Each of these steps will be discussed in turn in this chapter.

Training the Animals to Use the Bar-press Apparatus

Apparatus A standard experimental chamber of the type known as a Skinner box (Stoelting, University of Chicago design) was used. This chamber contained a single bar set into the far wall of the chamber at a position three inches from the right far corner and three inches above the level of the grid serving as a false door. The bar was connected to a dipper mechanism which supplied the animal with a drop of water each time the bar was pressed. The bar was also linked to a counter which automatically registered each bar-pressing response.

This standard experimental chamber was fitted to a ventilating system in such a way that all the air in the chamber entered through a rubber tube one inch in inside diameter. The tube was inserted into the floor of the box at the end farthest from the bar and dipper and ran along the floor to a position just under the bar. It was,

therefore, from this point under the grid that air entered the experimental chamber.

The air was drawn from the chamber through a series of minute holes drilled into the roof of the chamber at a point equidistant from the four walls. A latex hood placed over these air exits led to a ventilating fan which drew the air through the chamber at a steady rate. All other exits or adits were sealed by thick latex patches held in place with electrician's tape and lightly coated with paraffin.

Since the air entering the chamber came from a point directly under the bar and was drawn up and back away from the point of entry, it was impossible for the animal to face in any other direction than directly into the air stream while pressing the bar. This arrangement obviated the need for special training of the animal to face into the air stream as in the olfactometer of Pfaffmann, Goff, and Bare. The subject could not press the bar except by facing into the air stream.

Subjects for Bar-press Training

Thirty male albino rats of the Sprague-Dawley strain were used in this training procedure. They were 66 days old when training began and experimentally naive. Before beginning training each of these animals was well adapted to the laboratory. Each rat had been placed on a water deprivation schedule for five days preceding the onset of bar-press training. During these five days the animals were allowed 15 minutes access to water once a day but were fed on an ad lib schedule with Rockland Rat Diet pellets. Feeding and watering

took place at approximately the same hour (plus or minus two hours) each day.

Procedure for Bar-press training

Each animal was trained to press the bar in the experimental chamber

for a water reward. This training was accomplished in 12 daily 20 minute sessions in the experimental chamber. In addition to the water received in the experimental chamber each rat was given ten minutes access to water immediately after each training session and was continued on an ad lib feeding schedule. During these bar-press training sessions the ventilating system kept a steady stream of air coming through the apparatus but no odorant was introduced into the air stream. No restrictions other than those inherent in the mechanical limitations of the apparatus were imposed upon the response of the animals. A minute by minute record of the bar-presses registered by the automatic counter was kept during this entire period.

The animals used in this experiment were never handled manually by the experimenters. Transfer of the animals from their home cages to the experimental chamber was accomplished by placing the entire home cage on its side within the experimental chamber. At the beginning of bar-press training a considerable delay occurred before the animals ventured from their home cages into the body of the experimental chamber at the beginning of the training session and an even longer delay before they returned to the home cages when these were reinserted at the conclusion of the session. Before the fifth day of bar-press training was completed, however, all animals had learned to negotiate these transfers with little or no delay. This facility in transfer

was retained throughout subsequent experimentation by all animals used.

Results of Bar-press Training By the end of the 12 experimental training sessions the rate of response of the subjects had become stable in the case of every animal actually used for further experiment. No animal of the 30 failed to learn to press the bar for a water reward.

Selection of the Subjects for Retention Experiments

At the conclusion of the training sessions ten animals were selected for the study on retention. The selection was made on the basis of high bar-press rate during the training sessions. Of the 20 animals showing the highest number of responses per session while they were being trained to press the bar for water reward, ten were selected for the study on retention. Five were assigned to each of two groups in such a way that the groups were paired for response rate and for weight. Half of these animals constituted the experimental group for the study of retention (Experimental Group A), and half constituted the operated control group (Control Group A). It is to be noted that the entire set of 30 animals were already paired for age (all animals were born on the same day) and for sex (all animals were males). The treatment of these animals was rigorously standardized throughout the entire set of experiments.

Training of the Subjects for Olfactory Discrimination

Apparatus The experimental chamber used for training the animals to press the bar was used throughout the entire set of experiments of this study. This chamber and its ventilating system have been previously described in this chapter. During the

discrimination training the following additions were made to the intake part of the ventilating system. Latex expansion bulbs were fitted to the air intake in such a way that the experimenter could by opening or closing the valves on these bulbs introduce a measured quantity of odorant into the air stream just before the entry of air into the intake tube. Thus odorized air was introduced into the intake tube and drawn through the experimental chamber. These additions were at all times invisible to the subjects because they were situated under the floor of the bar-pressing box on a shelf constructed for that purpose. No other modifications of the apparatus was made.

Procedure for Discrimination Training

Each subject elected for the retention experiments

was trained in an olfactory discrimination by daily 20 minute sessions in the experimental chamber. Each rat was trained to respond by pressing the bar for water reward whenever the odor of extract of pine was introduced into the chamber and to refrain from pressing the bar when the odor of oil of hyacinth was present. This training in odor discrimination was accomplished by the simple expedient of replacing the water well from which the dipper was replenished by an empty well whenever the odor of oil of hyacinth was introduced into the apparatus. The full well was put in place under the dipper whenever the odor of extract of pine was present. This exchange could be effected without giving the subjects any auditory cue because a paper well was used from the beginning of preliminary training and throughout the discrimination training and

testing. This well could be maneuvered without making any noises. To avoid any possible visual cues the experimenter was careful to change wells only when the animal was not actually at the dipper. Since the electrical mechanism which set the dipper in operation made a distinctive noise it was necessary to employ these precautionary measures. If the dipper had simply been disconnected the presence or absence of the sound made by the mechanism would have constituted an auditory cue to the presence or absence of water. But the use of an empty well eliminated this difficulty since the dipper was always activated by a bar-press whether or not a water reward was forthcoming. This meant that the noise made by the dipper could not become a discriminative stimulus.

Each daily 20 minute session was equally divided into periods in which extract of pine was present in the chamber (at which time a bar-press was rewarded) and periods in which oil of hyacinth was present (at which time a bar-press received no reward). The time for changing odors was determined by recourse to a table of random numbers. Each number was taken to designate the time in minutes (from the start of the session) at which a change of odor was to be instituted.

Results of Discrimination Training

At the end of nine days of training in discrimination each of the ten animals was tested for differences in rate of response under the two sets of conditions constituted by the presence of two different olfactory discriminative stimuli. The

number of responses per minute for each of the experimental animals and the matched control animals for this twenty minute session can be seen in the bar-graphs on the three following pages in Figures one through ten. Simple inspection of the data shows strikingly that all animals did learn the olfactory discrimination. No animal gave as much as three responses per minute while the negative stimulus was present. The sharp drop in the number of responses at each introduction of the negative discriminative stimulus (oil of hyacinth) leaves no doubt that the discrimination was well learned. It is perfectly patent that all animals simply stopped responding when the negative stimulus was present and began responding when the positive stimulus was reintroduced into the box.

The results of this discrimination test may be evaluated in terms of the total number of responses made during the presence of the positive and negative discriminative stimuli. These data are presented in Table 1.

Table 1
 Number of Responses Made in the Presence
 of Positive and Negative Discriminative Stimuli

Subjects	Total number of responses made in the presence of	
	Extract of Pine (Positive stimulus)	Oil of Hyacinth (Negative stimulus)
Experimental Group A		
Rat # 1	152	10
Rat # 2	273	8
Rat # 3	197	12
Rat # 4	177	9
Rat # 5	204	11
Control Group A		
Rat # 6	191	10
Rat # 7	195	10
Rat # 8	109	10
Rat # 9	241	11
Rat #10	202	9



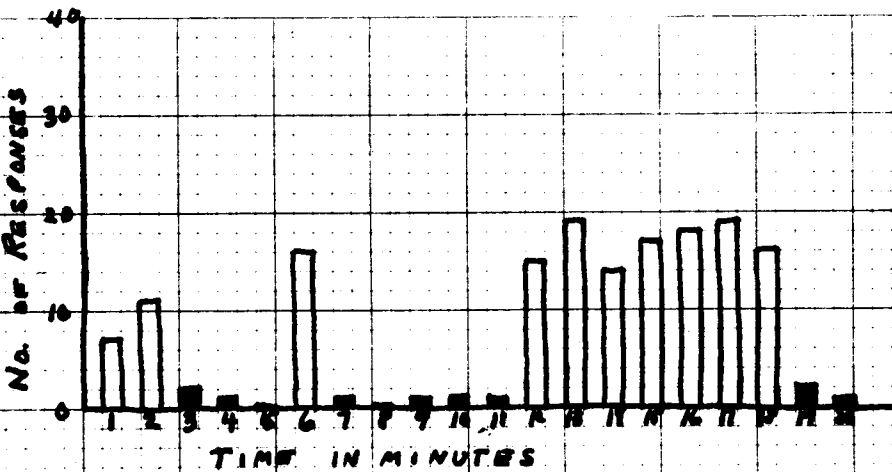


FIG. 1. NUMBER OF BAR PASSES MADE BY RAT #1 IN PREOPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE □ AND NEGATIVE ■ DISCRIMINATIVE STIMULI.

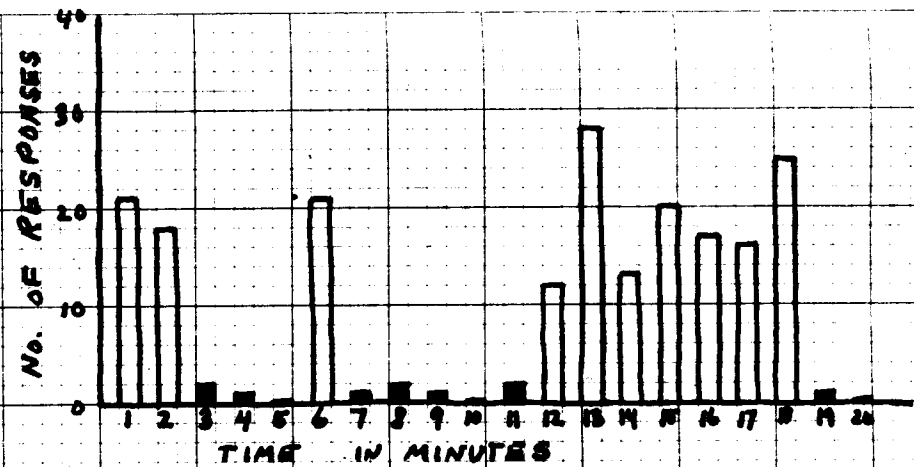


FIG. 2. NUMBER OF BAR PASSES MADE BY RAT #6 (PAIRED WITH RAT #1) IN PREOPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE □ AND NEGATIVE ■ DISCRIMINATIVE STIMULI.

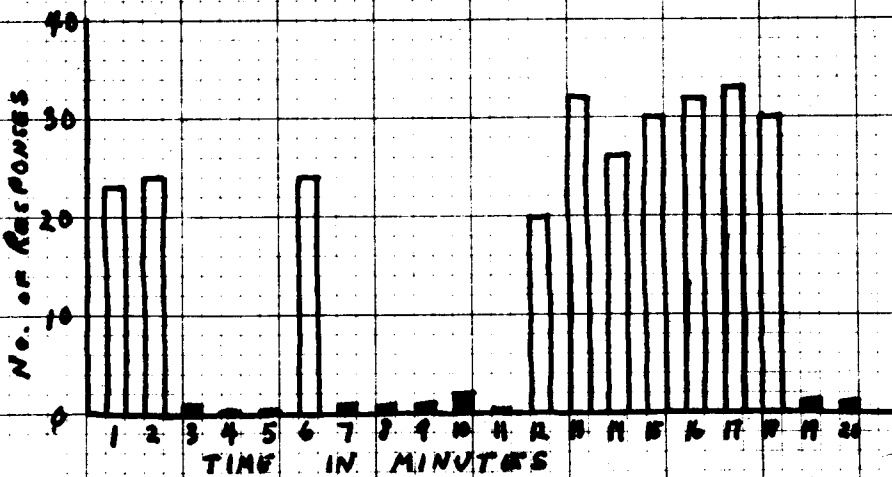


FIG. 3. NUMBER OF BAR PASSES MADE BY RAT #2 IN PREOPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE □ AND NEGATIVE ■ DISCRIMINATIVE STIMULI.

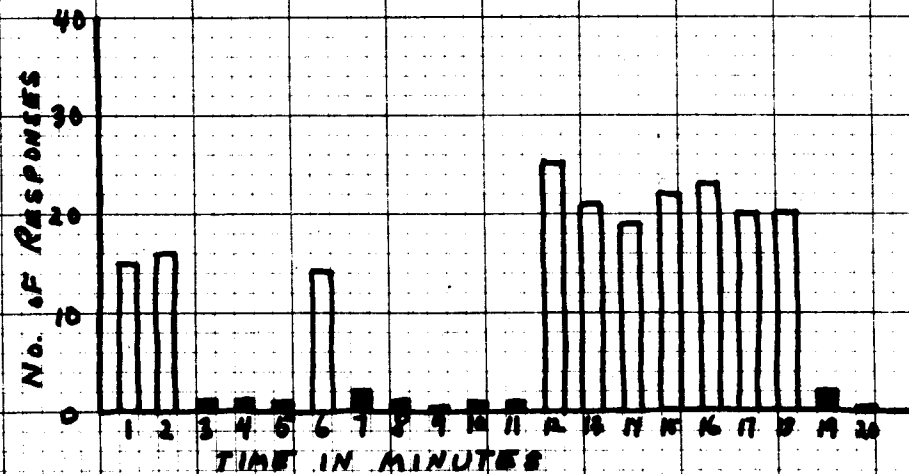


FIG. 4. NUMBER OF BAR PASSES MADE BY RAT #7 (PAIRED WITH RAT #2) IN PREOPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE □ AND NEGATIVE ■ DISCRIMINATIVE STIMULI.

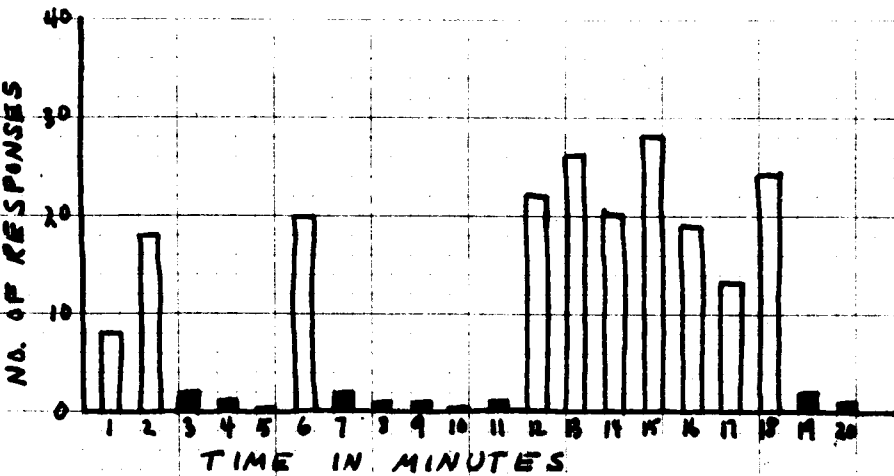


FIG. 5. NUMBER OF BAR PRESSES MADE BY RAT #3 IN PRE OPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE AND NEGATIVE DISCRIMINATIVE STIMULI.

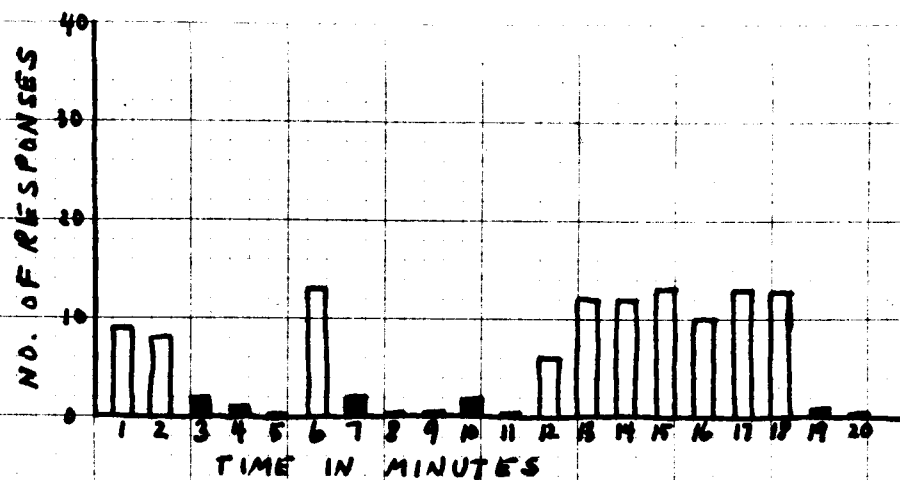


FIG. 6. NUMBER OF BAR PRESSES MADE BY RAT #8 (PAIRED WITH RAT #3) IN PRE OPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE AND NEGATIVE DISCRIMINATIVE STIMULI.

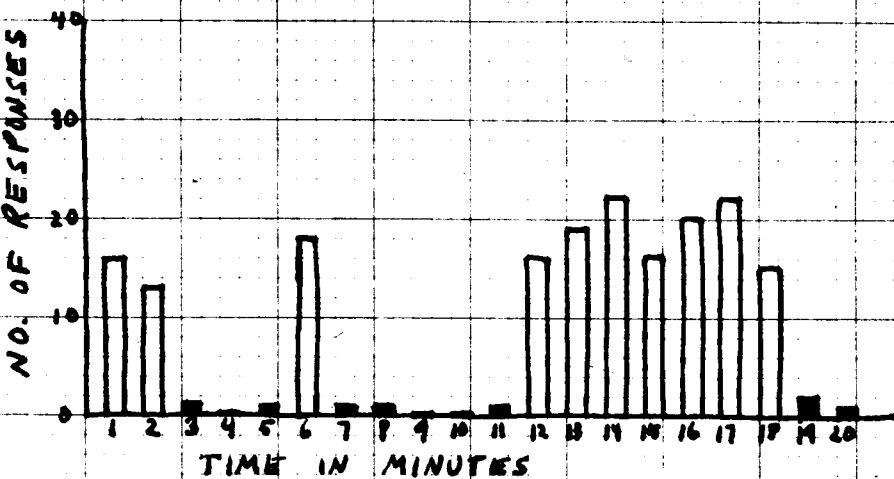


FIG. 7. NUMBER OF BAR PRESSES MADE BY RAT #4 IN PRE OPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE AND NEGATIVE DISCRIMINATIVE STIMULI.

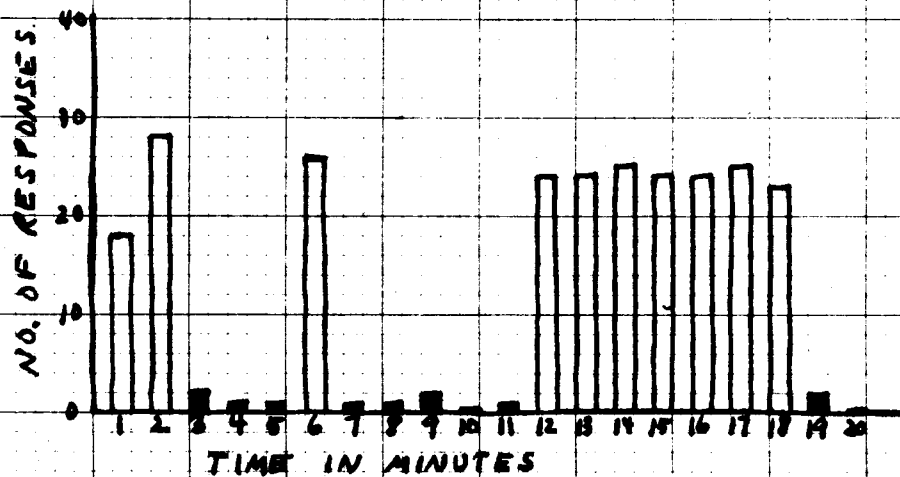


FIG. 8. NUMBER OF BAR PRESSES MADE BY RAT #9 (PAIRED WITH RAT #4) IN PRE OPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE AND NEGATIVE DISCRIMINATIVE STIMULI.

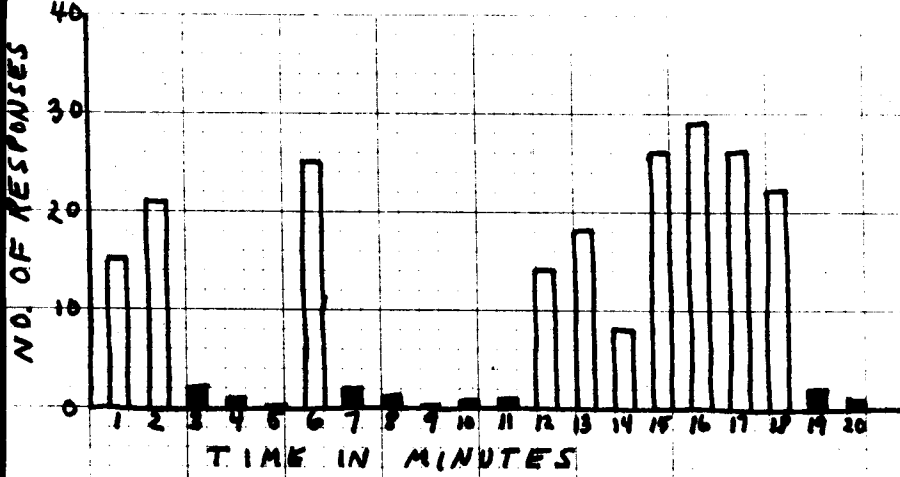


FIG. 9. NUMBER OF BAR PASSES MADE BY RAT #5 IN PREOPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE AND NEGATIVE DISCRIMINATIVE STIMULI.

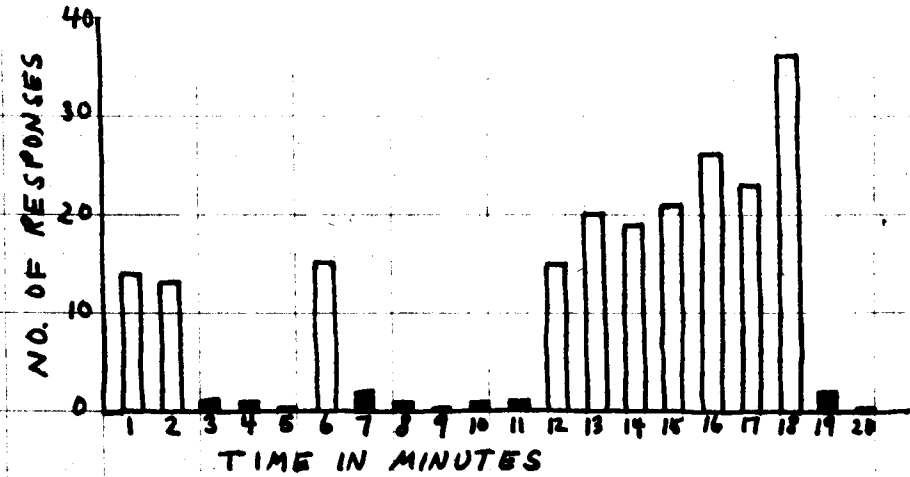


FIG. 10. NUMBER OF BAR PASSES MADE BY RAT #10 (PAIRED WITH RAT #5) IN PREOPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE AND NEGATIVE DISCRIMINATIVE STIMULI.

The significance of these distributions of response between negative and positive discriminative stimulus conditions was tested for each animal individually (using the total number of responses) in a chi-square test. Since the direction of the expected difference was implicit in the operational statement of the research hypothesis, the one-tailed version of the test was used.

The probabilities of values as large as the observed values of chi-square being considerably below the 0.001 level, it was concluded for each animal undergoing the discrimination test that he had learned the olfactory discrimination.

Indeed, the actual results of the experimental testing for olfactory discrimination are so striking that such a statement of fiduciary probability levels scarcely seems to do justice to the data. As a matter of fact the probabilities are null at much lower levels. Simple inspection of the Figures 1 through 10 and the data of Table 1 should suffice to make this point abundantly clear. In any event there seems no reasonable doubt that can be cast on the conclusion that all animals so trained actually learned the olfactory discrimination by the time of this preoperative discrimination test.

Surgery Aimed at Placing Lesions in the Hippocampal Rudiment

Subjects for Surgery

The ten animals which had learned the olfactory discrimination described above had been divided, as mentioned previously, into Experimental Group A, composed of rats # 1, 2, 3, 4, and 5, and Control Group A, comprised of rats # 6, 7, 8, 9, and 10. Each of these animals underwent

surgery in the following way.

Surgical Procedure

Each rat was anesthetized by sub-induction dosages of pento-barbital sodium (Nembutal) injected intra-peritoneally followed by administration of an ether-air mixture in an ether cabinet. The cabinet consisted of a cylindrical chamber with a capacity of five liters. A measured quantity of the mixture was pumped into this cabinet and allowed to escape through a small orifice at the side opposite to the point of entry of the pump.

When the anesthetic had taken effect surgery proceeded. The scalp on the dorsal surface of the skull was incised at the midline and the skull itself was cleared of galea and periosteum. Trepine holes were drilled at a point one millimeter rostral to the bregma and one millimeter to the right of the sagittal suture of the skull.

A Krieg Stereotaxic Instrument (Model # 51200, Stoelting) was used to insert a 29 gauge ready-varnished copper wire electrode with indifferent cathode. In order to minimize electrode track damage to the cingulate gyrus, the electrode was inserted at an angle rostral and left of the point of insertion following the procedure recommended by Krieg (1946). This method was intended to permit the electrode to slip more easily into the medial sagittal fissure. Since the structures aimed for are very small, a single electrode was believed sufficient to place a lesion which would interrupt the hippocampal rudiment bilaterally.

For all animals of Experimental Group A, 1.0 milliamperes of

direct current was applied to the electrode for a duration of 15 seconds. For the animals of Control Group A, the electrode was inserted in the same manner, but no current was applied.

When the lesions had been performed, the trephine holes were covered with Gelfoam padding and the incision was sutured.

Postoperative Care Each animal was allowed to recover from the effects of surgery for a period of 14 days during which he was maintained on an ad lib food and water regime. The animals were carefully observed but were left unmolested during this recovery period. No antibiotic injections were given in view of the evidence of LeMagnen that penicillin and other antibiotics have a marked influence on olfactory acuity (LeMagnen, 1948).

Testing the Animals for Retention of the Discrimination

Procedure At the end of 14 days of recovery in the home cages, each animal was subjected to three days of water deprivation during which he had access to water for 15 minutes a day. No change was made in the ad lib feeding schedule at this time. Finally, on the 18th day after surgery, each rat was tested for retention of the previously learned olfactory discrimination. Once more the table of random numbers was used to determine at what points in the testing the odors would be changed.

Results of the Retention Test The number of bar-press responses made by each experimental animal

can be seen in the bar-graphs given in Figures eleven through twenty on the three following pages. Each control animal's record is placed near the record of the corresponding matched animal of the experimental group for the purpose of facilitating comparison of performance. It is clear from these figures that the control animals all retained the olfactory discrimination which they had learned preoperatively. They show the same marked characteristics as in the preoperative discrimination test. By contrast, it is evident that the animals of Experimental Group A, each of whom had clearly discriminated between the two odors before surgery, did not retain the discrimination.

The results of this discrimination test may be evaluated in terms of the total number of responses made during the presence of the positive and negative discriminative stimuli. These data are presented in Table 2.

Table 2
 Number of Responses Made in the Presence
 of Positive and Negative Discriminative Stimuli

Subjects	Total number of responses made in the presence of	
	Extract of Pine (Positive stimulus)	Oil of Hyacinth (Negative stimulus)
Experimental Group A		
Rat # 1	133	148
Rat # 2	253	251
Rat # 3	224	241
Rat # 4	181	189
Rat # 5	192	207
Control Group A		
Rat # 6	135	8
Rat # 7	221	10
Rat # 8	137	9
Rat # 9	191	10
Rat #10	187	13

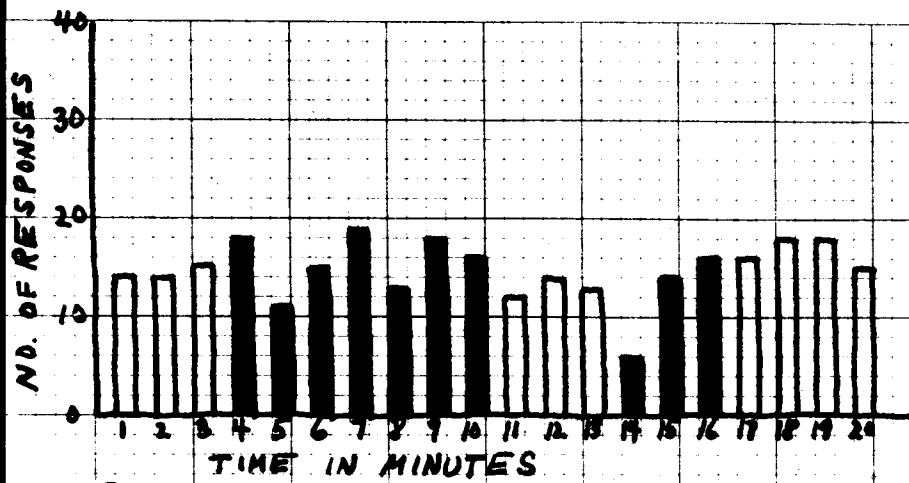


FIG. 11. NUMBER OF BAR PRESSES MADE BY RAT #1 IN POSTOPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE AND NEGATIVE DISCRIMINATIVE STIMULI.

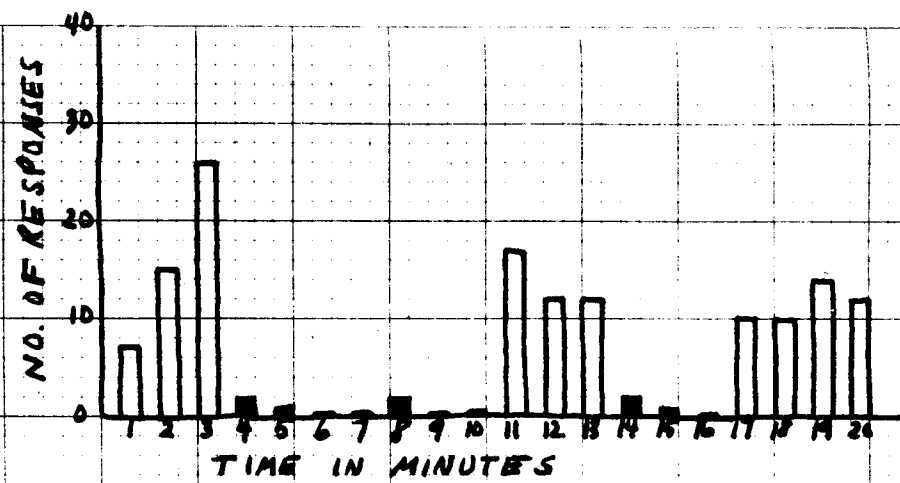


FIG. 12. NUMBER OF BAR PRESSES MADE BY RAT #6 (PAIRED WITH RAT #1) IN POSTOPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE AND NEGATIVE DISCRIMINATIVE STIMULI.

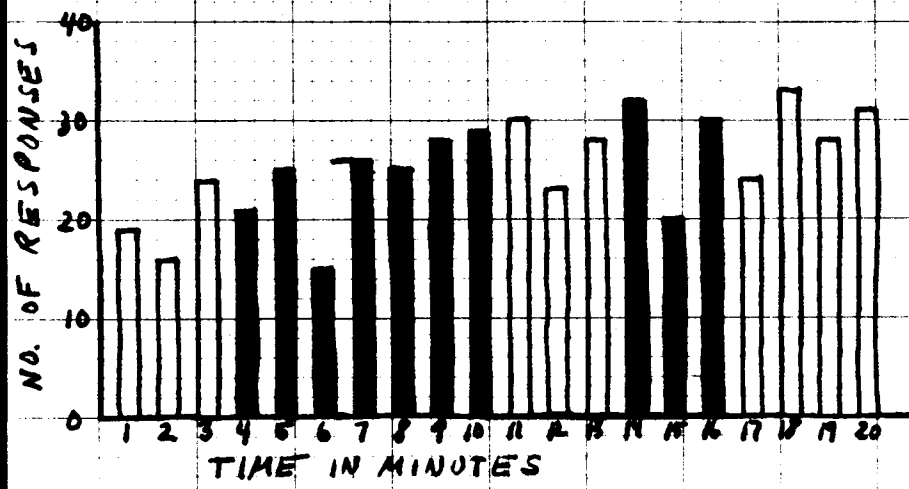


FIG. 13. NUMBER OF BAR PRESSES MADE BY RAT #2 IN POSTOPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE AND NEGATIVE DISCRIMINATIVE STIMULI.

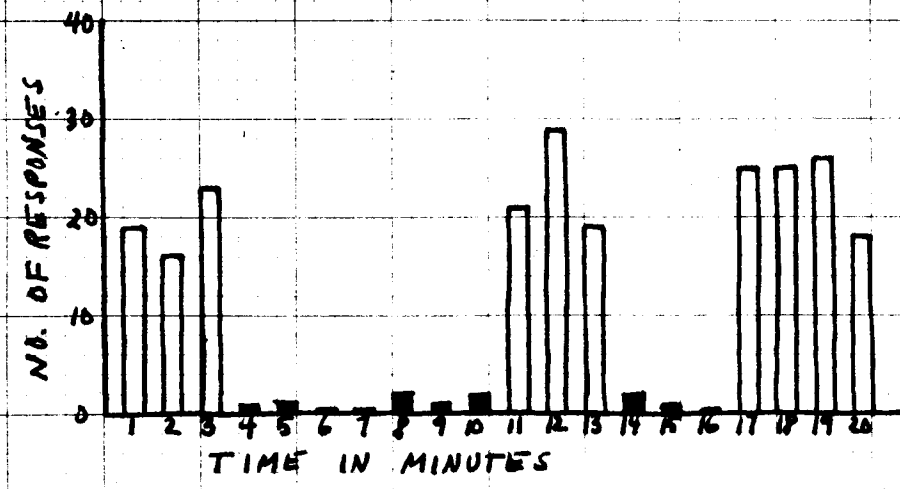


FIG. 14. NUMBER OF BAR PRESSES MADE BY RAT #7 (PAIRED WITH RAT #2) IN POSTOPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE AND NEGATIVE DISCRIMINATIVE STIMULI.

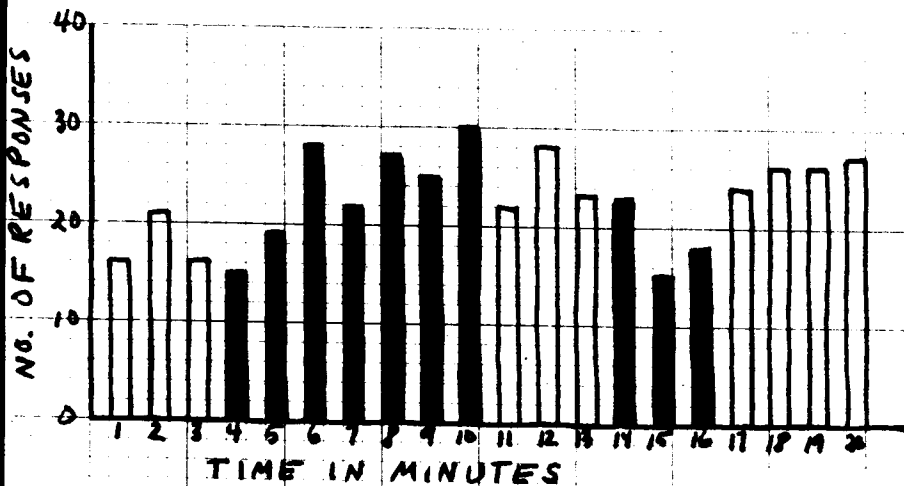


FIG. 15. NUMBER OF BAR PASSES MADE BY RAT #3 IN POSTOPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE AND NEGATIVE DISCRIMINATIVE STIMULI.

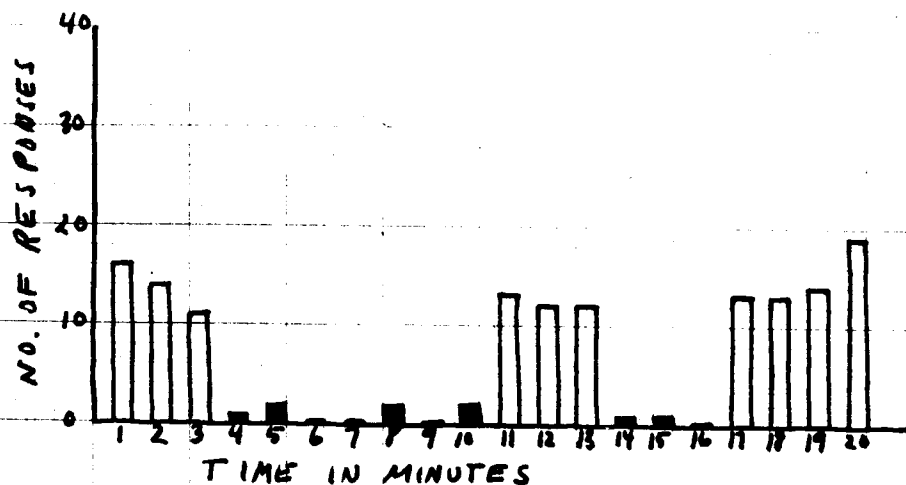


FIG. 16. NUMBER OF BAR PASSES MADE BY RAT #8 (PAIRED WITH RAT #3) IN POSTOPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE AND NEGATIVE DISCRIMINATIVE STIMULI.

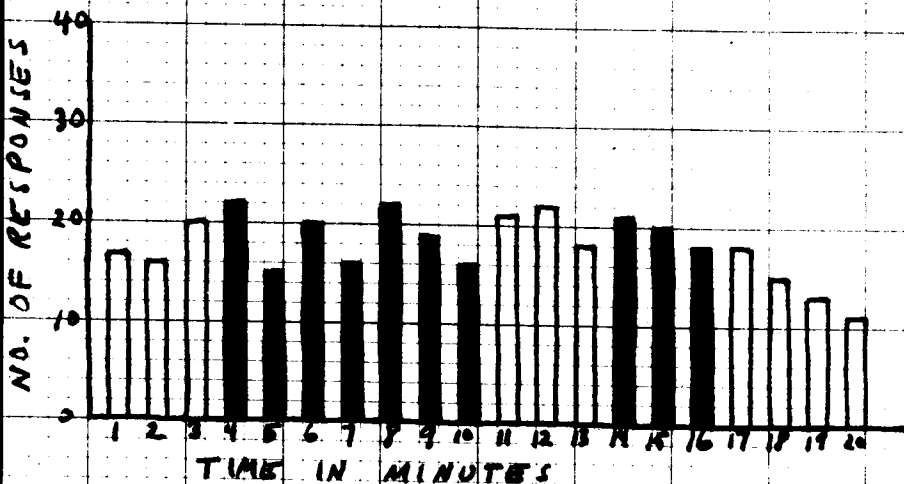


FIG. 17. NUMBER OF BAR PASSES MADE BY RAT #4 IN POSTOPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE AND NEGATIVE DISCRIMINATIVE STIMULI.

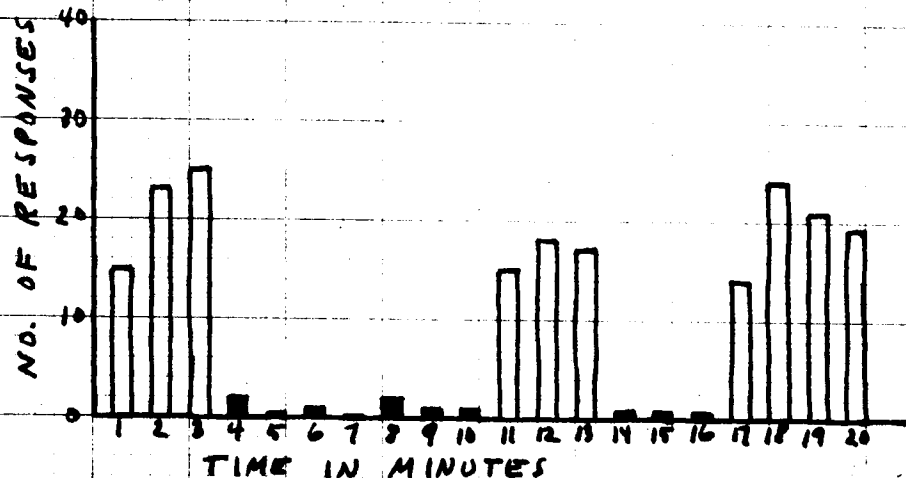


FIG. 18. NUMBER OF BAR PASSES MADE BY RAT #9 (PAIRED WITH RAT #4) IN POSTOPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE AND NEGATIVE DISCRIMINATIVE STIMULI.

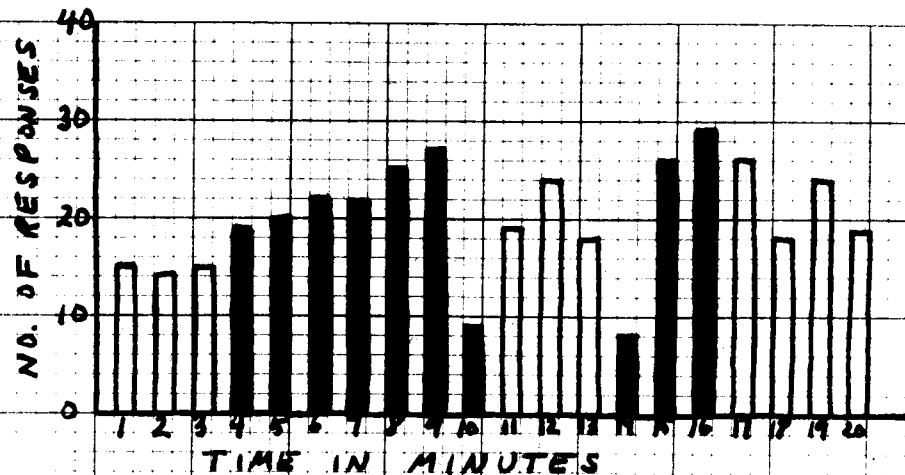


FIG. 19. NUMBER OF BAR PRESSES MADE BY RAT #5 IN POSTOPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE AND NEGATIVE DISCRIMINATIVE STIMULI.

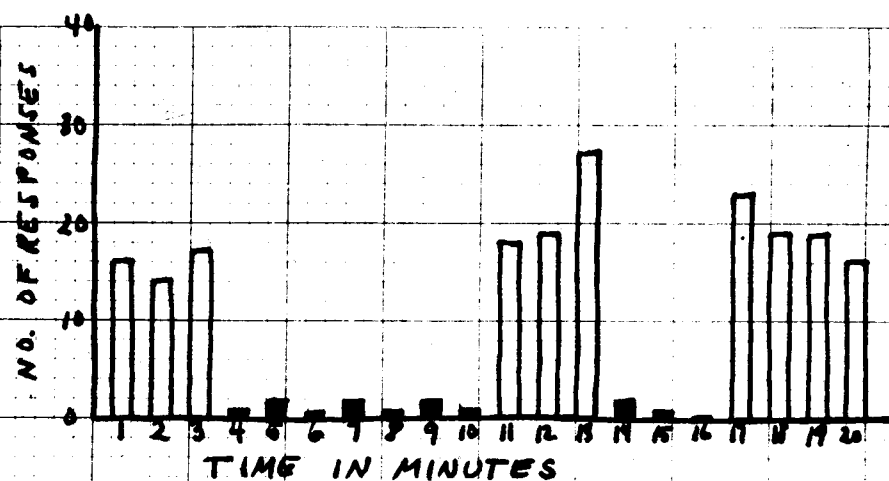


FIG. 20. NUMBER OF BAR PRESSES MADE BY RAT #5 (PAIRED WITH RAT #5) IN POSTOPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE AND NEGATIVE DISCRIMINATIVE STIMULI.

The significance of these distributions of response between negative and positive discriminative stimulus conditions was tested for each animal individually (using the total number of responses) in a chi-square test. Since the direction of the expected difference was implicit in the operational statement of the research hypothesis, the one-tailed version of the test was used.

For the experimental animals, it was found in each case that the probabilities of values as large as the observed values of chi-square was above the 0.25 level. It was concluded that none of these animals had retained the olfactory discrimination (which they had previously learned before surgery).

For the control animals, it was found in each case that the probabilities of values as large as the observed values of chi-square were quite considerably below the 0.001 level. It was concluded that these animals had retained the olfactory discrimination.

Inspection of the Figures 11 through 20 and the data of Table 2 show the clear cut difference in the performance in these animals, so that such statistical confirmation is hardly surprising.

Chapter 6

Experiments on Learning

The experiments on learning for this study were carried out in four successive steps which may be discussed in turn in this chapter: 1) training the animals to use the bar-pressing apparatus 2) choice of subjects for learning experiments 3) surgery aimed at placing lesions in the hippocampal rudiment 4) training the subjects in an olfactory discrimination.

Since several of the steps mentioned are essentially duplications of procedures already explained at length in Chapter 5 of this study, it should not be necessary to dwell upon them at length. Rather, they will be treated succinctly in the present chapter since the detailed explanation has already been presented.

Training the Animals to Use the Bar-Pressing Apparatus

It will be remembered that an original group of 30 rats were trained in bar-pressing without any odorant being introduced into the apparatus. (See Chapter 5, Experiments on Retention). Ten animals were selected from this larger group for the study on retention. For the study on learning, of which we are treating in this chapter, ten other rats of the group of 30 were selected. Since their training in the basic skill of bar-pressing is already described minutely at the beginning of Chapter 5 of this study, it will be sufficient at this point to stress that this preliminary training in no way involved any experience with odors as discriminative

stimuli.

Selection of the Subjects for Learning Experiments

The ten rats chosen for this experiment were selected on the basis of high bar-press rate during the sessions in which they learned to press the bar for a water reward. Five were assigned to each of two groups so that they were paired for response rate and for weight. Half of these animals constituted the experimental group for the study of learning (Experimental Group B) and half constituted the control group (Control Group B). As previously mentioned, these animals were already paired for age (since all were born on the same day) and for sex (since all were males). The treatment of these animals was rigorously standardized throughout the entire set of experiments which follow.

Surgery Aimed at Placing Lesions in the Hippocampal Rudiment

Subjects for Surgery

The five animals of the experimental group were rats # 11, 12, 13, 14, and 15. Each of these animals was submitted to surgery for the purpose of placing lesions in the hippocampal rudiment. The five animals of the control group were rats # 16, 17, 18, 19, and 20.

In view of the fact that (as will be shown later) the insertion of the electrode in the control animals of the retention group left no perceptible effects, these animals of Control Group B were not submitted to surgery. In view of the findings of Stern (1960) on after effects of anesthetic, however, each control animal was kept under anesthesia for the same period of time as experimental animals.

Surgical Procedure

Each animal of Experimental Group B as well as each control animal was anesthetized by the combination of pento-barbital sodium and ether as described under surgical procedure in the preceding chapter. By the same procedure given in detail in that chapter lesions were placed in the brains of each experimental animal. Control animals, as mentioned above, were not submitted to surgery. They were simply anesthetized to control for the effects of the anesthetic on later learning.

Training the Animals in an Olfactory DiscriminationApparatus for Discrimination Training

The experimental chamber used for training the animals of the retention experiment and adequately described in Chapter 5 of this study was employed without modification.

Discrimination Training Procedure

Each of the subjects selected for the learning experiments was trained in an olfactory discrimination by the use of daily 20 minute sessions in the experimental chamber. Each rat was trained to respond by pressing the bar for a water reward whenever the odor of oil of hyacinth was introduced into the chamber and to refrain from pressing the bar whenever the odor or extract of pine was present. This procedure represents a reversal of stimuli in comparison with the retention experiments. In the previous set of experiments the extract of pine was used as a positive stimulus and the oil of hyacinth was the negative stimulus. In this set

of experiments the oil of hyacinth was the positive stimulus and the extract of pine was the negative stimulus. This reversal was adopted to control for possible effects of the particular odors in question upon the discrimination.

The method of establishing the discrimination by exchanging the full well with an empty one in accordance with the nature of the discriminative stimulus present in the apparatus was adopted as explained in Chapter 5. The same controls were adopted to prevent auditory or visual cues from interfering with the learning of the olfactory discrimination.

At the end of 14 days of discriminative training each of the ten animals was tested for differences in the number of responses under the two different sets of conditions defined by the use of two different olfactory discriminative stimuli. Four of the animals in the experimental group of five,* which did not discriminate between the two conditions in this first postoperative test, were subjected to further training. This was done at the rate of one daily training session of twenty minutes duration for ten more days. The animals were then retested to see if they had learned the olfactory discrimination in these ten days of additional training. This retest occurred on the twenty-fourth day of the discrimination training. When no evidence of any discrimination was found despite

* The fifth rat in this group, rat # 14, which did learn to discriminate, was found to have a lesion in the left anterior cingulate gyrus, but this lesion did not damage the hippocampal rudiment, as will be shown in a later chapter.

this prolonged time of training, the experiment was terminated.

Results of the Discrimination Training

The number of responses per minute for each of the experimental animals and for their matched controls during the first postoperative test (conducted on the fifteenth day of training) are given in Figures 21 through 30 on pages 51 to 53 of this study. These bar-graphs show that all control animals did learn the olfactory discrimination. No control animal gave as many as three responses per minute while the negative discriminative stimulus was present in the apparatus. The sharp drop in the number of responses at each introduction of the negative stimulus leaves no doubt that the discrimination was well learned. These animals simply stopped responding when the negative stimulus was present and began to respond again when the positive stimulus was once more presented.

Of the experimental animals, only one showed discrimination. This animal, as will be shown in Chapter 7 of this study, was, by an error in placing the electrode, not damaged in the hippocampal rudiment. His performance was in no way inferior to that of the control animals. The remaining four animals clearly do not discriminate between the positive and negative stimuli. They continue to press in a manner that is not systematically related to the discriminative stimuli present in the apparatus. Their performance, by contrast to the paired control animals of Control Group B, shows no relationship to the discriminative stimuli. These animals were subjected to ten days further training and retested. The number of

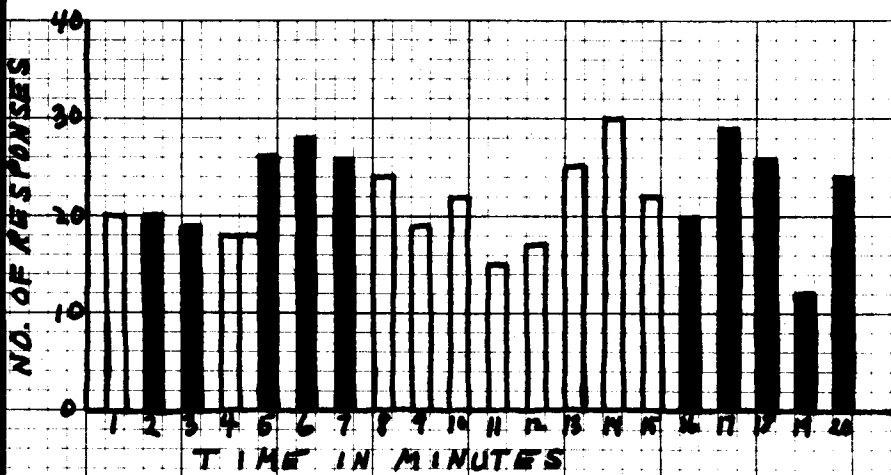


FIG. 21. NUMBER OF BAR PASSES MADE BY RAT #11 IN FIRST POST OPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE \square AND NEGATIVE \blacksquare DISCRIMINATIVE STIMULI.

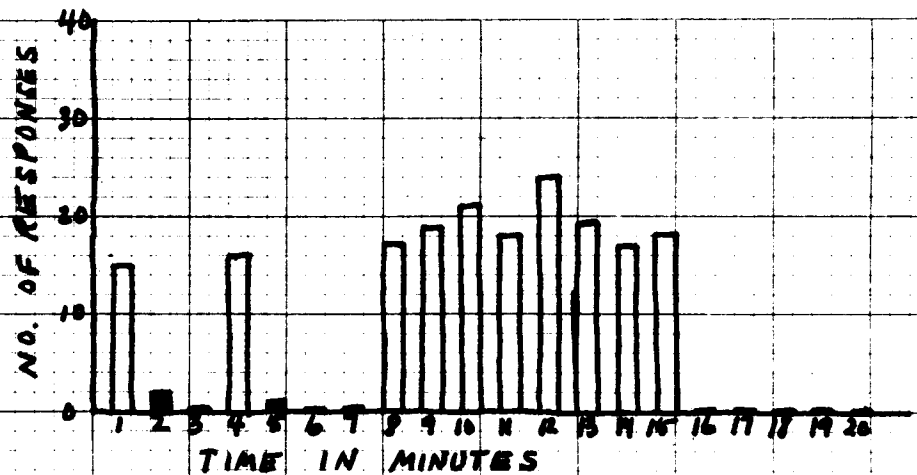


FIG. 22. NUMBER OF BAR PASSES MADE BY RAT #16 (PAIRED WITH RAT #21) IN POST OPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE \square AND NEGATIVE \blacksquare DISCRIMINATIVE STIMULI.

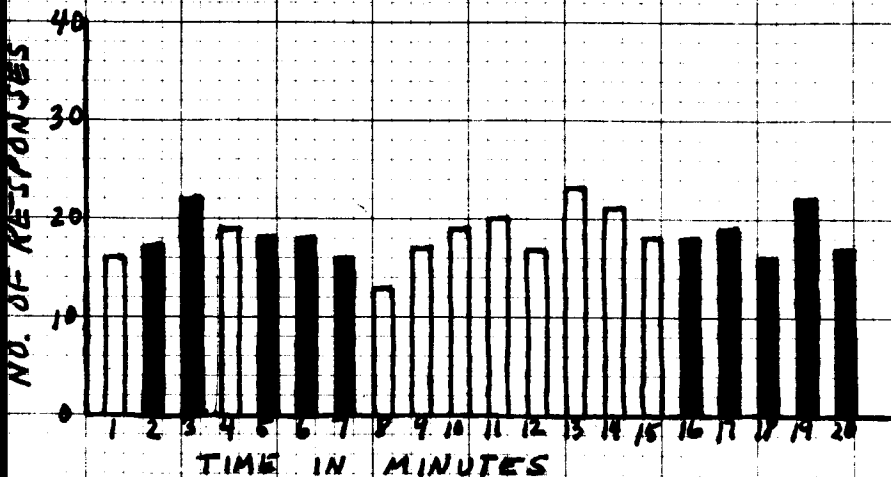


FIG. 23. NUMBER OF BAR PASSES MADE BY RAT #12 IN FIRST POST OPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE \square AND NEGATIVE \blacksquare DISCRIMINATIVE STIMULI.

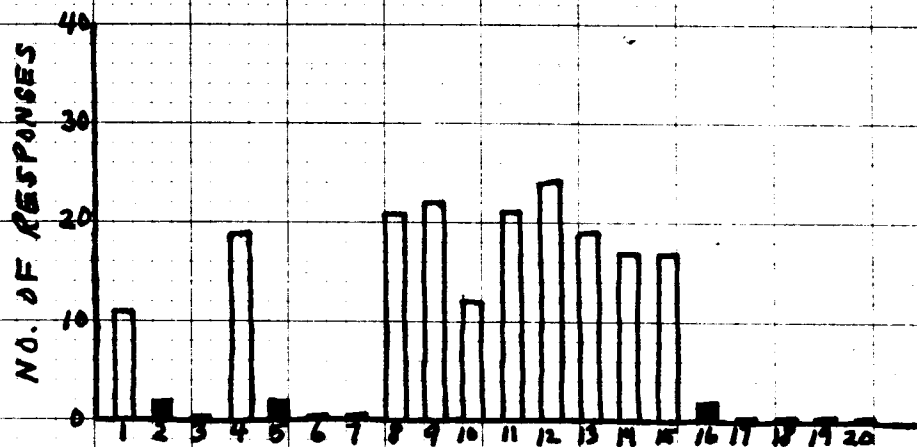


FIG. 24. NUMBER OF BAR PASSES MADE BY RAT #17 (PAIRED WITH RAT #12) IN POST OPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE \square AND NEGATIVE \blacksquare DISCRIMINATIVE STIMULI.

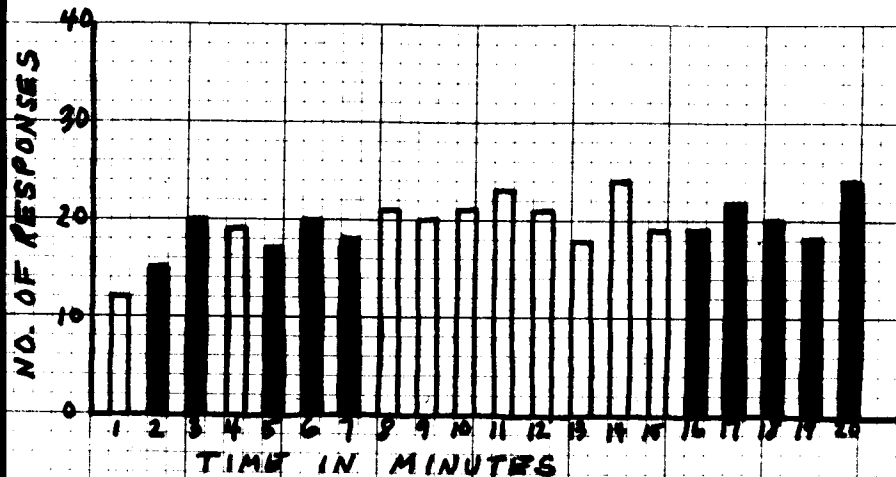


FIG. 25. NUMBER OF BAR PASSES MADE BY RAT #13 IN FIRST POSTOPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE AND NEGATIVE DISCRIMINATIVE STIMULI.

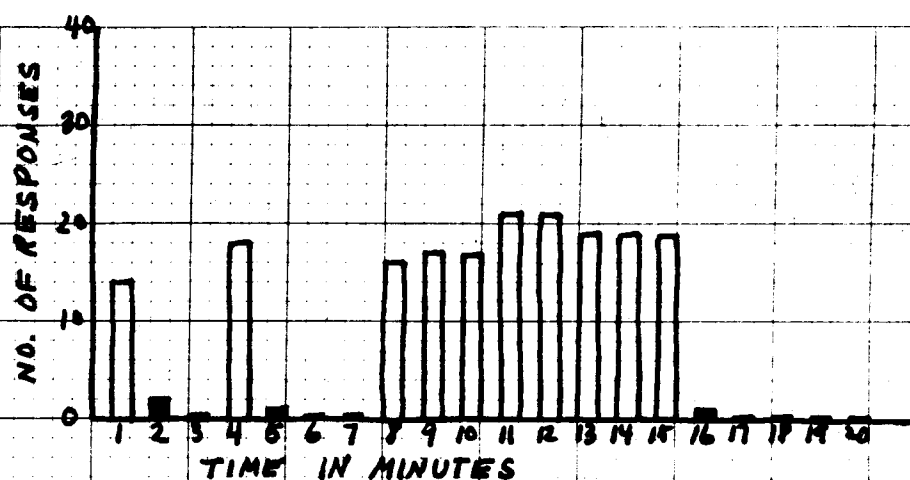


FIG. 26. NUMBER OF BAR PASSES MADE BY RAT #1 (PAIRED WITH RAT #13) IN POSTOPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE AND NEGATIVE DISCRIMINATIVE STIMULI.

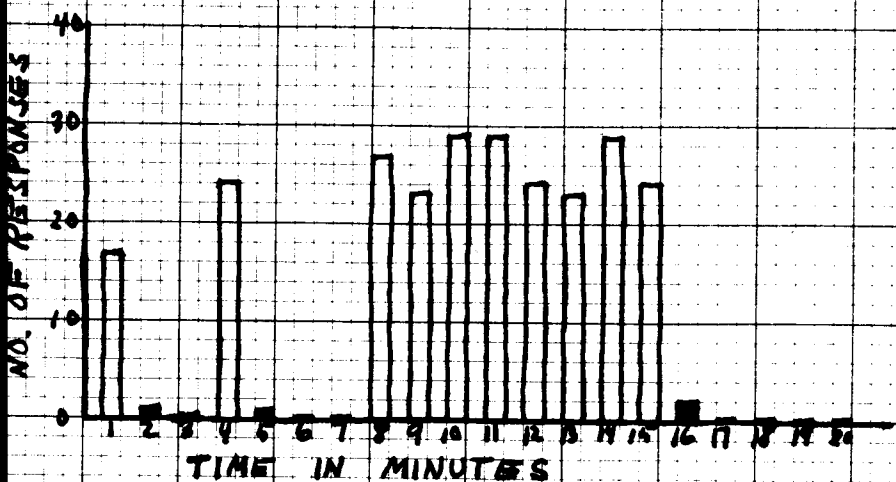


FIG. 27. NUMBER OF BAR PASSES MADE BY RAT #14 IN FIRST POSTOPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE AND NEGATIVE DISCRIMINATIVE STIMULI.

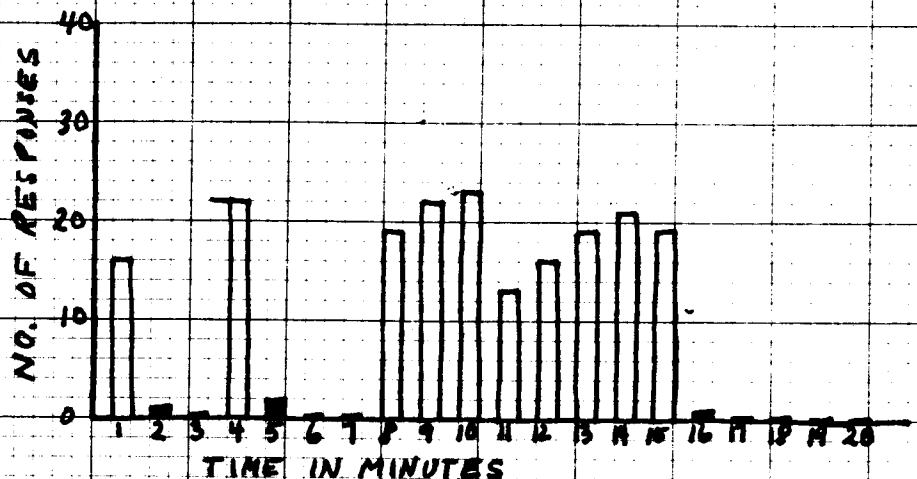


FIG. 28. NUMBER OF BAR PASSES MADE BY RAT #19 (PAIRED WITH RAT #14) IN POSTOPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE AND NEGATIVE DISCRIMINATIVE STIMULI.

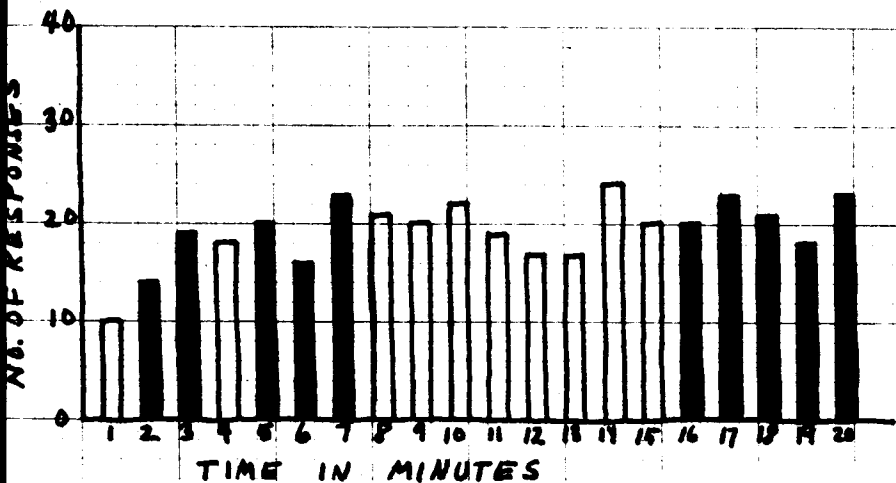


FIG. 29. NUMBER OF BAR PRESSES MADE BY RAT #15 IN FIRST POST OPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE \square AND NEGATIVE \blacksquare DISCRIMINATIVE STIMULI.

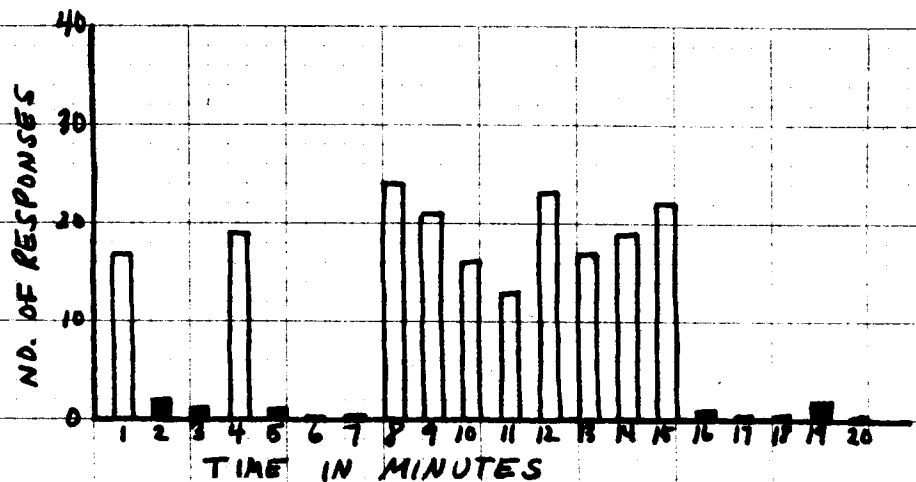


FIG. 30. NUMBER OF BAR PRESSES MADE BY RAT #16 (PAIRED WITH RAT #15) IN POST OPERATIVE DISCRIMINATION TEST IN THE PRESENCE OF POSITIVE \square AND NEGATIVE \blacksquare DISCRIMINATIVE STIMULI.

responses per minute during this final retest is given in Figures 31 through 34 on page 55 of this report. The retest shows no change in performance relative to the discriminative stimuli. They still do not discriminate.

The data in terms of the total number of responses made in the presence of the positive and negative stimuli are presented in Table 3.

Table 3
Number of Responses Made in the Presence
of Positive and Negative Discriminative Stimuli

Subjects	Total number of responses made in the presence of	
	Oil of Hyacinth (Positive stimulus)	Extract of Pine (Negative stimulus)
Experimental Group B		
Rat # 11	212	230
Rat # 12	183	183
Rat # 13	198	193
Rat # 14	250	4
Rat # 15	188	197
Control Group B		
Rat # 16	184	3
Rat # 17	183	5
Rat # 18	181	4
Rat # 19	191	5
Rat # 20	190	4

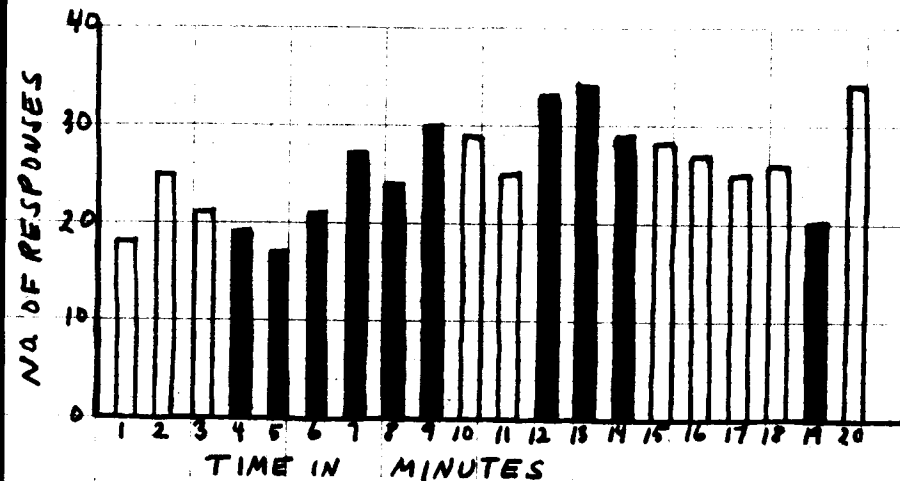


FIG. 31. NUMBER OF RESPONSES MADE BY RAT #11 IN FINAL DISCRIMINATION RETEST IN THE PRESENCE OF POSITIVE AND NEGATIVE DISCRIMINATIVE STIMULI.

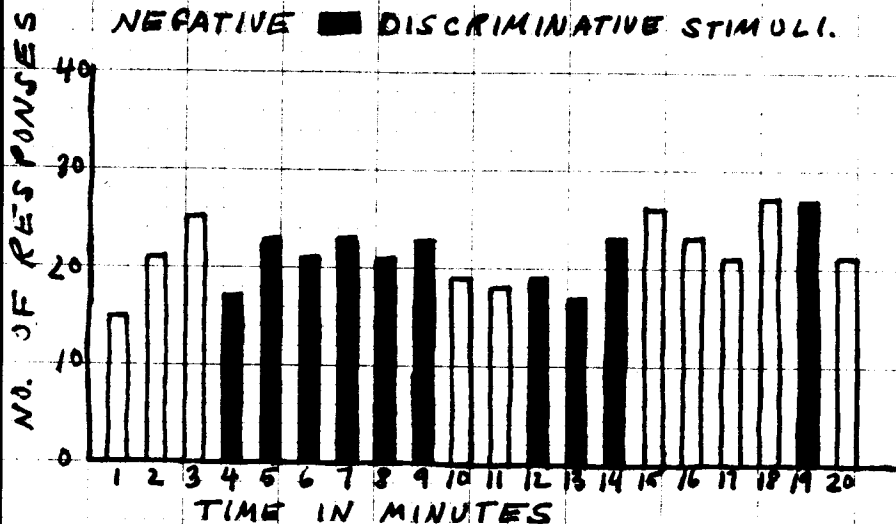


FIG. 33. NUMBER OF RESPONSES MADE BY RAT #13 IN FINAL DISCRIMINATION RETEST IN THE PRESENCE OF POSITIVE AND NEGATIVE DISCRIMINATIVE STIMULI.

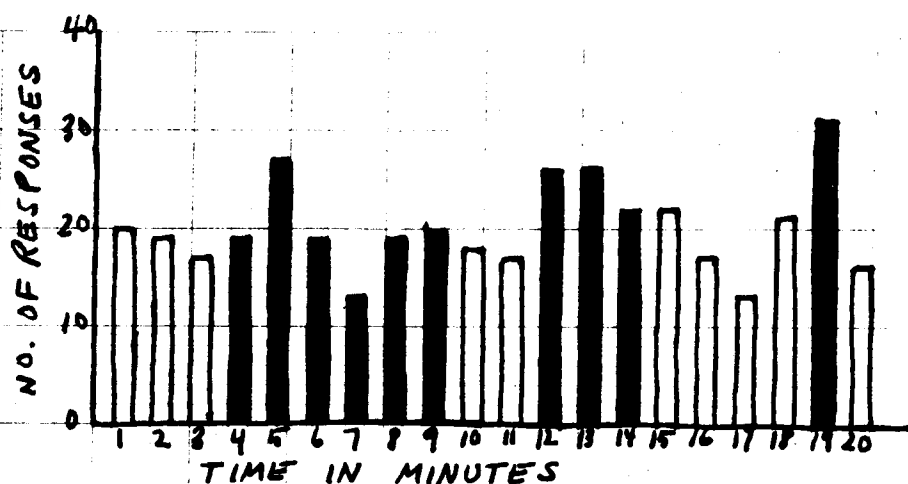


FIG. 32. NUMBER OF RESPONSES MADE BY RAT #12 IN FINAL DISCRIMINATION RETEST IN THE PRESENCE OF POSITIVE AND NEGATIVE DISCRIMINATIVE STIMULI.

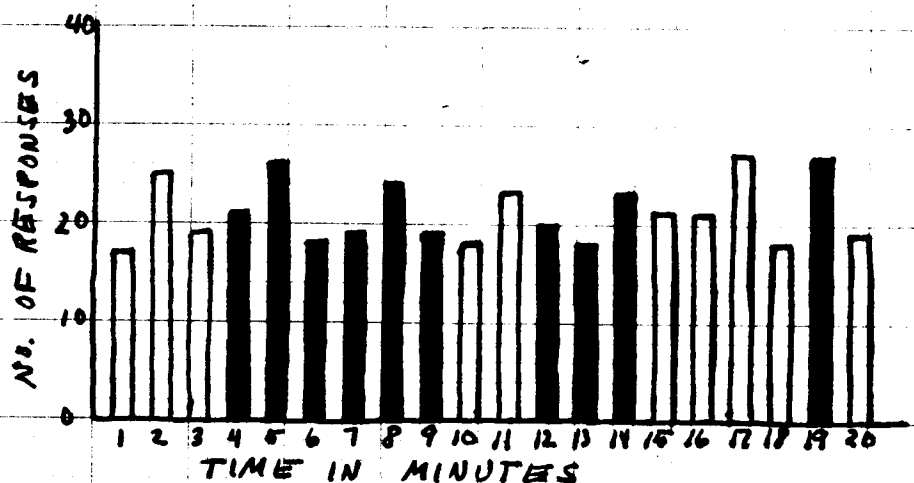


FIG. 34. NUMBER OF RESPONSES MADE BY RAT #15 IN FINAL DISCRIMINATION RETEST IN THE PRESENCE OF POSITIVE AND NEGATIVE DISCRIMINATIVE STIMULI.

The data for the final retest, whose graphs appear in the figures on page 55, are given in terms of the total number of responses in Table 4 below.

Table 4
Number of Responses Made in Retest
to Positive and Negative Discriminative Stimuli

Subjects	Total number of responses made in the presence of	
	Oil of Hyacinth (Positive stimulus)	Extract of Pine (Negative stimulus)
Rat # 11	258	253
Rat # 12	181	212
Rat # 13	216	214
Rat # 15	208	215

The significance of these distributions of response between positive and negative discriminative stimuli conditions was tested for each animal's performance individually, using the total number of response. The method of analysis used was the one-tailed version of the chi-square test.

The probabilities of values as large as the observed values of chi-square was well below the 0.001 level for all control animals and for rat # 14. It was concluded that each of these animals had learned the discrimination.

However, the probabilities of values as large as the observed values of chi-square for animals # 11, 12, 13, and 15 proved to be

above the 0.20 level in each case. The null hypothesis, therefore, that there is no evidence of discrimination by these subjects was accepted. This last hypothesis also held for the final retest, the probabilities being above the 0.30 level in each case.

Chapter 7

Post Mortem Studies

Upon conclusion of the experiments discussed in the preceding chapters, the brain of each animal submitted to surgery was removed. Each brain was fixed in formalin solution and sent to a professional technician for post mortem studies.

The post mortem report reveals that each brain was embedded in paraffin and sectioned at five micra. Every twentieth slice was stained with toluidene blue and eosin to bring out the cell bodies and unmyelinated fibers. This Nissl method was supplemented by Weigert stains of every fiftieth slice to bring out the myelinated fibers.

The report can be expressed as follows, using the three dimensional coordinates of Krieg's Atlas of Standard Coordinates for the Rat Brain (Krieg, 1946). These coordinates are expressed in millimetric intervals and include a dorsoventral dimension measured from 0 to 10, a posteroanterior dimension measured from 44 to 66, and a right to left dimension measured from 78 to 90. The units as stated by Krieg are millimeters in the fresh rat brain. The unavoidable shrinkage of tissue during the process of drying and embedding requires a correction, but the dimensions used here are in every instance the dimensions of the fresh brain.

Control Animals There is no perceptible lesion, not even an electrode track, in any operated control animal brain with the exception of rat # 9 of Control Group A, used in the

experiments on retention. In this case there was a narrow electrode track which injured a strip of cells in the left anterior cingulate gyrus, the area designated as # 24 by Krieg. This lesion angled forward and to the left from the position of entry (Krieg coordinates: 1.5, 83, 59) to the position where it terminated (Krieg coordinates: 3, 84, 60). There were no indications of any projection fibers due to degeneration emanating from this minute track.

Descriptively, this lesion may be characterized as a tiny scratch on the cortical surface of area # 24, about one and one half millimeters long. Since this animal's performance did not vary one whit from that of the other control animals the damage may be deemed negligible from the point of view of this study.

It may appear surprising that there was no evidence of electrode track lesions in these animals, but, in fact, the procedure of angling the electrode in order to allow it to slip into the medial longitudinal fissure, and the fact that very small gauge wire was used for the electrodes make this result quite plausible. See the discussion on the accurate placement of minute lesions in the rat brain by Krieg (1946).

Experimental Group for Retention

The animals of this group
(rats # 1, 2, 3, 4, and 5)

constitute Experimental Group A.

In each case a small lesion (about three-quarters of a millimeter in diameter and nearly spherical) in the upper part of

the corpus callosum appeared. These lesions were all placed in a position approximating closely the Krieg coordinates: 3, 83, 59.5.

In animals # 1, # 2, and # 3 this lesion also damaged the left cingulate gyrus in area # 24. Degeneration fibers from this damage moved caudalward along the left cingulate gyrus and vanished at a point near Krieg coordinate, 3, 83, 56 close to four millimeters back along the cingulate gyrus but never left that structure.

Other fibers, presumably from the corpus callosum damage, proceeded through the corpus callosum on both sides and moved upward toward the frontal lobes (in the direction of the posterior part of area # 10 in Krieg's atlas). These fibers, however, vanished shortly after leaving the corpus callosum and did not reach the cortex.

In animals # 4 and # 5, the damage done to the corpus callosum went deeper into that structure (as far as Krieg coordinate 3.5, 83, 59.5) but spared the cingulate gyrus entirely. More extensive degeneration of fibers in the corpus callosum appeared and went through the white matter of the frontal lobe up to the posterior part of area # 10. Other projection fibers from this degeneration appeared on the upper surface of the callosum and proceeded caudally for the space of two millimeters before vanishing, still on the surface of the callosum.

Descriptively, all the animals of Experimental Group A, none of whom proved able to retain the olfactory discrimination, were damaged in the upper part of the corpus callosum, including its dorsal surface where the hippocampal rudiment passes. Animals # 1, # 2, and #3 showed damage to the left cingulate gyrus, but less

damage to the corpus callosum, while animals # 4, and # 5 showed more extensive damage to the corpus callosum but no indication of damage to the cingulate gyrus.

The only damage common to these animals was in the upper part of the corpus callosum with its projection fibers moving in the direction of the prefrontal lobes. This area includes the surface of the callosum over which passes the hippocampal rudiment (indusium griseum and longitudinal striae). There appears little doubt that the rudiment was interrupted in each of these animals bilaterally. There was also, however, some damage to the corpus callosum. None of these animals, as has been said, retained the olfactory discrimination. See Chapter 5, Experiments in Retention.

Experimental Group for Learning

The animals of this group

(rats # 11, 12, 13, 14, and 15)

constituted Experimental Group B.

In general, the lesions placed in these animals, while still meriting the name small, were somewhat larger than those of Experimental Group A. They measured nearly one millimeter in diameter. The sole exception was animal # 11 whose lesion measured only about one half millimeter in diameter. These lesions were also somewhat higher in the brain than the previous lesions. More in detail the following remarks seem pertinent.

The lesion of the animal # 11 damaged only a small depth of the corpus callosum. It was placed nearly in the dead center of the callosum in a left-right dimension and slightly damaged both anterior cingulate gyri. Projection fibers from degeneration in

the cingulate proceeded caudalward on each side and vanished (still within the cingulate) only three millimeters behind the lesion itself. Projection fibers within the callosum moved to both sides but never left the callosum itself.

The lesions of animals # 12, # 13, and # 14 were all placed so that they damaged the cingulate gyrus of the left side and also the upper surface of the corpus callosum. Degeneration fibers moved back along the corpus callosum on its dorsal surface to a position approximately six millimeters behind the lesion (which was placed at Krieg coordinates: 2.5, 60, 83.5). The left-right spread of the lesions varied from 84.5 to 83 (Krieg coordinates). Degeneration fibers also ran along the cingulate cortex of the left side as far back as seven millimeters when they, too, vanished. Commissural fibers in the corpus callosum also degenerated although this degeneration was restricted to the callosum itself.

Animal # 14 showed a lesion about one millimeter in diameter placed entirely in the left cingulate gyrus. This lesion was placed at a depth of only 2.0 millimeters. (Krieg coordinates: 2, 83, 60). There was no perceptible damage to the corpus callosum from the lesion itself and there were no degenerated projections in the callosum or frontal lobes. The cingulate gyrus was heavily damaged on the left side. Degenerated fibers ran caudalward along the cingulum in a compact bundle and faded about seven millimeters behind the lesion. Faint traces of these fibers continued to the splenium of the callosum and vanished in the region of the fascia dentata near its juncture with the hippocampus.

It would appear that the electrode was angled too far forward and not deeply enough. The resulting damage did not, consequently, reach the corpus callosum, nor the overlying hippocampal rudiment, although extensive damage was done to the left cingulate gyrus. This animal, rather to the surprise of the experimenters, did learn the olfactory discrimination by the fifteenth day of training and his performance was in no way inferior to that of unlesioned controls.

Animals # 11, 12, 13, and 15 all showed damage to the corpus callosum, although this damage was not very deep in the case of animal # 11. It is clear that in all four of these animals the interruption of the hippocampal rudiment on the upper surface did take place bilaterally. It will be recalled from Chapter 6, Experiments in Learning, that none of these rats was able to learn the olfactory discrimination despite the fact that training procedures were continued until the final testing on the twenty-fourth day from the start of discrimination training.

In summary, it can be said that every experimental animal which failed to learn or retain the discrimination was damaged in the hippocampal rudiment. Control animals and experimental animal # 14, who did learn or retain the olfactory discrimination, were not damaged in the rudiment. The extensive damage suffered by rat # 14 in the left cingulate gyrus, seems to exclude that structure as critical for olfactory discrimination, for he performed very well in postoperative tests. There may be a question as to the effect of damaging the commissural fibers of the corpus callosum,

but the discussion of this question can best be postponed to the following chapter which is devoted to a discussion of the results and their possible significance.

Chapter 8

Discussion, Conclusions, and Summary

In evaluating the findings of this study attention should first be turned to the question of the structures damaged by the lesions placed in the brains of the experimental animals. As seen in the previous chapter, there can be no question that the hippocampal rudiment was interrupted bilaterally in the case of every experimental animal which failed to learn or retain the olfactory discrimination. The only other damage done seems to have been to the cingulate gyrus and the corpus callosum. That the damage done to the cingulate was critical seems effectively excluded by the fact that rats # 4 and # 5 in which there was no indication of cingulate damage both failed to retain the discrimination, while animal # 14, who was extensively damaged in the left cingulate gyrus, was able to learn the olfactory discrimination very well. The loss of ability to learn an olfactory discrimination cannot, therefore, be laid at the door of cingulate damage. This means that the only structure which could have been responsible for the deficit in discrimination besides the hippocampal rudiment is the corpus callosum.

It is well known that the corpus callosum is a commissural tract connecting the two hemispheres. See for example Peele (1961, pp. 354-356). The genu and rostral part of the callosum are composed of connecting fibers for the two frontal lobes (Bremer and Stoupe, 1957a, 1957b; Chang, 1953; Curtis, 1940;

McCulloch and Garol, 1941). Krieg (1947) points out that the callosal fibers from these areas form quite distinct fascicles that are limited in their position throughout their entire extent and have a location in the callosum which reflects their cortical connections exactly. He comments on how remarkable it is that at no point in the projection fibers in this area can any divergent fascicles or components be found.

Now the areas of damage and degeneration found in the post-mortem studies of the animals used in this study are very circumscribed and occupy precise positions within the rostral end of the corpus callosum. Their total spread does not exceed three millimeters within the callosum. They move to only one area of the cortex bilaterally, area # 10. There are no thalamic projections or projections to any known sensory part of the cortex or underlying nuclei.

The possibility that olfactory connections between the two hemispheres have been severed in these animals is excluded, for it is well known that in the rat these fibers go, not through the corpus callosum, but by way of the anterior commissure (Krieg, 1955, p. 170) which remained intact in every animal used in this study.

Furthermore, even in the case of somato-sensory fibers that do pass through the corpus callosum (although these pass in the splenium far caudal to the damage done in this study) no interference has been found in earlier studies with previously learned discriminations. It is true that animals trained to solve a problem with one paw without visual aid could not, after sectioning

of the callosum, "transfer" the learning to the other paw. But they did retain this learning in the original paw and they could be trained to perform it with the other paw also. (Ebner and Myers, 1960; Myers, 1960). The same effect has been reported previously for the visual modality (Sperry, Stamm and Miner, 1956), but this effect seems limited to these two modalities (Peele, 1961, p. 355).

Finally, this effect of damaging the callosum cannot be attributed to interference with the functioning of area # 10 of the frontal cortex (which is the only neo-cortical area damaged in any animal of this study), for Swann long ago removed this part of the cortex completely and found that it failed to interfere with olfactory discrimination (Swann, 1935). Similar results have been reported by Bard and Rioch (1937), Brown and Ghiselli (1938), Lashley and Sperry (1943) and Allen (1940).

On the basis of this evidence it would seem reasonable to conclude that the damage which interfered with the olfactory discrimination was indeed the interruption of the hippocampal rudiment.

The rudiment, of course, consists of a thin sheet of gray matter through which course three myelinated strands known as the medial and lateral longitudinal striae. On the basis of the work done here, it is not possible to assert whether the interference with olfactory discrimination resulted from the interruption of the striae or of the gray matter. It is virtually impossible in this part of the brain to damage one of these without damaging

the other since the striae actually run directly through the indusium griseum or gray matter of the rudiment. In any case, both of these structures are known to have connections with the septal area and the hippocampus (Peele, 1961, pp. 531-532). In the absence of any experimental evidence on the influence of either of these parts of the rudiment on discrimination or other processes, it can be concluded only that one or the other or both of these was involved in the effects reported in this study.

It may be concluded that lesions in the hippocampal rudiment prevented both retention and learning of a conditioned olfactory discrimination in the albino rat, thus confirming the hypothesis stated in Chapter 4 of this study (p. 23).

The experimental verification of this limited hypothesis by no means proves the general theory of hippocampal functioning from which it was derived, nor does it establish the functions of the various parts of the rhinencephalon which were discussed in an earlier chapter. Many further studies would be required for such a conclusion.

The present study, however, opens up several very promising avenues of research which would help to clarify the meaning and significance of these first experiments in the area.

Thus, it would be interesting to know if the interruption of the rudiment also prevented visual, motor or auditory discrimination. On the basis of observations made on lesioned animals which could neither learn nor retain an olfactory discrimination, it seems that both motor and visual memory was untouched. These rats still

retained the ability to press the bar for a reward; but they ran to the bar immediately and began pressing it without waiting for the odor signal.

This seems to indicate that the visual stimulus (the bar) recalled the appearance of the water and the movement that could make it appear (bar-press). According to Arnold's theory this visual and motor recall requires intact connections from the visual area to the hippocampus proper and from there to the frontal and occipital association areas. These connections actually were left intact in these animals. Since the connections from the olfactory area to the hippocampal rudiment were broken, the rats should not have been able to recall the one odor (extract of pine, for example) signalled water, and the other odor signalled "no water" and did not require a bar-press. The further implications of this line of thought could be formulated into many worthwhile experiments.

It would be interesting to investigate the problem of whether the positioning of the interruption of the rudiment would have differential effects on various sensory discriminations. Would interrupting the rudiment more caudally affect other discrimination processes? If so, which processes, and in what order? Would severing the fornix just rostral to the hippocampal commissure have the effect of preventing all learning or retentions of all discriminations? Does interrupting the rudiment really interfere with processes other than the sense of smell? This would appear the more probable hypothesis since direct connections between the olfactory tracts and the rudiment do not seem to exist (Peele, 1961, pp. 543-552),

but it would be desirable to put this question and all those suggested by it to the experimental test.

The significance of this study, therefore, lies more in the questions it raises than in the completeness of the answers it gives. It does give a clear and unequivocal answer to a limited question: does interrupting the rudiment interfere with olfactory discrimination? But perhaps more important, it opens the way for a whole series of further studies which bid fair to shed quite considerable light on the functioning of the hippocampal system and the rhinencephalon in general. In so doing, the study has contributed to its general purpose: shedding some light on the processes that intervene in the organism between stimulus and response.

Summary On the basis of Arnold's theory of functions and brain processes, it was hypothesized that lesions in the hippocampal rudiment would prevent learning and retention of a conditioned olfactory discrimination in the albino rat when tested by an operant technique.

Twenty albino rats were divided into two groups, one for the study of retention, the other for the study of learning.

"Retention" animals were first taught an olfactory discrimination, then submitted to surgery resulting in electrical lesions in the hippocampal rudiment. All experimental animals failed to retain the preoperatively learned discrimination. Operated control animals paired for age, weight, sex, and preoperative rate of response by bar-press all retained the discrimination.

"Learning" animals were submitted to surgery resulting in interruption of the hippocampal rudiment. Control animals paired for age, weight, sex, and preoperative rate of bar-press all learned the discrimination in 15 days of training. Those experimental animals that had lesions interrupting the hippocampal rudiment failed to learn the olfactory discrimination even though training was continued up to 24 days. One experimental animal with left cingulate gyrus damaged but no lesion in the hippocampal rudiment learned the olfactory discrimination as well as intact animals.

After discussion of the significance of the results, it was concluded that interruption of the connection between the septal area and the hippocampus via the hippocampal rudiment does prevent olfactory discrimination as predicted by Arnold, but it remains for further studies to clarify further the import of this interruption of the circuit in question.

Chapter 9

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APPROVAL SHEET

The dissertation submitted by Reverend Hacker J. Fagot, S.J. has been read and approved by five members of the Department of Psychology.

The final copies have been examined by the Chairman of the Department of Psychology 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.

Nov. 7, 1962
Date

V. H. Kern
Signature of Adviser