HIPPOCAMPAL ELECTRICAL ACTIVITY AND OPERANT CONDITIONING

HIPPOCAMPAL ELECTRICAL ACTIVITY AND OPERANT CONDITIONING IN THE DOG

By

ARTHUR JOSEPH LOUIS DALTON, M.A.

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree
Doctor of Philosophy

McMaster University
October 1968

DOCTOR OF PHILOSOPHY (1968)
(Psychology)

McMASTER UNIVERSITY
Hamilton, Ontario.

TITLE: Hippocampal Electrical Activity and Operant Conditioning in the Dog

AUTHOR: Arthur J. L. Dalton, B.Sc. (University of Alberta)

B.A. (University of Alberta)

M.A. (University of Alberta)

SUPERVISOR: Professor A. H. Black

NUMBER OF PAGES: vii, 135

SCOPE AND CONTENTS: EEG activity was observed during operant reinforcement of single-press, multiple-press, and passive, avoidance responses in order to identify hippocampal theta activity as a central correlate of avoidance behavior in dogs. A clear relationship between theta activity and active skeletal behavior was found. To determine whether the occurrence of the avoidance response and its associated proprioceptive feedback were necessary for operant reinforcement to take place, two attempts were made to operantly reinforce hippocampal electrical activity in the absence of the avoidance response. Hippocampal theta activity was readily conditioned in naive deeply curarized dogs. The second attempt, with paralyzed avoidance pre-trained dogs, suggested that theta can be operantly reinforced but failed to demonstrate a normal state transfer effect on the avoidance response.

ACKNOWLEDGMENTS

The author wishes to express his gratitude to Drs. A. H. Black and C. H. Vanderwolf whose support and help made this thesis possible. Thanks are also due to Mr. C. Batenchuk for his invaluable assistance in designing and maintaining the equipment, and to Miss Janet Harper who typed the manuscript.

TABLE OF CONTENTS

TABLES

FIGURES

APPENDICES

CHAPTER ONE

INTRODUCTION

The concern of this thesis is with the following question: which of the many events that immediately precede the application of an operant reinforcer must occur if the reinforcer is to have an effect? The usual answer to this question is the response of some effector organ, either muscle or gland. Some observable change in an effector organ must precede reinforcement in order for operant conditioning to occur. This effector organ activity however, is only one aspect of the operation of a complex control system only partially understood (Woodburne, 1967; Matthews, 1964). In addition to the change in the effector organ, feedback produced by the change, central processes at various levels which initiate and maintain movement, and impulses from the central nervous system to the effector organ are involved. It is therefore evident that the operant reinforcer actually follows a complex pattern of activity in the control system which is concerned with the initiation and maintenance of movement. The question then arises as to which of these correlates of effector organ activity are essential for operant reinforcement. Is a pattern of change in the intact fully-functioning system necessary or is activity in only certain components required?

The effect of blocking proprioceptive feedback from the skeletal musculature has been explored by Gorska and Jankowska (1961), and by Taub and Berman (1964) who found that operant conditioning of specified skeletal muscles occurred when feedback from these muscles was blocked. These results indicate that the reinforcement of central processes, impulses to the periphery, and the muscle movement are sufficient and that feedback is not necessary. There is little information however, on the role of central correlates of the skeletal response. Is the reinforcement of central processes sufficient to produce an increase in the probability of overt skeletal responses? The objective of this thesis was to provide some evidence bearing on this question for one central correlate of overt skeletal responding. The central correlate of interest consisted of regular rhythmical electrical activity in the dorsal hippocampus and was chosen on the basis of Vanderwolf and Heron's (1964) suggestion that such activity is involved in the initiation of overt skeletal movements. More recently, Vanderwolf (1967; 1968) has presented convincing evidence in support of this hypothesis.

The first experiments described in the thesis were designed to study the relationship between electrical activity in the dorsal hippocampus and operantly conditioned skeletal responding. The operant reinforcement of electrical activity was then carried out. Finally, the attempt was made to determine whether the operant reinforcement of hippocampal electrical activity is sufficient to produce the conditioning of skeletal responses. The procedure involved the operant reinforcement of hippocampal electrical activity in subjects whose skeletal musculature had been paralyzed with a curare-like drug and then observation of skeletal responding in the normal state following recovery from curarization. If such a demonstration is successful, then it can be concluded that neither feedback nor the overt skeletal muscle activity is necessary for operant reinforcement to occur and that the reinforcement of central processes is sufficient.

CHAPTER TWO

BACKGROUND

In this chapter, background material on two topics will be discussed. The first topic is the reinforcement of certain components of the skeletal movement control system when other components are blocked. The second topic is the relationship between hippocampal electrical activity and movement.

A. Operant reinforcement of components of the skeletal movement control system

A number of reports have appeared on the operant reinforcement of movement during partial elimination of sensory feedback. An experiment reported by Taub, Bacon and Berman (1965) is representative of this type of procedure. These investigators surgically sectioned the dorsal roots innervating the fore-arm of monkeys prior to the introduction of avoidance conditioning of an arm-flexion response. All of the response-produced feedback such as the activity of muscle spindle receptors, Golgi tendon organs, and receptors sensitive to the position of the arm was abolished. The limb used to perform the avoidance response had intact muscle innervation and could move, but no sensations could arise during such movement. Feedback from responding was further reduced by the use of a trace-CS and hiding the responding limb from direct vision by the subjects. The authors were able to show that an avoidance response could be learned by the monkeys under these conditions.

Similar findings have been reported in rats and cats (Jankowska, 1959) and in dogs (Gorska and Jankowska, 1961). Taub and Berman (1964) extended the area of deafferentation to include successively larger portions of the body in successive operations until total spinal deafferentation was achieved. These monkeys (pre-trained) made all of the movements of the partially deafferented animals and in the avoidance conditioning situation none showed any deficit, responding above the criterion level on the first testing day.

It would seem evident therefore, that learning can take place under a wide variety of conditions which produce extensive changes in the normal feedback associated with responding. The relevant question becomes the following: what other events within the organism must necessarily occur for reinforcement to take place?

One approach to this problem is to prevent all peripheral motor activity and associated feedback by the use of curare-like drugs. These drugs have their main effect at the neuromuscular junction and, therefore, leave central processes relatively intact. The technique of curarization, however, introduces complications not found in the deafferentation studies. Curarization interferes with the operant conditioning procedure itself because, normally the actual movement of the muscles or limb is used as the criterion indicator of when reinforcement ought to be presented. When sensory feedback is blocked, as in the dorsal-root deafferentation experiments, the occurrence of the skeletal movement can still be used as the criterion to define the moment when reinforcement should be applied. With blocked input to the muscles, the condition present during curarization, this criterion is no longer available for defining the moment of reinforcement application and a whole new approach must be developed. Two requirements must be met.

First, some event must be found to serve as the criterion indicator for the presentation of reinforcement. Second, the effect of reinforcing this criterion indicator when the response control system is intact must be determined in order to assess whether reinforcement of the indicator is equivalent to reinforcement of the normal system.

A partial step in meeting these requirements was taken by Black in a recent study (1967). He sought to demonstrate experimentally that a vestigial skeletal component of an avoidance response could be brought under operant control during curarization. He found that curarized dogs could be operantly trained to either make or to refrain from making small electromyographic responses in the limb normally used for executing a pedal-press avoidance response. In addition, he was able to show that such operant training under paralytic conditions was associated with transfer effects to behavior in the normal state. Dogs trained to increase electromyographic activity under curare increased their pedal-pressing rate in the normal state; dogs trained to refrain from making electromyographic activity under curare decreased their pedal-pressing rate in the normal state. The procedures which Black employed (1967) were administered under conditions of light paralysis, thereby reducing response-produced feedback but not completely eliminating it. If electromyographic activity is present under paralysis then the influence of proprioceptive feedback from muscle spindles can occur (Buchwald, Standish, Eldred, and Halas, 1964).

If the attempt is made to apply the design employed by Black (1967) in completely paralyzed animals, the question immediately arises as to what event or response component can be reinforced? One possibility could consist of an attempt to record electrical activity directly from the ventral roots innervating the dog's limb normally used for responding. Neurons in the ventral roots would be relatively unaffected by deep curarization and reinforcement could be made contingent on electrical activity from this source. A second possibility could involve the attempt to explore the rather vague suggestions that the motor cortex may mediate skeletal responding (DiCara and Miller, 1967; Taub, Bacon, and Berman, 1965). A third possibility, the one explored in this thesis, is based on the findings reported by Vanderwolf and Heron (1964) and more recently by Vanderwolf (1967). He has suggested that regular rhythmical electrical activity in the range of 6-9 Hz appearing in limbic and diencephalic structures in rats is regularly found to precede and accompany an avoidance response. This possibility appeared particularly attractive since he was also able to provide preliminary evidence indicating that alternative accounts of the function of this kind of brain activity could be rejected.

Perusal of the literature on electrophysiological studies of conditioning (e.g. John, 1961; Morrell, 1961) has failed to yield any reported attempts to apply operant conditioning procedures to hippocampal theta[1] waves. Only one study has been found which has any

---

[1] The term "theta" is used in this thesis to refer to slow, regular, rhythmical, sinusoidal electrical activity recordable from limbic and diencephalic structures.

direct bearing on such an attempt. Olds (1967) has reported some preliminary data in which the attempt was made to operantly change the probability of firing rate of single brain cells in hippocampal and pontine regions. The report-pulse of a cell-spike pattern-recognition device automatically delivered a food reinforcement to rats whenever the cell fired above a certain predetermined rate. Two major difficulties which were encountered make interpretation of the results entirely equivocal. First, movement-produced electromyographic activity produced spikes in the same frequency range as the cells and were not adequately filtered by the pattern-recognition device. Olds' attempt to control for this difficulty by use of the simple expedient of having time-outs from the training schedule when movements occurred failed because many smaller movements passed unrecorded by the movement detector. Secondly, when cells did come under operant control it was observed that the operant response included movements of the eyes or head (with reinforcement of pontine cells) and when hippocampal cells were conditioned the operant response included whisker and sniffing movements. The possibility arose that the operant contingencies acted on small skeletal movements alone and that the changes in the firing rate seen in pontine and hippocampal cells were simply a concomitant of the skeletal conditioning. Olds, in an attempt to circumvent the problem of skeletal conditioning, administered gallamine triethiodide (a curare-like drug) to a single rat in which a hippocampal cell had previously been conditioned in the normal state with electrical stimulation in the anterior lateral hypothalamus as a positive reinforcer. Under gallamine the previously established rate of cell firing disappeared, but with shaping, the firing rate was increased considerably and maintained for two minutes. Subsequent extinction and reacquisition failed to produce repeatable results and the animal died.

Interestingly, Olds does not report any attempt to operantly condition hippocampal theta waves although such an experiment would seem clearly feasible. There is evidence that the firing of single cells in the hippocampus is directly related to the sinusoidal pattern of the theta rhythm recorded with a macro-electrode in the same structure (Green and Machne, 1955) and that fixed phase relationships may be maintained between the spikes from single hippocampal cells and individual theta waves (Green, Maxwell, Schindler, and Stumpf, 1960; Harper, 1968). In addition, with intracellular techniques, Fujita and Sato (1964) have shown that within single pyramidal cells of the hippocampus there is a slow rhythmic change of the membrane potential which is synchronous with the EEG theta rhythm, and that burst discharges from the cells were often observed on the depolarization-going phase of the intracellular theta rhythm.

B. Hippocampal electrical activity and movement

Notwithstanding a large literature on the hippocampus and its possible functions (e.g. reviews by Green, 1964; Votaw, 1959), only in recent years has interest in the possible relationships between hippocampal theta waves and operantly conditioned behavior been reflected in the number of published reports.

The possibility that hippocampal theta waves may be a brain phenomenon associated with the learning process was first studied experimentally by Grastyan and his workers (Grastyan, Lissak, Madarasz, and Donhoffer, 1959; Lissak and Grastyan, 1960). These investigators have reported that hippocampal theta is associated with the early stages of training when orienting reflexes play an important role. Subsequently theta was found to disappear with the development of a well-learned, firmly established instrumental response.

Adey and his group (Adey, 1961; Adey, Dunlop, and Hendrix, 1960) and Pickenhain and Klingberg (1967) also report the presence of hippocampal theta in the early stages of instrumental learning. The former investigators examined the hippocampal activity occurring during the discriminative training of cats on a food-motivated response, while the latter observed rats in an avoidance situation and during the conditioning of a food-motivated running response. Whereas Grastyan, Lissak, Madarasz, and Donhoffer (1959) reported the disappearance of hippocampal theta with continued training, Adey (Adey, 1961; Adey, Dunlop, and Hendrix, 1960) and Pickenhain and Klingberg (1967) have found that hippocampal theta remains throughout training. Adey (1961) provided extensive evidence that theta persists in as many as 1200 to 1400 trials, while Pickenhain and Klingberg (1967) observed theta in rats after more than 200 paired presentations of the CS and UCS.

In a more recent report, Grastyan's workers (Karmos, Grastyan, Losonczy, Vereczkey, and Grosz, 1965) have provided evidence that the hippocampal theta rhythm is a regular concomitant of the orientation reaction. Adey (1961) denies that hippocampal theta is associated with the orienting reflex, as do Pickenhain and Klingberg (1967). Adey (1961) defines the orienting reflex much as Konorski (1948), with the important elements consisting of turning of the head and eyes towards a sudden novel stimulus without locomotion necessarily involved. This definition is also similar to the Pavlovian definition and corresponds to Grastyan's usage of the term in 1961 but not with his definition of 1959 (Grastyan, Lissak, Madarasz, and Donhoffer, 1959). Grastyan's 1959 usage of the term orienting reflex appears to be similar to that of Pickenhain and Klingberg (1967), in which the reflex is considered to consist of diffuse, non-specific searching movements involving perhaps locomotion towards a stimulus, a pattern of behavior which is largely characteristic of the early stages of instrumental learning.

As Adey has suggested (1961) perhaps the inconsistencies in the reports mentioned above are due to definitional problems. However, a more plausible explanation has been proposed by Vanderwolf (1968). He has suggested that theta activity might have disappeared in Grastyan's (1959) experiments because the amplitude of theta activity may be directly related to the vigor of the motor response. In the early stages of training the vigorous orienting reflex gives way to smoothly executed discrete responses and there may be a concomitant decrease in the amount of amplitude of theta. This possibility indicates that the adequacy of behavioral observations may be an important element in resolving apparent inconsistencies in the published reports. A second important element involves the localization of the recording electrode within the hippocampus. There are known regional differences in amplitude characteristics of hippocampal electrical activity (Radulovacki and Adey, 1965;

Vanderwolf, 1968). Though Grastyan has also observed (Grastyan, Lissak, Madarasz, and Donhoffer, 1959) regional differences in electrical activity he provided no data concerning the placement of electrodes in any but one or two of his animals. It is therefore difficult to determine whether histological evidence would have permitted an interpretation of his findings which might be more consistent with the reports of other investigators.

On the basis of their findings, Adey and his workers (Adey, 1961; Adey, Dunlop, and Hendrix, 1960) have offered the hypothesis that hippocampal theta is functionally involved in goal-directed behavior. A more recent statement of their position (Elazar and Adey, 1967a, 1967b) included the additional proposition that hippocampal theta is specifically correlated with the "connection" between the correct skeletal response and a CS which signalizes the requirement for an appropriate response. They suggested that theta is an electrical correlate of a process connected with the consolidation of learned behavior.

Pickenhain and Klingberg (1967) offer a hypothesis which appears to be highly similar to that of Adey. These authors state that theta in the dorsal hippocampus appears in all situations in which a display of motivated behavior is seen. Though the authors do not make the distinction between motivated behavior and motivation they do provide evidence bearing on this point. Ten, short-duration, intense shocks were delivered through a grid floor to rats at a rate of approximately two per second. The rats received a series of sets of ten shocks until they learned to jump to a freely hanging rod in order to avoid further shocks. No hippocampal theta was seen during the first applications of the series of shocks, even though the animals exerted chaotic efforts to escape. With repeated applications hippocampal theta began to appear during the shocks and during the rest of the time. Preceding and accompanying the first successful avoidance response on the sixteenth presentation of the shock series was a train of high amplitude hippocampal theta waves. It would appear, therefore, that fear motivation <u>per se</u> was not sufficient to elicit hippocampal theta but that the appropriate skeletal avoidance response had to occur.

Theta was also found to be absent during what Pickenhain and Klingberg (1967) considered "automatized motor acts". Although theta did accompany the locomotor approach behavior of a rat to a food tray, theta disappeared during the act of licking a glucose solution in the tray. This finding is consistent with similar observations made by Vanderwolf (1967). Vanderwolf (1967), in addition, has provided evidence that hippocampal theta in rats is largely absent when such movements as blinking, sniffing, grooming and chewing food occurred. Vanderwolf offered the hypothesis that hippocampal theta is functionally involved in a mechanism for the initiation of voluntary movement. He has recently (1968) suggested that hippocampal theta is associated with phasic somato-motor activity but not with tonic activity. Whether the "automaticity" of the behavior is a crucial determiner of the occurrence of hippocampal theta seems to be open to conjecture at the present time, in view of the relative paucity of observations available in the literature.

Vanderwolf's hypothesis appears to be consistent with the findings of earlier investigators (Adey, 1961;, Grastyan, Lissak, Madarasz, and Donhoffer, 1959; Pickenhain and Klingberg, 1967) to the extent that voluntary skeletal behavior can and does occur during the acquisition of a conditioned response. However, previous investigators all agree that hippocampal theta is involved directly in one way or another only in the learning process. Vanderwolf's hypothesis is at variance with the others to the extent that theta may be associated strictly with active skeletal activity and not necessarily dependent on the establishment of a learned connection between CS and a particular skeletal response.

Bremner (1964) sought to provide evidence that the presence of a CS during avoidance conditioning was not necessary for the occurrence of hippocampal theta. He trained rats on a Sidman avoidance lever-pressing task while electrical activity was recorded from the dorsal hippocampus. As the Ss were about to make the response the irregular hippocampal activity gave way to synchronized theta activity in the range 5-7 Hz. Theta was not found in "dummy trials" in which electrical activity was measured at points mid-way between two presses at least 14 seconds apart. The author concluded that hippocampal theta is correlated with overt behavior. He argued that the findings did not support the hypothesis of Grastyan, Lissak, Madarasz, and Donhoffer (1959) that the theta rhythm is a correlate of the orienting reflex. His argument rested on the proposition that since theta occurred in the absence of any exteroceptive stimulus it would be difficult to maintain that an orienting reflex could occur under these conditions. Further, he found that theta did not extinguish over hundreds of trials, a finding consistent with those of Adey (1961), Pickenhain and Klingberg (1967), and also Vanderwolf and Heron (1964).

Bremner (1964) advanced the hypothesis that the hippocampal theta rhythm is a correlate of attention or arousal as first proposed by Green and Arduini (1954). He also suggested that the difference between the arousal concept and Adey's "goal-directed" behavior hypothesis is largely a semantic one. The argument was made that in a learning situation animals have to be <u>attentive</u> to the cues to respond correctly. The results, however, are also consistent with Vanderwolf's hypothesis.

Evidence concerning the relationship between hippocampal theta and arousal comes from a number of sources. If hippocampal theta is involved in arousal or attention-like processes its presence should be observed during the occurrence of a startle response, for example. Pickenhain and Klingberg (1967) provided evidence that not every arousal is accompanied by hippocampal theta. Sleeping rats were presented with a single click. The animals displayed strong startle reactions. The electrical pattern in the hippocampus changed from a sleep pattern into a desynchronized record showing low-voltage fast activity. A click which did not produce a startle response likewise failed to be associated with hippocampal theta although the animal displayed behavioral arousal. Grastyan, Lissak, Madarasz, and Donhoffer (1959) reported the absence of hippocampal theta to entirely new stimuli presented for the first time to their subjects. Vanderwolf (1967) provided additional evidence that hippocampal theta was absent in an "alert" rat which was standing still.

This brief review of a number of hypotheses concerning the possible relationships between hippocampal theta activity and behavior by no means exhausts the literature on the subject. The focus has been restricted to those speculations which have direct bearing on the question as to whether hippocampal theta could be considered a central component of skeletal responses, particularly avoidance responses. The reader is referred to reviews by Green (1964) and by Votaw (1959) which explore the more general question concerning hippocampal functions rather than with the particular electrical activity recordable from this structure.

One final point which has not arisen in the literature but seems important enough to make is the following. Hippocampal theta may be directly involved in a "response control system". However, theta activity itself may not be the significant event. Rather, other electrical activity which normally masks hippocampal theta may have such a function or electrical activity in other neural structures may be the crucial event and hippocampal theta is merely an indicator of such activity. In the present thesis the term "hippocampal theta" will be used to include all of these possibilities.

C. Organization of the thesis.

The concern of this thesis, as indicated in the introductory chapter, was with a central component of a response control system. The questions of particular interest were: first, can a central component of the response control system be reinforced when other components (i.e., the overt skeletal movement, proprioceptive feedback from that movement) are blocked; secondly, will such reinforcement have an effect on the response? It was mentioned above that hippocampal theta appeared to be a good candidate for the central component of the response control system on the basis of evidence provided by Vanderwolf and Heron (1964) and Vanderwolf (1967; 1968). It was subsequently shown, however, that there are a number of alternative hypotheses available concerning the role of hippocampal theta in avoidance conditioning. One of the objectives of this thesis, therefore, was to provide empirical data obtained from dogs in our own experimental situation concerning the relationship between hippocampal theta and avoidance behavior. Chapter three of this thesis is a description of an experiment conducted with the specific aim of determining the extent of correlation between hippocampal theta and avoidance pedal-pressing behavior in dogs. A second experiment, described in chapter four, provides further evidence bearing on the relationship between hippocampal theta and the learning process.

The possibility that hippocampal theta may be a correlate of overt skeletal muscle responses in dogs makes the attempt to apply an operant reinforcer to the component feasible when the overt response is prevented by complete curarization. Such an attempt is described in chapter five of this thesis. Since the effects of curarization are reversible, the attempt was then made to demonstrate that reinforcement of hippocampal theta waves under deep paralysis may or may not be related to the overt complete avoidance response. This attempt is described in chapter six. Following recovery from paralysis a transfer test was applied to determine whether operant conditioning of the central component had any effect on the overt avoidance response.

CHAPTER THREE

EXPERIMENT ONE

The possible relationship between hippocampal theta activity and skeletal avoidance behavior has been studied in rats (Vanderwolf and Heron, 1964; Bremner, 1964; Vanderwolf, 1967; Pickenhain and Klingberg, 1967), and in cats (Grastyan, Lissak, Madarasz, and Donhoffer, 1959; Grastyan and Karmos, 1961; Karmos, Grastyan, Losonczy, Vereczkey, and Grosz, 1965). Similar investigations with dogs in an avoidance situation have not been reported though observations of naturalistic behavior in dogs and hippocampal activity have been made (Yoshii, Shimokochi, Miyamoto, and Ito, 1966).

The purpose of this chapter is to provide a description of a preliminary experiment in which the objective was to obtain evidence on the possible relationship between hippocampal theta and avoidance pedal-pressing in dogs. The method was simply to observe the relative frequency of occurrence of hippocampal theta activity during the performance of operantly reinforced pedal-press avoidance responses, and during the absence of such pedal-presses.

METHOD

Subjects

The subjects were fourteen adult mongrel dogs ranging in weight from twenty to thirty pounds. Each animal was housed individually and observed for a few days to assess the condition of health.

Apparatus

During all phases of the experiment each dog was confined in a sound-deadened chamber in a rubberized hammock and stood on a platform during training. The hindlegs were firmly strapped to the platform but the forelegs could move up to six inches vertically. Movements of the head were extensively restricted by its position in a stock-type wooden head-holder padded heavily around an opening for the neck. Shock was delivered through disc electrodes taped to shaved portions of both hindlegs. The pedal consisted of a 5" x 9" spring-loaded aluminum plate mounted at a forty-five degree angle from the perpendicular and situated within close reach of the dog's foreleg (about two inches).

Vertical leg movement, EMG activity and EEG activity were recorded on a Grass Model 5 polygraph located in a separate room. Vertical leg movements were measured by means of two potentiometers, each situated under the platform and connected to the dog's forelegs by means of long tapes. EMG activity from the extensor carpi radialis and the extensor digitorum muscles of each foreleg was taken by means of Grass needle electrodes held firmly in place by adhesive tape. EEG activity was recorded by means of a special six-foot cable consisting of nine, insulated, low-noise, individually-shielded wires normally used in phonograph turn-table arms. The cable was tightly covered with a flexible and waterproof tape with an Amphenol Min-Rac-17 connector at each end. One end of the cable was plugged into a board feeding to the polygraph. The other end of the cable was slipped through the neck-opening of the head-holder then passed underneath the dog's bonnet and fitted snugly into the connector permanently mounted on the dog's head. This arrangement minimized movement artifacts and permitted extensive re-use of the cable.

The auditory stimulus used as CS was delivered through a loudspeaker mounted 4.0 feet in front of the dog's head. The CS consisted of a 75 decibel white-noise. The equipment which automatically programmed the training schedule was located in a separate room and consisted of conventional relay-circuitry.

Procedure

All of the animals were placed through the following sequence of procedures:

1. pre-training,
2. regular avoidance training,
3. surgical operation plus 7-14 days recovery period,
4. post-operative regular avoidance training,
5. perfusion and histological examination of the brain.

1. Pre-training consisted of ten fixed-duration exposures to a 75 decibel white-noise stimulus. The duration of the stimulus was twenty seconds.[1]

2. Regular avoidance training was introduced on the day immediately following pre-training. The avoidance response consisted of pressing the pedal with the left foreleg. The CS was the same white-noise stimulus used in pre-training. On each trial, if the dog pressed the pedal during the CS-UCS interval (15 seconds in all but dog 6 for whom the in-

---

[1] One dog, number 6, received twenty rather than ten pre-training exposures to the stimulus, each of 30" duration.

terval was 30 seconds), the CS was terminated and shock avoided. If the dog failed to press during the CS-UCS interval then a series of 4- to 10-milliampere shocks each of one-tenth second duration were presented at five-second intervals until a pedal-press was made. Trials were spaced at intervals ranging from one minute to two minutes with an average intertrial interval of one- and one-half minutes. Fifty trials a day were given until each dog met a criterion of twenty consecutive avoidances.

3. Surgery was performed under "clean" but not aseptic conditions since experience revealed that the dogs could be expected to be highly resistant to infection. An electric scalpel (Bantam Bovie, J. F. Hartz Company) was used for incisions. Bi-polar twisted-nichrome formvar insulated wire electrodes (0.010 inches in cross-section) were implanted stereotaxically in the dorsal posterior hippocampus bi-laterally with the dog under deep sodium pentobarbital anesthesia. In addition to the factory applied formvar insulation, the electrodes were coated with epoxylite and baked three times to prevent leakage. The electrodes were then scraped for about one millimeter along the axis of each wire and the tips separated by about one- and one-half millimeters. A "ground" electrode assembly consisted of an Amphenol miniature pin connected with a three centimeter length of insulated nichrome wire and a jeweller's screw. The "ground" was embedded over the frontal sinuses in the mid-line approximately thirty to forty millimeters rostrally to the location of the hippocampal implants. Coordinates for the hippocampal implants were chosen on the basis of the examination of the brains of earlier dogs in which good hippocampal tracings had been obtained.

In some dogs two additional electrodes were aimed for the septal area and the medial thalamus. No dog had more than four electrodes implanted.

The pins from each electrode were fitted into an Amphenol, Min-Rac series 17, nine-contact, miniature female connector. The whole assembly was buried up to the collar of the connector in dental acrylic cement and held to the skull by means of three, 3/8" stainless-steel bone screws. Additional cement covered the sutures posterior and anterior to the connector which was centrally placed over the middle of the head. Finally, the head was sprayed with an antiseptic adhesive and covered with a Herculite bonnet, leaving only the upper portion of the connector exposed. [1]

4. Post-operative regular avoidance training was identical to that presented pre-operatively and consisted of fifty trials for all but two dogs (dogs 6 and 16 received 95 and 22 trials, respectively). During this phase of the experiment EEG activity was recorded as well as vertical leg movements and EMG activity.

5. Most of these dogs were subsequently involved in additional experiments. At the end of these experiments each dog was deeply anesthetized with sodium pentobarbital and the brain was perfused with ten percent formalin injected into the left ventricle of the heart. The brain was removed, sectioned at a thickness of forty micra, and sections through the electrode tracks from ten of the dogs were mounted on slides and stained with thionin.

---

[1] Covering the dog's head with a specially designed bonnet made of extremely tough fabric (Herculite) proved to be necessary. Dogs invariably scratched the head region for days after surgery and the bonnet prevented the development of local infection.

Measurements

The hippocampal records of the dogs obtained during the first day of post-operative reacquisition of the pedal-press avoidance response were analyzed. For purposes of analysis, hippocampal theta in dogs was defined as regular, roughly sinusoidal activity with very little or no superimposed "fast" activity. Hippocampal theta waves seldom appeared singly, were almost always of greater amplitude than other activity in the record, and were found in the frequency range of 4-6 Hz (refer to Figure 1). Frequency measurements were made of individual waves in ten dogs. Theta waves were examined under a magnifying glass fitted with a reticle which permitted measurements to the nearest 0.1 mm. The values so obtained were converted to frequency in cycles per second.

Analysis was carried out by visual inspection of the hippocampal tracings during two time periods before each avoidance trial and during a third time period consisting of the avoidance trial itself. The duration of each kind of inter-trial period was determined by the average duration of the avoidance trial for each dog. The number of inter-trial periods was determined by the number of avoidance trials for each dog which were usable in the analysis (Some avoidance trials were not usable either because the response latency was less than 0.4 seconds and therefore too short to permit a dependable observation, or because of the presence of movement artifacts [1]).

---

[1] Details of the unusable trials for each dog are provided in Appendix A.

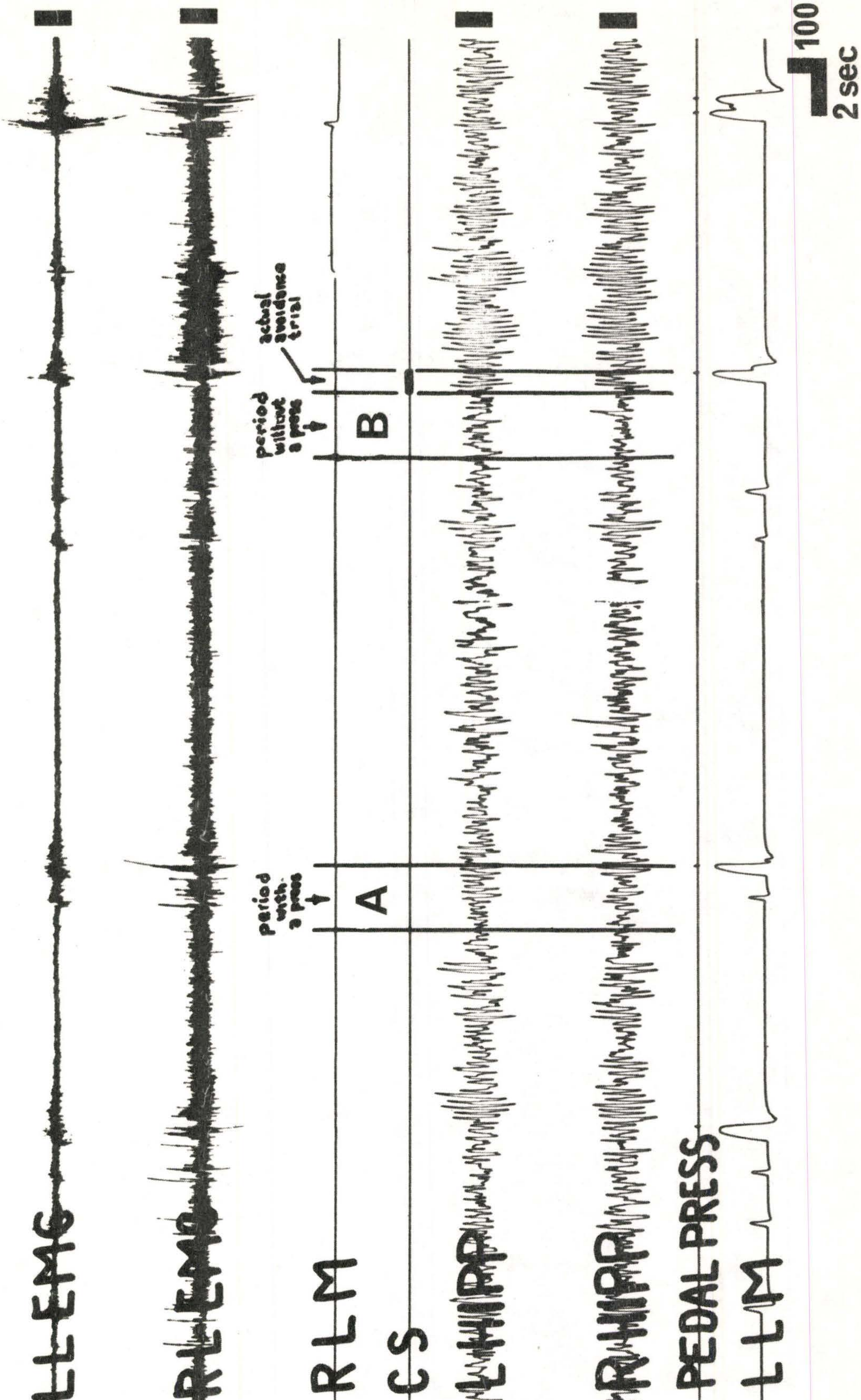
The two time periods immediately preceding each avoidance trial were chosen in the following manner. The first period preceding the CS without a pedal-press was selected and called the B period. Then the first period immediately preceding the B period, but with a pedal-press, was chosen and called the A period. The A period was chosen so that it terminated when a pedal-press occurred. (If no inter-trial pedal-press occurred there could be no A period, of course). Analysis of hippocampal theta during trials in which the CS was presented and an avoidance response failed to occur was not performed because there were only five such trials, one from each of five of the fourteen dogs. On the basis of the visual inspection of each period a yes-no decision was made as to presence-absence of at least one complete theta wave, each "yes" standing for a time period in which at least one theta wave occurred. The number of "yes" decisions for each dog was converted to a percentage of time periods with at least one theta wave and was computed for each of the three types of time periods.

Figure 1 shows the typical kind of record obtained during the post-operative reacquisition day and illustrates the A and B periods which entered into the analysis. Additional examples are to be found in the figures of Appendix B.

Figure 1 about here

Figure 1 Typical record obtained during post-operative reacquisition. An inter-trial time period with a press (A), an inter-trial time period without a press (B), and an actual avoidance trial are shown in the segments separated by perpendicular lines.

The uppermost channel is a continuous tracing of the electromyographic activity taken over the extensor carpi radialis and the extensor digitorum muscles of the left foreleg (LL EMG). The second channel, RL EMG, is a similar tracing of electromyographic activity taken over the extensor carpi radialis and the extensor digitorum muscles from the right foreleg of the dog. The third channel, RLM, is the tracing of gross perpendicular movement of the right foreleg. The fourth channel is an event marker indicating the occurrence of the training CS as a solid line. The fifth channel, L HIPP, is a tracing of the electrical activity from the left hippocampus. The sixth channel, R HIPP, is a similar tracing of electrical activity from the right hippocampus. The seventh channel is the record of occurrences of pedal presses which are indicated as brief deflections. The channel identified as LLM, is the tracing of gross perpendicular movement from the left foreleg.


LLEMG
RLEMG
RLM
CS
LHIPP
RHIPP
PEDAL PRESS
LLM
period with 3 pres
A
period without 3 pres
B
actual avoidance trial
100
2 sec

RESULTS

The results of the analysis are presented in Table 1.

Table 1 about here

Numbers in the second column indicate the number of usable time periods (avoidance, A and B periods) during the first day of post-operative reacquisition for each subject identified in the first column. The third column shows the average duration in seconds of avoidance trials for each dog. The fourth column shows the percentage of avoidance trials with hippocampal theta for each dog. The percentage of A periods in the presence of a spontaneous pedal-press with hippocampal theta is indicated in the fifth column. The sixth column shows the percentage of B periods with hippocampal theta occurring in the absence of a press.

A Friedman two-way analysis of variance (Siegel, 1956) was performed and indicated that the occurrence of hippocampal theta was highly related to the kind of time period in which theta was observed (chi square = 23; $p = .001$ with $df = 2$, $N = 14$, and $k = 3$).

Closer examination revealed that for eight of the fourteen dogs in which A periods were sampled at least one complete theta wave appeared in all of the A periods. During B periods, hippocampal theta appeared much less frequently than at any other time period sampled. Hippocampal theta appeared more frequently during actual avoidance trials and during A periods for all dogs when compared to the frequency of occurrence of hippocampal theta during the B periods. There was no overlap in the results. Statistical comparison of A periods against actual avoidance trials indicated that hippocampal theta was

TABLE ONE

Percentage of A periods (with a press), B periods (without a press), and actual avoidance trials correlated with theta during the first day of post-operative reacquisition of the pedal-press response.

Dog[1] No.	No. of trials	Average trial length in sec.	Percentage of periods with theta: avoidance with press	A period with press	B period without press
6*	92	2.3	74	(27)100	57
15	40	4.0	93	100	23
16	22	1.0	77	95	50
17	47	2.2	81	98	17
23	46	2.0	93	100	74
24*	50	1.4	98	(33)100	64
25	50	2.3	94	100	40
26*	50	1.8	86	(19) 95	40
29	48	2.8	96	100	77
32	47	1.9	64	57	43
33	47	3.4	62	100	43
36	43	2.9	100	98	67
39	47	2.7	83	100	60
42	47	1.0	87	81	45

---

[1] The dogs identified by an asterisk made too few inter-trial pedal-presses to permit the appropriate number of inter-trial A periods. For these three dogs the calculations of percentage theta shown in column five are based on the number of samples shown in the brackets. While the remaining calculations are based on the total number indicated in column two.

associated significantly more often with the former than with the latter trials (Wilcoxon matched-pairs signed-ranks test; $T = 13$ and $p = .01$ for two-tailed test with $N = 14$). This finding suggests that hippocampal theta was associated with spontaneous inter-trial presses significantly more often than with avoidance trial presses.

Photographic enlarged reproductions of brain sections from eleven of the fourteen dogs are presented in Appendix C. The sections shown were taken from the point of deepest penetration of the electrode on each side. Gross inspection revealed that of twenty-two electrode placements all but four were well within the body of the hippocampal formation. Eight electrodes were located in the hippocampus and ten in the dentate gyrus - CA 4 area. No electrical activity was recordable from a placement in the fimbria in dog 17 (right side), from the lateral geniculate nucleus (dog 29, left side), or from the fimbria on the left side in dog 33. The right side implant in dog 33 was also in the fimbria, but hippocampal theta was recorded from this site, though of very low amplitude (60 micro-volts).

DISCUSSION

The results shown in Table 1 indicate that hippocampal theta can be considered a correlate of the avoidance response in dogs in our experimental situation. It is apparent in addition, that spontaneous occurrences of the pedal-press response during inter-trial intervals may be even more closely associated with hippocampal theta than avoidance pedal-presses. Conceivably this result could be explained on the assumption that the presence of hippocampal theta is a direct function of the amount of skeletal activity occurring. Observation of the dogs during training by closed-circuit television indicated that the avoidance movement tended to become more discrete, precise, and efficient as training progressed, suggesting that perhaps less skeletal activity is involved in the response to the CS. Perusal of the tracings of electromyographic activity during early versus late training also tends to support this observation in a gross way. On the other hand, spontaneous inter-trial presses tend to involve more postural adjustments, head movements and other kinds of movements than what is seen during avoidance trials. If it is assumed that the presence of theta is a direct function of the amount of voluntary skeletal activity which occurs then theta could be expected to accompany spontaneous inter-trial presses more frequently than avoidance presses.[1]

---

[1] Alternatively, amplitude of theta might be associated with amplitude of movement. Careful measurements of theta amplitude of single waves preceding trial presses, inter-trial presses, and inter-trial movements not involving a press were made in two dogs (85 observations). In one dog theta was of greater amplitude during trial presses than at other times, while in the other dog theta was of greater amplitude during inter-trial movements.

CHAPTER FOUR

EXPERIMENT TWO

The results of experiment one suggested that hippocampal theta can be considered a correlate of avoidance behavior in our dogs but left open the question concerning the possible functional significance of hippocampal theta. The purpose of the experiment described in this chapter was to attempt to specify more precisely the events to which hippocampal theta are related. The procedures established two antagonistic responses to two different CSs. The contingencies of reinforcement (CS termination and shock-punishment) were arranged appropriately to lead to the acquisition of active pedal-pressing avoidance behavior in one CS and passive, "holding-still" or "not-pedal-pressing" behavior in the other CS. Under these experimental conditions only Vanderwolf's hypothesis would predict that hippocampal theta would accompany the active avoidance behavior and would not accompany the passive avoidance response. In his view, hippocampal theta is an accompaniment of a voluntary, phasic, active, skeletal response and not of a voluntary, tonic, "holding-still" response (Vanderwolf, 1968).

Bremner (1964) who has explored in an avoidance situation with rats Green and Arduini's 1954 arousal hypothesis, would have to predict that hippocampal theta would appear both during the active and passive avoidance trials of the present experiment because in both stimuli "arousal" or "attention" is approximately equated.

Adey (1961) and Elazar and Adey (1967a) would also predict equal probability of occurrence of hippocampal theta in active and passive avoidance trials because in their view theta is correlated with the establishment of a "learned connection" between the CSs and the responses. In the present experiment two such connections are established differing only in the characteristics of the somatomotor response which is instrumental in producing reinforcement. Grastyan, Lissak, Madarasz, and Donhoffer (1959) would make a similar prediction since "orientation" would occur in the presence of both stimuli.

Likewise, Pickenhain and Klingberg (1967) would predict equal probability of occurrence of hippocampal theta during both active and passive avoidance trials because in their view hippocampal theta is correlated with all motivated behavior. In the present experiment both the active avoidance response and passive avoidance response are motivated to approximately the same degree by appropriate scheduling of CS termination and shock punishment.

METHOD

Subjects

The subjects were four adult mongrel dogs ranging in weight from 23 to 33 pounds. Each animal was housed individually and observed for a few days to assess state of health. Two other dogs were preliminary subjects treated in somewhat different ways and therefore are not included in the following discussion and analyses.

Apparatus

The experimental chamber, recording apparatus, and conditioning equipment have already been described previously.

Procedure

The four dogs were placed through the following sequence of procedures:

1. surgical operation plus two weeks recovery period,
2. pre-training,
3. regular avoidance training,
4. multiple avoidance training,
5. active-passive avoidance training,
6. perfusion and histological examination of the brain.

1. Surgical procedures and electrode implants were comparable to those used in the previous experiment. In addition to a "ground" electrode, a second, indifferent electrode, identical in construction to the "ground" electrode, was screwed into the skull some five to ten millimeters posteriorly to the "ground" electrode to permit monopolar recording from the hippocampal leads under gallamine triethiodide paralysis.

2. Pre-training consisted of ten fixed-duration exposures to a 75 decibel white-noise and a pure-tone signal chopped ten times per second, also at 75 decibels. The duration of each stimulus was fifteen seconds. The first four stimulus exposures were presented in an ABBA order, the remainder in fixed irregular order.

3. Regular avoidance training was introduced on the same day immediately following pre-training. The CS, identified as S-1, was the same white-noise used in pre-training. The CS-UCS interval was ten seconds. The UCS was a brief, 1/10th second shock delivered across the hind legs every five seconds with a single pedal-press response producing termination of the CS and omission of the next scheduled shock.

Trials were spaced at intervals ranging from one minute to two minutes with an average inter-trial interval of 1.5 minutes. The dogs were trained on this procedure to an easy criterion of five consecutive avoidances in a row then switched to the multiple avoidance training procedure.

4. Multiple avoidance training consisted of shaping a relatively high rate of pedal-pressing. The dogs were required to press the pedal at least once per second for six seconds in order to avoid shock and produce CS termination. Pedal-pressing at rates lower than once per second were punished. The CS-UCS interval was ten seconds. If the dog failed to avoid shock within this interval, it received a brief pulse of shock after the end of the interval and after one second had passed without a response. These pulses of shock continued at ten-second intervals as long as no responses occurred. Each response delayed the next shock by one second if the response occurred during the second immediately preceding the time the shock would have been delivered in the absence of a response. If the dog responded for six seconds with inter-response times of less than one second, the CS terminated and the trial ended. Multiple avoidance training was continued until a criterion of five avoidances in a row was met. The average duration of the criterion trials was 6.0 seconds in which a rate was maintained of one press per second or better.

4. On the day following achievement of the multiple avoidance criterion the animals were introduced to active-passive avoidance training. Two CSs were used with operant contingencies in both. In the presence of S-1 (white-noise CS) the operant schedule used in multiple

avoidance training of the previous day was in effect. In the presence of S-2 (chopped-tone stimulus) the dog could avoid shock and produce CS termination at the end of six seconds by refraining from pressing the pedal during the CS[1]. Every pedal-press was instantly punished by a brief, 1/10th second shock at any time such a press occurred following onset of the chopped-tone stimulus.

Both the duration of active pedal-press responding and of passive "holding-still" were shaped. In both instances the length of time required for producing the appropriate response was gradually increased till on the last or criterion day of training the average duration of S-1 active avoidance trials was 7.4 seconds and 6.7 seconds on S-2 passive avoidance trials. The stimulus durations were not constant because the shaping procedures were tailored to the individual differences in responsivity of each dog and because the dogs had control of the duration of the CS within a small range (1-2 seconds) immediately following CS onset and just prior to CS offset.

Fifty trials per day were given in this phase of the experiment for up to nine days. On any given day the order of stimulus presentations on any block of ten trials was in fixed irregular order constrained only by the application of the following rule. If the number of escapes

---

[1] The chopped-tone was used as S-1 in a preliminary dog with no effect due to the CS. In the present experiment counterbalancing of the CSs was not carried out.

in each stimulus (S-1 and S-2) was the same then the block of ten trials consisted of five presentations of each stimulus, in fixed irregular order. If, on the other hand, on any given block of ten trials there were more escapes in one stimulus than in the other, then in the next block of ten trials that stimulus was presented a correspondingly greater number of times. For example, if there were two escapes more in S-1 than in S-2 on the first block of ten trials, then in the next block of ten trials there would be two more presentations of S-1 and two less of S-2. The animals were trained for fifty trials a day until the day on which they met a criterion of ten consecutive active avoidances in S-1 and ten consecutive passive avoidances in S-2 during a block of twenty consecutive trials. Some of the dogs were then over-trained to varying degrees.

6. Following active-passive avoidance training the dogs were involved in additional experiments while under paralysis. At the end of these experiments each dog was deeply anesthetized with sodium pentobarbital and the brain was perfused with ten percent formalin via injection into the left ventricle of the heart. The brain was removed, sectioned at a thickness of forty micra, and sections through the electrode tracks were mounted on slides and stained with thionin.

Measurements

The hippocampal records obtained from the dogs during the last or criterion day of active-passive avoidance training were analyzed. The analysis was restricted to this day for two reasons. First, the skeletal and emotional behavior occurring at this stage of training had considerably stabilized thus providing data which could yield a "cleaner" measure of the extent of the relationship between hippocampal theta and active and passive avoidance responses. Secondly, we knew from previous experience that shocks elicit theta both in the normal and paralyzed state and that these effects occasionally persist between trials. Because of the very low incidence of shocks late in training this effect was thereby minimized.

For purposes of analysis hippocampal theta was defined in the same way as in the previous experiment, that is as regular, rhythmical, sinusoidal activity in the range of 4-6 Hz.

Analysis consisted of visual inspection of the hippocampal tracings during periods preceding and immediately following each avoidance trial. Similar inspection was made during each active and passive avoidance trial. The total number of seconds of theta occurring during these three time periods was measured by visual examination and use of a ruler. The length of the pre- and post-trial periods was determined by the average duration of actual avoidance trials. The total number of pre-and post-trial periods of each kind was determined by the number of avoidance trials which were analyzed.

Figure 2 shows a typical record for an active avoidance trial and for a passive avoidance trial in one dog. Comparable segments were examined for all avoidance trials on the last or criterion day of active-passive avoidance training from the four dogs.

Figure 2 about here

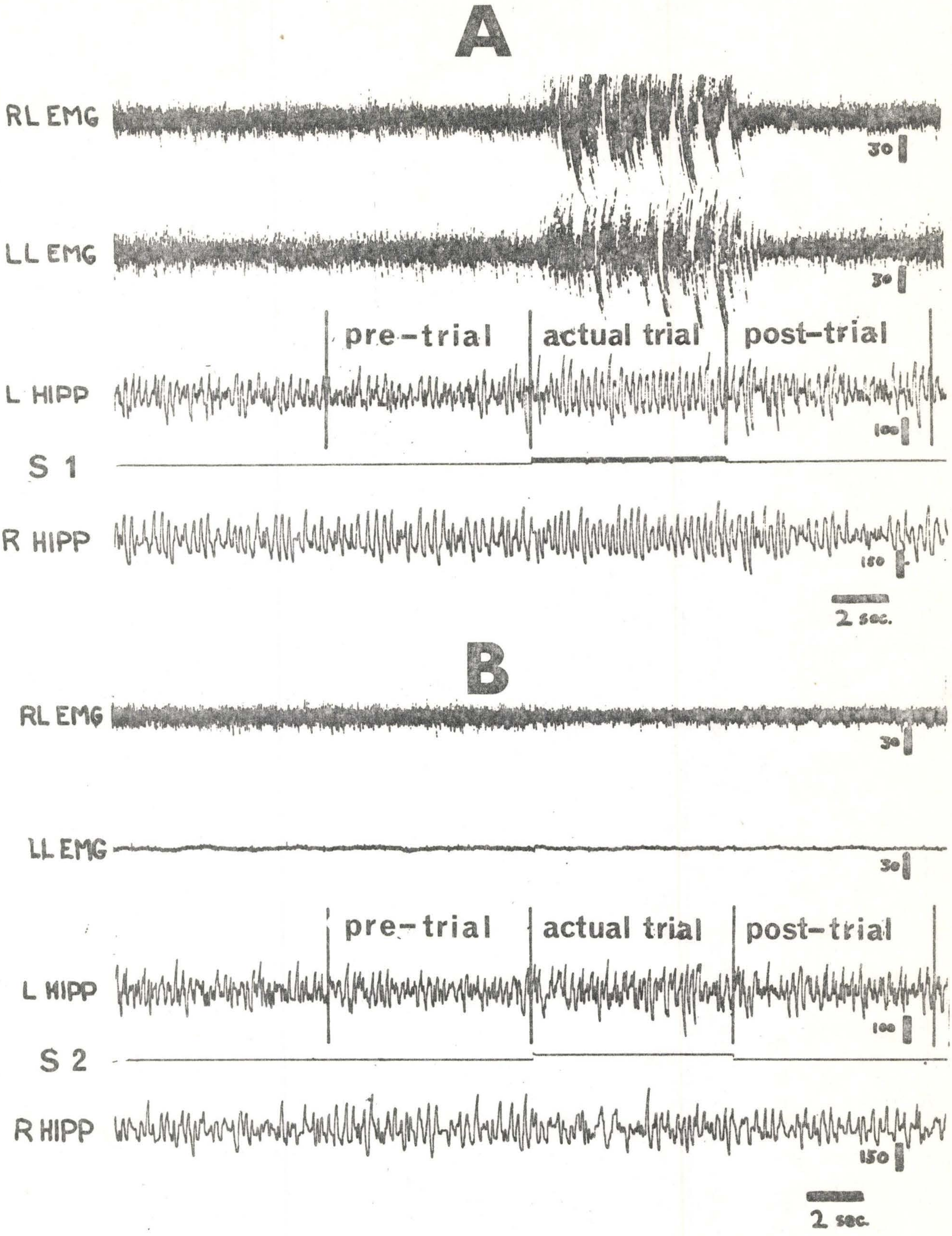
Figure 2. Typical record obtained during the criterion day of active-passive avoidance training in one dog.

Pre- and post-trial trial periods are indicated by the segments separated by perpendicular lines.

Part A of Figure 2 is the record of the last active avoidance trial and Part B is the record of the last passive avoidance trial in the same dog on the same day.

The uppermost channel identified as, _RL EMG,_ is a continuous tracing of the electromyographic activity from the right foreleg with the polygraph amplifier set at maximum gain. _LL EMG,_ is a similar tracing of EMG activity from the left foreleg. The third channel, _L HIPP,_ is the continuous tracing of electrical activity from the left hippocampus. The fourth channel, _S-1,_ indicates the onset and duration of an S-1 trial, requiring multiple pedal-press avoidance responding. The small interruptions in the solid mark indicate the occurrences of pedal-presses. The fifth channel, _R HIPP,_ is the continuous tracing of the electrical activity obtained from the right hippocampus.

In Part B, the fourth channel, _S-2_ identifies as an upward deflection (which is maintained throughout the trial) the occurrence of an S-2 or passive avoidance trial. If pedal-presses had occurred on this trial they would have been indicated as brief deflections similar to those seen in channel four of Part A.


A
RL EMG
30
LL EMG
30
pre-trial
actual trial
post-trial
L HIPP
100
S 1
R HIPP
150
2 sec.
B
RL EMG
30
LL EMG
30
pre-trial
actual trial
post-trial
L HIPP
100
S 2
R HIPP
150
2 sec.

RESULTS

All four dogs achieved the active and passive avoidance criterion within four training days. Table 2 provides a summary of the acquisition results for these dogs.

Table 2 about here

The number of escape trials and the total number of trials provide indices of the ease of acquisition of the two avoidance responses (multiple-pedal-pressing and "not-pedal-pressing"). Three of the four dogs required a greater number of exposures to S-2, the stimulus requiring a passive avoidance response (not-pressing) to achieve the avoidance criterion. A greater number of escape trials occurred in the stimulus requiring a passive avoidance response than in the stimulus requiring an active avoidance response (multiple-pressing). All four animals received more shocks for pedal-pressing in S-2 than they received for "not-pedal-pressing" in S-1. The average number of pedal-presses which constituted the active avoidance response for each dog is indicated in item 4 of Table 2.

Two features of the experimental procedures can perhaps account for the differential ease of training the two avoidance responses. First, all of the animals had to be initially trained to pedal-press so that they could be punished for so doing later when active-passive avoidance training was introduced. Secondly, during active-passive avoidance training, the punishment for making incorrect responses was different in S-1 than it was in S-2. In S-1 a shock was delivered only after every ten seconds of "holding still" occurred (the incorrect response in S-1). In S-2,

TABLE 2

Summary of the acquisition results obtained with four dogs trained in an active-passive avoidance situation

		Dog No.	to S-1	to S-2	to both stimuli
1.	number of trials to avoidance criterion	66	93	112	205
		67	32	50	82
		68	88	63	151
		69	37	75	112
2.	number of escape trials to criterion	66	19	34	53
		67	12	24	36
		68	31	5	36
		69	14	40	54
3.	mean number of shocks per escape trial	66	1.7	15.9	
		67	1.7	2.9	
		68	2.3	5.5	
		69	2.4	3.3	
4.	mean number of presses per avoidance trial	66	6.5	0.0	
		67	9.3	0.0	
		68	8.0	0.0	
		69	5.7	0.0	

every incorrect response, that is every pedal-press, was punished.

The course of acquisition of the two avoidance responses is graphically presented in Figure 3. The percentage of active avoidance trials and the percentage of passive avoidance trials in each successive block of twenty trials are plotted for each of the four dogs. It is evident that with three of the four dogs the level of performance of the active avoidance response in S-1 was superior to the level of performance of the passive avoidance response in S-2 in the early portion of training.

Figure 3 about here

Results of the analysis of the EEG records obtained from the dogs during the last or criterion day of active-passive avoidance training are presented in Table 3. The average number of pedal-presses, and the average number of seconds of theta occurring before, during, and after each active and passive avoidance trial are shown for each dog. The duration of the average avoidance trial, and therefore the length of the pre- and post-trial periods in which theta was measured, is shown in column two. The number of such time periods is shown in the last column identified as number of trials. The results are shown separetely for S-1 (active avoidance) trials and for S-2 (passive avoidance) trials.

Table 3 about here

There was very little pedal-pressing before and after each trial. The dogs, during active avoidance trials, however, pressed regularly

Figure 3. Course of acquisition of active and passive avoidance responses in four dogs plotted as percent avoidance trials occurring in successive blocks of twenty trials for active and passive avoidances separately.

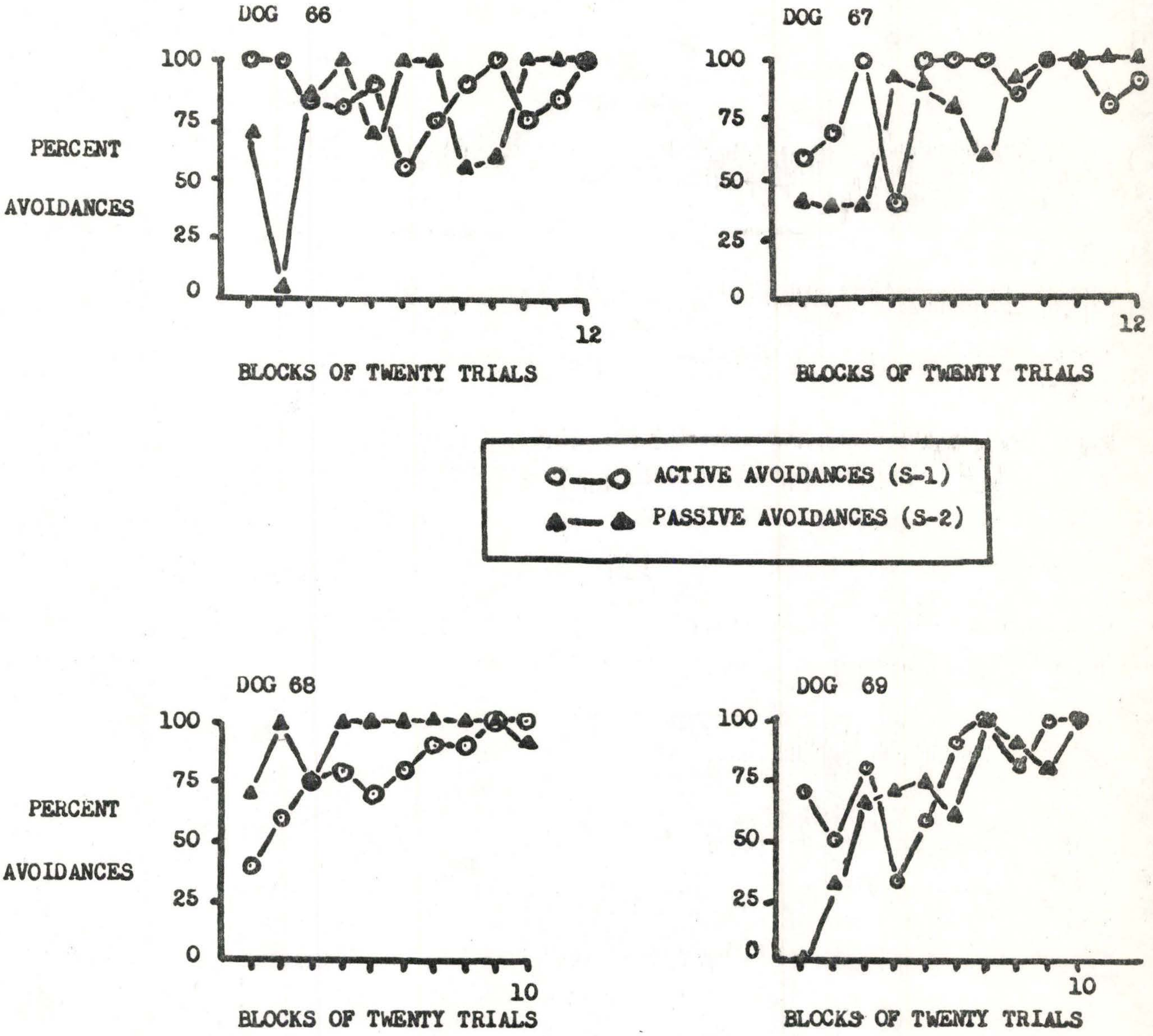

DOG 66
DOG 67
DOG 68
DOG 69
PERCENT AVOIDANCES
100
75
50
25
0
12
10
BLOCKS OF TWENTY TRIALS
ACTIVE AVOIDANCES (S-1)
PASSIVE AVOIDANCES (S-2)

TABLE 3

Average number of seconds of theta occurring before, during, and after each active and passive avoidance trial

(active avoidance)

dog	time	before	during	after	no. of S-1 trials
66	7.3	0.4	3.3	1.6	21
67	8.3	2.8	5.5	1.5	22
68	7.4	1.3	6.5	2.8	24
69	6.6	0.6	1.1	1.3	21

(passive avoidance)

		before	during	after	no. of S-2 trials
66	4.0	0.1	0.9	0.1	24
67	8.2	3.0	1.5	1.2	25
68	7.3	1.4	0.5	0.3	24
69	7.3	1.1	0.7	0.9	21

Average number of pedal presses occurring before, during, and after each active and passive avoidance trial

(active avoidance)

dog	time	before	during	after	no. of S-1 trials
66	7.3	0.0	6.5	0.1	21
67	8.3	0.3	9.3	0.0	22
68	7.4	0.0	8.0	0.0	24
69	6.6	1.0	5.7	0.4	21

(passive avoidance)

		before	during	after	no. of S-2 trials
66	4.0	0.0	0.0	0.0	24
67	8.2	0.1	0.0	0.0	25
68	7.3	0.0	0.0	0.0	24
69	7.3	0.3	0.0	0.2	21

during the CS.

It is evident that within each dog the amount of theta occurring just prior to a trial requiring an active avoidance response (multiple pedal-pressing) was essentially identical to the amount of theta occurring just prior to a trial requiring a passive avoidance response ("holding-still" for a number of seconds). During a trial requiring an active avoidance response all four dogs show an increase in the amount of hippocampal theta correlated with multiple pedal-pressing, while only one dog showed an increase in passive trials. In each case, the amount of hippocampal theta was greater during active than during passive trials. Also, the little theta that did occur in passive trials was associated with movements other than pedal-pressing (e.g. head movements, postural adjustments). The amount of theta occurring just after an active avoidance trial, (1) tended to return to the pre-trial level, and (2) was greater than the amount of theta occurring during the same post-trial period following a passive avoidance trial. There was no overlap in any of the dogs.

Photographic reproductions of brain sections from the dogs are presented in Appendix D. Gross inspection of the points of deepest penetration of the electrodes revealed that in dogs 66, 67, and 68, all of the implants were well within the body of the hippocampal formation. Of these, three electrodes were located in the hippocampus and four in the dentate gyrus - CA 4 area. One dog (number 69), showed one electrode on the left side which was located in the fimbria and partly in the left ventricle.

Voltage measurements were taken of the theta waves having maximum amplitude on the first and last active avoidance trials for each dog. Dog 68 ranked highest in amplitude of theta (275-340 microvolts) while dog 69 ranked lowest on the same measure (65-100 microvolts). The former dog showed the greatest increase in the amount of hippocampal theta correlated with multiple pedal-pressing, while the latter dog showed the smallest increase (Table 3). It is possible therefore, that the extent of the relationship between hippocampal theta and active avoidance behavior may be partly determined by the amplitude of hippocampal theta activity.

DISCUSSION

Having established a relationship between hippocampal theta and regular avoidance conditioning in the first experiment with fourteen dogs (chapter three), an active-passive avoidance experiment was performed in order to provide additional evidence bearing on this relationship.

A number of conclusions can be advanced. First, hippocampal theta under the conditions of this experiment was found to accompany a voluntary avoidance response which entailed active, energetic, skeletal behavior and was largely absent when the voluntary avoidance response consisted of "holding-still" or "not-pedal-pressing" for a number of seconds.[1] The results would therefore appear to be in keeping

---

[1] The last five criterion passive avoidance trials were examined for each of the dogs. No leg movements were observed in three of the four dogs and in the remaining dog small movements of less than two cms.

with Vanderwolf's (1968) notion that hippocampal theta may be a manifestation of a mechanism for the initiation of voluntary skeletal movement that is active.

Secondly, the possibility that hippocampal theta may be involved primarily in an arousal or attention-like process (Green and Arduini, 1954; Bremner, 1964) seems to be ruled out by the finding that hippocampal theta was largely absent during successful passive avoidance trials. The particular training procedures employed in the present experiment called for repeated presentations of two stimuli in an irregular order and required the performance of incompatible responses. Incorrect responses in each stimulus were punished with intense shocks. It seems intuitively obvious that animals trained under these conditions could be expected to pay particular attention to both stimuli from the very first moment of onset of either stimulus. Therefore hippocampal theta should have appeared with equal probability in

---

were present on two of the five passive avoidance trials analyzed. The EMG records from both forelegs in each dog were also examined during the last five criterion passive avoidance trials. Small, 20 micro-volt EMG activity occurring in one dog during inter-trial periods decreased during the passive avoidance trials (to 10 micro-volts). No observable EMG activity was present in the passive avoidance trials in two of the remaining three dogs. EMG activity of less than 50 micro-volts was present continuously during one of the passive avoidance trials sampled in the last dog (number 69), and EMG activity with an amplitude of 150 micro-volts was found associated with the small leg movements occurring during two of the five passive avoidance trials in the same dog. This evidence indicates that the dogs were indeed "holding-still" during the passive avoidance trials rather than making other skeletal responses such as struggling without hitting the pedal. That the dogs actually learned a voluntary "holding-still" response was further evidenced by the fact that there were explicit contingencies of reinforcement associated with its occurrence and that the response required more shocks to establish than the active avoidance response. This means that the passive avoidance response was instrumental and not just a simple discrimination.

both stimuli according to the arousal or attention hypothesis. The results obtained were inconsistent with this expectation.

Thirdly, the results obtained also appear to be at variance with Adey's most recent hypothesis (Elazar and Adey, 1967a). Adey seems to believe that hippocampal theta is not correlated with somatomotor response (as Vanderwolf maintains, 1967) but with the "connection" between this pattern and a conditional stimulus (Elazar and Adey, 1967a, p. 235). In the present experiment it would appear that the presence of a "connection" between any particular pattern of skeletal activity and a CS was not crucial. The training procedures called for the establishment of two different connections, one between S-1 and multiple-pedal-pressing, and a second connection between S-2 and "holding-still" or "not-pedal-pressing". The animals all met the avoidance criterion for both the active and passive avoidance responses, indicating that the two "connections" had been established. However, theta appeared only or mainly during the stimulus requiring active avoidance responding, suggesting that the instrumental significance of the two CSs did not determine the presence-absence of hippocampal theta. If and when hippocampal theta did occur during passive avoidance trials, television observation suggested that such theta was accompanied by head-turning movements and postural adjustments.

Finally, the possibility that hippocampal theta may be involved primarily in a motivational system (Pickenhain and Klingberg, 1967) seems likewise to be inconsistent with the finding that hippocampal theta was largely absent during successful passive avoidance behavior.

An attempt was made in the present experiment to schedule shocks in such a way that the conditioned aversiveness of the two stimuli, S-1 and S-2, would be approximately equivalent, thereby equating the motivational component associated with each stimulus. Results shown in Table 2 indicated that a greater number of escapes and a higher incidence of shocks occurred in S-2, the stimulus requiring passive avoidance behavior, than in S-1, the stimulus requiring multiple-pedal-press avoidance responding. If hippocampal theta was an electrical manifestation of conditioned fear then more theta should have appeared in S-2 than in S-1. The results obtained were inconsistent with this view.

CHAPTER FIVE

EXPERIMENT THREE

The overall concern of this thesis is with the relationship between hippocampal theta waves and operantly conditioned skeletal responses. In the previous chapters evidence was presented from two experiments. The results suggested first, that hippocampal theta is a correlate of avoidance pedal-pressing in dogs and could be considered a central component of the active avoidance response when either a regular avoidance training procedure or a procedure requiring multiple-pedal-pressing as the avoidance response was employed. Secondly, it was shown that hippocampal theta was a correlate of the skeletal activity involved in making the pedal-press and not with attention or arousal processes nor with the particular connections established between the CS and the response, nor with the motivational features associated with the training procedures.

Since hippocampal theta activity is correlated with active voluntary skeletal responses the question can be raised whether it is an essential component of the movement control system involved in operant conditioning. Is the operant reinforcement of hippocampal theta sufficient to produce a change in the probability of the skeletal movement when the system is intact? In order to determine whether this question can be answered convincingly, it would seem necessary to reinforce hippocampal theta when no overt movement is possible, and then to study the effects of this reinforcement subsequently when overt movement is permitted. The curarization technique provides an obvious method for achieving this objective.

Theta can first be reinforced when the overt response is prevented by deep curarization. Following recovery from paralysis a transfer test can be applied to reveal whether operant conditioning of the central component had any effect on the overt response.

The purpose of this chapter is to describe an experiment conducted to determine whether the first step of the procedures outlined above was feasible. Can hippocampal theta be operantly conditioned in naive deeply paralyzed dogs? The attempt to demonstrate that hippocampal theta conditioning under paralysis is related to overt avoidance responding is described in a subsequent chapter.

METHOD

Subjects

The subjects were six naive mongrel dogs housed in individual cages.[1]

Apparatus

The experimental chamber, recording apparatus and conditioning equipment has already been described in a previous chapter.

Procedure

Stainless-steel, 26 gauge, mono-polar electrodes were implanted bilaterally in the posterior dorsal hippocampus of the dogs. Following four to five days of post-operative recovery the dogs were paralyzed with gallamine triethiodide to a depth where electromyographic activity continuously monitored in both forelegs was absent (70 to 100 mgms. per

---

[1] Preliminary work with nine other dogs was conducted with similar objectives prior to the present experiment.

kgm. over the experimental session of six to ten hours).

All dogs received a series of adaptation trials to a predetermined criterion with fixed duration, random-fixed order of presentations of two auditory stimuli (white-noise and chopped-tone each of 75 decibels as in previous experiments) to be used subsequently in the discrimination phase of the experiment. The first four adaptation trials were presented in ABBA order and all adaptation trials lasted for fifteen seconds. Adaptation trials were continued until a criterion was met of six presentations of each stimulus without a single "theta response" in twelve consecutive trials.[1]

An operant discrimination procedure was then applied. The white-noise was used as S+ for all dogs. Failure to make seven theta responses (a "response-unit") in S+ produced the delivery of a single, 1/10th second, eight milliampere shock across the hindlegs coincident with termination of S+ at the end of fifteen seconds. A similar failure to make seven theta responses (a "response-unit") in S-, the chopped-tone stimulus, also produced termination of S- at the end of fifteen seconds, but the shock was never paired with this stimulus. If the dog made seven theta responses in less than fifteen seconds in <u>either</u> S+ or S- then the stimulus terminated automatically and simultaneously with the occurrence of the seventh response and shock was omitted. Termination of S+ and S- according to the same contingency rule meant that there was equal opportunity for hippocampal theta to occur in both stimuli. Therefore the estab-

---

[1] A "theta response" was defined as the report-pulse of a binary-coded decimal counter described in the measurement section of this chapter.

lishment of a hippocampal theta discrimination could not be explained in terms of a differential opportunity for responding.

S+ and S- trials were presented in fixed irregular order with the only restriction that five presentations of each stimulus had to occur within any given block of ten trials. The same order of presentation was given to each dog. The dogs were trained in this fashion to a criterion of twenty consecutive shock-free (avoidance) trials. Training in some dogs was then continued beyond this point in order to obtain some estimate of the ease or difficulty in maintaining the discrimination.

At the end of the experiment each dog was anesthetized with sodium pentobarbital and the brain was perfused and removed. Sections through the electrode tracks were then mounted on slides and stained with thionin as in previous experiments.

Measurements

The EEG signal from the "best" (in terms of amplitude) hippocampus in each dog was fed both to the ink-writing system of the Grass polygraph and to a Krohn-hite band-pass filter (model 330N). The low-pass side of the filter was set at 4 Hz and the high-pass side at 6 Hz. The filter output was then fed back to a second pen-assembly unit on the polygraph and also to a Schmidt trigger (Fersch, model 119, Montreal). The output pulses of the Schmidt trigger were then fed continuously through a Digibit counting circuit. The circuit consisted of four cascaded binary-coded decimal counters so that each pulse was fed into the previous cumulated count and also of itself initiated a new counting period. Each of the counters was capable of producing a report-pulse and of resetting all of the counters. Whenever four input pulses arrived within a period of one second or less, then a single report-pulse would occur and all the partial counts stored during the time period prior to the report-pulse would instantly clear.[1]

Each report-pulse was defined as the occurrence of one theta response. The report-pulse operated on conventional electromechanical circuitry which automatically programmed all of the events of the operant procedure and it was also fed to a marker system on the polygraph, each report-pulse appearing as a brief deflection.

Some difficulties with the automated equipment were encountered. First, the band-pass filter coupled to the binary-coded decimal counter system was sensitive to transient electrical discharges in the building. Secondly, the band-pass filter itself was somewhat inadequate at amplifying the particular frequencies of interest (4-6 Hz), because the band selected by the experimenter was too narrow for optimal functioning of the filter.

Figure 4 shows the typical kind of record obtained during the

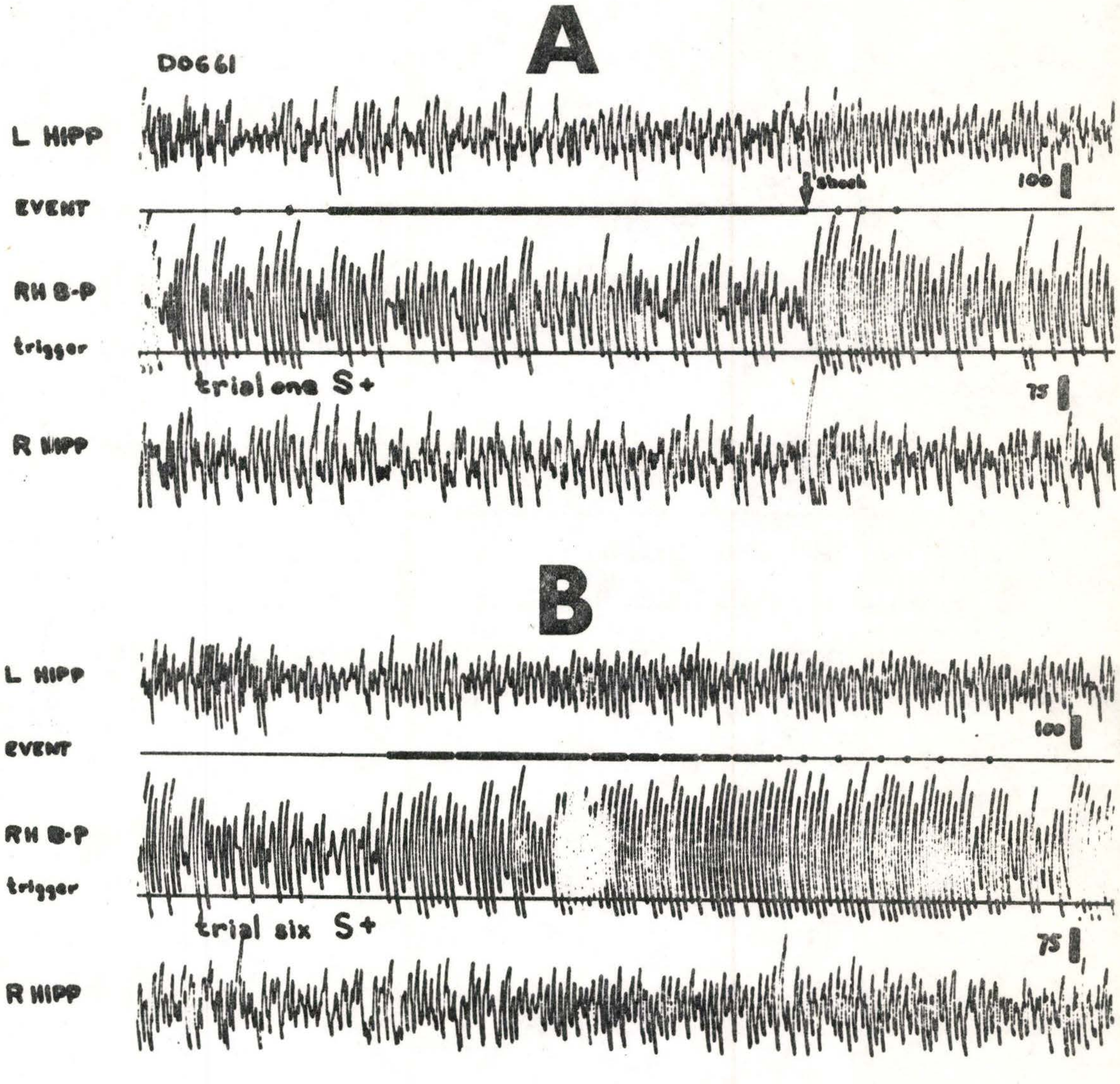
Figure 4 about here

early phase of the operant discrimination training under deep paralysis in one dog. The first S+ trial paired with shock is shown (A) as well as the first "avoidance" trial in S+ (B).

---

[1] A debt to Cy Dixon who designed and built the counting circuit is gratefully acknowledged.

Figure 4. A. The first S+ trial paired with shock. B. The first "avoidance" trial in S+. The uppermost channel, L HIPP, is the continuous record of the electrical activity from the left hippocampus. Channel two, EVENT, indicates S+ as a solid bar, and the small interruptions (in S+ of B) or deflections indicate the occurrence of a report-pulse-defined theta response. The arrow just above the event marker at the end of S+ indicates the delivery of shock. Channel three, identified as RH B-P, shows the continuous tracing of the filtered (4-6 Hz) EEG signal from the right hippocampus. It shows the amplification of a narrow band of waves (4-6 Hz) taken from the raw EEG signal of the tracing appearing just below which is identified as R HIPP (right hippocampus). The channel labelled, trigger, indicates which waves were processed by the Schmidt trigger. Each wave which crossed this line was converted to a square-wave pulse by the Schmidt trigger and was fed through the Digibit counting circuit. Waves which did not cross this line did not produce an output-pulse in the Schmidt trigger and thus were not counted.


A
DOG61
L HIPP
EVENT
shock
100
RH B-P
trigger
trial one S+
75
R HIPP
B
L HIPP
EVENT
100
RH B-P
trigger
trial six S+
75
R HIPP

RESULTS

The results obtained during the course of adaptation are shown in Table 4.[1] The six dogs required from 14 to 38 adaptation exposures to the two stimuli in order to meet the criterion of twelve consecutive trials without a single theta response (as defined by a single report-pulse from the Digibit counting circuit). A "response-unit" of hippocampal theta (seven theta responses) occurred in three dogs during the adaptation phase. Two theta response-units occurred in dog 62 and one theta response-unit occurred each in dog 63 and 64. During the very first presentation of S+, when this stimulus had maximum novelty, little theta appeared in all but one dog. Little or no theta appeared during subsequent adaptation trials. The theta which did appear was characteristically irregular in amplitude and consisted of much shorter bursts than were seen later during the discrimination phase of the experiment.

Table 4 about here

The results obtained during the discrimination training phase of the experiment are shown in Table 5. The total number of S+ presentations required to achieve the avoidance criterion and the total number of shock trials in S+ to the avoidance criterion provide two measures of

Table 5 about here

---

[1] Appendix E provides the raw data showing the average number of theta responses per three-trial blocks for S+ and S- trials separately.

TABLE 4

Summary of results obtained in six dogs
during the course of adaptation
under deep paralysis

	Dog number 59	60	61	62	63	64
1. Total trials to adaptation criterion	26	38	26	14	21	22
2. No. of trials with a "response-unit" (i.e., 7 theta responses)	0	0	0	2	1	1
3. No. of single theta responses in first S+	5	1	1	0	2	3
4. No. of single theta responses in first S−	0	3	3	8	3	8

TABLE 5

Summary of the results obtained in six dogs during the course of application of a discriminated-trials procedure under deep paralysis to hippocampal theta

	Dog number					
	59	60	61	62	63	64
1. Total S+ trials to first criterion avoidance	13	92	59	250+	8	15
2. Total shock trials in S+ to criterion	4	48	16	?	5	5
3. Trial of first avoidance in S+	3	4	4	3	4	3
4. Longest run of consecutive avoidances in S+	49	28	25	3	20	35
5. Total no. of S- trials with a response-unit	21	4	2	0	1	1

the ease of acquisition of the theta response-unit (the avoidance response) to S+. A third such measure is the trial number of the first avoidance in S+. All of the dogs made the first avoidance in S+ on or before the fourth presentation of S+. The longest run of consecutive avoidances in S+ provides a measure of the stability of the theta response-unit. A clear differential in theta performance could be maintained for as many as 49 consecutive exposures to S+ (with an equal number of interpolated S- trials). Only one dog provided results indicating that this discrimination could not be established within the arbitrary upper limit of 250 trials set by the experimenter at the outset. In dog 60 the discrimination was established with some difficulty, the dog requiring 47 shocked presentations of S+ before achieving the first criterion avoidance trial.

The total number of S- trials with the occurrence of a theta response-unit gives a measure of the extent of stimulus generalization. Only one dog produced a response-unit of theta on more than four exposures to S- during discrimination training.

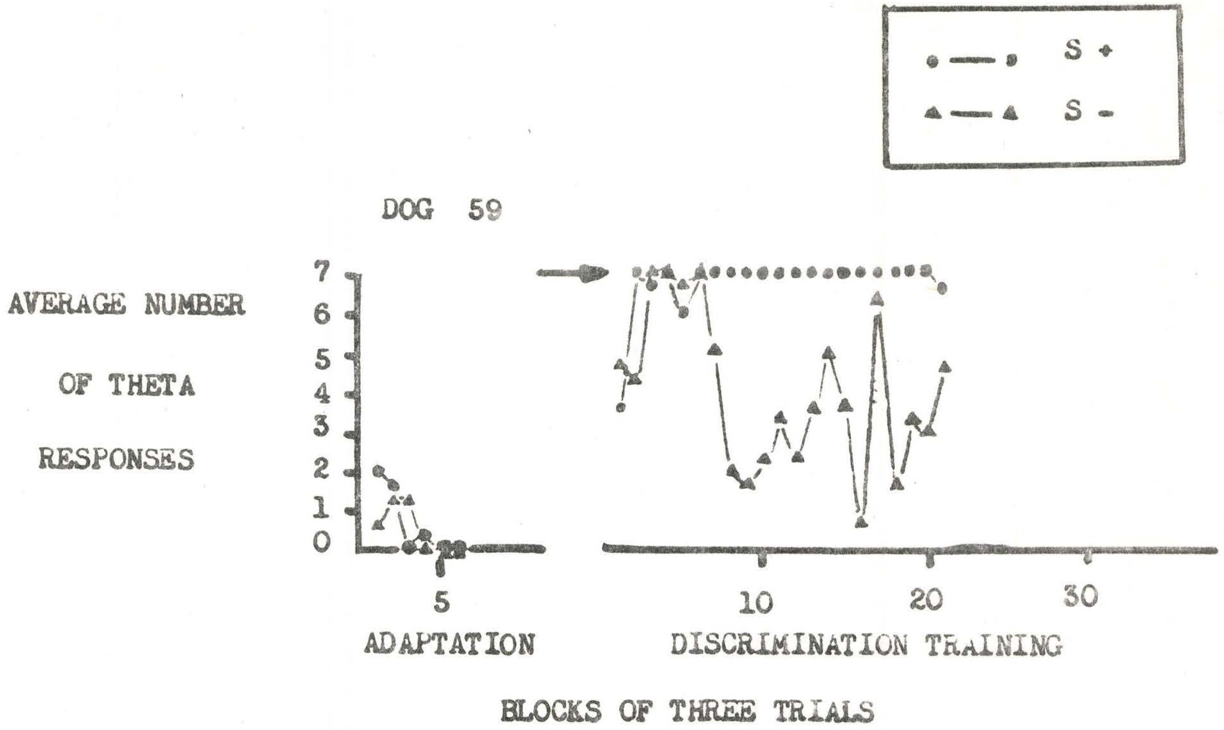
The course of acquisition of the hippocampal theta discrimination is shown graphically for each dog in Figures 5 and 6. The average number of hippocampal theta responses per successive three-trial block of each stimulus is plotted.[1] Separate values in S+ and S- trials are shown. Graphical presentation of the results during adaptation are

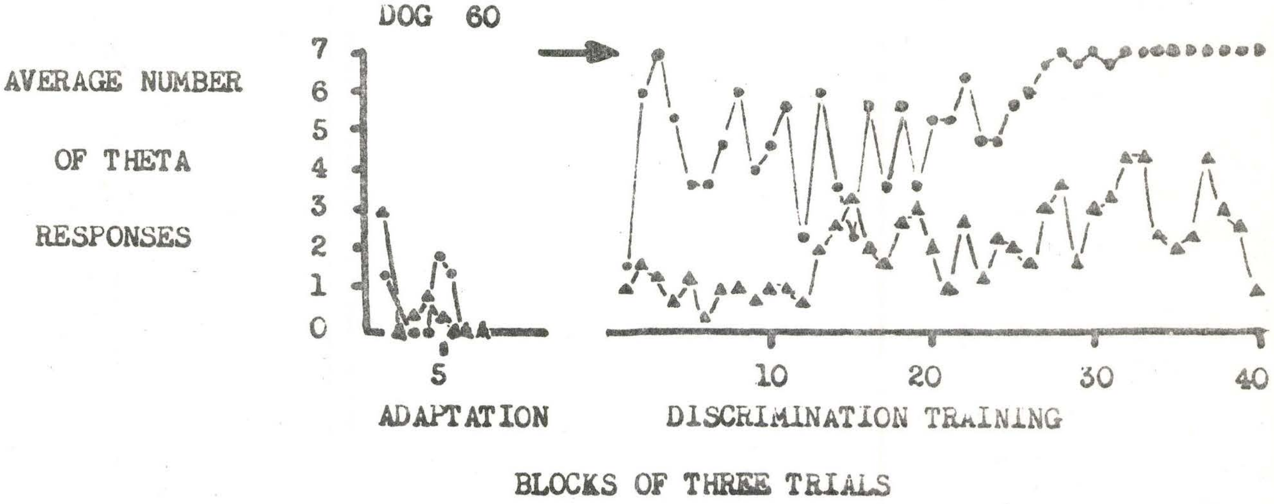
Figures 5 and 6 about here

---

[1] Appendix F provides the raw data showing the average number of hippocampal theta responses per three-trial block for S+ and S- separately.

Figure 5. Course of acquisition of a hippocampal theta discrimination plotted as average number of theta responses (single report-pulses) in blocks of three trials for S+ and S- separately. Dogs 59, 60, and 61. The arrows indicate the upper limit of opportunity for making theta responses was seven responses beginning in the discrimination phase of the experiment.


●——● S +
▲——▲ S −
DOG 59
AVERAGE NUMBER OF THETA RESPONSES
0
1
2
3
4
5
6
7
5
10
20
30
ADAPTATION
DISCRIMINATION TRAINING
BLOCKS OF THREE TRIALS


DOG 60
AVERAGE NUMBER OF THETA RESPONSES
0
1
2
3
4
5
6
7
5
10
20
30
40
ADAPTATION
DISCRIMINATION TRAINING
BLOCKS OF THREE TRIALS

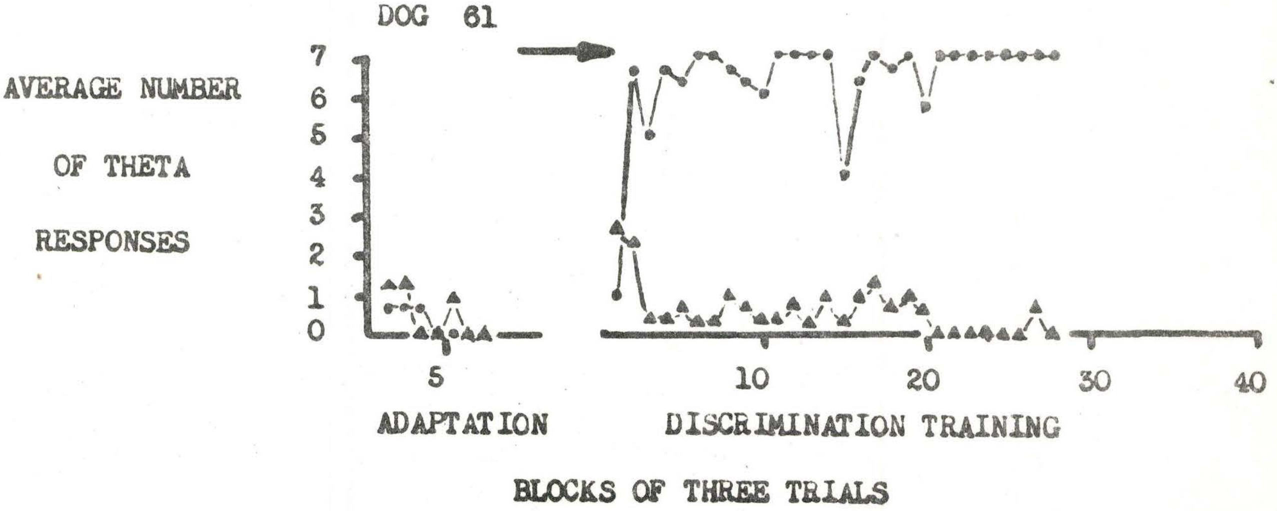
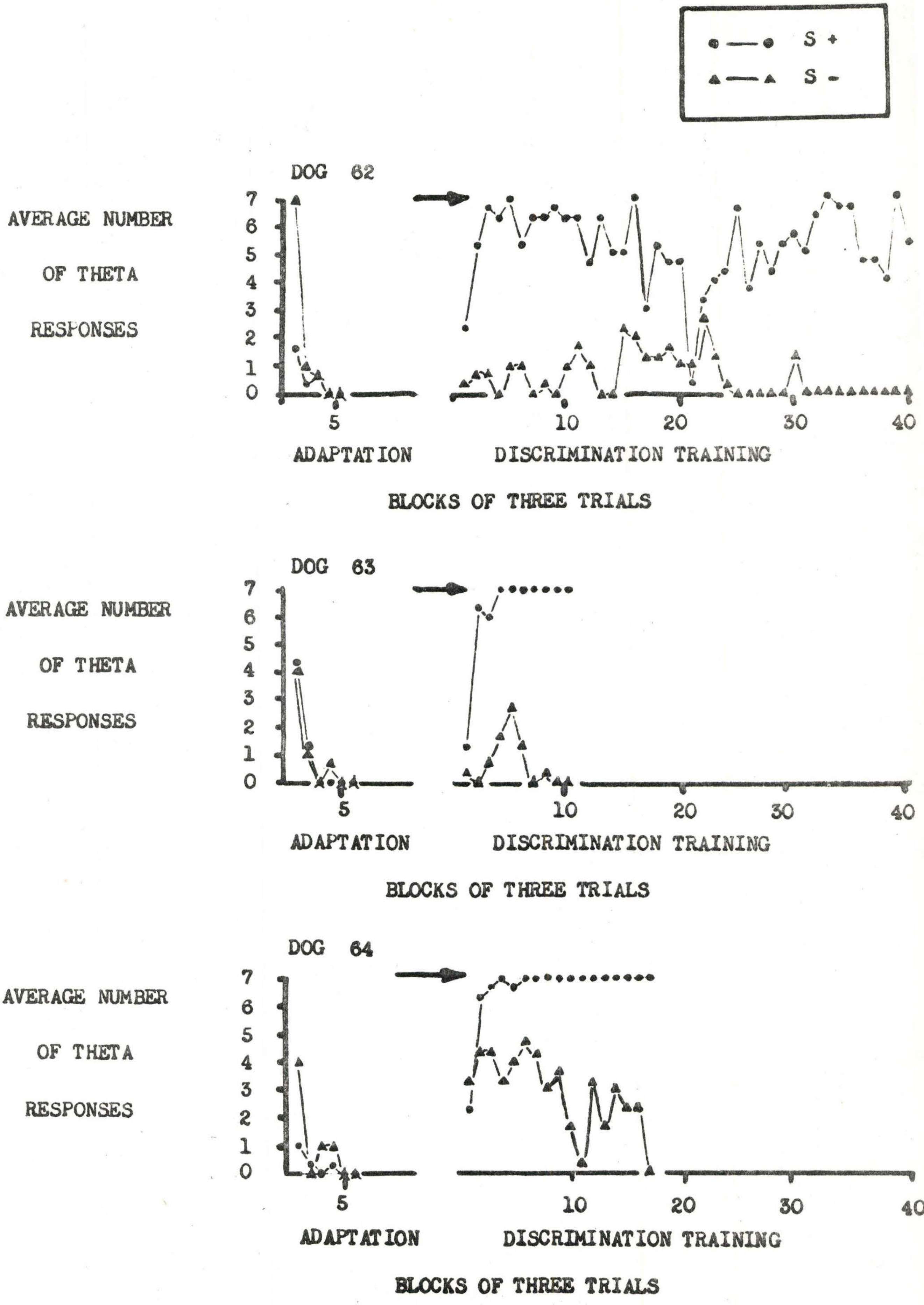

DOG 61
AVERAGE NUMBER OF THETA RESPONSES
0
1
2
3
4
5
6
7
5
10
20
30
40
ADAPTATION
DISCRIMINATION TRAINING
BLOCKS OF THREE TRIALS

Figure 6. Course of acquisition of a hippocampal theta discrimination plotted as average number of theta responses (single report-pulses) in blocks of three trials for S+ and S- separately. Dogs 62, 63, and 64. The arrows indicate the upper limit of opportunity for making theta responses was seven responses beginning in the discrimination phase of the experiment.


S +
S -
DOG 62
AVERAGE NUMBER OF THETA RESPONSES
7
6
5
4
3
2
1
0
5
10
20
30
40
ADAPTATION
DISCRIMINATION TRAINING
BLOCKS OF THREE TRIALS
DOG 63
AVERAGE NUMBER OF THETA RESPONSES
7
6
5
4
3
2
1
0
5
10
20
30
40
ADAPTATION
DISCRIMINATION TRAINING
BLOCKS OF THREE TRIALS
DOG 64
AVERAGE NUMBER OF THETA RESPONSES
7
6
5
4
3
2
1
0
5
10
20
30
40
ADAPTATION
DISCRIMINATION TRAINING
BLOCKS OF THREE TRIALS

shown in the first segment for each dog. The pre-operant level of theta responses was zero for all dogs in both stimuli by the end of adaptation. The second segment depicts the results obtained during the discrimination phase of the experiment. It is apparent that the hippocampal theta discrimination was established very rapidly in three of the six dogs. In a fourth dog, number 59, the early phase of discrimination was marked by complete generalization of the hippocampal theta response-unit to both S+ and S- till the sixth block of trials, then the response-unit dropped from S- trials while remaining in subsequent S+ trials. The number of single hippocampal theta responses never dropped-out entirely from S- during the course of the experiment with dog 59.

During the first block of discrimination training trials three of the six dogs had a higher average number of theta responses (single report-pulses) in the three-trial block of S- presentations than in the comparable first block of S+ presentations (dogs 59, 61, 64). In the last three-trial block of adaptation training there were no hippocampal theta responses in any of the dogs. Some generalization of single theta responses therefore occurred to S- presentations at the earliest stage of the discrimination procedure. Single theta responses tended to remain in S- throughout discrimination training in both dogs 59 and 61 but did drop out in the remaining dogs. Dog 62 which failed to meet the avoidance criterion showed a clear differential nonetheless in the frequency of occurrence of average number of theta responses per three-trial block in S+ and S-.

Photographic enlarged reproductions of brain sections from the dogs are presented in Appendix G. Five of the electrode placements were located in the dentate gyrus - CA 4 area, two in the hippocampus, two in the fimbria, two in the stria terminalis, and one in the corpus callosum. Theta activity in the 4-6 Hz range (60 - 470 micro-volts) was recordable from all placements, perhaps because mono-polar rather than bi-polar electrodes were used.

Comparison of the voltages of theta activity having the maximum amplitude during the first and last avoidance trials in S+ indicated that there was no significant changes in this amplitude during the course of the experiment, suggesting a negligeable effect of deep paralysis maintained for up to ten hours on this factor. In addition, there was no significant relationship between trials to first criterion avoidance and maximum amplitude of theta activity (Spearman rank correlation coefficient, $r_s = -.37$, for $N = 6$).

DISCUSSION

The results obtained during adaptation demonstrate that hippocampal theta under the conditions of this experiment was not an unconditioned response to the auditory stimuli and that hippocampal theta was not simply a reaction of the brain structure involved to novel auditory stimuli.

The results obtained during the discrimination phase indicate that the hippocampal theta discrimination was established with relative ease and rapidity. Some evidence was obtained which suggested that the ease of attaining the avoidance criterion may have been partly determined by the amount of hippocampal theta which was elicited by the brief shocks delivered at the end of "escape" trials in S+ (see A of Figure 4 which illustrates this feature). The presence-absence of one or more single theta responses was determined for the period immediately following every shocked S+ trial in each dog. Such an analysis revealed that one or more theta responses occurred after every single shock in the four dogs which achieved the avoidance criterion with relative ease (numbers: 59, 61, 63, and 64). Such theta responses, however, occurred only following ten percent of the shocks delivered to dog 62 and following 43 percent of the shocks received by dog 60. The former dog failed to achieve the avoidance criterion while the latter attained it only with some difficulty. Post-shock theta does not appear to be related to the electrode placement nor to the strength of the hippocampal theta signal.

The rapidity with which the discrimination was established could have been accounted for if hippocampal theta simply failed to appear at any time during the S- trials. This possibility seems to be ruled-out by two pieces of evidence. First, the theta response-unit (seven theta responses) occurred at least once in an S- trial for all of the dogs which met the avoidance criterion. Secondly, from 48 to 90 percent of the S- trials during training were found to contain at least one hippocampal theta response.

The possibility that the incidence of response-units of theta in S+ could be due to a simple effect of shock which carried over throughout the remainder of the experiment was ruled-out by the fact that complete response-units of hippocampal theta seldom occurred during S- trials. If a "sensitization" effect of this kind due to shock was present then response-units of hippocampal theta should have occurred in S- with equal probability to their appearance in S+.

In addition, the hippocampal theta discrimination performance cannot be accounted for in terms of a differential amount of theta appearing in each stimulus prior to the start of discrimination training because the adaptation procedures guaranteed that the "pre-operant" level of theta in each of the two stimuli was identical.

In this experiment no attempt was made to determine which feature or features of the conditioning procedure may have been responsible for the establishment of the hippocampal theta discrimination. That the reinforcing stimuli controlled the incidence of hippocampal thata seems evident. However, this control could have been due to the contingency between the discriminative stimuli and the shock (classical conditioning) or to the contingency between reinforcing stimuli and the avoidance response (operant conditioning).

The dogs in this experiment were unable to make movements of any kind and the depth of paralysis was such that no electromyographic activity was visibly present either. The establishment of a hippocampal theta discrimination under these conditions provides evidence that hippocampal theta responses did not depend on feedback from the peripheral musculature. In a sense then, the occurrence of hippocampal theta in a completely paralyzed animal could reflect the animal's attempt to make a movement, a belief consistent with Vanderwolf's (1968) hypothesis that such theta is involved in a mechanism for the initiation of voluntary skeletal movements.

CHAPTER SIX

EXPERIMENT FOUR

The third question that was raised in the introductory chapter of this thesis can now be considered. What is the effect of operantly reinforcing hippocampal theta activity under conditions of deep paralysis on the overt response after recovery from curarization? An affirmative answer to this question would indicate that the overt skeletal avoidance response is not essential for operant reinforcement of that response to take place. Furthermore, such transfer, if it were positive, would further strengthen the hypothesis concerning the close relationship between hippocampal theta activity and overt skeletal movement.

In the preliminary experiment to be reported in this chapter, the subjects were pre-trained to perform a pedal-pressing avoidance response in the normal state before operant conditioning of theta under paralysis. This was done for two reasons. First, the procedure for operantly conditioning theta involved training animals to increase the incidence of theta activity under curare and under other stimulus conditions, to refrain from theta activity under curare. By pre-training the animals in the normal state it was hoped to guarantee that the amount of hippocampal theta activity would be roughly the same in all subjects at the beginning of training. The second purpose of pre-training the subjects was as follows. Even if hippocampal theta is a close and necessary correlate of overt skeletal movement it is not possible from a consideration of hippocampal theta activity alone to indicate with which particular overt skeletal response the theta activity is correlated.

If the notion is accepted that the occurrence of theta in a completely paralyzed subject indicates an attempt to make some skeletal movement, it is not easy to determine which movement the subject is attempting to make. Because of this ambiguity it is difficult to decide which measures of skeletal responding should be employed as indicators of positive transfer. The pre-training was conducted in order to narrow the range of alternatives. It seemed reasonable to assume that animals pre-trained to make a particular pedal-pressing response to a CS would also attempt to make that response when the CS was presented in the curarized state. The transfer test could then be made by measuring the pedal-pressing response after recovery from curarization.

METHOD

Subjects

The subjects were twelve adult mongrel dogs. Seven of these dogs were subjects in experiment one of this thesis. The remaining five subjects were avoidance pre-trained in exactly the same way as the other subjects and therefore all Ss had an identical experimental history prior to introduction of theta training under paralysis.

Apparatus

The experimental chamber, recording apparatus and conditioning equipment have already been described in a previous chapter.

Procedure

Bi-polar twisted nichrome wire electrodes (described previously) were implanted bilaterally in the posterior dorsal hippocampus of all dogs and in the medial thalamus as well in five of the dogs (numbers 36, 39, and 43). The sequence of pre-training, operation, recovery, and post-operative reacquisition have been described in detail in chapter three of this thesis.

The procedures used under curare were identical to those used by Black in his most recent EMG study (1967).

The two procedures used in the curare phase of the experiment were called "reinforce-theta" and "reinforce-non-theta". While paralyzed, dogs in Group-One were given a number of presentations of the CS used in the regular pedal-press avoidance training previously. In the CS the absence of hippocampal theta (medial thalamic theta in dogs 38 and 40) was punished by brief shocks and termination of the CS was made contingent on the occurrence of a number of seconds of theta (reinforce-theta procedure). Achievement of a criterion of twenty avoidances in a row or the administration of one hundred trials signalled the end of the curare phase of the experiment. The dogs were allowed to recover from paralysis for 48 hours then were tested in extinction to determine the effects of the curare procedure on the previously acquired pedal-press response.

The extinction test consisted of fifty exposures to the CS in the absence of shock. On each extinction test trial, the CS terminated only after ten seconds had passed without a pedal-press. This special extinction procedure was employed in order to reduce the amount of time required for between-group differences to emerge. In previous work (Black, 1967; Black and Dalton, 1965) it had been found that the response was extremely difficult to extinguish when the usual extinction procedures were used in which a single pedal-press produces CS termination.

Also, this special extinction procedure provided a measure of the curare training effects on the pedal-press response before the results were contaminated by the reinforcing effects of CS termination.

Dogs in a second group, Group-Two, were treated in identical fashion under curare except that the occurrences of theta were punished with brief shocks and CS termination was made contingent on the appearance of "non-theta" for a number of seconds (reinforce-non-theta procedure). Non-theta was defined as any electrical activity in the tracing other than 4-6 Hz, regular, rhythmical, sinusoidal waves and included low-voltage, desynchronized fast activity, and large amplitude slow "sleep" waves with a wavelength of 3.5 Hz or longer. Dogs in Group-Two were likewise tested in extinction following recovery from paralysis.

At the end of the experiment the dogs were killed with an overdose of sodium pentobarbital, the brains were perfused, sectioned, and sections through the electrode tracks were mounted on slides and stained with thionin.

Measurements

The EEG signal from the "best" hippocampal tracing in terms of amplitude was visually monitored throughout the curare phase. Since automated equipment was not available at the time of the experiment theta responses were defined by an experimenter hand-held micro-switch. A single theta response consisted of the occurrence of one second (estimated) of regular, rhythmical, sinusoidal activity in the range 4-6 Hz.

In two dogs (38 and 40) the hippocampal tracings were unusable and the definition of theta was based on regular, rhythmical, sinusoidal activity in the 4-6 Hz range observed from the paper-tracing of activity in the dorso-medial nucleus of the thalamus.

Non-theta was similarly defined by means of visual inspection of the moving paper record (one cm./sec.) during curarization. Any activity other than synchronized 4-6 Hz waves occurring for a period of one second was considered an occurrence of one non-theta response.

Figure 7 shows samples of a theta avoidance trial (A) and a non-theta avoidance trial (B). The theta avoidance trial consists almost entirely of continuous high amplitude theta activity while only two or three theta waves of low amplitude appear in the non-theta avoidance trial.

Figure 7 about here

RESULTS

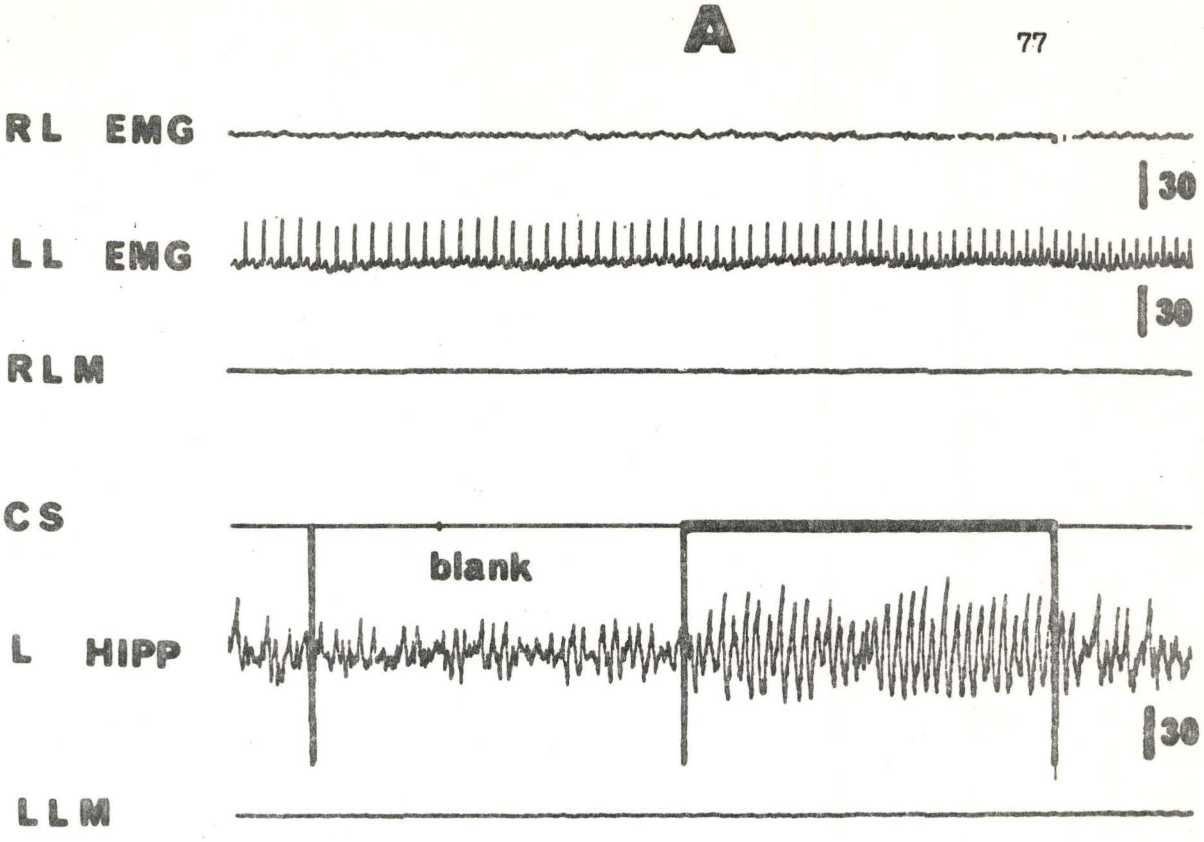
During the pre-operative regular avoidance training phase nine of the twelve dogs made the first avoidance pedal-press response on or before the fourth acquisition trial. Ten of the twelve dogs made nine or fewer escapes during the entire pre-curare phase which included pre-operative and post-operative pedal-press pre-training.

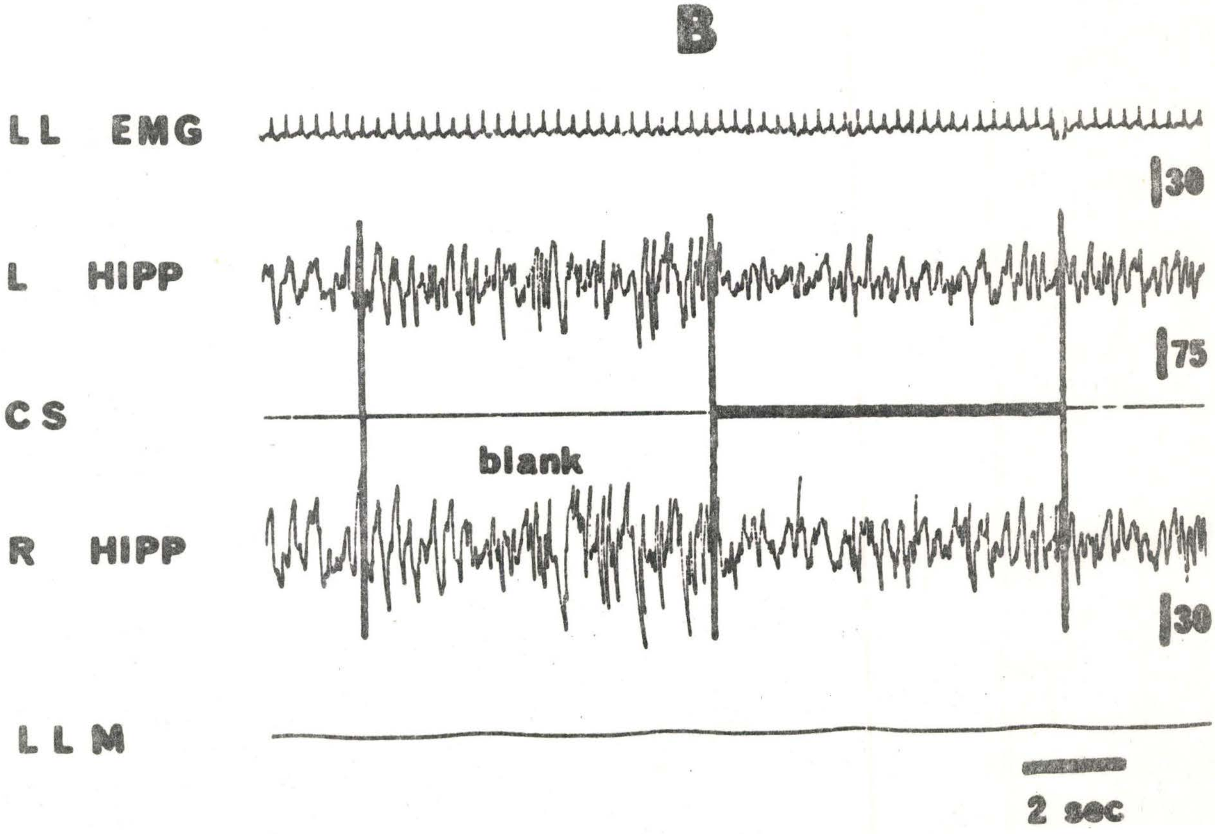
During training under curare, all of the Group-One dogs which received the reinforce-theta procedure achieved the theta avoidance criterion within the arbitrary upper limit of one hundred trials set by the experimenter at the outset. Only one dog in Group-Two failed to meet the non-theta avoidance criterion during curarization in which the reinforce-non-theta procedure was used. No statistically significant differences were found when comparisons were made between Group-One and Group-Two on the variables of number of curare trials, number of shock

Figure 7. A. Sample "theta avoidance" trial obtained with the "reinforce-theta" procedure (dog 17). B. Sample "non-theta avoidance" trial obtained with the "reinforce-non-theta" procedure (dog 43). Both samples were obtained after the avoidance criterion was met.

RL EMG, is a tracing of the electromyographic activity taken over the extensor carpi radialis and the extensor digitorum muscles of the right foreleg. LL EMG, is a similar tracing of electromyographic activity taken over the extensor carpi radialis and the extensor digitorum muscles from the left foreleg of the dog. Heart rate artifacts are present in the tracings of LL EMG in both A and B because the amplifiers were set at maximum gain. RLM and LLM are tracings of the gross perpendicular movement from the right and left forelegs respectively. L HIPP and R HIPP are tracings of the electrical activity from the left hippocampus and the right hippocampus respectively.

Pre-trial segments of the hippocampal tracings which were examined are shown in the segments labelled blank. The avoidance trial itself is identified by the solid line in the channel identified as, CS, in A and B.


A
RL EMG
30
LL EMG
30
RLM
CS
blank
L HIPP
30
LLM


B
LL EMG
30
L HIPP
75
CS
blank
R HIPP
30
LLM
2 sec

trials, and number of total shocks.[1]

Table 6 provides a summary of the course of acquisition of the two avoidance responses for all dogs under curare.

Table 6 about here

The percentage avoidances of each kind (theta and non-theta) occurring in successive quarters of the curare session is shown for both groups.

All of the dogs in Group-One were already "performing" at or nearly at the one hundred percent level for theta avoidances during the first quarter of curarization with the reinforce-theta procedure.

Similarly, four of the seven dogs in Group-Two were already "performing" at or nearly at the one hundred percent level for non-theta avoidances during the first quarter of curarization with the reinforce-non-theta procedure. Of the three Group-Two dogs at a low level of non-theta avoidances during the early stages of training one failed to meet the avoidance criterion.

Table 7 provides a summary of the transfer test data obtained 48 hours following recovery of the dogs from paralysis. The total number of pedal-presses made during the first extinction test-trial is shown for

Table 7 about here

---

[1] The raw data obtained during the curare session for each S is presented in Appendix H.

Table 6

Per cent avoidances (theta or non-theta) by quarters of training sessions under curare with "reinforce-theta" and "reinforce-non-theta" procedures for dogs in Group-One and Group-Two respectively

	dog no.	Per cent avoidances for quarters of curare session			
		1	2	3	4
Group-One (reinforce-theta)	17	100	86	100	100
	24	83	100	100	100
	29	100	89	100	100
	36	88	94	81	100
	39	78	78	72	100
Group-Two (reinforce-non-theta)	23	68	100	100	100
	26	100	88	100	100
	32	100	100	100	100
	33	100	42	92	100
	*38	46	88	46	87
	*40	10	00	00	14
	43	00	00	43	100

*

Both of these dogs had poorly aimed hippocampal placements but good theta was recorded from the medial thalamus. Activity in the medial thalamus was used as the response for these dogs.

Table 7

Transfer test in the normal state:

Number of presses on first extinction test-trial

	dog no.	test-trial presses
	17	2028
	24	110
Group-One	29	17
	36	63
	39	32
	23	49
	26	23
	32	198
Group-Two	33	16
	38	815
	40	0
	43	128

each subject identified by a dog number.

The number of pedal-presses occurring during the first extinction test-trial in the normal state provides an indication of the transfer effect from curare to normal state. This measure provides the best estimate of the possible relationship between operant reinforcement of the theta component of the avoidance response and the complete response itself.

The transfer results yielded a median number of pedal-presses for Group-One dogs of 63 presses while for Group-Two dogs (reinforce-non-theta during curare training) it was 49 presses. This difference though in the expected direction was not found to be statistically significant (Mann-Whitney U = 11.0; p = .421).

Photographic enlarged reproductions of brain sections from nine of the twelve dogs are presented in Appendix C (because these dogs were also used in experiment one). Similar reproductions for the remaining three dogs (38, 40, and 43) are presented in Appendix I along with a photographic reproduction of the medial thalamic placement in dog 39.

Voltage measurements were taken of the theta waves having maximum amplitude during the first trial of post-operative reacquisition of the pedal-press avoidance response and during the first trial under curare prior to the introduction of reinforcing contingencies for each dog. A comparison of the theta amplitude in the normal state with theta amplitude in the curarized state was then performed. No significant change in the amplitude characteristics of theta in the two states was found, suggesting that paralysis had a negligeable effect on this factor. In addition, there was no significant relationship between trials to first criterion avoidance during curare training and maximum amplitude of theta (Spearman rank correlation coefficient, $r_s = .20$, for $N = 12$).

DISCUSSION

The attempt was made in the present experiment to reinforce operantly "attempts to pedal-press" as indicated by occurrences of theta in the hippocampus (medial thalamus in two dogs) under conditions where no movements or EMG activity was visibly present. The attempt was also made to determine whether such reinforcement of "attempted movement" had any effect on the complete avoidance pedal-press response. The overall objective was to determine experimentally whether the occurrence of the overt response and its associated gamma motor feedback were necessary for an operant reinforcer to have an effect on the response.

A bi-directional control design (Black, 1967; Miller and DiCara, 1967) was used under curare to reinforce hippocampal activity (medial thalamic activity in two dogs) and a post-paralysis transfer test was used to assess the effects of reinforcing such activity.

The results obtained during curarization suggested that changes in the probability of theta and non-theta occurrences occurred while the animals were deeply paralyzed. However, a number of difficulties were encountered which suggest that the curare results should be viewed with caution. Group-One dogs were almost "already there" in terms of the probability of theta during the early stages of the "reinforce-theta" procedure (Table 6). This could be explained in terms of the high probability of transfer of theta from the normal to the curarized state. Since the CS used in both states was the same as the one originally used for pedal-press pre-training then the avoidance correlate (theta) should transfer to the curare state and appear with the presentations of the CS.

A similar transfer of theta from the normal to the paralyzed state did not occur for four of the seven dogs in Group-Two when they were introduced to the curare training procedure. Therefore application of the "reinforce-non-theta" procedure was confounded by the fact that four of the seven dogs were "already there" in terms of the high probability of non-theta during the early stages of the curare session.

Of the three Group-Two dogs which did not have a "pre-operant" bias in probability of non-theta during the first quarter of curare training two did show an increase in probability of non-theta from the start to the end of training (Table 6) and both achieved the non-theta avoidance criterion, indicating that a decrease in the probability of occurrence of theta did occur during curare training. These results taken with those of the previous experiment (chapter five) suggest that theta can be operantly conditioned.

Data presented in Table 7 indicated that there was no significant transfer from paralysis to the overt avoidance response. However, it is not clear that the results to the normal state were due to the effects of the training procedures under curare. A number of difficulties were encountered. There was a "pre-operant bias" in most of the dogs at the outset of curare training. Furthermore, experimenter-produced sources of uncontrolled bias may have been introduced because of the unavailability of fully automated procedures.

These difficulties mean that any number of alternative explanations could almost equally well account for the results obtained in the present experiment. For example, it might be argued on the basis of the failure to obtain transfer that theta is not a correlate, or at least a necessary correlate, of overt skeletal responding. On the other hand it might be argued that the avoidance response was an inappropriate measure of overt skeletal responding because the assumption that theta under curare represented an attempt to make this pedal-press response was wrong. A measure of general activity might have been more appropriate. Alternatively, it could be argued that the pre-training with the avoidance pedal-press response led to the occurrence of a conditioned response in the normal state which was so pre-potent that it washed out the effects of training under curare. This argument suggests that naive animals should be employed. While alternative explanations such as these can be accepted, the findings are sufficiently suggestive to merit exploration of this problem in further research.

CHAPTER SEVEN

CONCLUSION

The specific concern of this thesis has been with three questions related to a single aspect of how an operant reinforcer has its effect on a response. First, is hippocampal theta activity a correlate of the overt skeletal avoidance response? Secondly, can this central correlate of the response be operantly reinforced in the same way as the overt response when the overt response is blocked? Third, will such reinforcement of a central correlate have an effect on the overt response (or more accurately, the complete response) when the response control system is functioning in a normal manner?

The first two experiments described in this thesis therefore sought to obtain evidence bearing on the first of these questions. In the first experiment hippocampal theta was found to be a correlate of avoidance pedal-pressing in dogs. It was found in addition that spontaneous occurrences of the pedal-press response during inter-trial intervals were even more closely associated with hippocampal theta than avoidance trial pedal-presses. The speculation was advanced that hippocampal theta may be closely linked to the total amount of gross skeletal activity involved in the response. Pedal-presses which could be characterized as stereotyped and discrete whether occurring during actual avoidance trials or during inter-trial periods may be less frequently associated with hippocampal theta activity. A second experiment was then conducted with the aim of providing further supporting evidence for the findings obtained in experiment one and to rule out some of the currently available alternative explanations concerning the function of hippocampal theta. In experiment two, hippocampal theta was found to accompany an avoidance response which consisted of multiple presses of the pedal at a rate of one press or more per second. Hippocampal theta was largely absent when the avoidance response consisted of "refraining-from-pressing" (passive avoidance) for a number of seconds.

The results were found to be inconsistent with Grastyan's 1959 notion (Grastyan *et al.*, 1959) that hippocampal theta is a concomitant of the orienting reflex. Orienting, that is, diffuse, nonspecific searching movements, disappeared very rapidly in our dogs, yet hippocampal theta was found to persist throughout four to five days of training including up to 250 trials.

The results were also found to be inconsistent with an arousal hypothesis (Green and Arduini, 1954; Bremner, 1964) because the contingencies of training were such that the animals could be expected to pay particular attention to both of the CSs since punishment for incorrect responding occurred in both stimuli.

The results were also considered to be at variance with the notion that hippocampal theta is functionally involved with the establishment of a "connection" between a skeletal response and a conditional stimulus (Elazar and Adey, 1967a). The dogs in experiment two of this thesis all met an avoidance criterion for both an active and a passive avoidance response. However, hippocampal theta appeared only or mainly during the stimulus requiring active avoidance responding, suggesting that the instrumental significance of the two CSs did not determine the presence-absence of hippocampal theta.

Finally, the training procedures called for shock punishment in both CSs thereby approximately equating the motivational component associated with each stimulus. If hippocampal theta is an electrical manifestation of motivated behavior, as Pickenhain and Klingberg (1967) maintain, then hippocampal theta should have appeared in both stimuli. The results were inconsistent with this view, appearing mainly during the CS requiring an active avoidance response, and relatively infrequently during the CS requiring a passive avoidance response.

The conclusion was advanced that the results are consistent with Vanderwolf's (1967; 1968) notion that hippocampal theta may be a manifestation of a mechanism for the initiation of phasic, active, voluntary movement as opposed to tonic muscular activity in which a position is held. The question does remain open however, as to what particular feature or features of movement are related to hippocampal theta. Is hippocampal theta simply related to a change of state from immobility to movement or from movement to immobility? Is hippocampal theta related directly to the amount of skeletal activity, as was suggested in chapter three of this thesis? These and related questions appear to merit further experimental study.

In summary, the results of both experiment one and experiment two permitted the identification and specification of a central nervous system event which could be considered a correlate of skeletal responding.

The next question which was considered was whether this central correlate of movement could be operantly reinforced when the overt response was blocked. In experiment three, an operant discrimination procedure was carried out in naive deeply paralyzed dogs. The hippocampal theta discrimination was established rapidly and could be maintained with relative ease during a single paralytic training session. The conditions of the experiment were such that the discrimination could not be explained away as a "sensitization" effect due to shock, or as a differential in "pre-operant" level of theta to the two stimuli, nor as an "unconditioned reaction" of the brain structures involved to novel auditory stimuli. In this experiment, no attempt was made to determine which feature or features of the conditioning procedure (i.e., classical or operant) may have been responsible for the establishment of the hippocampal theta discrimination. The data of experiment four, however, in which decreases in the probability of theta were produced by operant reinforcement, suggest that the operant reinforcement procedure is effective in controlling theta.

The results of experiment three indicated that feedback from the skeletal system is not necessary for the occurrence of hippocampal theta. The suggestion was made, on the basis of Vanderwolf's hypothesis (1967; 1968), that the occurrence of hippocampal theta in the discriminative stimulus under deep paralysis could be taken as an indicator of the animals's "attempt to move" even though the overt behavior was not possible. Conceivably, reinforcement of attempts to respond can occur without the participation of the peripheral motor system.

Experiment four was conducted in order to answer the third and final question, concerning transfer from the curarized to the normal state. What transfer effects occur from operantly reinforced theta under curare to overt responding in the normal state? During the curare phase, a bi-directional control design (Black, 1967) was used in an attempt to train increases or decreases in the occurrences of hippocampal theta. The aim was to demonstrate that the operant contingencies present rather than other features of the conditioning situation could be considered responsible for the changes in probability of hippocampal theta. In post-paralysis extinction tests, an attempt was made to determine whether reinforcement of hippocampal electrical activity had any effect on the previously learned avoidance response.

The results of the curare procedures and transfer testing in experiment four were suggestive rather than conclusive. Tentatively, the first two of the three questions raised in the opening paragraph of this thesis could be answered affirmatively. First, it does appear that hippocampal theta is a correlate of overt skeletal activity. Second, hippocampal theta, a central component of the avoidance response can be operantly reinforced. The third question remains unanswered because of the ambiguity of interpretation of the curare results of experiment four.

Should transfer of the type studied in experiment four be demonstrated in future experiments, then a number of implications could be considered. The results would have direct bearing on much of the "learning under paralysis" research reported in the literature. For example, Black (1958) was able to show more rapid extinction of an avoidance response following curarized presentations of the training

CS than without such presentations. Conceivably, the avoidance response was modified by the "extinction" of the hippocampal correlate of the response in the presence of the CS alone under paralysis. Solomon and Turner (1962) argued that the responding which occurred following recovery from paralysis in their experiment was due to the pavlovian features present under curare. It is conceivable, however, that punishment of hippocampal theta occurred in their subjects and that the effects of this punishment of the response correlate transferred to the normal state subsequently. Their choice of the curare technique as a control procedure for operant conditioning effects could thus be questioned. Similarly, Black in his most recent EMG study (1967) was able to show operant reinforcement of small electromyographic responses under conditions of light curarization. Conceivably, the conditioning of these small EMG responses was mediated by the reinforcement of hippocampal theta activity. The occurrence of hippocampal theta could be considered a manifestation of an "attempt to move" and this attempt was reflected as EMG activity in the appropriate limb.

REFERENCES

Adey, W. R. Studies of hippocampal electrical activity during approach learning. In J. F. Delafresnaye (Ed.), *Brain Mechanisms and Learning: A Symposium*. Toronto: Ryerson, 1961, Pp. 577-588.

Adey, W. R., Dunlop, C. W., & Hendrix, C. E. Hippocampal slow waves; distribution and phase relations in the course of approach learning. *A.M.A. Arch. Neurol.*, 1960, *3*, 74-90.

Black, A. H. The extinction of avoidance responses under curare. *J. comp. Physiol. Psychol.*, 1958, *51*, 519-524.

Black, A. H. Transfer following operant conditioning in the curarized dog. *Sci.*, 1967, *155*, 201-203.

Black, A. H., & Dalton, A. J. The relationship between the avoidance response and subsequent changes in heart rate. *Acta Biol. Exp.*, 1965, *25*, 107-119.

Bremner, F. J. Hippocampal activity during avoidance behavior in the rat. *J. comp. Physiol. Psychol.*, 1964, *58*, 16-22.

Buchwald, J. S., Standish, M., Eldred, E., & Halas, E. S. Contribution of muscle spindle circuits to learning as suggested by training under flaxedil. *Electroenceph. clin. Neurophysiol.*, 1964, *16*, 582-594.

DiCara, L. V., & Miller, N. E. Changes in heart rate instrumentally learned by curarized rats as avoidance responses. *J. comp. Physiol. Psychol.*, 1967, in press.

Elazar, Z., & Adey, W. R. Electroencephalographic correlates of learning in subcortical and cortical structures. *Electroenceph. clin. Neurophysiol.*, 1967a, *23*, 306-319.

Elazar, Z., & Adey, W. R. Spectral analysis of low frequency components in the electrical activity of the hippocampus during learning. *Electroenceph. clin. Neurophysiol.*, 1967b, *23*, 225-240.

Fujita, Y., & Sato, T. Intracellular records from hippocampal pyramidal cells in rabbit during theta rhythm activity. *J. Neurophysiol.*, 1964, *27*, 1011-1025.

Gorska, T., & Jankowska, E. The effects of deafferentation on instrumental (Type II) conditioned reflexes in cats. *Acta Biol. Exp.*, 1961, *21*, 219-233.

Grastyan, E., Lissak, K., Madarasz, I., & Donhoffer, H. Hippocampal electrical activity during the development of conditioned reflexes. Electroenceph. clin. Neurophysiol., 1959, 11, 409-430.

Green, J. D. The hippocampus. Physiol. Rev., 1964, 44, 561-608.

Green, J. D., & Arduini, A. A. Hippocampal electrical activity in arousal. J. Neurophysiol., 1954, 17, 533-557.

Green, J. D., & Machne, X. Unit activity of rabbit hippocampus. Amer. J. Physiol., 1955, 181, 219-224.

Green, J. D., Maxwell, D. S., Schindler, W., & Stumpf, C. Rabbit EEG "theta" rhythm: its anatomical source and relation to activity in single neurons. J. Neurophysiol., 1960, 23, 403-420.

Harper, R. M. Behavioral and Electrophysiological Studies of Sleep and Animal Hypnosis. Unpublished doctoral dissertation. Psychology Department, McMaster University, 1968.

Jankowska, E. Instrumental scratch reflex of the deafferented limb in cats and rats. Acta Biol. Exp., 1959, 19, 233-247.

John, E. R. Higher nervous functions: brain functions and learning. Ann. Rev. Physiol., 1961, 23, 451-484.

Karmos, G., Grastyan, E., Losonczy, H., Vereczkey, L., & Grosz, J. The possible role of the hippocampus in the organization of the orientation reaction. Acta Physiol. Acad. Sci. Hung., 1965, 26, 131-141.

Konorski, J. Conditioned Reflexes and Neuron Organization. New York: Cambridge University Press, 1948.

Lissak, K., & Grastyan, E. The changes of hippocampal electrical activity during conditioning. In H. H. Jasper and G. D. Smirnov (Eds.), Moscow Colloquium on Electroencephalography of Higher Nervous Activity. Suppl. 13. Electroenceph. clin. Neurophysiol. Amsterdam: Elsevier, 1960, Pp. 271-279.

Matthews, P. B. C. Muscle spindles. Physiol. Rev., 1964, 44, 219-288.

Morrell, F. Electrophysiological contributions to the neural basis of learning. Physiol. Rev., 1961, 41, 443-494.

Olds, J. The limbic system and behavioral reinforcement. In W. R. Adey and T. Tokizane (Eds.), Progress in Brain Research. Vol. 27. Structure and Function of the Limbic System. Amsterdam: Elsevier, 1967.

Pickenhain, L., & Klingberg, F. Hippocampal slow wave activity as a correlate of basic behavioral mechanisms in the rat. In W. R. Adey and T. Tokizane (Eds.), Progress in Brain Research. Vol. 27. Structure and Function of the Limbic System. Amsterdam: Elsevier, 1967.

Radulovacki, M., & Adey, W. R. The hippocampus and the orienting reflex. Exp. Neurol., 1965, 12, 68-83.

Siegel, S. Nonparametric Statistics for the Behavioral Sciences. New York: McGraw-Hill, 1956.

Solomon, R. L., & Turner, L. H. Discriminative classical conditioning in dogs paralyzed by curare can later control discriminative avoidance responses in the normal state. Psychol. Rev., 1962, 69, 202-219.

Taub, E., Bacon, R. C., & Berman, A. J. Acquisition of a trace-conditioned avoidance response after deafferentation of the responding limb. J. comp. Physiol. Psychol., 1965, 59, 275-279.

Taub, E., & Berman, A. J. Avoidance conditioning in the absence of relevant proprioceptive and exteroceptive feedback. J. comp. Physiol. Psychol., 1963, 56, 1012-1016.

Vanderwolf, C. H. Behavioral correlates of "theta" waves. Paper presented at Canadian Federation of Biological Societies meeting, Montreal, Canada, 1967.

Vanderwolf, C. H. Hippocampal electrical activity and voluntary movement in the rat. Technical Report No. 17. Psychology Department, McMaster University, 1968.

Vanderwolf, C. H., & Heron, W. Electroencephalographic waves with voluntary movement: Study in the rat. A.M.A. Arch. Neurol., 1964, 11, 379-384.

Votaw, C. L. Certain functional and anatomical relations of the Cornu Ammonis of the macaque monkey. J. comp. Neurol., 1959, 112, 353-382.

Woodburne, L. S. Partial analysis of the neural elements in posture and locomotion. Psychol. Bull., 1967, 68, 121-131.

Yoshii, N., Shimokochi, M., Miyamoto, K., & Ito, M. Studies on the neural basis of behavior by continuous frequency analysis of EEG. In T. Tokizane and J. P. Schade (Eds.), Progress in Brain Research. Vol. 21A. Correlative Neurosciences: Fundamental Mechanisms. Amsterdam: Elsevier, 1966, Pp. 217-250.

APPENDIX A

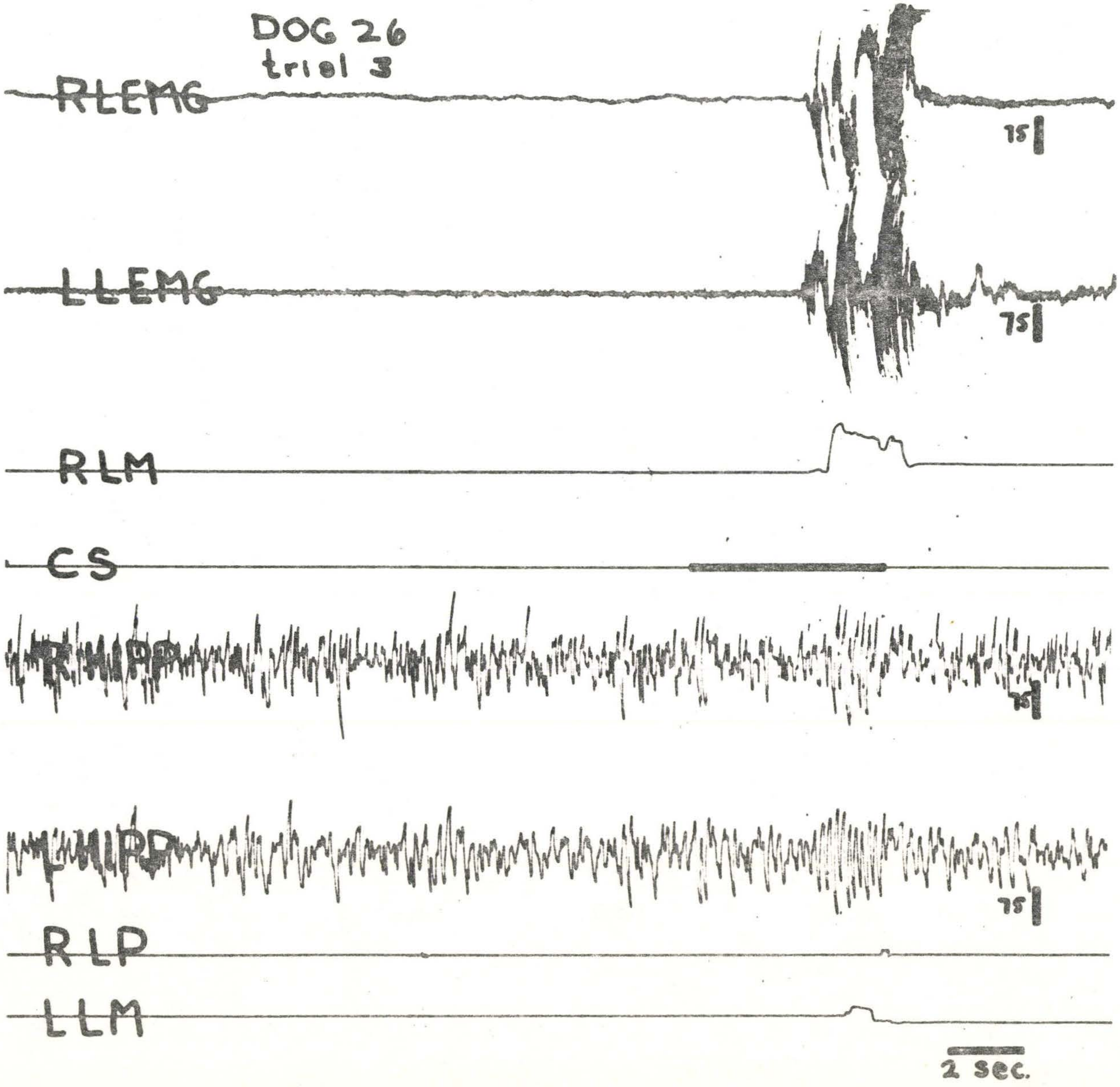
Unusable trials in the analysis of Experiment One

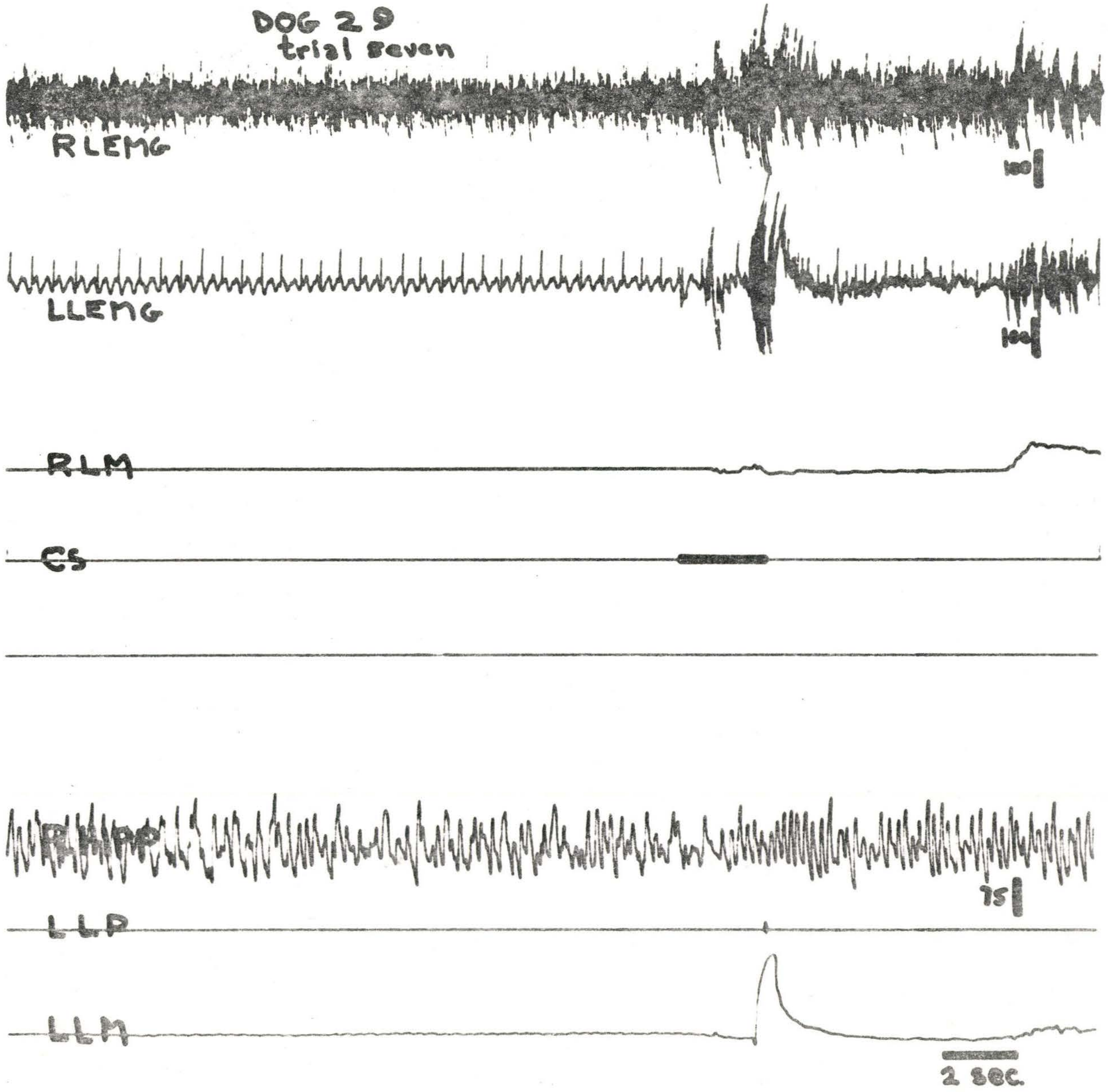
Details of unusable trials in the analysis of hippocampal theta and avoidance behavior in experiment one

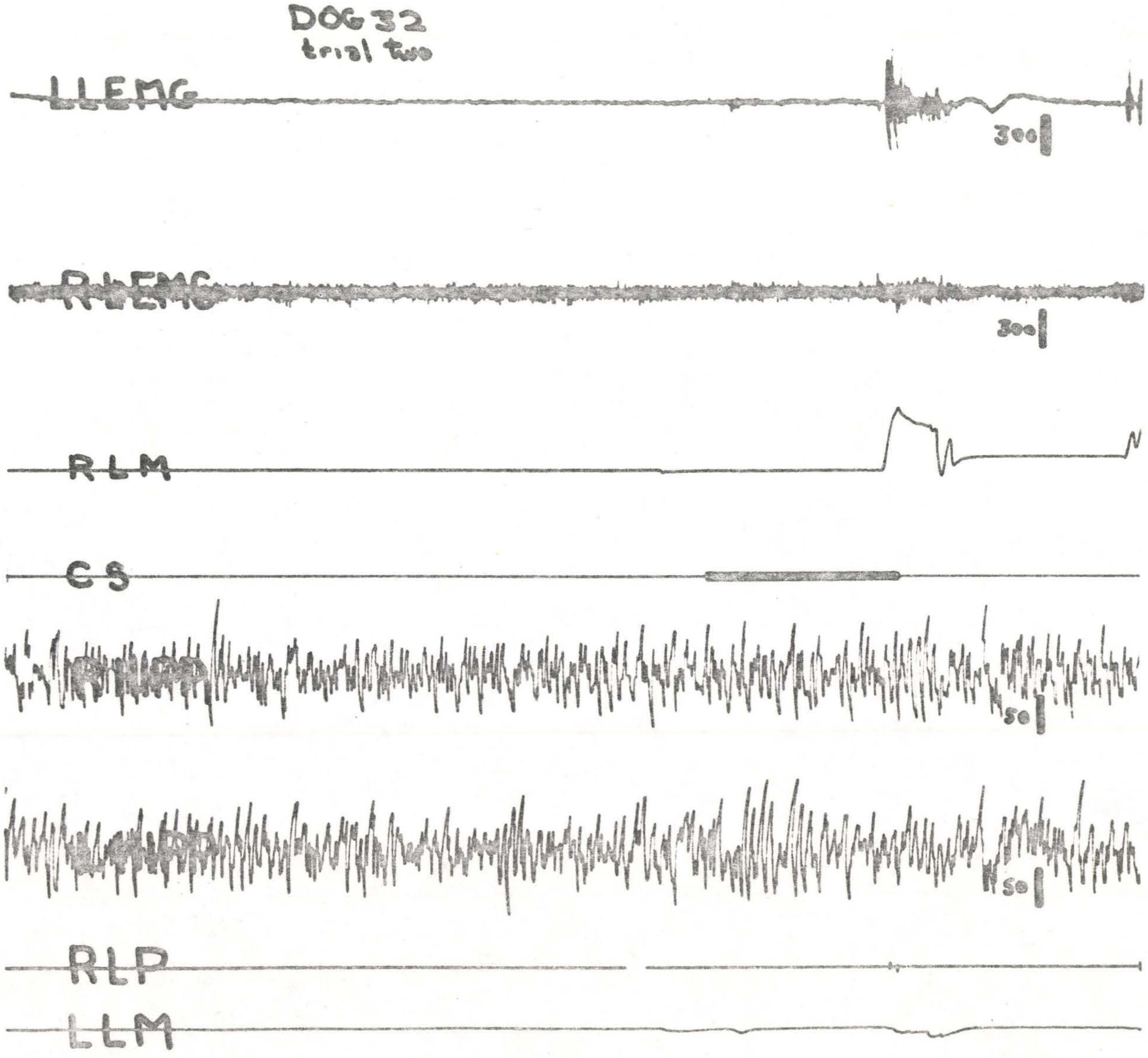
Dog No.	Total trials	escape trials	trials with movement artifact	trials with CS duration less than .4"	Total unusable trials
6	92	0	0	0	0
15	40	1	0	0	0
16	22	1	0	0	0
17	50	0	1	2	3
23	50	1	0	4	4
24	50	0	0	0	0
25	50	0	0	0	0
26	50	0	0	0	0
29	50	0	1	1	2
32	50	1	0	3	3
33	50	0	0	3	3
36	50	1	7	0	7
39	50	0	0	3	3
42	50	0	2	1	3

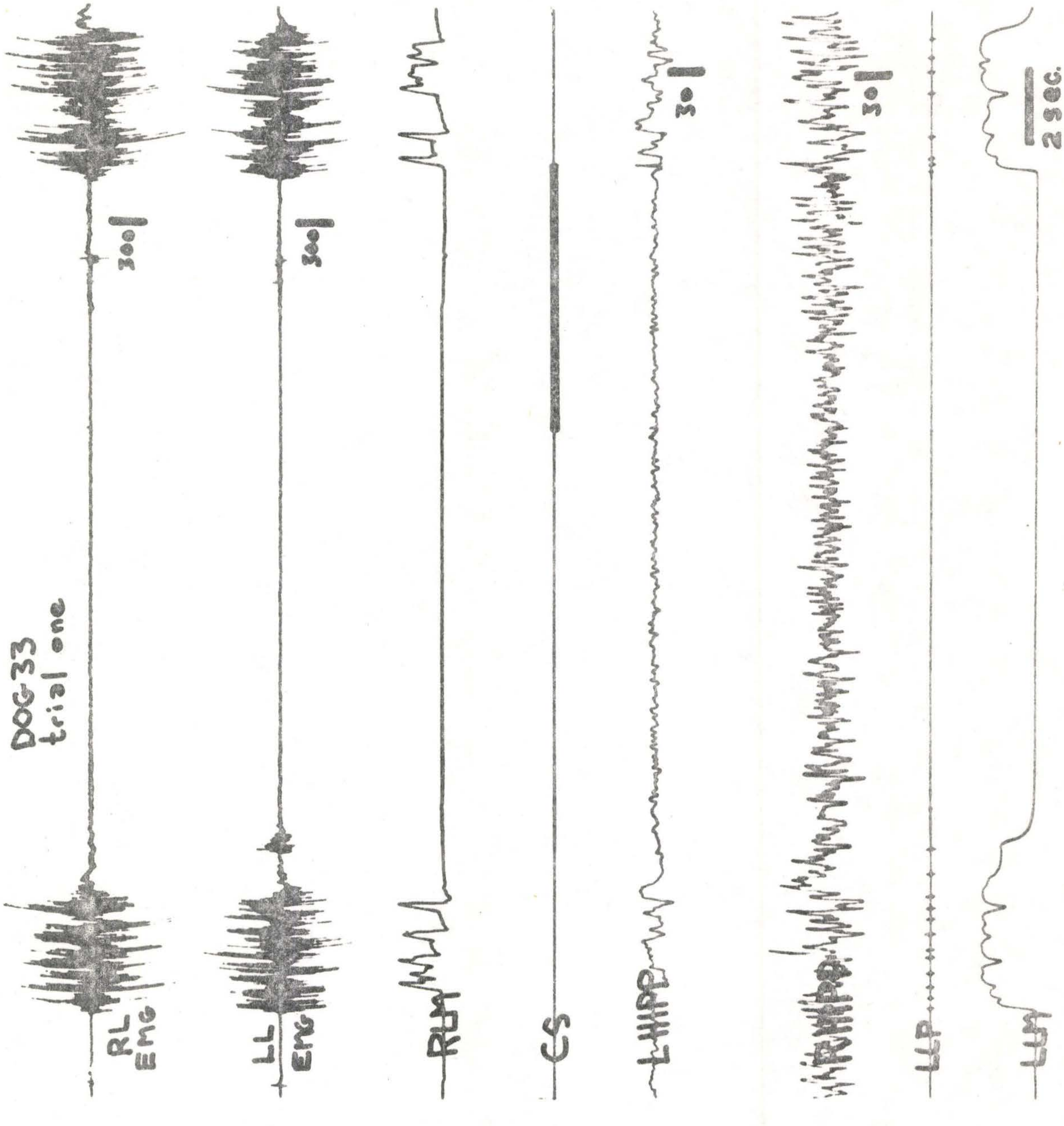
APPENDIX B

Sample records of post-operative avoidance trials in different dogs from Experiment One


DOG 26
trial 3
RLEMG
LLEMG
RLM
CS
RLP
LLM
75
75
75
75
2 sec.


DOG 29
trial seven
RLEMG
100
LLEMG
100
RLM
CS
RHPP
75
LLP
LLM
2 sec.

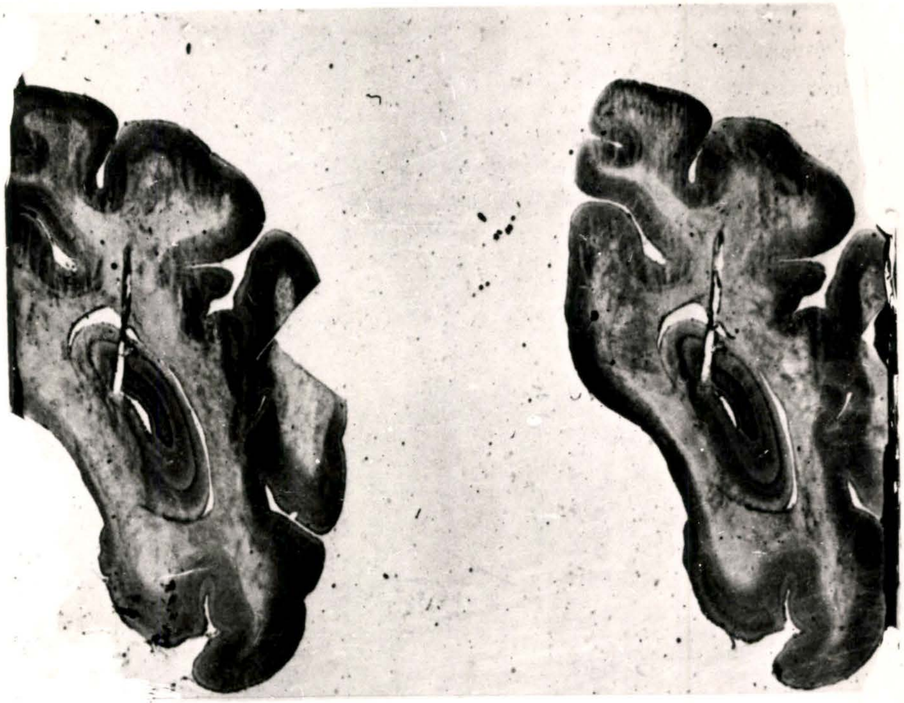

DOG 32
trial two
LLEMG
300
RLEMG
300
RLM
CS
50
50
RLP
LLM


DOG-33
trial one
RL EMG
LL EMG
RLM
CS
LHIPP
RHIPP
LLP
LLM
30
30
30
30
2 sec.

APPENDIX C

Photographic enlarged reproductions of brain sections from dogs in Experiment One

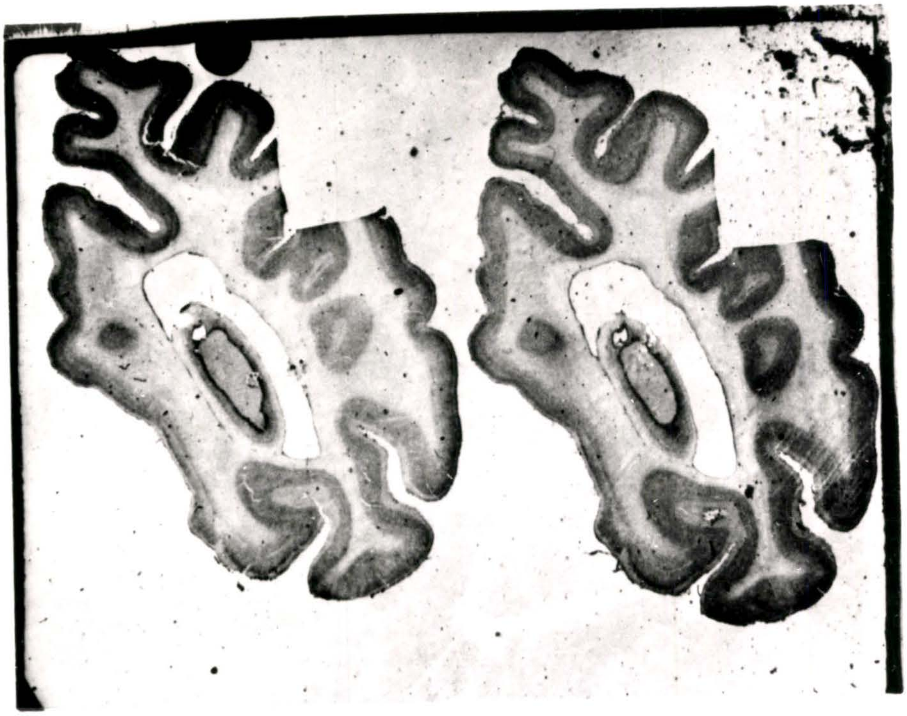
DOG 17



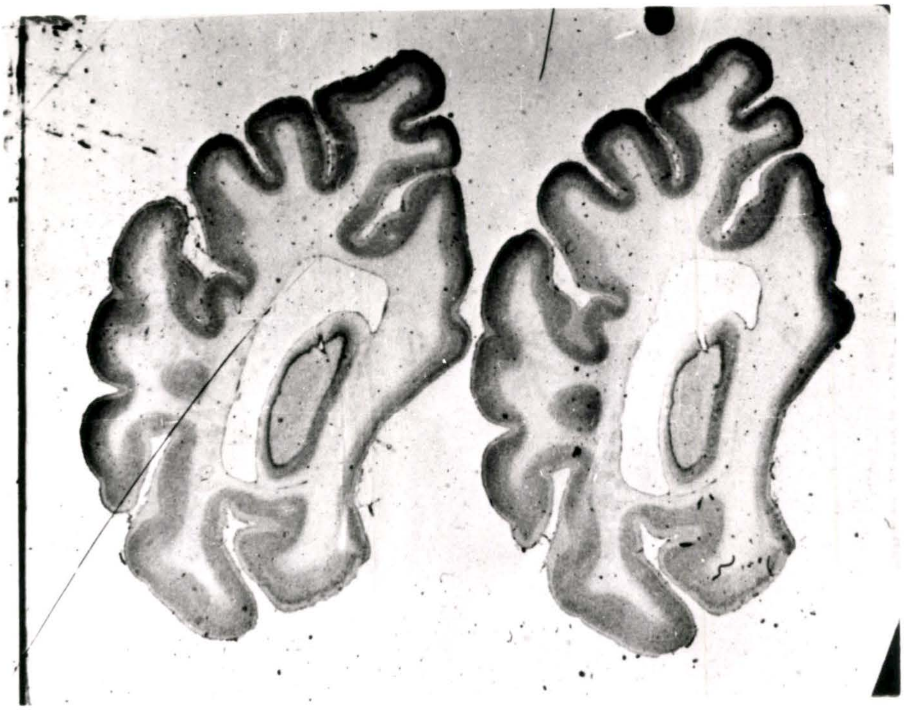
Left Hippocampus

Fimbria

DOG 23



Left Hippocampus



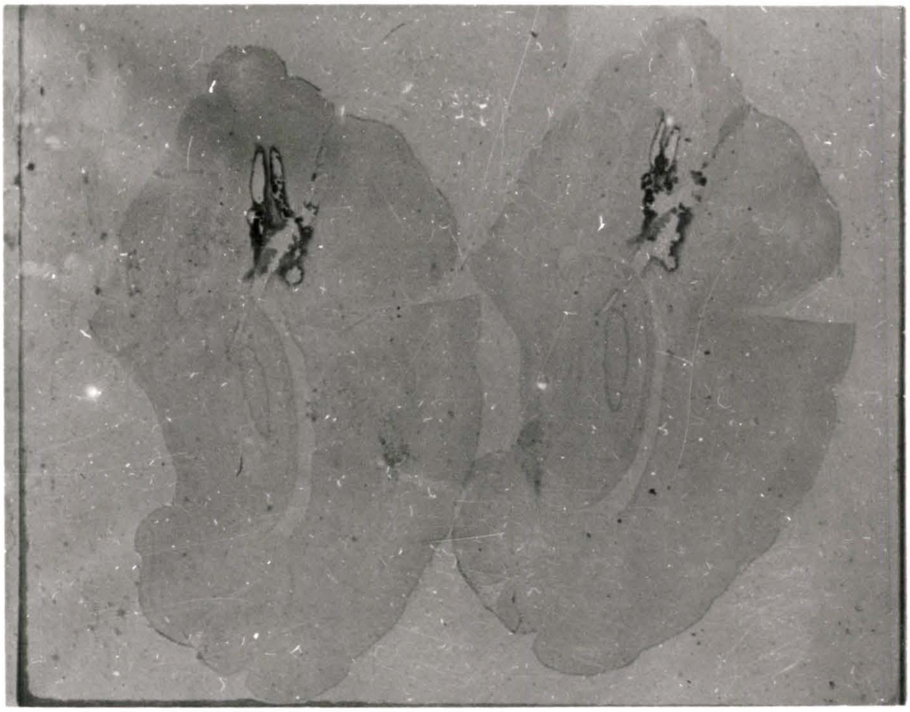
Right Hippocampus

DOG 24

Left Hippocampus

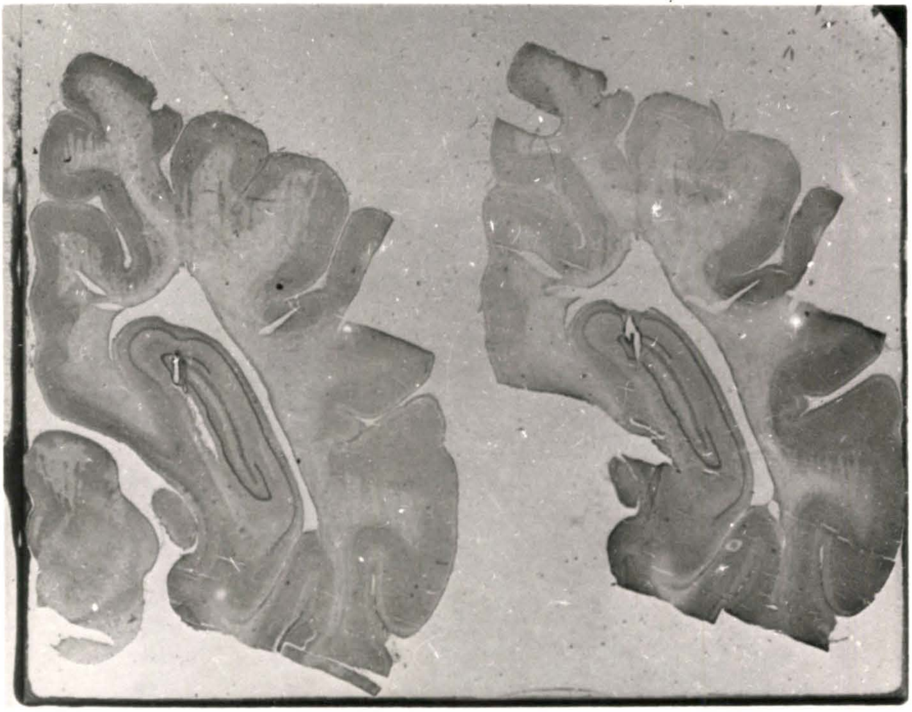
Right Hippocampus

DOG 25

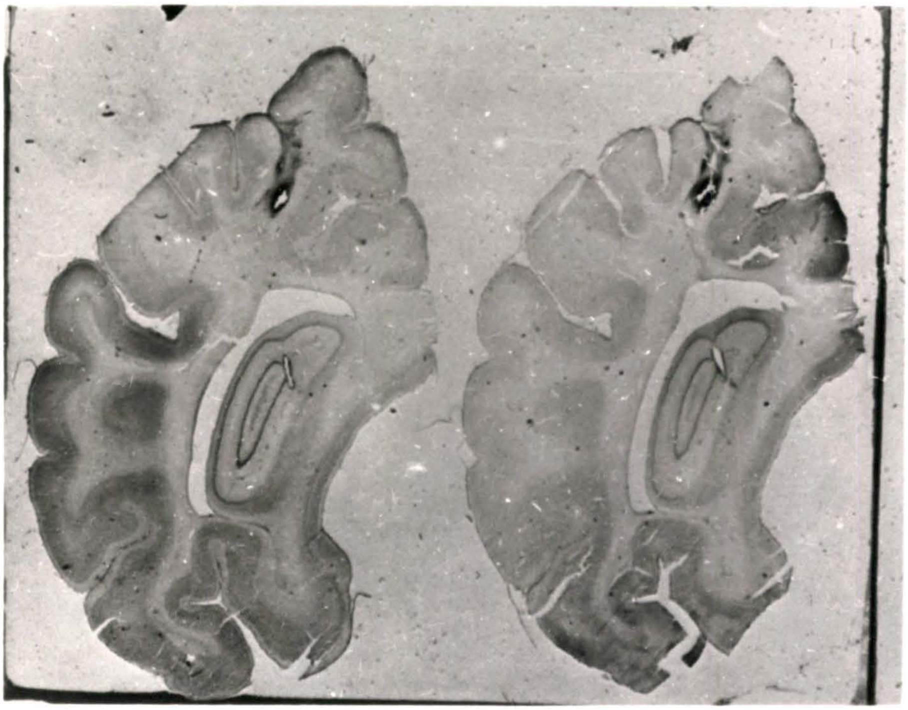


Left Hippocampus

DOG 26

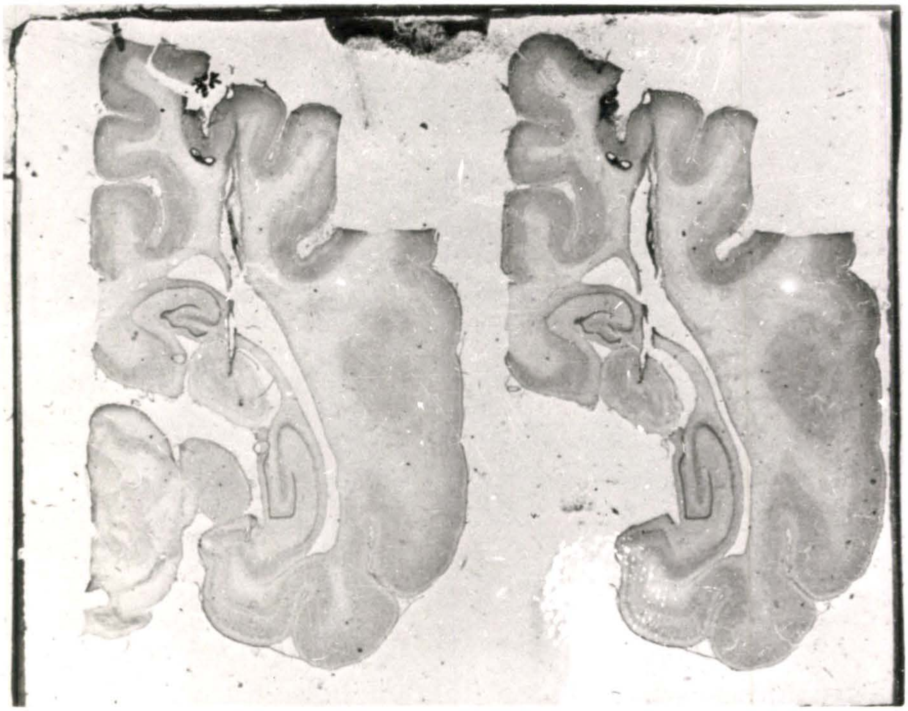


Left Hippocampus

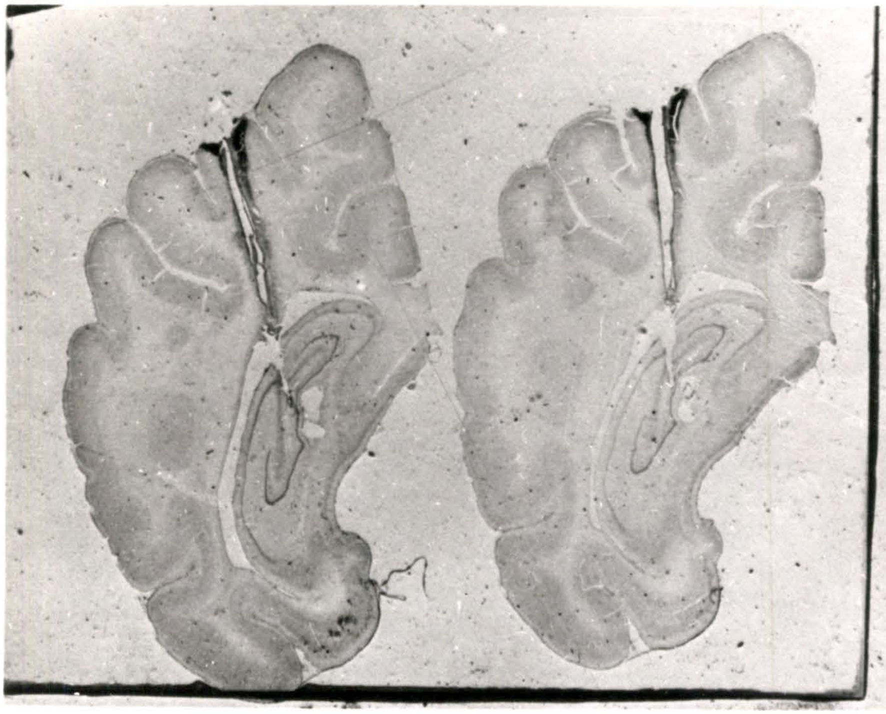


Right Hippocampus

DOG 29



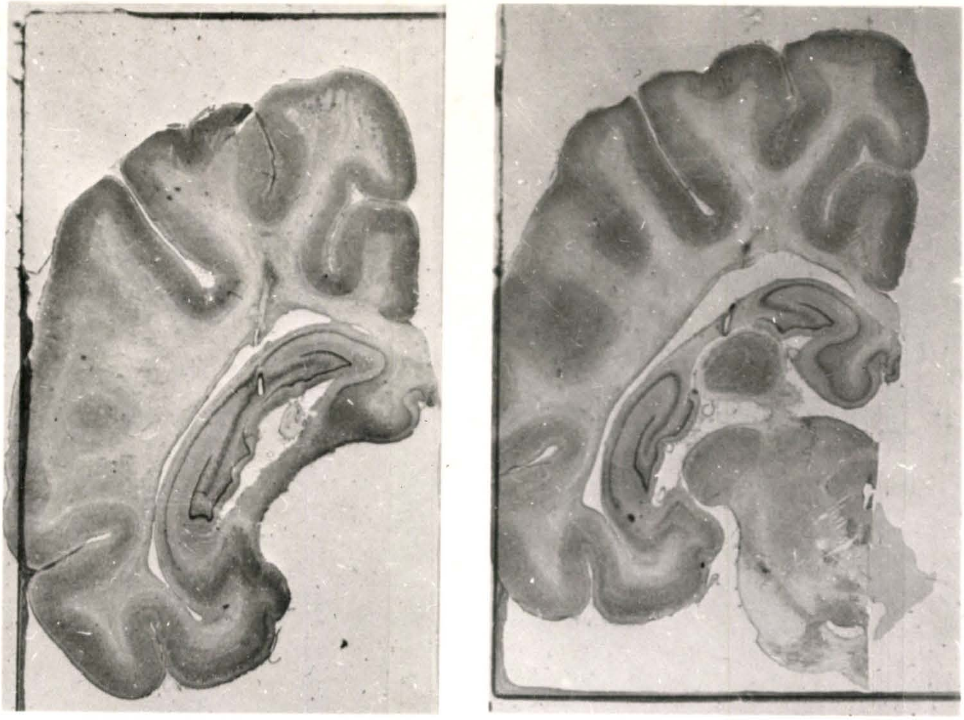
Lateral geniculate nucleus



Right Hippocampus

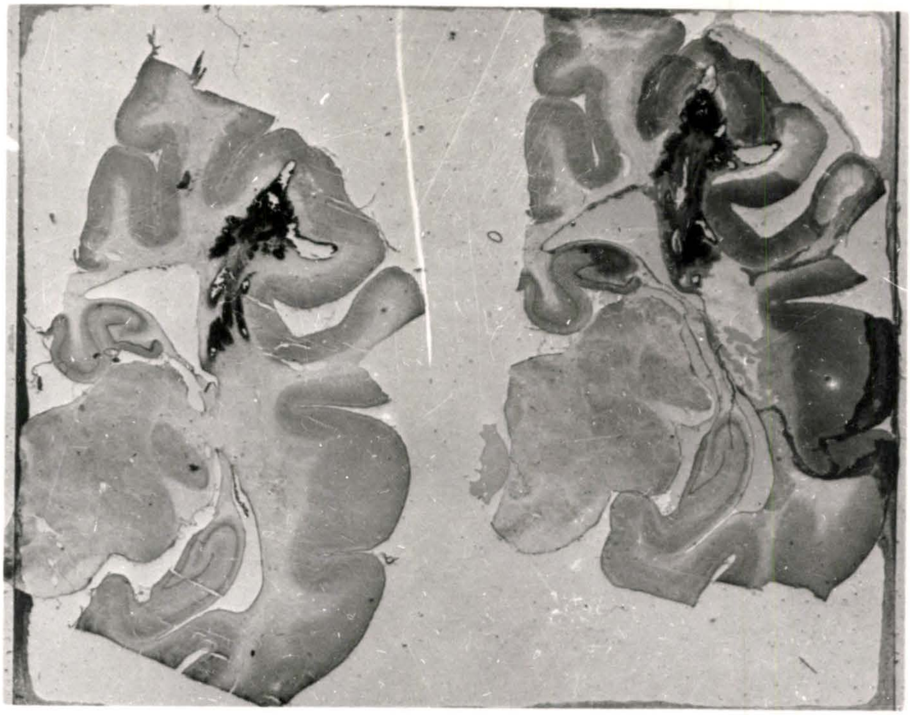
DOG 32

Left Hippocampus



Right Hippocampus

DOG 33



fimbria (infected)

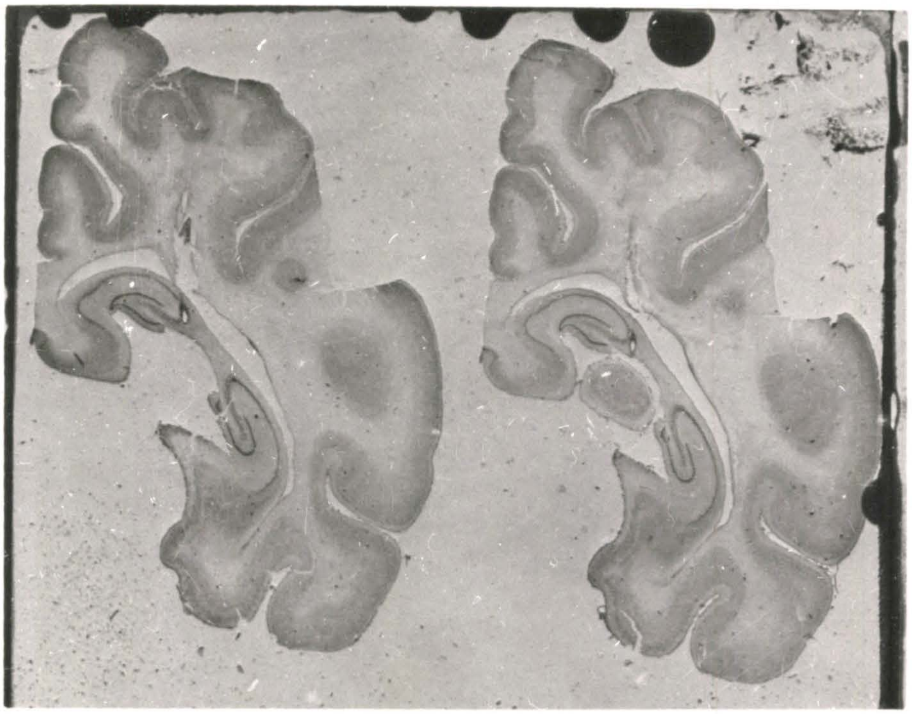
Right Hippocampus

DOG 36

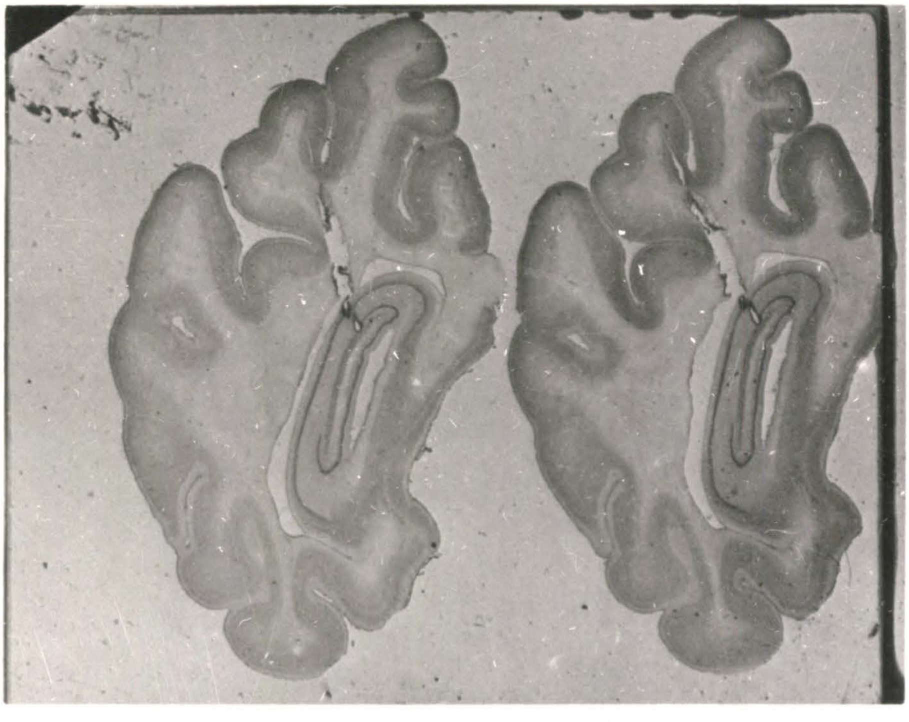
Left Hippocampus

Right Hippocampus

DOG 39

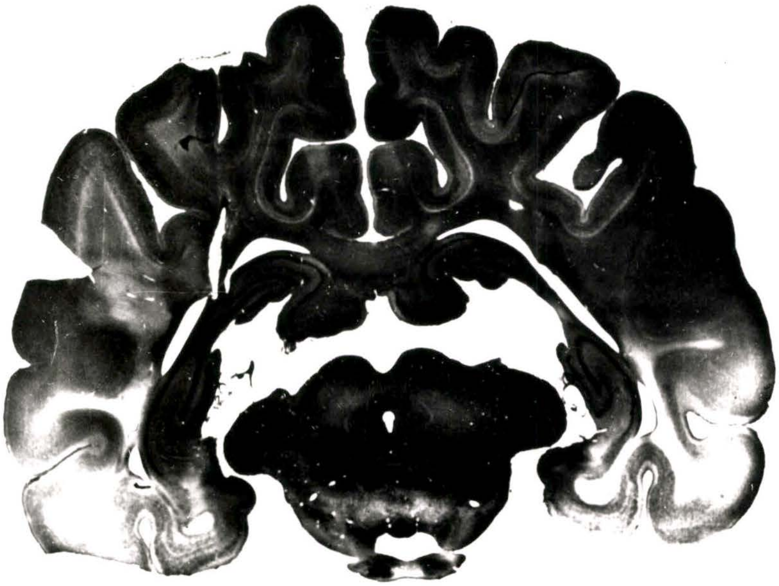


Left Hippocampus



Right Hippocampus

DOG 42



Left Hippocampus

Right Hippocampus

APPENDIX D

Photographic enlarged reproductions of brain sections from dogs in Experiment Two

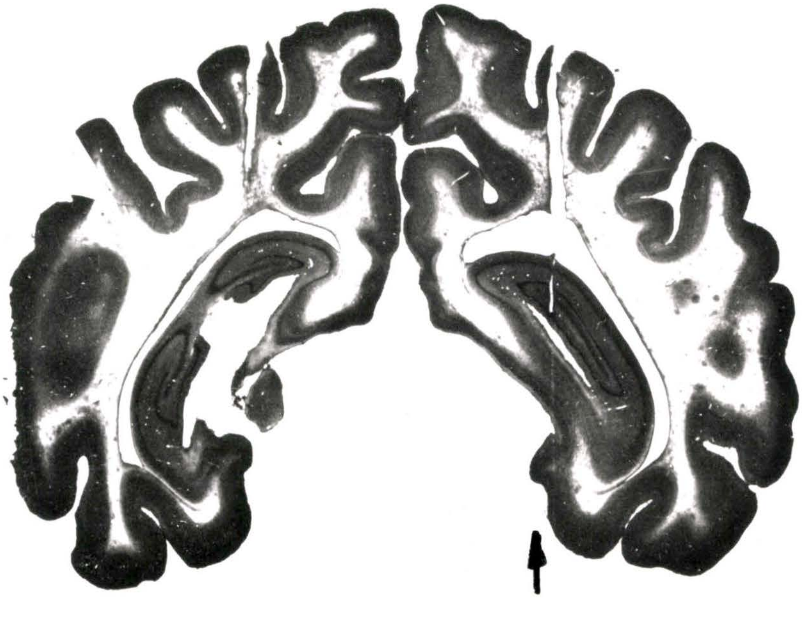
DOG 66

Left Hippocampus

Right Hippocampus

DOG 67

Right Hippocampus



Left Hippocampus (reversed)

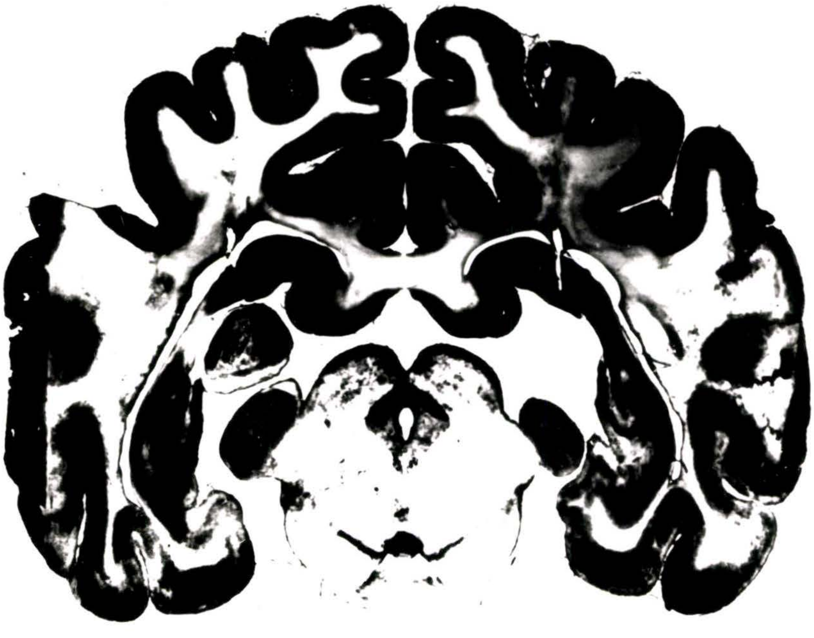
DOG 68

Left Hippocampus

Right Hippocampus

DOG 69

Left Hippocampus



Right Hippocampus

APPENDIX E

Raw data during the adaptation phase of Experiment Three

EXPERIMENT THREE

Adaptation results. Average number of hippocampal theta responses per three-trial block for S+ and S- trials separately in seconds

Block no.	59		60		61		62		63		64	
	S+	S-	S+	S-	S+	S-	S+	S-	S+	S-	S+	S-
1	2.0	0.7	1.3	3.0	0.7	1.3	1.7	7.0	4.3	4.0	1.0	4.0
2	1.7	1.3	0.0	0.0	0.7	1.3	0.3	1.0	1.0	1.0	0.3	0.0
3	0.0	1.3	0.0	0.3	0.7	0.0	0.7	0.7	0.0	0.0	0.0	1.0
4	0.3	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.7	0.3	1.0
5	0.0	0.0	1.7	0.3	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	1.3	0.0	0.0	0.0			0.0	0.0	0.0	0.0
7			0.0	0.0	0.0	0.0						
8			0.0	0.0								

APPENDIX F

Raw data during the discrimination phase of Experiment Three

EXPERIMENT THREE

Discrimination data. Average number of hippocampal theta responses per three-trial block for S+ and S- trials separately in seconds

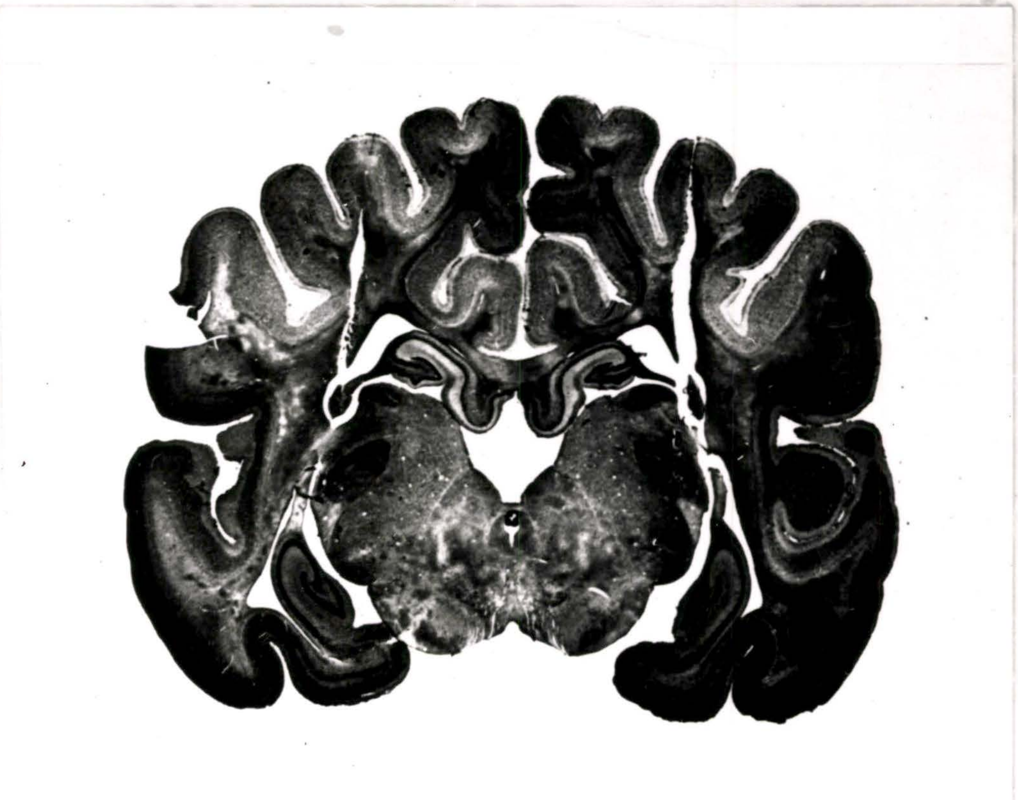
Block No.	59		60		61		62		63		64	
	S+	S-	S+	S-	S+	S-	S+	S-	S+	S-	S+	S-
1	3.7	4.7	1.7	1.0	1.0	2.7	2.3	0.3	1.3	0.3	2.3	3.3
2	7.0	4.3	6.0	1.7	6.7	2.3	5.3	0.7	6.3	0.0	6.3	4.3
3	6.7	7.0	7.0	1.3	5.0	0.3	6.7	0.7	6.0	0.7	6.7	4.3
4	7.0	7.0	5.3	0.7	6.7	0.3	6.3	0.0	7.0	1.7	7.0	3.3
5	6.0	6.7	3.7	1.3	6.3	0.7	7.0	1.0	7.0	2.7	6.7	4.0
6	7.0	7.0	3.7	0.3	7.0	0.3	5.3	1.0	7.0	1.3	7.0	4.7
7	7.0	5.0	4.7	1.0	7.0	0.3	6.3	0.0	7.0	0.0	7.0	4.3
8	7.0	2.0	6.0	1.0	6.7	1.0	6.3	0.3	7.0	0.3	7.0	3.0
9	7.0	1.7	4.0	0.7	6.3	0.7	6.7	0.0	7.0	0.0	7.0	3.7
10	7.0	2.3	4.7	1.0	6.0	0.3	6.3	1.0	7.0	0.0	7.0	1.7
11	7.0	3.3	5.7	1.0	7.0	0.3	6.3	1.7			7.0	0.3
12	7.0	2.3	2.3	0.7	7.0	0.7	4.7	1.0			7.0	3.3
13	7.0	3.7	6.0	2.0	7.0	0.3	6.3	0.0			7.0	1.7
14	7.0	5.0	3.7	2.7	7.0	1.0	5.0	0.0			7.0	3.0
15	7.0	3.7	2.3	3.3	4.0	0.3	5.0	2.3			7.0	2.3
16	7.0	0.7	5.7	2.0	6.3	1.0	7.0	2.0			7.0	2.3
17	7.0	6.3	3.7	1.7	7.0	1.3	3.0	1.3			7.0	0.0
18	7.0	1.7	5.7	2.7	6.7	0.7	5.3	1.3				
19	7.0	3.3	3.7	3.0	7.0	1.0	4.7	1.7				
20	7.0	3.0	5.3	2.0	5.7	0.7	4.7	1.0				
21	6.7	4.7	5.3	1.0	7.0	0.0	0.3	1.0				
22			6.3	2.7	7.0	0.0	3.3	2.7				
23			4.7	1.3	7.0	0.0	4.0	1.3				
24			4.7	2.3	7.0	0.0	4.3	0.3				
25			5.7	2.0	7.0	0.0	6.7	0.0				
26			6.0	1.7	7.0	0.0	3.7	0.0				
27			6.7	3.0	7.0	0.7	5.3	0.0				
28			7.0	3.7	7.0	0.0	4.3	0.0				
29			6.7	1.7			5.3	0.0				
30			7.0	3.0			5.7	1.3				
31			6.7	3.3			5.0	0.0				
32			7.0	4.3			6.3	0.0				
33			7.0	4.3			7.0	0.0				
34			7.0	2.3			6.7	0.0				
35			7.0	2.0			6.7	0.0				
36			7.0	2.3			4.7	0.0				
37			7.0	4.3			4.7	0.0				
38			7.0	3.0			4.0	0.0				
39			7.0	2.7			7.0	0.0				
40			7.0	1.0			5.3	0.0				

APPENDIX G

Photographic enlarged reproductions of brain sections from dogs in Experiment Three

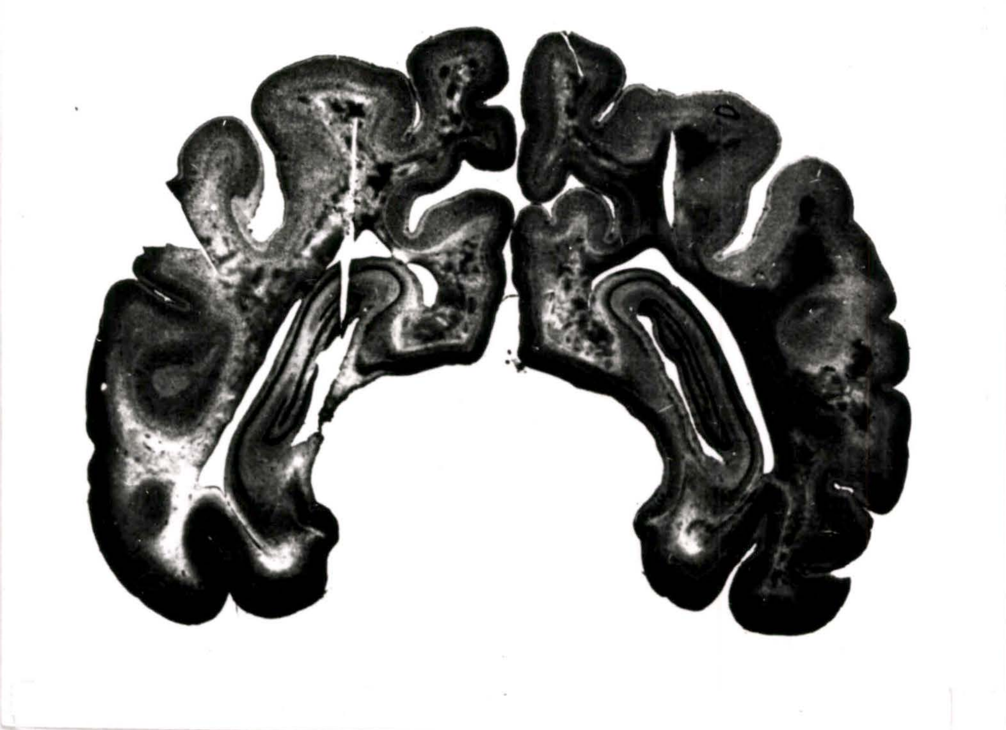
DOG 59

Stria terminalis

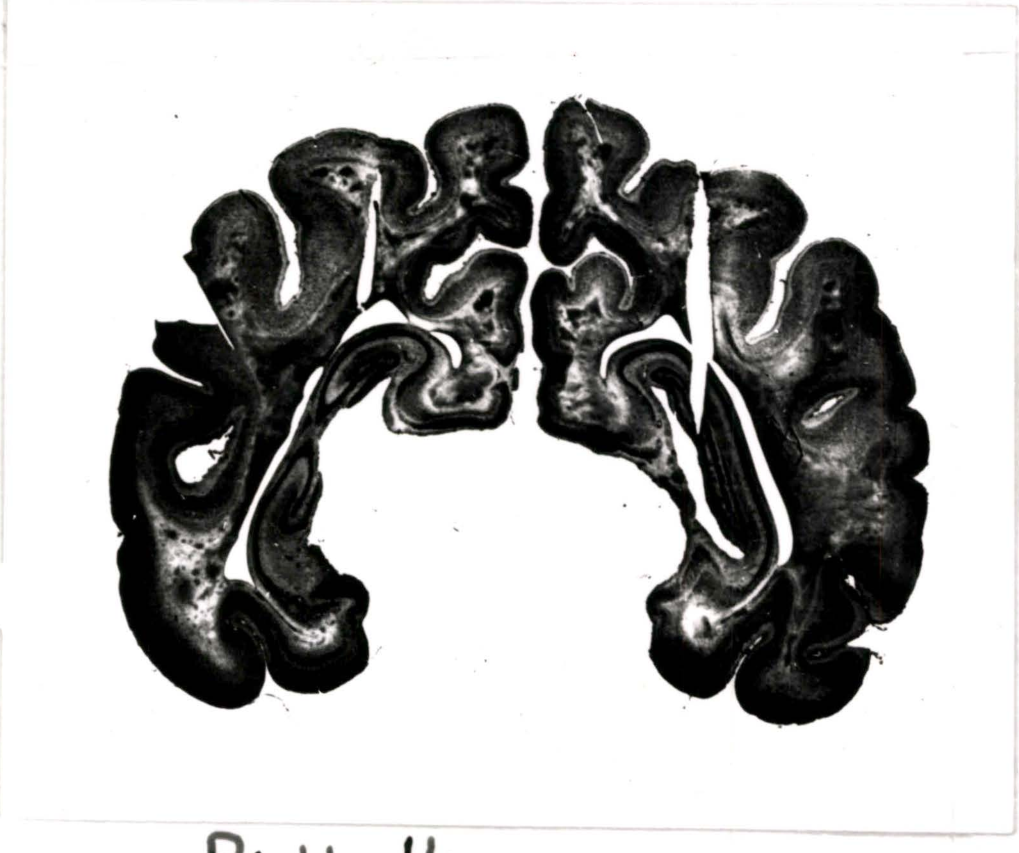


Stria terminalis

DOG 60



Left Hippocampus



Right Hippocampus

DOG 61

Left Hippocampus

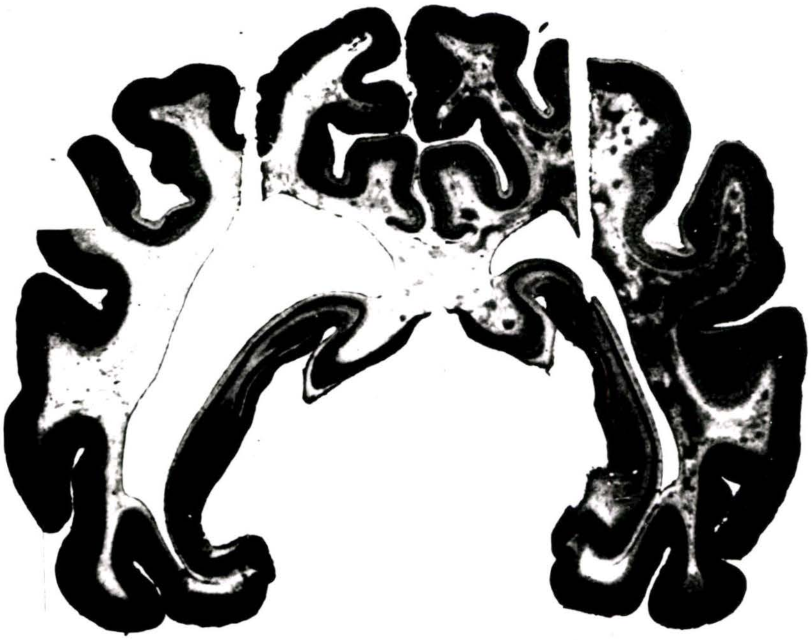
Right Hippocampus

DOG 62

Callosum

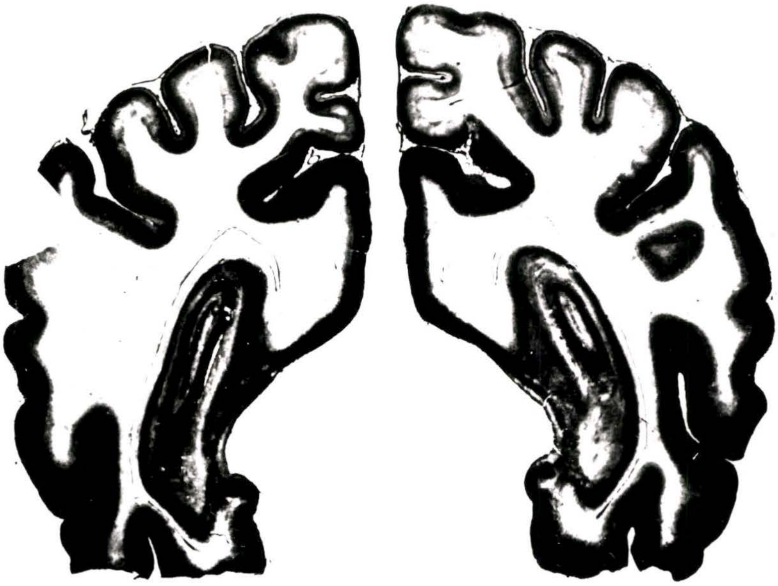
Right Hippocampus

DOG 63



Right Hippocampus

DOG 64



Left Hippocampus

Right Hippocampus

APPENDIX H

Summary of curare data obtained in Experiment Four

EXPERIMENT FOUR

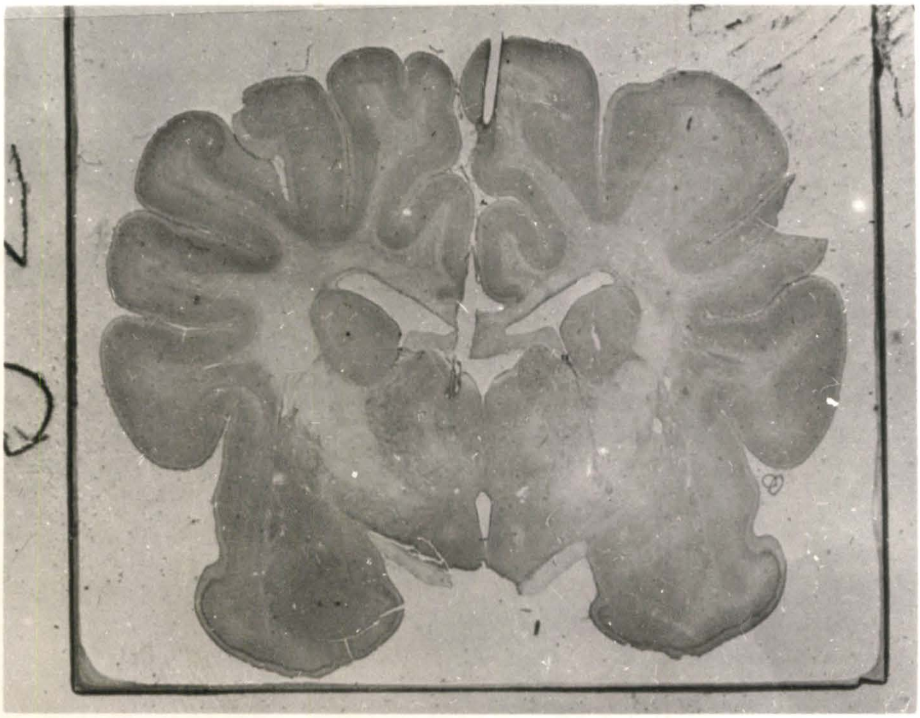
Summary of curare data for all dogs

Dog no.	trial duration first	last	total curare trials	total shock trials	Trial number of first shock	last shock	total number shocks
17	7.5	10.4	30	1	10	10	1
24	8.2	5.6	27	1	6	6	1
29	5.4	5.4	37	1	17	17	1
36	11.1	4.4	66	6	1	46	8
39	7.7	7.9	71	13	3	51	19
23	30.7	5.1	75	6	1	8	8
26	11.0	4.5	32	1	11	11	1
32	6.8	5.6	20	0	0	0	0
33	10.9	5.9	49	8	15	29	13
38	42.9	7.0	96	31	1	76	138
40	135.6	22.1	85	81	1	85	467
43	162.0	6.9	54	34	1	34	225

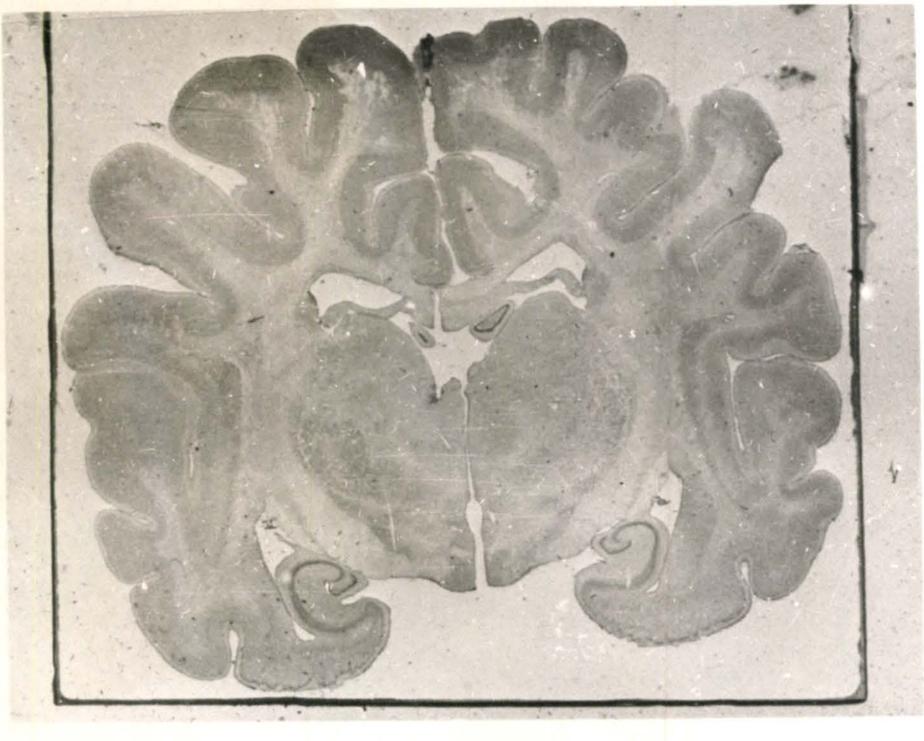
APPENDIX I

Photographic enlarged reproductions of brain sections from four of the dogs in Experiment Four

DOG 38



Medial Thalamus

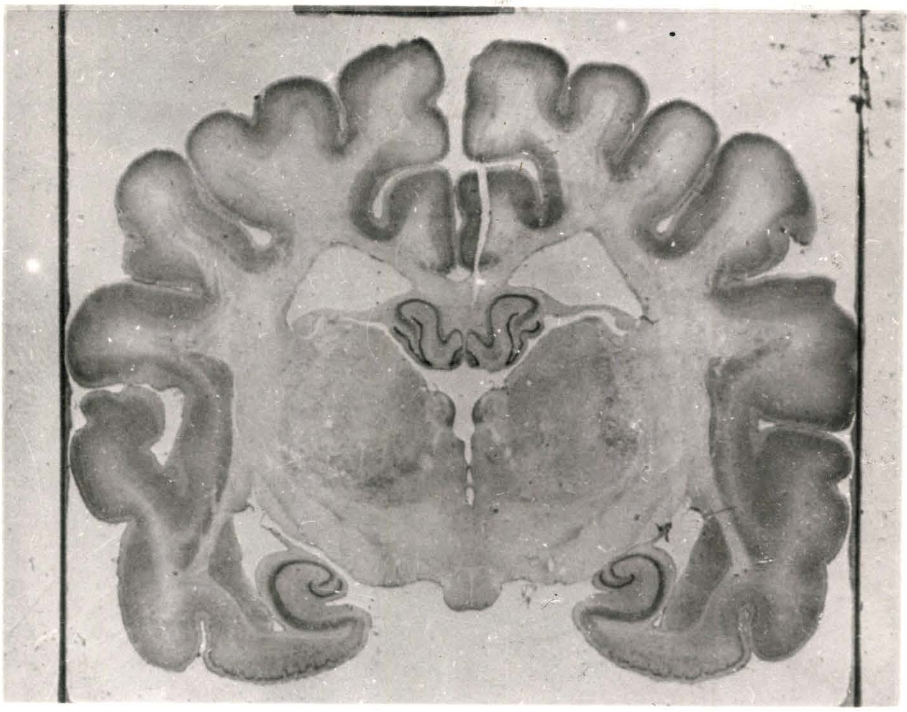


Medial Thalamus

DOG 39

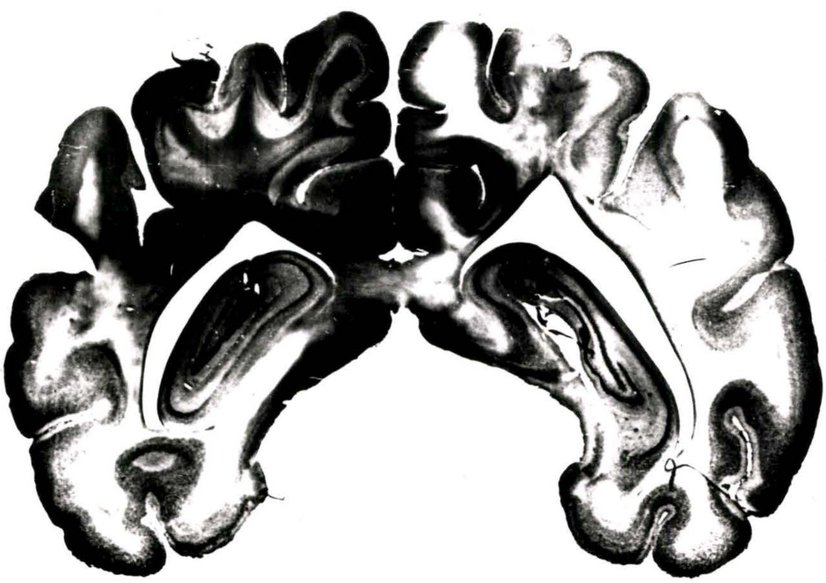
Medial Thalamus

DOG 40



Medial Thalamus

DOG 43



Left Hippocampus

Right Hippocampus

APPENDIX J

Raw data obtained in Experiment Two

EXPERIMENT TWO

Proportion of avoidances in S-1 and S-2 in successive blocks of twenty trials (ignoring days)

BLOCK	S-1	S-2	S-1	S-2	S-1	S-2	S-1	S-2
1	10/10	7/10	6/10	4/10	4/10	7/10	5/7	0/13
2	4/4	1/16	7/10	4/10	9/15	5/5	1/2	6/18
3	5/6	12/14	4/4	6/16	4/12	6/8	4/5	10/15
4	8/10	10/10	3/8	11/12	11/14	6/6	2/6	10/14
5	9/10	7/10	11/11	8/9	9/13	7/7	7/12	6/8
6	7/13	7/7	10/10	8/10	8/10	10/10	9/10	6/10
7	8/11	9/9	10/10	6/10	9/10	10/10	8/8	12/12
8	10/11	5/9	6/7	12/13	9/10	10/10	8/10	9/10
9	3/3	10/17	10/10	10/10	10/10	10/10	10/10	8/10
10	8/11	9/9	10/10	10/10	10/10	9/10	9/9	11/11
11	9/11	2/2	8/10	10/10				
12	3/3		4/5	5/5				
13								
14								

Dog 66
Last day of active-passive avoidance training

TRIAL	CS	DUR	SECONDS OF THETA		
			PRE-	TRIAL	POST-
26	+	8.2	0	1	1
27	-	4.0	0	1	0
28	-	4.0	0	2	0
29	-	4.0	0	1	0
30	-	4.0	0	0	0
31	+s				
32	-	4.0	0	1	0
33	-	4.0	0	0	0
34	+	11.0	0	5	1
35	+s				
36	-	4.0	0	1	0
37	+	5.4	0	3	2
38	-	4.0	0	1	1
39	-	4.0	0	0	0
40	+	13.5	0	6	1
41	+	10.5	1	4	1
42	-	4.0	1	1	0
43	+s				
44	+	4.9	1	2	3
45	-	4.0	0	2	0
46	+	6.4	0	3	3
47	+	10.5	0	5	1
48	-	4.0	1	1	0
49	-	4.0	1	1	0
50	+	11.5	1	5	3
51	+s				
52	+	9.5	1	5	2
53	-	4.0	1	1	1
54	+	10.2	1	6	1
55	+s				
56	-	4.0	0	2	0
57	-	4.0	0	0	0
58	+	3.8	0	3	3
59	-	4.0	0	0	1
60	-	4.0	0	1	1
61	+	4.2	0	2	1
62	+	4.5	1	2	2
63	-	4.0	1	1	1
64	+	4.5	0	1	1
65	+	6.0	1	3	1
66	+	4.9	0	4	2
67	-	4.0	0	0	0
68	-	4.0	0	1	0
69	+	6.8	1	3	2
70	-	4.0	0	0	0
71	+	4.6	1	1	2
72	-	4.0	0	2	0
73	+	6.2	1	4	2
74	-	4.0	0	1	0
75	+	6.0	0	1	2

Dog 67
Last day of active-passive avoidance training

			SECONDS OF THETA		
TRIAL	CS	DUR	PRE-	TRIAL	POST-
1	-	8.2	1	2	2
2	-	8.2	1	2	0
3	+	7.8	4	4	1
4	-	8.2	1	1	1
5	+	7.5	4	1	3
6	+	7.8	2	2	1
7	+	7.5	2	4	4
8	-	8.2	6	1	0
9	-	8.2	7	1	2
10	+	8.1	3	1	1
11	-	8.2	7	4	1
12	-	8.2	1	0	0
13	+	9.3	1	4	0
14	-	8.2	3	2	2
15	+	9.0	4	4	2
16	+	9.2	4	7	3
17	-	8.2	7	1	2
18	-	8.2	2	0	1
19	+	9.7	1	7	1
20	+	7.5	1	7	0
21	-	8.2	2	1	3
22	+	8.5	4	7	0
23	-	8.2	7	2	4
24	+	8.0	7	5	1
25	-	8.2	4	2	1
26	+s	10.0			
27	+	8.8	1	9	2
28	-	8.2	1	0	0
29	-	8.2	2	1	1
30	+	9.9	3	7	2
31	+s	42.0			
32	+	5.6	0	5	1
33	-	8.2	5	2	0
34	-	8.2	3	1	1
35	+	8.5	2	7	1
36	-	8.2	3	2	0
37	+	9.4	1	8	2
38	+	8.6	4	5	1
39	-	8.2	3	2	2
40	-	8.2	1	2	0
41	-	8.2	1	1	1
42	+	8.1	3	6	1
43	+s	10.1			
44	-	8.2	2	2	3
45	+	7.7	3	6	3
46	-	8.2	1	3	0
47	+	7.8	2	7	1
48	-	8.2	2	0	0
49	+	8.0	3	8	0
50	-	8.2	0	2	3

Dog 68

Last day of active-passive avoidance training (day 4)

			SECONDS OF THETA		
TRIAL	CS	DUR	PRE-	TRIAL	POST-
1	+s				
2	+	6.0	0	6	2
3	+	6.4	1	6	3
4	-	6.4	3	1	2
5	-	7.3	0	0	0
6	+	8.8	1	7	3
7	+	7.8	2	8	2
8	-	7.3	0	1	0
9	-	7.3	2	0	0
10	-	7.3	1	0	0
11	+	7.7	1	7	5
12	+	8.0	2	6	4
13	-	7.3	2	1	0
14	-	7.3	1	0	0
15	+	6.3	1	6	4
16	+	7.2	2	6	2
17	-	7.3	2	1	0
18	-	7.3	1	0	0
19	+	6.7	0	6	2
20	-	7.3	1	1	1
21	+	7.1	0	6	4
22	+	6.8	3	6	5
23	+	6.5	2	5	2
24	-	7.3	1	0	0
25	-	7.3	1	0	0
26	-	7.3	1	0	0
27	+	6.8	0	6	4
28	+	6.8	2	6	4
29	-	7.3	3	1	0
30	-	7.3	1	1	0
31	+	6.8	2	5	3
32	+	8.5	2	8	3
33	-	7.3	0	0	1
34	-	7.3	2	0	0
35	+	8.1	2	7	2
36	+	9.1	1	8	2
37	-	6.3	2	0	0
38	+	6.8	2	6	3
39	-	7.3	1	0	0
40	-	7.3	0	0	1
41	-	7.3	3	0	1
42	+	7.9	1	7	3
43	+	6.9	1	6	2
44	-	7.3	2	1	1
45	-	7.3	1	1	0
46	+	8.2	1	7	1
47	-s				
48	+	8.6	2	8	3
49	+	7.0	3	7	3
50	-	7.3	4	4	1

Dog 69
Criterion day of active-passive avoidance training (day 3 of 4)

			SECONDS OF THETA		
TRIAL	CS	DUR	PRE-	TRIAL	POST-
1	-	6.3	1	3	3
2	-	7.4	1	0	1
3	+s				
4	-s				
5	-	7.4	2	0	1
6	+	3.8	0	2	2
7	+	5.8	4	1	2
8	-s				
9	+	5.1	1	1	4
10	+	8.3	0	2	1
11	-s				
12	-s				
13	+	5.8	1	1	0
14	+	6.7	1	1	0
15	-	7.4	2	1	0
16	+	6.8	0	1	0
17	-	7.3	0	1	0
18	+	9.8	1	1	1
19	+	4.8	0	1	0
20	-	7.3	3	0	0
21	-	7.3	3	0	0
22	-	7.3	1	0	0
23	+	8.0	0	2	1
24	-	7.3	3	1	3
25	+	7.4	1	0	3
26	+	4.9	0	0	4
27	-	7.3	0	0	0
28	-	7.3	2	1	0
29	+	10.1	0	1	0
30	-	7.3	3	1	0
31	-	7.3	1	1	0
32	+	9.8	0	2	0
33	+	5.9	0	1	5
34	+	7.4	1	1	1
35	-	7.3	0	0	0
36	-	7.3	4	0	0
37	+	4.8	0	1	2
38	-	7.3	0	0	0
39	+	6.0	0	1	1
40	-	7.3	0	0	4
41	-	7.3	0	3	3
42	-	7.3	0	3	3
43	-	7.3	0	0	3
44	+s				
45	+	5.7	2	1	4
46	unusable				
47	-s				
48	+	3.5	0	1	0
49	+	8.2	0	2	0
50	-	7.3	0	0	0