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The Effects of Hippocampal Lesions on Short Term Memory in the Rat

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THE EFFECTS OF HIPPOCAMPAL LESIONS ON SHORT TERM MEMORY IN THE RAT

by

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INTRODUCTION

The purpose of this experiment was to study the effects of bilateral hippocampal lesions on short term memory in the rat.

The functional significance of the hippocampus has been speculated for many years. Ferrier (1876) concluded, on the basis of lesion studies with monkeys, that the hippocampus was the center of cutaneous sensitivity. Thereafter, hypotheses have been made involving the hippocampus in emotion, olfaction, memory, and arousal, (Milner and Penfield, 1955; Russell and Nathan, 1946; Hirsh, 1970).

Papez (1937) suggested that the hippocampus functioned in an emotional capacity, but as was the case for many early suggestions there was little direct evidence to support such a suggestion, and even Papez's conclusions along with subsequent similar ones, appeared to be based mainly on the anatomical relationship between the hippocampus and other limbic system structures, and the hypothalamus. Some evidence for an emotional function has been inferred from the studies of avoidance learning, but these unfortunately were confounded with the learning variable.

Anatomical considerations seem to have brought forth speculation dealing with olfactory function, but the hippocampus has been shown to be of trivial importance in olfactory discrimination (Swann, 1934, 1935; Allen, 1940, 1941).

The involvement of the hippocampus in memory has been suggested by Penfield (Penfield, 1955; Penfield and Milner, 1958) and

currently is one of the concepts being seriously considered. The role of the hippocampus in learning and memory has been scrutinized extensively in recent years by investigators implementing lesion, stimulation, and electrophysiological recording techniques.

Lashley (1950) was one of the first investigators to collect extensive data on surgical brain lesioning techniques. He brought cerebral neurology away from the neurology that applies only at the periphery of the nervous system toward a field of dynamics of the brain.

A great deal of additional data concerning memory disruption has been produced from the use of amnesic treatments (Jarvik, 1970; Coons and Miller, 1960; Russell and Nathan, 1946; Chorover and Scheller, 1965). In this instance memory deficits result following electroconvulsive shock, trauma, etc.

There have been conflicting results reported on the effects of hippocampal lesions on active avoidance learning. Reports by Thomas and Otis (1958a) said that there was an impairment of avoidance learning in rats; however, faster avoidance learning was shown by Isaacson, Douglas, and Moore (1961). According to Hunt and Diamond (1959), no effects on avoidance learning in cats were found, but they did show a behavioral decrement when the lesions followed learning. This decrement, however, was abolished by pre-lesion overtraining. Consistent impairment of passive avoidance (Kimura, 1958; Isaacson and Wickelgron, 1962; Kimble, 1962) has been revealed to result from hippocampal lesions in rats. Impaired learning following amygdala-hippocampal lesions in monkeys has been shown by Stepien, Cordeau,

and Rasmussen (1960) and Orbach, Milner, and Rasmussen (1960). Leaton (1965) using a T-maze for rats showed that a deficit in habituation exists following hippocampal lesions and concluded that this deficit was in an inhibitory mechanism. A few studies involving the use of human subjects have shown memory disturbances following bilateral damage to the hippocampus (Glees and Griffith, 1952; Scoville, 1954; Scoville and Milner, 1957; Penfield and Milner, 1958).

During learning, stimulation of the hippocampus has been shown to have little ultimate effect on response acquisition (Flynn and Wassman, 1960; Correll, 1957; Weiskrantz, Mihailovic, and Gross, 1962; Stamm, 1961). However, Correll did prolong extinction of a running-bar-pressing response by hippocampal stimulation. Additional studies have shown a relationship between the role of the hippocampus in memory and both protein synthesis (Flexner et. al. 1962) and acetylcholinesterase activity (Liebowitz, 1968).

Because a growing number of investigators have dealt with the pursuit of evidence either supporting or rejecting the role of the hippocampus in short term memory, new and very interesting experiments have been devised as avenues for investigation. It is to these avenues that this experiment was primarily directed.

Short term memory, that is, the ability to receive, transmit, and store data and recall this data at an instance within a time span of 72 hours, presents a major problem for testing. That major problem lies in the selection of a behavioral paradigm because it must scrutinize necessarily small fluctuations in behavior. Single trial avoidance paradigms have been successfully employed to examine

changes in response rate recovery following an aversive consequence, (Jarvik and Essman, 1960; Jarvik, 1964; Essman and Alpern, 1964). In order to measure subtle variations in the acquisition of short term memory, this present study employed a one trial suppression type learning paradigm described by Gault and Hunt (1971). It involved a process in which rats were trained to lever press for food on a variable interval 30 second (VI 30) schedule of reinforcement. After this behavior was conditioned, one session of extinction followed and on the next day the rats were allowed to make one lever press which resulted in a foot shock. Reacquisition testing was employed 48 hours later.

GENERAL METHOD

Subjects

The subjects were fifteen naive, male albino rats, of Sprague-Dawley strain, approximately ninety days old, weighing between 280 and 320 grams at the beginning of the experiment. They were all individually housed in cages with free access to water and maintained at 80% ad lib weight.

Apparatus

A clear plexiglas "operant test chamber", with a response lever, food dispenser, and floor shock grid was housed in a sound attentuated chamber.

The apparatus was programmed and the responses recorded by standard electromechanical equipment. By means of a scrambler, foot shock was provided by a Grason Stadler (E60708) shock generator.

Hippocampal lesions were made electrolytically by means of a Radionics solid state radio frequency lesion generator model RFG-4.

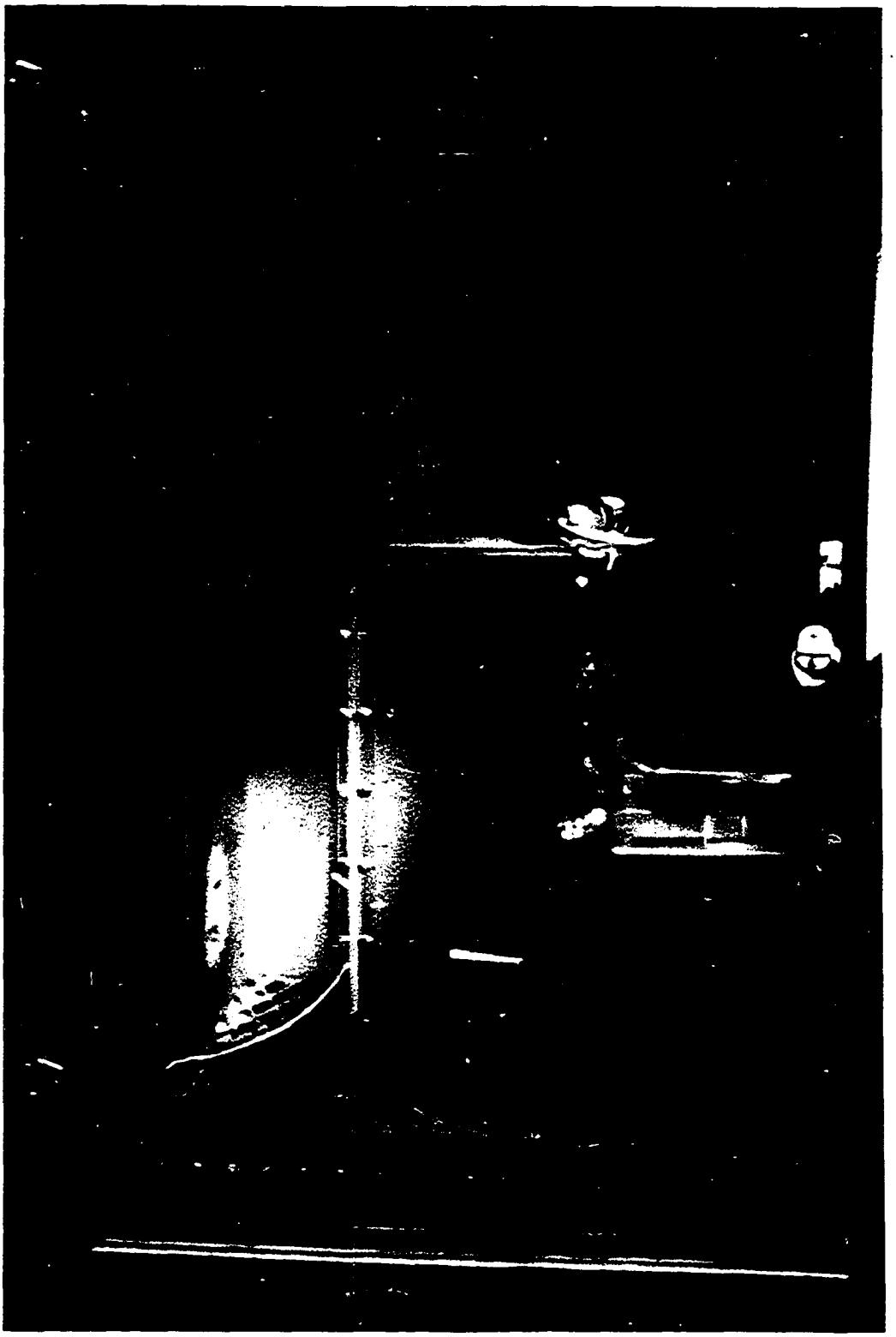
Surgery

All animals were operated under clean, but not aseptic surgical techniques. They were anesthetized with sodium pentobarbital with atropine added to reduce respiratory secretion.

The hippocampal lesions were stereotaxically oriented using De Grott coordinates (Pellegrino and Cushman, 1967), of anterior 3 mm.,

FIGURE 1A

View of operant test chamber.



lateral 2.4 mm., and depth 2.0 mm. and the animal was fixed in a stereotaxic instrument. Two holes were drilled into the skull at the above coordinates and the temperature probe was lowered to the prescribed depth. A temperature of 60 degrees centigrade was attained for the area surrounding the tip of the probe and held there for approximately sixty seconds. Thus bilateral lesions were made. The holes in the bone were filled with bone wax and the skin was approximated and sutured with 00 silk thread. The edges of the wound were swabbed with tincture of merthiolate and each animal was given a 0.10 cc. intramuscular injection of bicillin. The animal was returned to the cage for a two day recovery period.

Procedure

All rats were placed on food deprivation until they reached 80% ad lib weight. They were then shaped into pressing the lever for food reinforcement on a continuous reinforcement schedule through successive approximation. Daily thirty minute sessions were employed for five days, or until the subjects had reached a stable baseline of responding (125 $\pm$ 15 responses within a session). After this baseline period was reached they were then switched over to VI 30 schedule.

Daily sessions were held until the subjects' responding level reached stabilization (300 $\pm$ 50 responses), on the VI 30 schedule. All but five were then exposed to a thirty minute extinction period where they received no reinforcement. Subsequent to the extinction session, the rats were randomly divided into three respective groups

of five, (1) control, (2) lesioned, and (3) spare. The lesioned group received treatment different from the non-lesioned and spare.

The lesioned on the next session was allowed to make one lever press, which triggered a 3.0 milliamp foot shock for 0.75 seconds. They were removed from the apparatus and within five minutes received an injection of an anesthetic consisting of sodium pentobarbital and atropine; and bilateral hippocampal lesions were made. They were given two days to recover from surgery.

Contrary to the lesioned group, the control group received no surgery, but was shocked and then anesthetized and placed back into their cages for a two day recovery period. The spare group was not touched because their only specific use was for replacement in case any of the animals from the other group died.

Following the 48 hour recovery period both lesioned and control subjects were returned to the VI 30 schedule with responses being recorded every thirty seconds during the entire session. Comparisons of the lesioned and control groups were made in terms of the time lapse as associated with initial and total responses within the session (see figures 2 through 7).

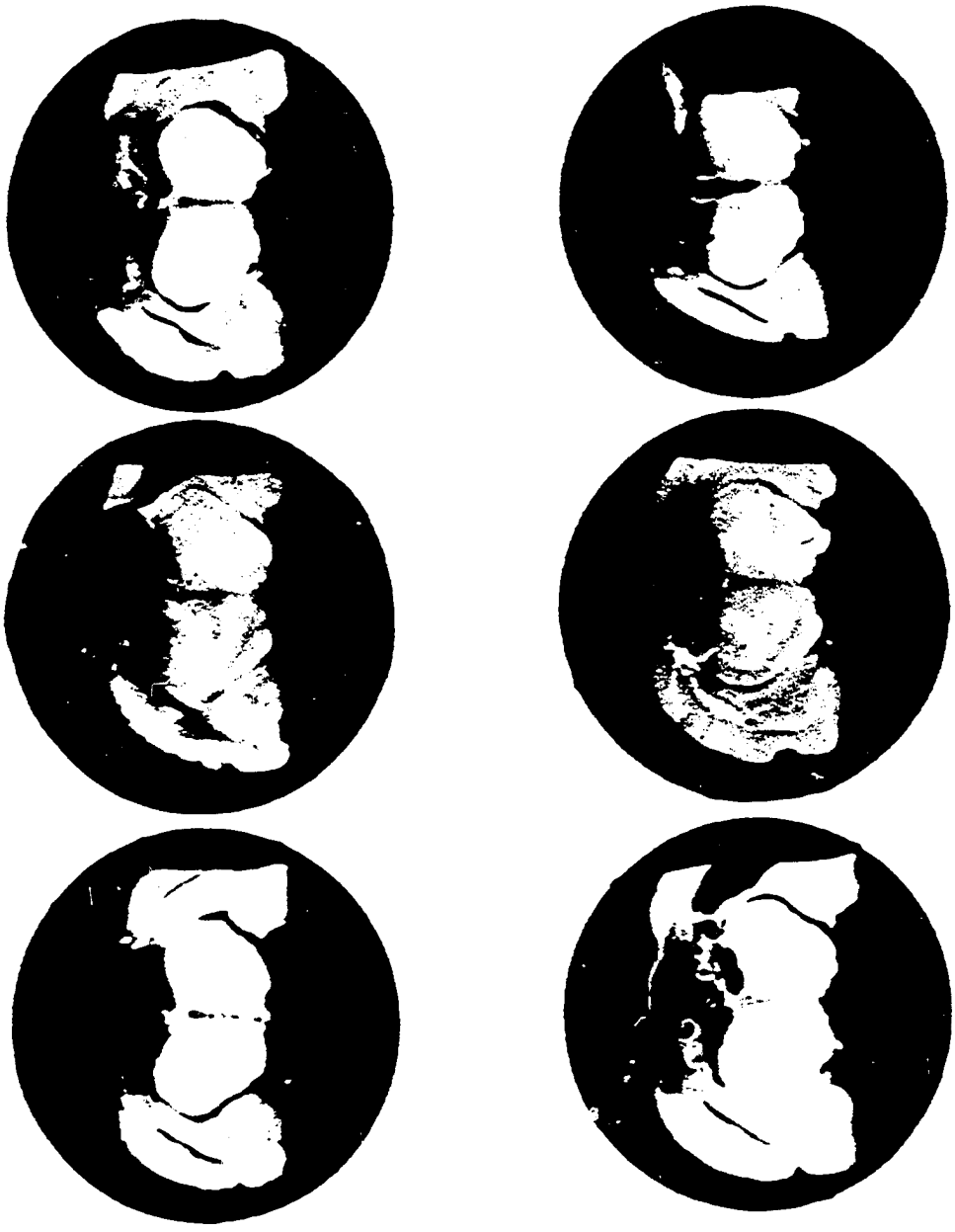
Anatomy

Following the experiment, the animals were anesthetized with sodium pentobarbital and perfused with 10% formalin and saline solutions. Frozen sections were cut at 25 micra to verify hippocampal lesioning (see figure 1). The lesions were localized in the rat

atlas of Cushman and Pellegrino (1967). Each animal's lesion varied very little from that shown in the figure.

FIGURE 1B

Representative histological sections.



RESULTS

In this paradigm rats were exposed to several days of training for a particular task followed by an extinction session. Twenty-four hours later they were placed in a single trial non avoidance situation where a lever press resulted in a sudden single, novel, noxious stimulus. They were subsequently tested 48 hours later for their ability to remember the single trial and respond appropriately. Thus behavior indicative of short term memory consolidation was examined.

Figures 2 through 7 show the response rate for the pre-shock and post-shock sessions. Table 1 indicates the results of the statistical analysis of the first ten minutes and total responses of both groups in the final session. It also shows that the differences between the lesioned and control groups were significant ($p. .05$) using "t" test.

Cumulative curves shown in figure 2 compare the lesioned group versus the control group in the pre-shock session. In this case the data indicate no major differences in their response rates for the first 300 seconds. There was no statistical significance between both groups.

The cumulative curve of the control group compared to the lesioned group for the first 300 seconds in the post-shock recovery session shows a difference as is indicated by figure 3. The rates of the lesioned group are significantly higher than the rates of the control group ($p. .001$).

Figure 4 shows that the cumulative response rate of the pre-shock

session in the first ten minutes are relatively the same for both groups. There was no statistical significance between both groups.

Figure 5 reveals that the cumulative response rates in the post-shock session during the first ten minutes for the lesioned group is much higher than the control (p. .001).

Figure 6 shows that the average cumulative response rates in the pre-shock session during the entire 30 minutes are relatively the same for both groups. There was no statistical significance between both groups.

Figure 7 reveals that the average cumulative response rates in the post-shock session during the entire 30 minutes is much higher for the lesioned group than the control (p. .001).

Table 1

Levels of Statistical Significance

Session for Group Comparison	P
Pre-shock session first 300 seconds	N.S.
Post-shock session first 300 seconds	.001
Pre-shock session first 10 minutes	N.S.
Post-shock session first 10 minutes	.001
Pre-shock session entire 30 minutes	N.S.
Post-shock session entire 30 minutes	.001

FIGURE 2

Pre-shock response rate of subjects during the first 300 seconds of the session.

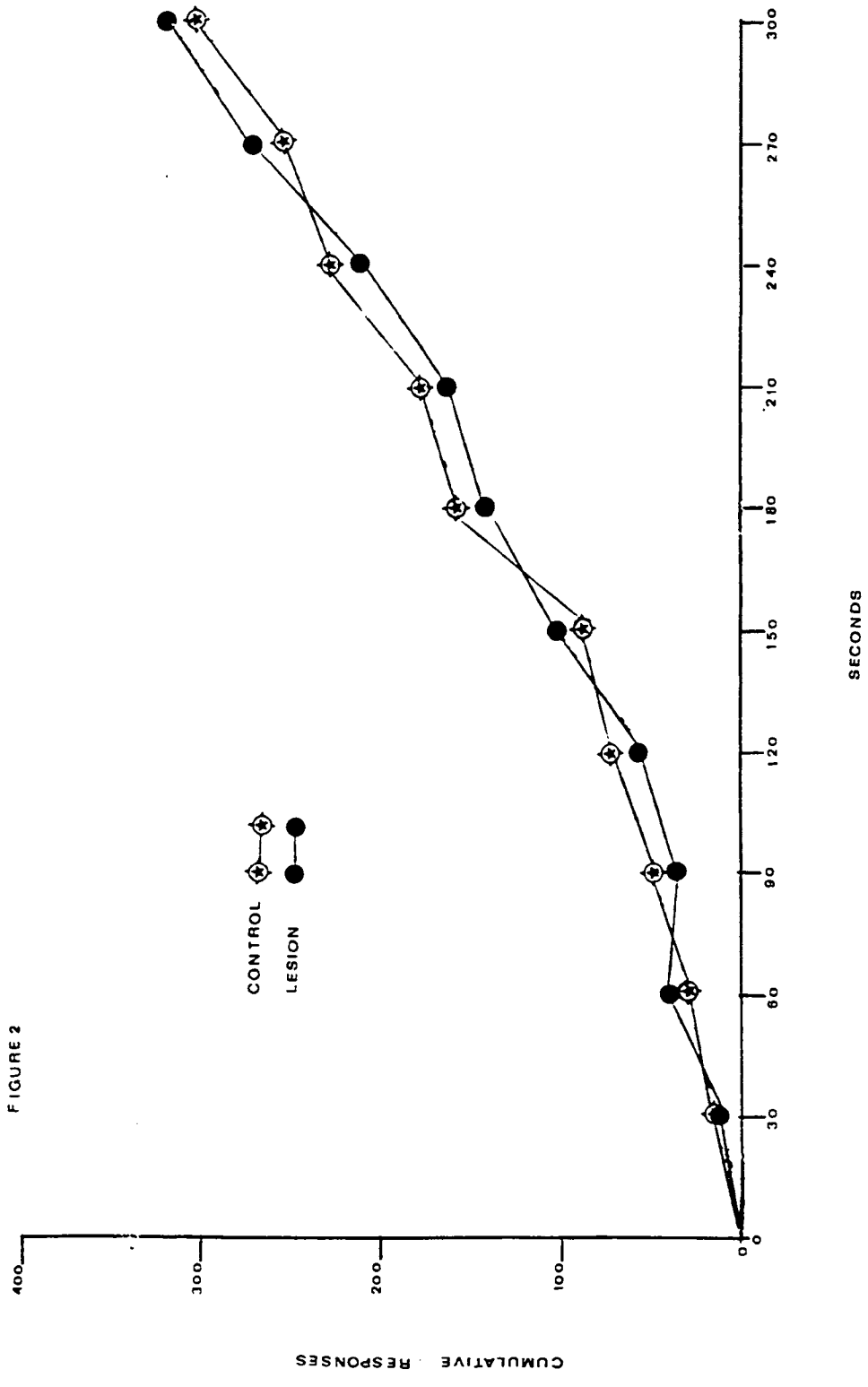


FIGURE 2

FIGURE 3

Post-shock response rate of subjects during the first 300 seconds of the session.

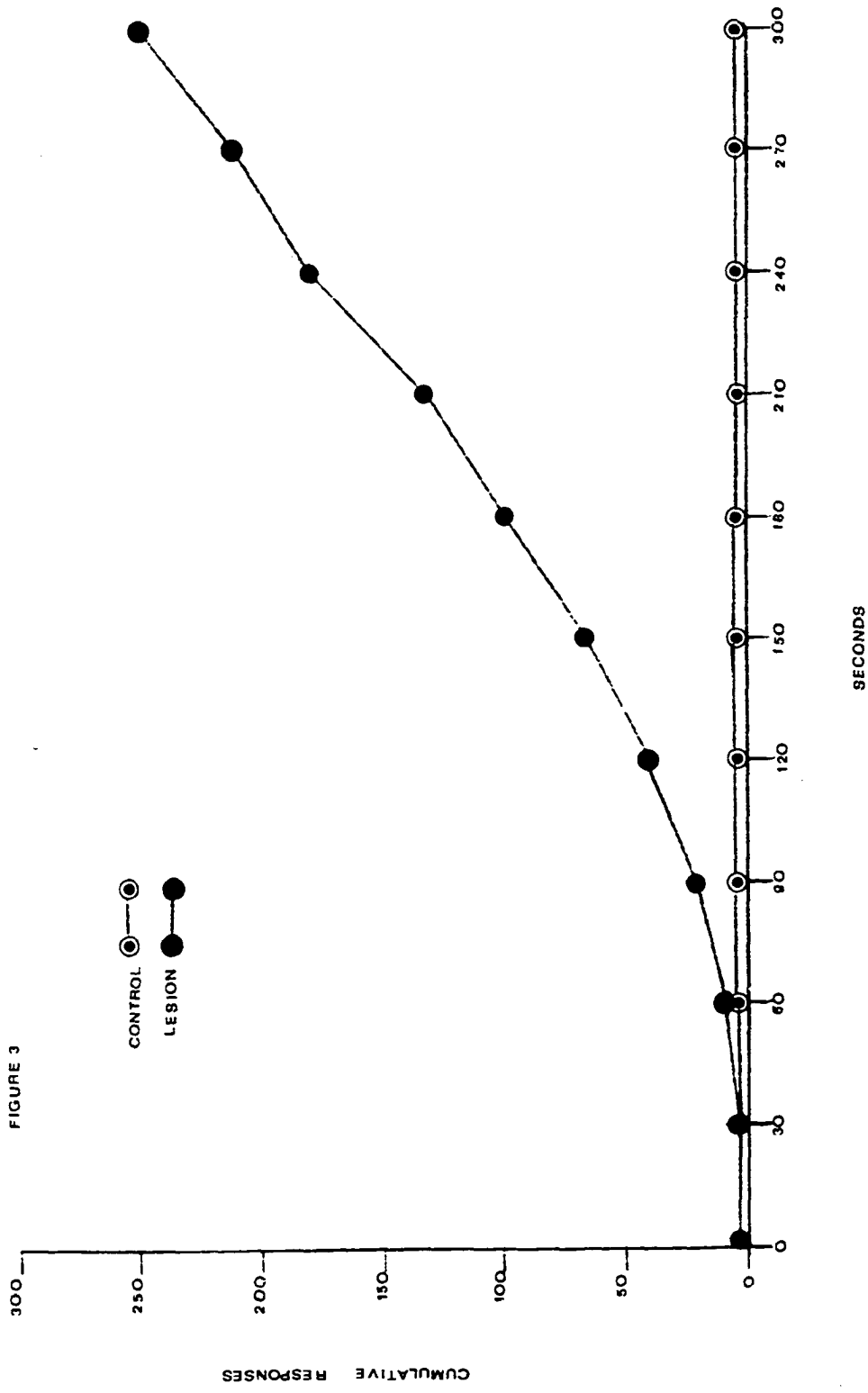


FIGURE 3

FIGURE 4

Pre-shock response rate of subjects during the first 10 minutes of the session.

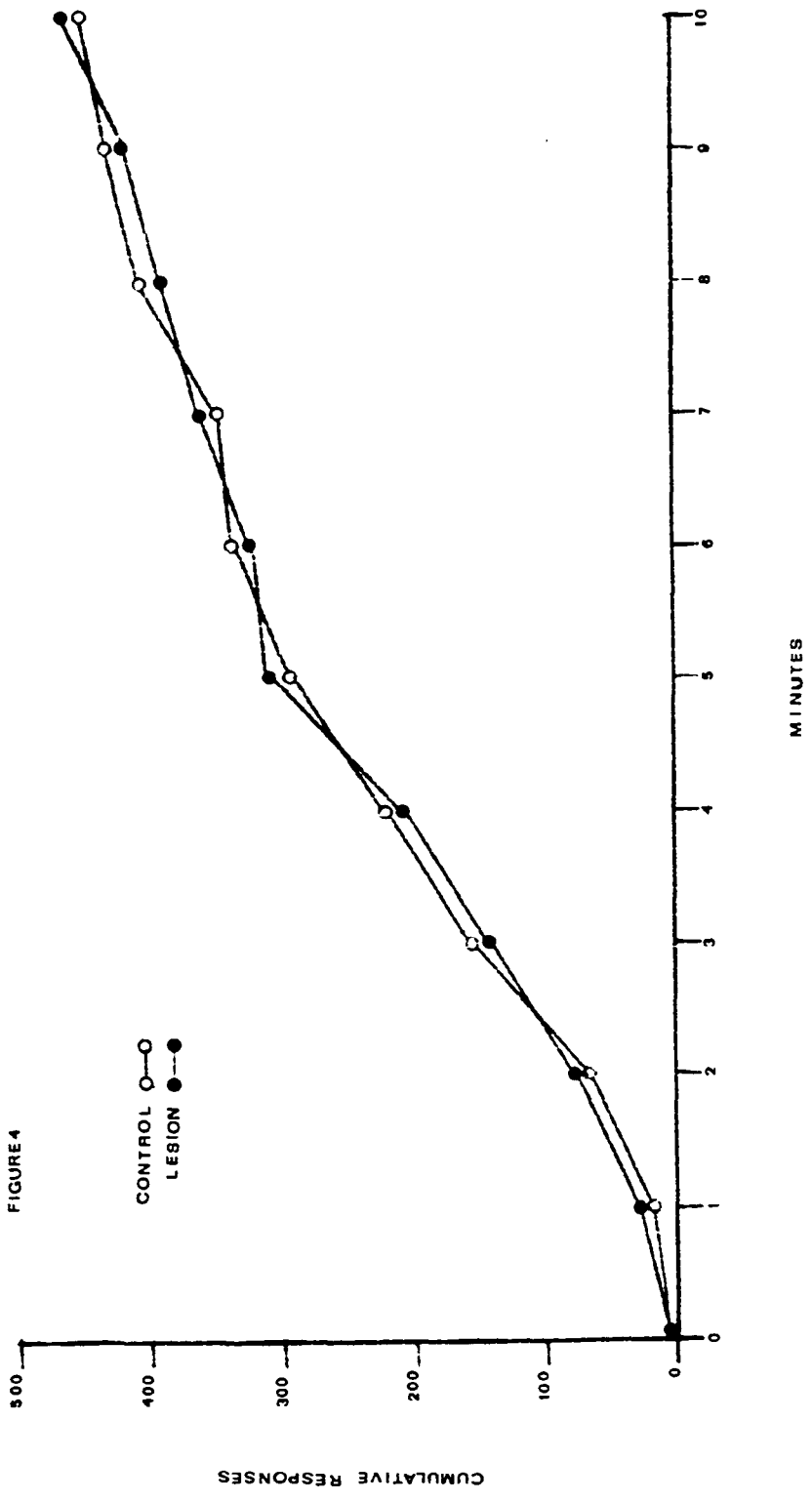


FIGURE 4

FIGURE 5

Post-shock response rate of subjects during the first 10 minutes of the session.

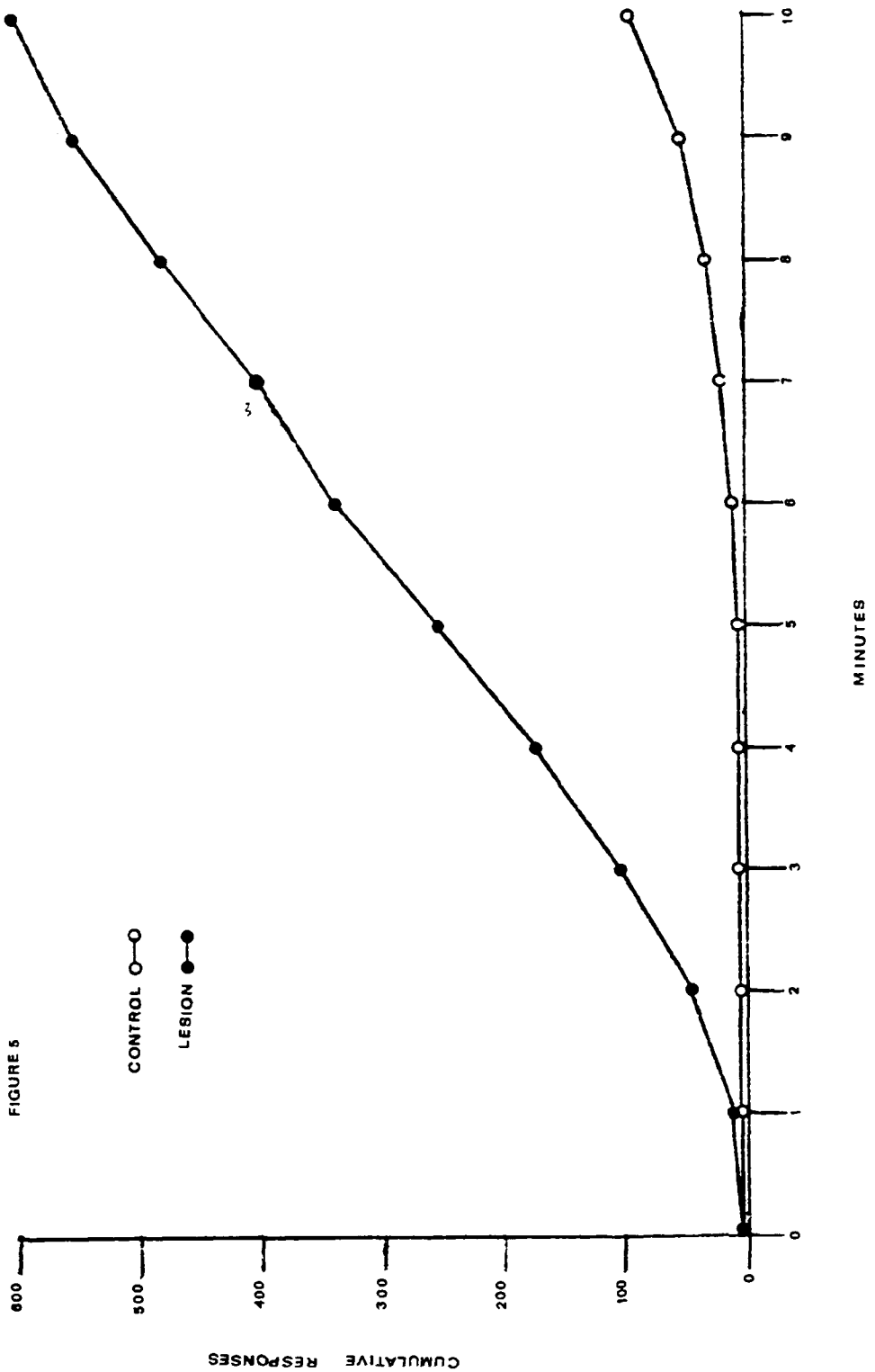


FIGURE 5

FIGURE 6

Pre-shock response rate of subjects during the entire 30 minutes of the session.

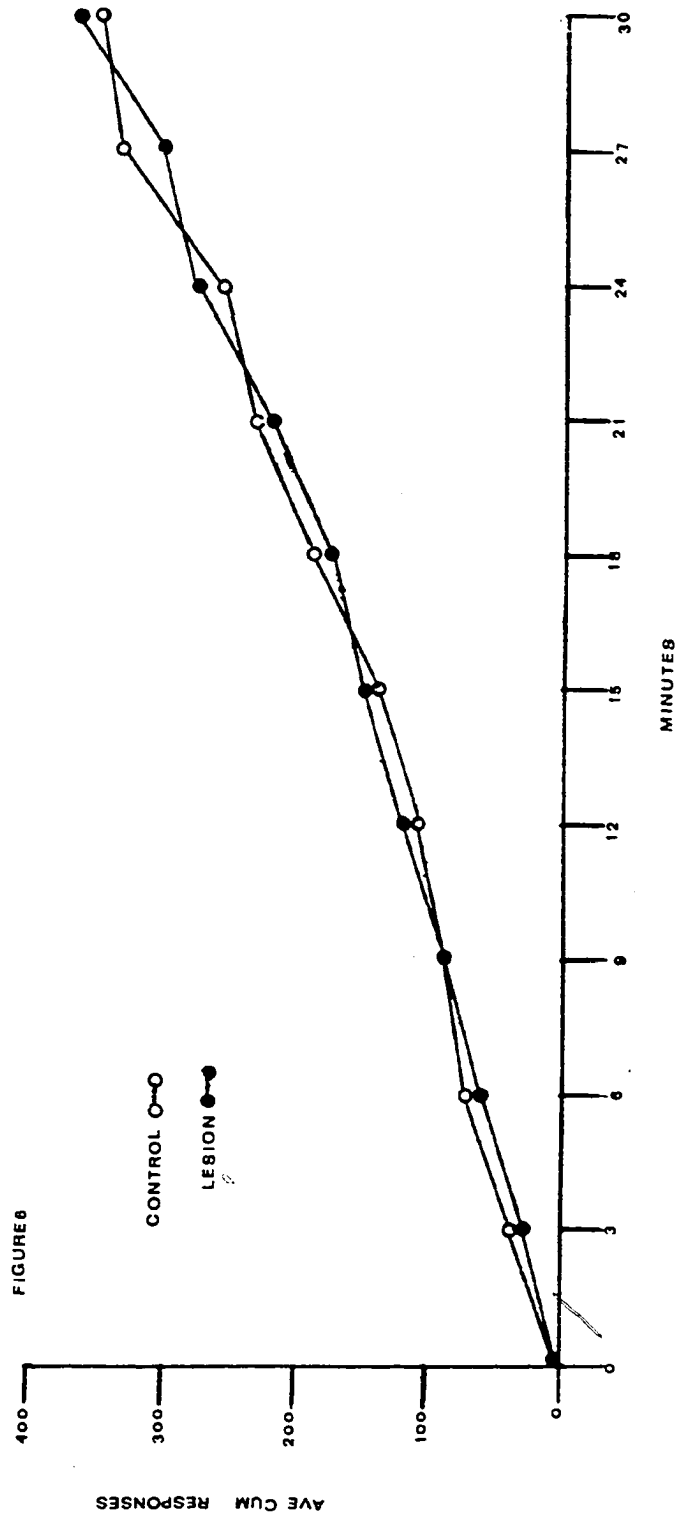


FIGURE 8

FIGURE 7

Post-shock response rate of subjects during the entire 30 minutes of the session.

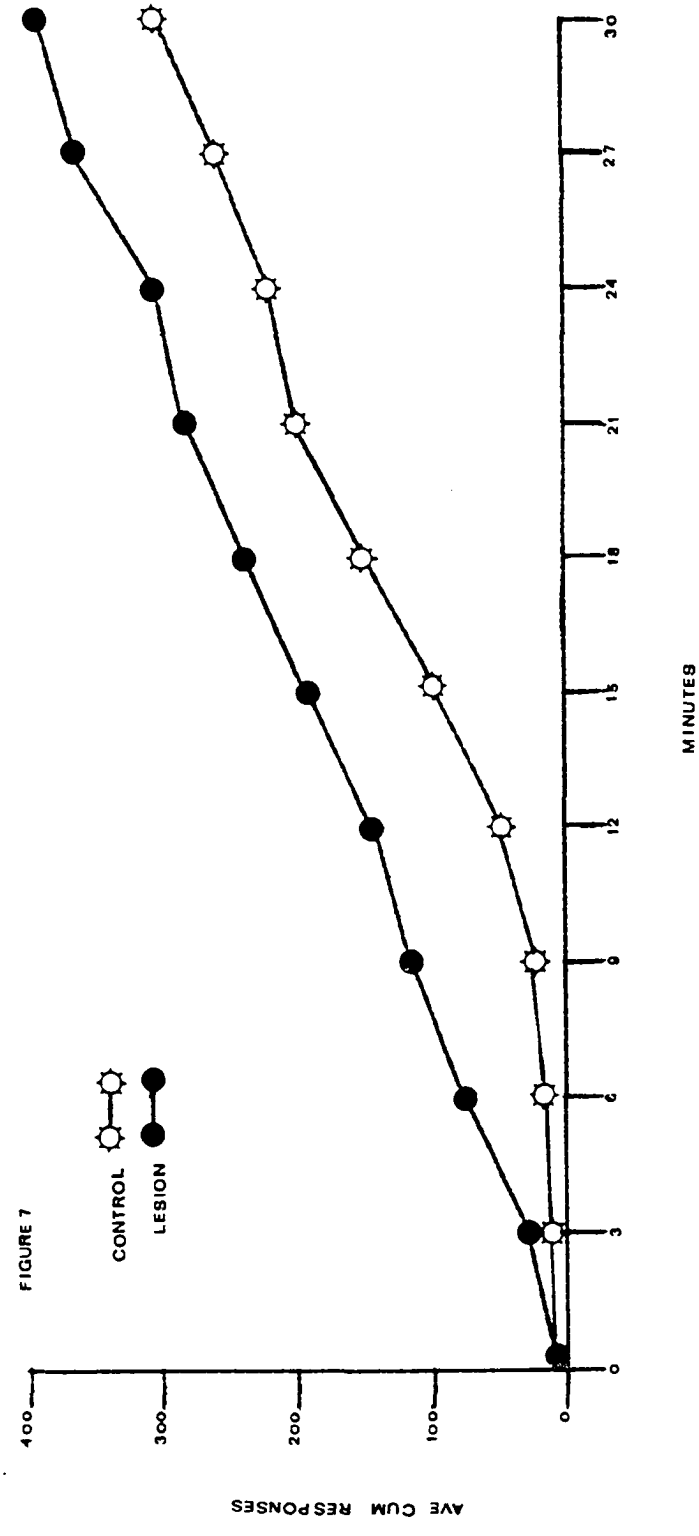


FIGURE 7

DISCUSSION

Results of these experiments, taken as a whole, suggest that hippocampal lesions impair an animal's ability to exhibit short term memory. These data are partially consonant with results reported (Douglas, 1967; Milner and Penfield, 1955; Isaacson and Wikelgron, 1962; Kimble, 1963; Thomas and Otis, 1968; Leaton, 1963; Husich, 1970).

There may be many factors which are unknown at present, but which contribute to this phenomenon. However, in considering what is now known it would be safe to say with some degree of confidence that the hippocampus does play some type of role in short term memory.

At least two processes are involved in habituation to novelty (Roberts, Dember, and Brodwick, 1962), and a deficit in either or both of them could lead to an impaired rate of habituation. In order to habituate to a novel stimulus, a memory of the stimulus must be retained from one exposure to the next, and this memory must act through some mechanism to inhibit the exploration or conditioned responding to the stimulus. Short term memory is dependent upon the time interval between exposures. The time interval involved in some instances negate the results and conclusions drawn up on that particular experiment.

The type of behavioral paradigm used to test short term memory is very critical. In some instances the design of the experiment

excludes any significant results that may come from the experiment itself.

In this particular experiment, the animals were able to retain a simple stimulus-response connection, but their specific memory of the box could have been impaired. This specific memory would require the retention of more information than would the stimulus-response connection; therefore, a lesion-produced memory deficit could spare the latter while disrupting the former.

Another possible result of hippocampal lesions is a disruption of pre-lesion memories with or without a concomitant decrement in post-lesion recent memory. Because the response rate was relatively the same in pre-lesion sessions and post-lesion sessions for the experimental group and much more different for the control in both sessions, it can be concluded that the hippocampal lesions could have disrupted pre-lesion memories.

There was no overlapping of data in comparing the results obtained for both groups which may imply good results. One aspect which could have been overlooked is the number of subjects used.

The effects found in the hippocampal group in the present experiments cannot be attributed exclusively to the hippocampus, without reservations. The cingulate cortex, as well as other cortical areas, was consistently damaged in the hippocampal group. Although the effects cannot be attributed to the cortical damage alone, the effects could have resulted from combined cortical and hippocampal damage. The hippocampal group lost a large amount of total tissue

It is therfore possible that the effects could be attributed to a non-specific occuring action. Because cortical lesions were not made exclusively, it may be a possibility for specific or related study in the testing of short term memory in the future as set up according to this present behavioral paradigm. The possibility, however does exist that the effects could have resulted from some combination of damage.

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