THE EFFECTS OF HIPPOCAMPAL ABLATION ON THE INHIBITORY CONTROL OF OPERANT BEHAVIOR IN THE RAT

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The effects of hippocampal ablation on some forms of response inhibition associated with nonreinforcement or delay of reinforcement were investigated in the rat. In the first experiment, hippocampal ablation restored the preoperatively extinguished bar-pressing response to a significant degree. In the second experiment, hippocampally ablated animals responded more frequently than did the neocortically ablated animals during the extinction of a differential bar-pressing response. In the third experiment, hippocampal ablation increased the rate of bar pressing to negative stimuli without affecting the response rate to positive stimuli. In the final experiment, the decremental change in runway performance due to the delay of reinforcement was much slighter in the hippocampally ablated animals than in the neocortically ablated animals.

These results suggest that the hippocampus is important for the inhibitory control of operant behavior.

In recent years an increased theoretical attention has been paid to the neural mechanisms which play an important role in the inhibitory control of behavior (Diamond et al., 1963). Deficits in the inhibition of responses following the brain ablation have been reported by several investigators.

The experimental evidences suggesting the hippocampal involvement in the inhibitory control of behavior are as follows:

The hippocampally ablated animals responded deficiently in passive avoidance situation (Kimura, 1958, Isaacson & Wickelgren, 1962) and they also showed an increased resistance to extinction of certain types of learned response, e.g. an active avoidance response (Isaacson et al., 1961), a running response in the runway (Niki, 1962; Jarrard et al., 1964).

The present study investigates the effects of hippocampal ablation on some forms of response inhibition associated with non-reinforcement or delay of reinforcement under a variety of conditions.

The purpose of the first experiment in the present study is to investigate the effect of hippocampal ablation on an extinguished bar-pressing response. The second experiment is undertaken to determine whether a greater resistance to extinction following the hippocampal ablation as was found in the runway would also occur in a choice situation. In the third experiment, the ability of the hippocampally ablated animals to inhibit responses to negative stimuli is investigated. The aim of the fourth experiment is to study the effect of hippocampal ablation on a performance decrement due to the delay of reinforcement.

GENERAL METHOD

Surgery. All operations were performed under Nembutal anesthesia (40 mg/kg). The Ss
were held in a stereotaxic instrument during the operation. The aspiration technique was used to ablate either the hippocampus or the neocortex overlying the hippocampus. After a midline incision of the scalp, suitable holes were drilled in the skull and enlarged with rongeurs. The dura was then opened and the desired portion of the brain was removed by aspiration. In the experimental group the hippocampus was sucked bilaterally through the neocortex as much as possible, both medially and ventrally, care being taken to spare the underlying thalamus. In the operative control Ss the neocortex overlying the hippocampus was sucked bilaterally approximately to the same extent in the case of hippocampal ablation. After bleeding ceased, the scalp was closed. The Ss received intra-muscular injections of penicillin for two days postoperatively. A recovery period of three or four weeks was allowed before testing.

Histology. Following the termination of testing, the animals were sacrificed and the standard procedure followed in preparing the brains for a histological study. The brains were removed, and perfused with 10 per cent formalin for about a week. Then, these were embedded in paraffin, sectioned frontally 25 micra, and every fifth section was stained with hematoxylineosin. The sections were examined for the evidence of damage.

**Experiment I: Restoration of an Extinguished Bar-Pressing Response Following the Hippocampal Ablation**

There is a striking parallelism between the behavioral effects of hippocampal ablation and those of electro-convulsive shock (ECS). The loss of recent memory is one of the parallel effects (Duncan, 1949; Penfield & Milner, 1958). Considering the susceptibility of the hippocampus to seizure discharges, the parallel effects of hippocampal ablation and ECS would not be surprising. In regard to the inhibitory control of operant behavior, Kessler & Gellhorn (1943) found that the extinguished avoidance responses in rats can be restored by ECS. This result has been interpreted to mean that Pavlovian internal inhibition is specifically disturbed. ECS was also effective in re-establishing an extinguished lever-pressing habit (Griffiths, 1961). The first experiment was designed to investigate the effect of hippocampal ablation on an extinguished bar-pressing response.

**Method**

Subjects. The Ss were 16 naive male albino rats about three months old at the start of the experiment. They were housed individually throughout the experiment.

Apparatus. The apparatus was a Skinner box (25 cm × 18 cm × 16 cm), constructed of wood. A T-shaped metal bar was mounted on the center of the wall 6.5 cm from the floor. To the right of the bar was a food hopper through which a 0.05 g pellet was delivered to the dish immediately below the bar.

Illumination was provided by a white 10-c.p. bulb which was mounted at the center of the lid of the Skinner box.

Procedure.

<Preliminary Training> The Ss were first adapted to a 22-1/2-hr schedule of food deprivation for twelve days. Water was continuously available. The final four days of pre-training were used to adapt the Ss to the Skinner box. During the first two of these days, each S was placed in the Skinner box and remained there until ten pellets were eaten. This procedure was repeated twice a day for two days. On the final two days of pre-training, 30 pellets were daily delivered to the S one at a time by the experimenter's activation of the delivery mechanism. By this procedure the Ss were trained to approach the food tray at the sound of the magazine click associated with the delivery of food.

<Training> After the completion of preliminary training the Ss were trained to press the bar on the continuous reinforcement schedule until they obtained 90 reinforcements (30 trials per day for three days).

<Extinction> Following the training procedure mentioned above the bar-pressing response was extinguished by the lack of food
The effect of hippocampal ablation on an extinguished bar-pressing response

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Preoperative extinction</th>
<th>Postoperative test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number of responses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>77.4</td>
<td>54.1</td>
</tr>
<tr>
<td></td>
<td>(55-105)</td>
<td>(28-78)</td>
</tr>
<tr>
<td>H</td>
<td>78.3</td>
<td>53.4</td>
</tr>
<tr>
<td></td>
<td>(31-106)</td>
<td>(30-62)</td>
</tr>
<tr>
<td>Number of responses to criterion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>63.9</td>
<td>37.4</td>
</tr>
<tr>
<td></td>
<td>(39-105)</td>
<td>(16-64)</td>
</tr>
<tr>
<td>H</td>
<td>66.7</td>
<td>42.7</td>
</tr>
<tr>
<td></td>
<td>(32-126)</td>
<td>(10-94)</td>
</tr>
<tr>
<td>Time to criterion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>686.6</td>
<td>477.3</td>
</tr>
<tr>
<td></td>
<td>(384-1200)</td>
<td>(228-634)</td>
</tr>
<tr>
<td>H</td>
<td>685.3</td>
<td>556.6</td>
</tr>
<tr>
<td></td>
<td>(440-1200)</td>
<td>(413-724)</td>
</tr>
</tbody>
</table>

Note: Values in parenthesis indicate range. Abbreviations used are CC, cortical control and H, hippocampal.

reinforcement. Auditory cues associated with the delivery of food were also omitted. The extinction procedure consisted of daily 20-min period for three successive days. The criterion of extinction was the lapse of a 3-min period during which there were no bar presses. On the 19th day, half the animals were assigned to the experimental (hippocampally ablated) group while the remaining half to the control (neocortically ablated) group on the basis of their scores during the extinction. All animals were then subjected to operation.

<Postoperative Test> After the three weeks recovery period, the Ss were again put on the deprivation schedule for seven days and were then tested on the spontaneous recovery of the preoperatively extinguished bar-pressing response for two days. The test procedure was the same as the extinction procedure used preoperatively.

Results and Discussion

The results are presented in Table 1. There were no group differences during the preoperative extinction for each of the measures. It can be seen, however, that the hippocampally ablated animals showed a higher rate of bar pressing, requiring more responses and time in reaching the criterion of extinction than did the control (neocortically ablated) animals during the post-operative test period. These differences are all statistically significant (p < .01—Mann-Whitney U Test 2).

It is also evident from Table 1 that hippocampal ablation led to the restoration of the previously extinguished bar-pressing response, whereas neocortical ablation as a control was not so effective. Although the degree of recovery was small, it was statistically significant (responses to the criterion, p < .01—Wilcoxon matched-pairs signed-ranks test 2) except for one measure, that is, total number of responses.

It can thus be said that hippocampal

2 Siegel (1956)
ablation resulted in "disinhibition" of the previously extinguished bar-pressing response. This result should be interpreted to mean that hippocampal ablation produced a loss of inhibition normally associated with nonreinforcement.

Obviously, we find here a remarkable parallelism between the effect of hippocampal ablation and that of ECS in restoring the inhibited conditioned responses.

**EXPERIMENT II: THE EFFECT OF HIPPOCAMPAL ABLATION ON ACQUISITION AND EXTINCTION OF A DIFFERENTIAL BAR-PRESSING RESPONSE**

The second experiment deals with the effect of hippocampal ablation upon the acquisition and extinction of a differential bar-pressing response. Previous reports have indicated that hippocampal ablation results in a greater resistance to extinction under certain experimental conditions (Isaacson et al., 1961; Niki, 1962; Jarrard et al., 1964). The aim of this experiment is to determine whether an increased resistance to extinction following the hippocampal ablation would occur in a differential bar-pressing situation.

**Method**

**Subjects.** The Ss were the same 16 animals used in Experiment I. They were approximately 135 days old at the start of this experiment.

**Apparatus.** The apparatus was a modified Skinner box (30 cm × 30 cm × 36 cm) with two bars and two pellet-dishes (4 cm square). The bar was T-shaped, consisting of an aluminum cylinder, and was placed 3 cm off the floor. The top of the apparatus was closed with a translucent glass cover, above which was mounted a light source (a 10-c.p. bulb).

**Procedure.** The procedure may be summarized as follows:

Days 1–2. Preliminary training. The Ss were adapted to the test apparatus, both bars taken out. On the second day one bar was inserted and the Ss were trained to press the bar until they received 20 reinforcements. Half of the Ss were given access to the right bar and the other half to the left.

Day 3. Differential bar-pressing training. At this point both bars were inserted in the apparatus and the Ss were required to depress one of the two bars until they received 60 reinforcements. The bar used in the pretraining was this time irrelevant, its pressing being never reinforced.

Day 4. Extinction. On the fourth day each S was placed in the apparatus and allowed to remain for 20 min during which the food magazine did not operate.

**Results and Discussion**

Performance curves during the acquisition of differential bar-pressing response are presented in Fig. 1. As indicated in this figure, there was little difference between the two groups during the acquisition. During the extinction, however, the hippocampally ablated animals responded much more frequently than did the neocortically ablated control group. Fig. 2 is a graphic presentation of the number of bar-pressing responses to relevant and irrelevant bars during the extinction. Statistical analysis indicates that the differences between the two groups are significant (relevant response, \( p < .01 \); irrelevant response, \( p < .05 \)—Mann-Whitney \( U \) Test).

![Fig. 1. Median number of irrelevant responses during acquisition.](image)
The hippocampally ablated animals, therefore, depressed both relevant and irrelevant bars more often during the extinction than did the neocortically ablated animals.

In order to evaluate the over-all resistance to extinction of differential bar-pressing response, the percentages of relevant responses throughout extinction were calculated for each animal. Table 2 shows the mean percentages of relevant responses during the extinction. It can be seen that the hippocampal group is superior to the cortical control group during the extinction \((p<.05—\text{Mann-Whitney } U \text{ Test})\).

**EXPERIMENT III: THE EFFECT OF HIPPOCAMPAL ABLATION ON BAR-PRESSING RESPONSES DURING NONREINFORCEMENT**

In order to obtain a further information on the role of the hippocampus in the inhibitory control of operant behavior, the third experiment was undertaken. In this experiment, the ability of the hippocampally ablated animals to inhibit responding during nonreinforcement was investigated.

**Method**

**Subjects.** Twenty-six naive male albino rats were used as Ss. All were three months old at the time of surgery. They were randomly assigned to one of the three groups: I. Hippocampally operated \((N=7)\); II. Cortically operated control \((N=9)\); III. Sham-operated control \((N=10)\).

**Apparatus.** The apparatus was the same Skinner box used in Experiment I.

**Procedure.** Following recovery from surgery the Ss were put on a 22-hr food deprivation schedule one week before training. On the first two days of experimentation the operant level of responding was measured for 30 min each day. No significant differences in operant level among the groups were found. They were then trained to press the bar under the continuous reinforcement schedule until a stable rate of bar pressing was shaped. Other details of the preliminary training procedure were
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Almost the same as employed in Experiment I. All of the preliminary training required 10–14 days.

Following the preliminary training, the Ss received discrimination training for 12 days with a bright light (10 watt) serving as a discriminative stimulus.

A 4-min nonreinforced period intervened between the two 2-min reinforced periods in the daily experimental session.

Results and Discussion

Fig. 3 represents the median number of bar presses emitted by the three groups of animals during the daily reinforced and nonreinforced periods. By referring to Fig. 3, it will be seen that the response rate during the nonreinforced period was higher in the hippocampally ablated animals than in the other control animals although the rate of responding during the reinforced period was similar for different groups. The Mann-Whitney U Test was used to analyze these data. Table 3 shows the U values of various group comparisons. The higher response rate of the hippocampally ablated animals during the nonreinforced period seems to reflect their inability to inhibit responding to nonreinforced stimuli. These findings agree with those of Jarrard (1965) and Clark & Isaacson (1965), who found that hippocampectomized rats could not adjust their responding to fit the schedule of reinforcement and consistently pressed at higher rate than controls under VI and DRL.

In order to evaluate the discriminatory behavior of the animals, a discrimination

<table>
<thead>
<tr>
<th>Groups compared</th>
<th>1st 2 min non-reinforced period</th>
<th>2nd 2 min non-reinforced period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U</td>
<td>p</td>
</tr>
<tr>
<td>H : C</td>
<td>1.5</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>H : CC</td>
<td>2.5</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Table 3
Significance tests for intergroup comparisons during nonreinforcement

Fig. 3. Median number of bar-pressing responses during reinforced and nonreinforced periods. (C = control Ss; CC = cortical control Ss; H = hippocampal Ss).
ratio was calculated each day for every animal. The discrimination ratio was defined as the ratio of the rate of responding during the nonreinforced period over that of responding during the reinforced period.

The median discrimination ratio of the last three days of discrimination training provided a single index of each S’s discriminatory behavior. The group medians based on this measure were 1.06, 0.25, and 0.20 respectively for the hippocampal, cortical control, and sham-operated control groups. The differences between the hippocampal group and each of the two control groups are significant by the Mann-Whitney U test (Hippocampal vs Cortical Control, \( p < .001 \); Hippocampal vs Sham-operated Control, \( p < .001 \)).

Thus, the hippocampally ablated animals showed a consistent impairment on discrimination. It would appear that the impairment was closely associated with significantly higher rate of nonreinforced responding without concomitant increases in reinforced response rate. In other words, the hippocampally ablated animals cannot react adequately to the nonreinforced component of the discrimination.

**Experiment IV: The Effect of Hippocampal Ablation on a Decline in Runway Performance due to the Delay of Reinforcement**

In the fourth experiment, hippocampally ablated animals are compared to cortical and sham-operated controls in regard to their performance decrement due to the delay of reinforcement. Considering the greater resistance to extinction of the hippocampally ablated animals in a runway situation, it is reasonably expected that the runway performances of the hippocampally ablated animals would be less affected by the delay of reinforcement.

**Method**

*Subjects.* The Ss which were the same as those in Experiment III were reduced in number to 7 in the cortical control and 7 in the sham-operated control group. They were approximately five months old at the beginning of this experiment at which time they were placed on a 22-hr feeding schedule for ten days.

*Apparatus.* The apparatus consisted of an alley (100 cm x 10 cm x 28 cm), a start box (22 cm x 30 cm x 28 cm) and a goal box (22 cm x 30 cm x 28 cm). Two guillotine doors were used to prevent retracing. One was placed between the start box and the runway, the other being located between the runway and the goal box. In front of the food cup in the goal box, there was a vertical sliding door which was lowered during the 30 sec delay interval.

*Procedure.*

<Adaptation> Prior to training each animal was placed in the apparatus without food reward in the goal box for 30 min and was allowed to explore freely. On the two days preceding training, each animal was placed in the goal box for 5 min access to food.

<Training> Each animal received 10 training trials per day for five days with an inter-trial interval of 10 min. The reward given was a 0.5 g piece of cheese.

<Test> After 50 training trials the test trials were begun. Thirty test trials, ten per day, were given with an inter-trial interval of 10 min. The only change in procedure during the test was that the animals had to wait in the goal box for 30 sec before they were given access to food reward. Starting times (ST) and running times (RT) were measured on all training and test trials.

*Results and Discussion*  

The results of the training and test trials are summarized in Figs. 4 and 5, where median starting and running times have been plotted for successive days. As indicated in these figures there was no difference between the groups in training trials; in test trials, however, the Ss in the hippocampal group had shorter ST and RT than those Ss in the other two control groups. In the analysis of these data, the ratios of the median ST (RT) of the 1st, 2nd, and 3rd 10 test trials to the median
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FIG. 4. Median starting time during training and test. (H = hippocampal Ss; CC = cortical control Ss; C = control Ss.)

ST (RT) of the last 10 training trials, were computed for each animal. With this ratio measure, differences between the groups taken two at a time were tested by the Mann-Whitney U Test. Table 4 shows the U values of various group comparisons.

With ST ratio measure, we find that the hippocampal group is significantly different from each of the other two control groups except for the first day.

In terms of RT ratio measure, all the differences between the hippocampal group and each of the two control groups are found to be significant.

Thus, hippocampal ablation seems to interfere with the development of response inhibition associated with the delay of reinforcement.

ANATOMICAL RESULTS

Fig. 6 presents a diagrammatic summary of the typical brain lesions for the hippocampal and neocortical groups. The primary lesions were located in the standard atlas of de Groot (1959). In general, the destruction of both hippocampus and neocortex was much greater than in the previous study (Niki, 1962). The extent of hippocampal damage varied from about 30% to 80% in Experiments I & II and 25% to 75% in Experiments III & IV. A correlation between behavioral changes and the degree of hippocampal damage was not found in this study. In no case was the hippocampus completely ablated, but the middle portion of the hippocampus was constantly ablated for all experimental animals. The most rostral and ventral portions were spared. The overlying cortical lesions in the hippocampal group chiefly involved the visual projection area.

The attempt to equate the total volume of destruction in the hippocampal and neocortical groups was fairly successful.

TABLE 4
Mann-Whitney U values for intergroup comparisons during test trials

<table>
<thead>
<tr>
<th>Groups compared</th>
<th>ST Test trials</th>
<th>RT Test trials</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-10 11-20 21-30 1-30</td>
<td>1-10 11-20 21-30 1-30</td>
</tr>
<tr>
<td>H : C</td>
<td>14 4 11 6.5</td>
<td>2.5 0 0 0</td>
</tr>
<tr>
<td>H : CC</td>
<td>17 11 8 11</td>
<td>0 3 5 3</td>
</tr>
</tbody>
</table>

Note: Critical value required for significance at .01 (.05) is 6 (11).
Damage to other structures was slight in both groups.

Damage in the cortical control group was largely restricted to the dorsal neocortex. The extent of the cortical lesion in the control group was consistently larger than in the hippocampal group.

**General Discussions**

The results of this study are as follows:

1. Hippocampal ablation resulted in "disinhibition" of the previously extinguished bar-pressing responses.
2. Hippocampal ablation produced the increased resistance to extinction of a differential bar-pressing response.
3. Hippocampal ablation selectively increased the rate of responding to negative stimuli without a concomitant increase in the response rate to positive...
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stimuli.

4. The runway performances of the hippocampally ablated animals were less affected by the delay of reinforcement. From these results it is clear that the hippocampus is important for the inhibitory control of operant behaviors under a variety of experimental conditions.

Since the hippocampally ablated animals showed no increase in food intake (Niki, 1962; Jarrard, 1963), it would be difficult to account for the present results on the basis of an increase in food motivation. Although an increase in general activity has been reported (Niki, 1962; Kimble, 1963; Teitelbaum & Milner, 1963; Douglas & Isaacs, 1964), the present results cannot be attributed to an over-all increase in activity. The following observations would be relevant here:

First, the data for operant level of bar pressing showed no group differences (Experiment III).

Second, the hippocampally ablated animals showed no increase in the rate of reinforced bar-pressing response (Experiment III).

Third, the hippocampally ablated animals failed to run faster than controls during the acquisition of running responses in the runway (Niki, 1962; Jarrard et al., 1964; Experiment IV in this study).

The involvement of other neural structures such as the amygdala (Brutkowski et al., 1960; Schwartzbaum et al., 1964a), the septal area (McCleary, 1961; Kaada et al., 1962; Ellen et al., 1964; Schwartzbaum et al., 1964b), and the frontal cortex (Lichtenstein, 1950; Brutkowski, 1959; Butter et al. 1963; Glickstein et al., 1964) have been implicated in the inhibitory control of operant behaviors. For example, passive avoidance performance is impaired by frontal lesions in dogs (Lichtenstein, 1950). Septal lesions in rats have a similar effect (McCleary, 1961; Kaada et al., 1962). An inability to inhibit responding to negative stimuli in certain types of discriminations has been found following lesions in the amygdala (Brutkowski et al., 1960; Schwartzbaum et al., 1964a), the septum (Schwartzbaum et al., 1964b), and the frontal cortex (Brutkowski, 1959).

Finally, a similar inability to withhold responses during the delay period under DRL is also observed following either septal or frontal lesion (Ellen et al., 1964; Glickstein et al., 1964). It is quite possible that these neural structures as well as the hippocampus form a common diffuse system which exerts an inhibitory control over the behavior. It would be therefore of value to study possible similarities and differences among the effect of each lesion in these structures.

On the other hand, there is a remarkable parallelism between the effect of hippocampal ablation and that of cholinergic blocking agents (scopolamine, atropine) on the inhibitory control of operant behavior (Carlton, 1963). Increased responding during the nonreinforced component of a multiple-schedule or discrimination was reported following the administration of scopolamine or atropine (Herrnstein, 1958; Brady, 1959; Hearst, 1959; Carlton, 1961). Since administration of atropine was found to eliminate the hippocampal arousal pattern, it is possible that the hippocampus would be depressed by atropine. Consequently, the failure in adequate inhibitory control of operant behaviors following the administration of cholinergic blocking agents may be related to the functional depression of the hippocampus.

Finally, it should be borne in mind that although the present data support the hypothesis of a hippocampal role in the inhibitory control of behavior, they do not exclude the possible involvement of the hippocampus in other functions, such as emotion and memory.

In conclusion, it can be stated that one of the functions of the hippocampus in rats is the inhibitory control of behavior.
REFERENCES


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(Received Sep. 7, 1965)