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Pup Survival and Development Following Hippocampal Lesions in the Female Rat

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ABSTRACT. Animals with hippocampal lesions, induced by aspiration, are inferior to cortical control and normal animals in providing some aspects of maternal care needed for the proper survival of their young. Pup survival of animals with hippocampal lesions is significantly lower than pup survival of cortical control and normal animals. Nest construction of dams with hippocampal lesions is inferior to the cortical control and normal groups. Female animals with hippocampal lesions retrieve sensory altered pups more slowly than cortical control and normal animals. The deficits in retrieval behavior in animals with hippocampal lesions may be linked to defects in their ability to properly sequence cues. On day 5 after parturition, pups born to animals with hippocampal lesions weigh significantly less than those born to cortical control and normal animals. By day 14 after parturition, there are no significant differences between pup weights of animals born to hippocampally lesioned dams and those born to normal dams. If pups survive to 21 days after parturition, there are no significant differences in a number of serum components when pups born to hippocampally lesioned animals are compared to control pups. Serum levels of the following are similar in all three groups of pups: TSH, total T₄, free T₄, Na, K, Cl, P, Fe, glucose, uric acid, blood urea nitrogen, creatinine, cholesterol, triglycerides, total protein, albumin, and bilirubin. If pups born to mothers with hippocampal lesions survive until weaning, endocrinological and biochemical abnormalities are not apparent.

INTRODUCTION

It has been hypothesized that the hippocampus is the core of a neural memory system providing an objective spatial framework within which the items and events of an organism's experience are located and interrelated (O'Keefe and Nadel 1978). Available neuroanatomical, neurophysiological, and behavioral data suggest that several different behavioral processes may be affected when the hippocampus is experimentally altered (Jarrard 1973). There is strong clinical evidence that the hippocampus is involved in human memory (Milner 1968). Evidence from animal research suggests a role for the hippocampus in response perseveration (Douglas 1967, Kimble 1968). The hippocampus also has a significant influence on nonspecific changes in activation or arousal, and possibly some specific types of incentive motivation such as thirst and hunger (Jarrard 1973).

Mammalian maternal behavior is a complex behavioral pattern that is affected by both internal and external factors. The maternal behavior of the rat has been studied extensively. Components such as licking, nursing, nesting, and retrieving have been measured under both normal and abnormal conditions. Disturbances of
normal maternal behavior have occurred following: 1) experience of the rats, and 2) changes in physiology in these animals.

Changes in maternal experience of the rat have been brought about by deprivation of a particular aspect of maternal behavior. For example, female rats raised without any nest building experience since birth, fail to build nests when they become pregnant (Riess 1950). Female rats raised with rubber collars around their necks so as to eliminate the opportunity for self-licking or sniffing, fail to lick or sniff their own pups and eat them instead (Birch 1956). These experiments suggest that maternal behavior is influenced by early experience and learning. Disruption of maternal behavior may, therefore, involve impairment of recall or use of memory from past experience.

Changes in brain physiology have also been found to affect the maternal care given by the female rat. Neuronal control of maternal behavior has been studied by stimulating or ablating areas of the rat brain and noting the resulting behavioral changes. Since stimulation of brain areas is difficult because it must be applied continuously, lesions are usually the preferred method of study in experiments on maternal behavior. Both cortical and subcortical electrolytic and aspiration lesions have been used, and behavioral changes have often resulted in a deficit or improvement of maternal care. Aspiration lesions of the median cortex have produced deficits in nest building and reconstruction, nursing, and pup retrieval (Stamm 1955). Damage to areas in the limbic system have also produced profound deficits in maternal behavior of the rat. Rats with electrolytic lesions of the septum, inflicted prior to mating, fail to nurse or retrieve their pups properly (Fleischer and Slotnick 1978). Instead of proper retrieval, the dams carry their pups and drop them in a disorderly manner about their cages. Damage to the fimbria, an area closely associated with the hippocampus, causes disorderly nest building as evidenced by nests and pups which are generally scattered in several sites around their observation boxes (Terlecki and Sainsbury 1978). The hippocampus is an area of the limbic system which has been found to be necessary for proper maternal response (Kimble et al. 1967). In the study of Kimble et al., bilateral dorsal aspiration lesions of the hippocampus were performed prior to mating. The maternal behavior of the dams was measured primarily by allowing the dams to carry on their normal behavior without interruptions during the first five days after parturition. During this time, rats were observed during two periods each day and the time spent in different activities was recorded. It was found that animals with hippocampal lesions spent significantly less time nursing and nest building, but spent a greater amount of time exploring, grooming, and licking pups. Direct tests of retrieving were also conducted and the performance of dams with hippocampal damage was slightly inferior to control dams.

The present study was designed to extend the findings with regard to the effects of lesions of the hippocampus on the maternal behavior of the rat, and to examine the role of experiential factors in these effects. In a more general sense, one emphasis of the experiment was on learning based upon the ability to form discriminations, that is, how an animal comes to identify the relevant aspects of an environment and how it chooses adaptive behaviors. The choice of adaptive behavior is crucial for the survival of animals. This principle also applies to the human who must adapt to environmental circumstances.

The present study was conducted in order to assess the effects of large (dorsal and ventral) bilateral hippocampal lesions on maternal behavior. Previous studies of maternal behavior were modified in order to study the effects of hippocampal lesions on discriminatory retrieving in which the sensory stimuli (visual, olfactory, and tactile) of the pups were altered. The other behaviors involved in maternal care such as licking, nesting, nursing, and retrieving were rated daily for the first five days after parturition. Pup weights, pup survival rates, and pup blood chemistry were examined in order to determine the effects of such lesions on offspring born to these brain damaged animals.

**MATERIALS AND METHODS**

**SUBJECTS.** The subjects used were 60 female Sprague-Dawley rats weighing between 250 and 300 g at the time of the operation. These animals were weighed and randomly divided into three groups of 20 animals each. The first group received bilateral hippocampal lesions. The second group served as cortical control, and the third group served as normal controls (N). Surgery was performed under ether anesthesia 10 days after the arrival of the animals at the laboratory. For the hippocampal lesioned and cortical control animals, a midline incision was made on the scalp. All animals in these two groups had the bone between bregma and lambda removed with a trephine, thus exposing that part of the cortex overlying the dorsal hippocampus. All lesions were made by aspiration with a 22-gauge blunt-tipped needle. Following removal of the exposed cortex and corpus callosum by aspiration in the hippocampotectomedized group, bilateral aspiration of the dorsal, lateral, and ventral hippocampus was performed. In the cortical control group, only the cortex and corpus callosum overlying the hippocampus were bilaterally aspirated. In these cortical control animals the hippocampus was exposed but not damaged. Normal controls received no surgical treatment. In both hippocampotectomedized and cortical control animals gel foam was inserted into the lesioned areas and the scalp was closed with stainless steel wound clamps. Animals were allowed to recover from the surgery for three weeks before being tested. All animals were maintained and used in accordance with the Guide for the Care and Use of Laboratory Animals (DHEW).

**HISTOLOGICAL PROCEDURE.** At the conclusion of the experiment the animals were sacrificed by decapitation. The brains were immediately removed and embedded in paraffin and sectioned at 15 μ. Every 10th section through the lesion was mounted and stained according to the method of Klüver and Barrera (1953). For the cortical control group, the same area of cortex was exposed as in the animals with hippocampal lesions. The medial border of cortical lesions was 1 to 1¼ mm from the midline. The cingulate gyrus was not damaged. The lateral border of cortical lesions was approximately 6 mm from the midline. The anterior-posterior ablation was from coordinates anterior 3.0 to anterior 1.3 (Pellegrino and Cushman 1967). For animals with hippocampal lesions, the anterior-posterior ablation of the hippocampus was from coordinates anterior 4.5 to anterior 0.5. The dorsal and lateral portions of the hippocampus, including the subiculum, were completely removed in all animals, but some variability among lesions occurred with respect to the ventral extent of the ablations. The anterior ventral portion was typically spared. The fimbria was transected in all animals. Extent of damage to the hippocampus ranged from 75% to 90% of the structure.

**EXPERIMENTAL PROCEDURE.** In the experiment, the female animals were kept in individual mating cages with ad libitum access to food and water. They were placed in alternating periods of light and dark each day. The lights went on at 7:00 a.m. and off at 7:00 p.m. The female rats were placed in the mating boxes two days prior to the introduction of male rats. The mating boxes were 25 cm × 45.5 cm × 15.5 cm. The sides and bottom of the boxes were opaque white plastic. The top of the boxes consisted of metal bars which were slanted at one end so that the water bottle and Purina rat chow pellets could be placed on the top of the bars. Twenty normal male rats were rotated among the females for 30 days, allowing each female to be with a male rat for 10 days (about two cycles). After removal of the male rats, five Scott Brand Utility Wipes were placed between the
bars at the top of the box so that the female rats could pull them into the box. Within 19 to 40 days, 16 of 20 hippocampally lesioned, 18 of 20 cortically lesioned, and 15 of 20 normal females delivered pups. Any females that did not deliver pups were excluded from further experimentation. When a female was discovered delivering pups or with a litter, the date of discovery was counted as day 1. All litters were discovered within 12 hours of delivery.

For the first five days after parturition, indirect indices of maternal performance were taken. Nursing, nest building, licking, and retrieving were rated twice each day; once between 7:30 and 7:45 a.m. and a second time between 6:15 and 6:30 p.m. An exact 12-hour observation schedule was not kept in order to maintain the rats on a 12-hour light-dark cycle. When rating the dam, the observer stood to the side of the box and noted the behavior of the rat upon immediate observation. Licking behavior was rated by noting if the dam was licking any of her pups. The number of times this occurred was recorded. Next, the number of live pups in the observation box were counted and recorded. If all of the pups were not visible, the mother was moved gently until all of the pups were visible. To rate nursing behavior, the number of pups which were attached to a nipple were counted, and this number was divided by the total number of live pups in the observation box.

The quality of the nest was graded according to a rating scale similar to one used by Stammi (1955), while studying the effects of median cerebral cortex lesion on maternal behavior. The following index of nesting was used:

0 — Rat ignored nesting material completely.
1 — Rat pulled nesting materials into box and moved them around.
2 — Towels were assembled for a simple covering in a limited area (no nest construction).
3 — A crude nest assembled, most of paper towels used, some torn and arranged in a limited floor area. (This rating also applies when two or more small nests were constructed in different corners of the box.)
4 — A real nest using all paper towels was constructed. The towels were torn and the nest appeared compact with a good floor and solid sides.
5 — An excellent compact nest was constructed. Generally all available materials including the smallest bits of paper towels, rat pellets, and even feces were used in nest structure and the floor of both compartments appeared very clean.

Retrieving behavior was rated by counting the number of pups that were entirely within the boundaries of the nest. All of the dams arranged the paper towels in some fashion and the boundaries of these arrangements were considered as the boundaries of the nest. No part of the pup, including the tail, could be partially or entirely outside of the nest for the pup to be considered retrieved.

Direct tests of retrieving behavior were conducted within 18 to 24 hours after parturition. In order to test discriminatory retrieving ability of the mother, four pups were removed from the nest with clean forceps wiped with alcohol. The pups were then placed on a paper towel and one pup was covered with a thin coat of vaseline on all areas except the eyes and nose. One pup was covered with a light coat of food powder made from finely ground Purina Chow Pellets. The third was placed in a box with a nonexperimental female rat for one minute. The fourth pup was left untouched. (Thus, the first three pups had altered sensory stimuli.) The pups were returned to the observation box by placing the pup 1 inch (2.54 cm) from the outside border of the middle of the nest. The time it took for the dam to return the entire pup to the nest was recorded. If the dam did not return the pup within five minutes, the trial was terminated and the pup was returned to the nest near the mother. The order of pups returned to the observation box was randomized in order to avoid testing of retrieval order.

After the testing and rating of each rat was completed, the females were allowed to care for their pups without intervention. Body weights were recorded for all pups at 5, 14, and 21 days after parturition.

At 21 days of age the pups were sacrificed by decapitation and the following values were determined: TSH, total T₄, free T₄, Na, K, Cl, P, Fe, glucose, uric acid, blood urea nitrogen, creatinine, cholesterol, triglycerides, total protein, albumin, and bilirubin. Serum thyroid stimulating hormone (TSH) and total thyroxine (T₄) levels were determined by radioimmunoassay. Rat TSH standard was provided by Dr. A. F. Parlow through the National Institute of Arthritis, Metabolism, and Digestive Diseases. The other tests on the serum were done in the Technicon SMAC system (an automated system used by Consolidated Biomedical Laboratories).

Statistical comparisons of the three different groups of rats with respect to survival, various behavioral measures, and plasma constituents in offspring were made by an analysis of variance (ANOVA). If statistical differences were found among the three groups, ANOVA was followed by a protected t-test to determine significant differences between groups.

RESULTS

Pup survival to 21 days after parturition was significantly different for the three groups (Fig. 1). From the daily rating scores of maternal behavior, it was found that pup survival of hippocampally lesioned dams was significantly lower than pup survival of cortically lesioned controls (p < .01) and normal controls (p < .01).

Nursing scores also showed some differences among the groups (Fig. 2). Nursing scores were significantly different when comparing hippocampally lesioned dams and cortically lesioned dams (p < .01). There were no significant differences between hippocampally lesioned dams and normal dams or between cortically lesioned dams and normal dams.

Nest construction data also indicated impairment in animals with hippocampal lesions (Fig. 3). The nest construction of hippocampally lesioned dams was inferior to nest construction of cortical controls (p < .05) and normal animals (p < .01).

Pup licking behavior was also recorded, though dams were seldom found licking their pups (15 minutes X 10 observations periods = 150 minutes of observation). The total of all licking scores for animals with hippocampal lesions was seven times; for cortical controls it was six times, and for normal controls it was nine times.

![Figure 1. Maternal behavior of rat dams as evidenced by survival of pups to 21 days. (Bars on all figures indicate S.E.M.)](image-url)
Data on retrieval behavior revealed interesting and unexpected results (Fig. 4). During the retrieving tests no significant differences were found between retrieval scores of hippocampally lesioned, cortically lesioned, or normal dams when the rats were retrieving an untreated pup. The hippocampally lesioned dams were significantly slower than control groups when retrieving a vaseline covered (H vs C, p < .05; H vs N, p < .05), a food powder covered (H vs C, p < .01; H vs N, p < .01), or a scent contaminated pup (H vs C, p < .01; H vs N, p < .05). Thus, sensory alteration of pups influenced retrieval behavior.

Pup weights were recorded on days 5, 14, and 21 after parturition. Differences between and among groups were found on days 5 and 14 after birth (Fig. 5). On day five, pups of hippocampally lesioned dams weighed significantly less than cortical control (p < .05) and normal dams (p < .01). On day 14, pup weights of cortical control animals were significantly higher than normal animals. On day 14, there were no significant differences between animals with hippocampal lesions and normal animals. No significant differences were found among the three groups 21 days after birth.

There were no significant differences among 21-day-old pups born of mothers with hippocampal lesions, cortical control, and normal animals in any of the serum constituents that were measured (TABLE 1). The pups born to mothers with hippocampal lesions that survived to 21 days of age did not show the endocrinological and biochemical abnormalities typically found in animals with hippocampal lesions.

**DISCUSSION**

The present experiment, as well as the previous research of Kimble et al. (1967), indicates that bilateral hippocampal lesions disrupt maternal behavior and pre-
vent normal pup survival during the early preweaning period. Deficits in nursing behavior do not correlate with the study of Kimble et al., since they found significant differences in nursing behavior when animals with hippocampal lesions were compared with normal dams. Differences between the experiment of Kimble et al. and this experiment include: experimental method (e.g., anesthesia and time after operation to testing), extent of hippocampal lesion (e.g., dorsal vs dorsal-lateral-ventral lesions), and laboratory conditions and procedure (e.g., previous training in a maize vs naive subjects). Despite the differences in the experiment of Kimble et al. and the present experiment, both studies demonstrate that hippocampal lesioned rats are inferior to control rats in providing some aspect or aspects of maternal care needed for the proper survival of their young.

Low survival rates of pups born from hippocampal lesioned dams could be the result of poor nest construction. Nest construction is an important factor in pup survival. Failure to construct a proper nest which provides warmth can be fatal to pups which are poikilothermic during the early preweaning period (Terlecki and Sainsbury 1978). Kim (1960) found that hippocampal lesions produced a deficit in male rats subjected to low temperatures. The same may be true for female rats with hippocampal lesions. Kimble et al. (1967) found that hippocampal lesions produce a deficit in nest building in maternal rats; the present study supports that finding.

Low survival scores could be caused by other factors, including maternal cannibalism or improper nursing. Maternal cannibalism was never observed during this experiment. Observation time during this experiment was somewhat short and cannibalism may have occurred during non-observation times, but there were no pieces of young found and there were no mysteriously missing pups. Kimble et al. (1967) found that maternal cannibalism occurred in five out of seven of the dams with hippocampal lesions.

Improper nursing behavior could be a cause of low pup survival. Kimble et al. (1967) noted that dams with hippocampal lesions expressed abnormal nursing behavior by crouching in a nursing posture away from the pups. Crouching outside of the nest and other abnormal nursing behaviors occurred in dams with hippocampal lesions in the present study. Occasionally the dams were found sitting on the pups instead of assuming a crouched position, and sometimes dams were found crouching in a nursing posture over nesting material.

There are several other factors which may have contributed to the poor survival rate of pups born to dams with hippocampal lesions. These include: poor milk quality, congenital defects, improper licking, or violence between pups during the later stages of develop-

**Table 1**

<table>
<thead>
<tr>
<th>Blood constituent</th>
<th>Hippocampal lesioned</th>
<th>Cortical controls</th>
<th>Normal controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH (µg/dl)</td>
<td>322.81 ± 32.25</td>
<td>348.04 ± 32.45</td>
<td>332.01 ± 24.76</td>
</tr>
<tr>
<td>Total T (µg/dl)</td>
<td>2.23 ± 0.21</td>
<td>2.52 ± 0.12</td>
<td>2.48 ± 0.13</td>
</tr>
<tr>
<td>Free T (ng/ml)</td>
<td>0.96 ± 0.05</td>
<td>1.04 ± 0.06</td>
<td>0.99 ± 0.04</td>
</tr>
<tr>
<td>Na (meq/l)</td>
<td>137.67 ± 1.04</td>
<td>137.44 ± 0.71</td>
<td>139.07 ± 0.33</td>
</tr>
<tr>
<td>K (meq/l)</td>
<td>7.98 ± 0.16</td>
<td>8.33 ± 0.16</td>
<td>8.03 ± 0.10</td>
</tr>
<tr>
<td>Cl (meq/l)</td>
<td>100.17 ± 0.71</td>
<td>98.89 ± 0.54</td>
<td>100.40 ± 0.35</td>
</tr>
<tr>
<td>P (mg/dl)</td>
<td>8.68 ± 0.13</td>
<td>8.94 ± 0.18</td>
<td>8.81 ± 0.14</td>
</tr>
<tr>
<td>Fe (mg/dl)</td>
<td>518.33 ± 81.56</td>
<td>420.33 ± 45.54</td>
<td>438.58 ± 30.72</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>163.17 ± 2.61</td>
<td>165.78 ± 2.10</td>
<td>160.33 ± 4.41</td>
</tr>
<tr>
<td>Uric acid (mg%)</td>
<td>1.42 ± 0.06</td>
<td>1.37 ± 0.11</td>
<td>1.21 ± 0.05</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dl)</td>
<td>18.50 ± 0.47</td>
<td>16.11 ± 0.48</td>
<td>16.53 ± 0.60</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.38 ± 0.02</td>
<td>0.42 ± 0.02</td>
<td>0.41 ± 0.02</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>76.67 ± 3.86</td>
<td>71.11 ± 2.39</td>
<td>73.73 ± 2.18</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>116.50 ± 26.20</td>
<td>113.29 ± 9.71</td>
<td>124.10 ± 41.37</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>4.70 ± 0.14</td>
<td>4.49 ± 0.28</td>
<td>4.51 ± 0.05</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.93 ± 0.05</td>
<td>2.87 ± 0.02</td>
<td>2.89 ± 0.03</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>0.08 ± 0.02</td>
<td>0.12 ± 0.02</td>
<td>0.05 ± 0.02</td>
</tr>
</tbody>
</table>
ment. There are, however, insufficient data from the present study or previous studies to support any of these propositions.

Animals with hippocampal lesions retrieved sensory altered pups (covered with vaseline, or food powder, or scent contaminated) more slowly than control animals. Delay or failure to retrieve pups may be related to the following factors:

1 — The dam ignored her pup and made no attempt to retrieve.
2 — The dam carried the pup around the box and dropped it in different areas of the box.
3 — The dam rebuilt a nest around the unretrieved pup.
4 — The dam removed more pups from the nest.
5 — The dam licked, sniffed, or manipulated the pup excessively.

Dams with hippocampal lesions demonstrated all of these behaviors during retrieval experiments. Cortical control and normal dams demonstrated only points 1 and 5 of these retrieving behaviors.

Wiesner and Sheard (1933) found that normal dams did not display discriminatory behavior when retrieving their own or alien young. Their method of returning the pup to the nest was similar to the method used in the present experiment except that pups were dropped down a chute into the observation box instead of being returned with forceps. Beach and Jaynes (1956) found, however, that normal rats displayed some discrimination between retrieval of normal and sensory altered pups, when the dam was presented with more than one pup.

Many hypotheses have been proposed in order to explain the deficits in maternal and other behaviors produced by hippocampal lesions. It has been suggested that deficits in behavior are caused by defects in the animal's ability to properly sequence cues (Terlecki and Sainsbury 1978). This hypothesis seems to most closely correlate with the behavior observed during the present experiment. It is possible that dams with hippocampal lesions were unable to conduct the sequence: recognition of the pup, picking up of the pup, and return of the pup to the nest. When compared to the control groups, animals with hippocampal lesions most frequently interrupted this sequence of events. An example of a dam with hippocampal damage retrieving a vaseline covered pup is as follows: After the pup was returned to the observation box, the dam left the nest, sniffed and licked the pup, picked up the pup and carried it around, put the pup down outside of the nest, moved away from the pup, returned to the pup, licked and sniffed the pup, and returned it to the nest. Since this dam and other dams with hippocampal lesions were unable to carry out the normal retrieving sequences, it seems that this disruption in behavior could be the result of a sequencing deficit. Cortical control and normal animals did not exhibit these behaviors. The control animals picked up the pup and returned it to the nest. Further experimentation on sequencing tasks and responses to cues would be helpful in determining the nature of the deficits in animals with hippocampal lesions.

In the endocrinological and biochemical aspect of the present study, a number of chemicals were measured in the serum of the pups. The tests were conducted 21 days after birth and there were no significant differences among pups born of animals with hippocampal lesions, or cortical control and normal animals in any of the chemical substances measured. It thus appears that if pups born to mothers with hippocampal lesions can survive until weaning, they will not show endocrinological and biochemical abnormalities.

**LITERATURE CITED**

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