Hippocampal Lesions in Rhesus Monkeys Disrupt Emotional Responses but Not Reinforcer Devaluation Effects

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Background: Although the role of the hippocampus in emotional behavior has long been recognized, the extent to which the hippocampus plays a role in the regulation and expression of emotion in rhesus monkeys has not been systematically explored.

Methods: Rhesus monkeys (*Macaca mulatta*) with excitotoxic lesions of the hippocampal formation and unoperated control animals were assessed on two different types of emotional processing: defensive reactions to a potential predator (experiment 1) and ability to update the value of positive reinforcers, in this case food (experiment 2). Monkeys with aspiration lesions of the perirhinal cortex were also included in this study as an operated control group.

Results: In experiment 1, whereas both operated groups showed reduced latencies to retrieve food located near an innately fear-provoking stimulus, a fake snake, only monkeys with hippocampal lesions displayed reduced defensive reactions to the snake. In experiment 2, both operated groups performed as well as control animals when choosing objects flexibly based on the current value of a food.

Conclusions: These findings dissociate the hippocampus and perirhinal cortex in fear expression and specifically implicate the hippocampal formation in generating responses to stimuli that are potentially threatening.

Key Words: Devaluation, emotion, fear, hippocampus, monkey, perirhinal cortex, reward, snake

Studies examining the neural circuitry subserving the expression and regulation of emotion in nonhuman animals provide a foundation for understanding the neuroanatomical and neuropathological correlates of human mood and anxiety disorders. Such studies have focused primarily on the role of the amygdala, which is a critical site for both the acquisition and storage of fear memory (1–3) as well as for generating certain types of unconditioned defensive responses (4,5). Although a contribution of the hippocampus to emotional behavior has long been recognized (6–8), the extent to which it plays a role in the acquisition and expression of defensive behavior has not been systematically explored. Given the recent upsurge of interest in hippocampal contributions to the psychopathophysiology of certain clinical disorders such as depression and posttraumatic stress disorder (9–11), further investigation of hippocampal function in nonhuman primates is warranted.

Much of the evidence regarding the putative role of the hippocampus in defensive behavior is derived from rodent studies. First, in standard tests of conditioned fear, rats with hippocampal lesions are deficient in using information about specific stimuli (e.g., tones) as well as experimental contexts to generate defensive responses (1,12–16). Second, lesions of the ventral but not dorsal hippocampus produce robust anxiolytic effects such as reduced freezing in fear conditioning tasks, increased open arm exploration in elevated plus mazes, and reduced defensive reactions in response to threat stimuli such as cat odor (15–19). Given the paucity of information in monkeys, we evaluated rhesus monkeys with bilateral, selective, excitotoxic lesions of the hippocampus and unoperated control animals on two types of emotional processing. In experiment 1, we evaluated monkeys' emotional responses to an artificial snake and spider, common objects of fear and anxiety in humans (20). In experiment 2, we used a reinforcer devaluation (RD) task to assess monkeys' abilities to associate objects with the value of food rewards. Work in rats and monkeys has suggested that the amygdala but not the hippocampus is important for RD effects (21–23). Inasmuch as food valuation is provided by an affective tag (24), this task can be considered a test of emotional processing for positive, nonthreatening items. Given the findings cited above, we expected that hippocampal lesions would disrupt unconditioned emotional responses but not reinforcer devaluation effects. We also studied monkeys with perirhinal cortex lesions as an operated control group. Because Meunier *et al*. (25) showed that perirhinal cortex lesions had no effect on monkey's emotional reactions to an artificial snake, we expected the lesion to be without effect on this task.

Methods and Materials

Subjects

A total of 17 adult male rhesus monkeys (*Macaca mulatta*), ranging in weight from 6.7 to 12.3 kg at the start of behavioral training, were used for this study. The same monkeys served as subjects in both experiment 1 and experiment 2. They were housed individually in temperature-controlled rooms (76°–80°F) under diurnal conditions (12-hour light/dark cycle). There were eight monkeys with selective hippocampal lesions (group H), four monkeys with perirhinal cortex lesions (group PRh), and five unoperated control animals (group Con). All monkeys were fed a controlled diet of primate chow (catalog number 5038, PMI Feeds Inc., St. Louis, Missouri) supplemented with fresh fruit and vegetables. Water was available ad libitum. All procedures were approved by the National Institute of Mental Health (NIMH) Animal Care and Use Committee.

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Surgery

Excitotoxic Hippocampal Lesions. Monkeys were anesthetized with isoflurane gas (1%–3% to effect) and received injections of the excitotoxin *N*-methyl-D-aspartate (NMDA) bilaterally into the hippocampus in a single-stage surgery. Each monkey received injections into the uncal portion of the hippocampus via a dorsal approach and throughout the remaining rostrocaudal extent of the hippocampus via a longitudinal approach (26). At each site, 2.0 μL (.2 mol/L) of NMDA was injected. The lesion was intended to include the dentate gyrus, cornu ammonis (CA) fields, and subicular complex. Detailed methods are provided in Supplement 1.

Aspirative Perirhinal Cortex Lesions. Monkeys were anesthetized with isoflurane gas (1%–3% to effect) and received bilateral removals of the perirhinal cortex by subpial aspiration. The lesion included the cortex on the lateral bank of the rhinal sulcus as well as 2 to 3 mm of the cortex lateral to the sulcus. The medial boundary of the lesion was the fundus of the rhinal sulcus. Detailed methods are provided in Supplement 1.

Lesion Assessment

Hippocampal Lesions. The extent of the lesion in the monkeys with hippocampal lesions was assessed in two different ways. The brains of four monkeys (H1–H4) underwent traditional histological processing. The size of the lesion was then estimated by expressing the extent of damage to the hippocampal formation (dentate gyrus, CA fields, subicular complex) relative to the volume of the same region in a standard rhesus monkey brain. The NMDA produced cell loss in 45% of the hippocampal formation. The four remaining monkeys in this group (H5–H8) are participating in ongoing studies. For these monkeys, we estimated the volume loss of the hippocampal formation by comparing preoperative and postoperative structural T1-weighted magnetic resonance imaging (MRI) scans. The volume reduction was then converted to an estimated percent cell loss using a regression function generated by Malkova *et al.* (27), who directly compared the volume reduction determined by MRI scans with percent cell loss determined by microscopic examination of Nissl-stained brain sections from the same set of monkeys (26). We estimated that monkeys H5 to H8 sustained damage to 64% of the hippocampal formation. Altogether, monkeys H1 to H8 sustained an average of 54.5% (range 28%–83%) damage to the hippocampal formation (Table 1). The damage was distributed roughly equally between the rostral and caudal hippocampus. Perirhinal, entorhinal, and parahippocampal cortical fields appeared to be completely intact. Photomicrographs of sections from one representative case are shown in Figure 1. For details, see Supplement 1.

Perirhinal Cortex Lesions. Based on postoperative T1-weighted MRI scans, we estimated that the operated monkeys sustained an average of 92% (range 79%–96%) damage to the perirhinal cortex (Table 1). As intended, the lesions spared the entorhinal cortex, areas TE and TF/TH (21). For details, see Supplement 1.

Apparatus

All testing was conducted in the Wisconsin General Test Apparatus (WGTA). For experiment 1, a clear Plexiglas box measuring 11.4 cm (width) × 71.1 cm (length) × 11.4 cm (height) was located inside the test compartment of the WGTA. Ten stimulus objects, all novel at the beginning of testing, were used: eight neutral junk objects, one grey/green colored rubber snake measuring 50.8 cm in length and approximately 2 cm in diameter; and one black, "hairy," rubber spider which measured 10 cm (width) × 13.5 cm (length) × 2.5 cm (height) made to jump using an air bladder. Food rewards consisted of fruit snacks (Giant Food, Inc., Landover, Maryland) or chocolate M&M's (Mars Candies, Hackettstown, New Jersey).

Table 1. Estimated Percent Damage to Hippocampal Formation (H1–H8) or the Perirhinal Cortex (PRh1–PRh4)

	Estimated Percent Damage (by volume)		
Monkey	Left	Right	Mean
H1	47.6	46.7	47.2
H2	53.5	44.8	49.2
H3	48.5	48.1	48.3
H4	34.2	35.7	35.0
H5	75.4	91.2	83.3
H6	61.4	68.1	64.6
H7	72.7	87.2	79.9
H8	20.1	35.6	27.9
PRh1	96.6	95.1	95.8
PRh2	87.4	70.6	79.0
PRh3	95.4	97.1	96.2
PRh4	97.6	91.7	94.6

For monkeys H1 to H4, percent damage represents the extent of the lesion based on direct microscopic examination of Nissl-stained sections. For monkeys H5 to H8, the extent of the lesion was based on hippocampal volume reduction, which is directly related to percent damage (see lesion assessment for details). In each of the cases H1 to H8, the percent damage reflects the extent of lesion in the dentate gyrus, hippocampus proper, and subicular complex considered together.

H, monkeys with bilateral excitotoxic hippocampal lesions; Left, left hemisphere; PRh, monkeys with bilateral perirhinal cortex lesions; Right, right hemisphere.

For experiment 2, the test compartment contained a tray with two food wells (diameter, 3.8 cm; depth, .6 cm) located 29 cm apart, center to center, on the midline of the tray. The stimuli for visual discrimination learning were 120 junk objects that varied in color, shape, and size. Each monkey was assigned two different foods (food 1 and food 2) that were roughly equally palatable as determined from food preference tests (Supplement 1). The two foods were selected from the following six foods: banana-flavored pellets (P.J. Noyes, Inc., Lancaster, New Hampshire), half-peanuts, raisins, sweetened dried cranberries (Craisins, Ocean Spray, Lakeville-Middleboro, Massachusetts), fruit snacks (Giant Food, Inc.), or chocolate M&M's (Mars Candies).

Experiment 1: Responses to a Rubber Snake, Jumping Spider, and Neutral Objects

Main Task. Each session comprised 10 trials. In eight trials, one of the neutral objects was placed in the center of the Plexiglas box. Each object was used once per session. In the remaining two trials, the spider or snake was placed inside the box. The snake and spider trials occurred pseudorandomly in the sequence of 10 trials with the constraint that neither occurred on the first trial of the session. Trials were separated by 20 seconds.

On each trial, an opaque screen that separated the monkey from the test compartment was raised. The monkey was free to reach over the object to retrieve a food reward located on the edge of the box furthest from the monkey. If the monkey took the food, the trial was immediately terminated by lowering the screen. If the monkey failed to take the food within the 30-second limit, the trial was terminated. Monkeys were tested every other day for a total of five sessions.