Cell Number and Neuropil Alterations in Subregions of the Anterior Hippocampus in a Female Monkey Model of Depression

Stephanie L. Willard, David R. Riddle, M. Elizabeth Forbes, and Carol A. Shively

Background: The anterior hippocampus is associated with emotional functioning and hippocampal volume is reduced in depression. More women are clinically depressed than men, yet the depressed female brain is little studied. We reported reduced anterior hippocampal volume in behaviorally depressed adult female cynomolgus macaques; the mechanisms contributing to that reduction are unknown. The present study represents the first systematic morphological investigation of the entire hippocampus in depressed female primates.

Methods: Cellular determinants of hippocampal size were examined in subregions of anterior and posterior hippocampus in antidepressant-naïve, adult female monkeys characterized for behavioral depression and matched on variables that influence hippocampal size ($n = 8$ depressed, 8 nondepressed). Unbiased stereology was used to estimate neuronal and glial numbers, neuronal soma size, and regional and layer volumes.

Results: Neuropil and cell layer volumes were reduced in cornu ammonis (CA)1 and dentate gyrus (DG) of the anterior but not the posterior hippocampus of depressed compared with nondepressed monkeys. Glial numbers were 30% lower in anterior CA1 and DG of depressed monkeys, with no differences observed in the posterior hippocampus. Granule neuron number tended toward a reduction in anterior DG; pyramidal neuron number was unchanged in any region. Size of pyramidal neurons and glial densities tended to be reduced throughout the whole hippocampus of depressed monkeys, whereas neuronal densities were unchanged.

Conclusions: The reduced size of the anterior hippocampus in depressed female monkeys appears to arise from alterations in numbers of glia and extent of neuropil, but not numbers of neurons, in CA1 and DG.

Key Words: Glial number, hippocampal volume, *Macaca fascicularis*, neuron number, soma size, unbiased stereology

Hippocampal morphometric alterations are implicated in the pathophysiology of depression, as evidenced by numerous reports of reduced hippocampal volume in depressed patients (1). The mechanisms contributing to these reductions are poorly understood. Given that standard pharmacotherapeutics help less than half of patients, treating depression poses a substantial challenge (2). Increased understanding of the underlying neurobiology is essential to generating novel therapeutic interventions.

The therapeutic effects of some antidepressants appear to be associated with hippocampal neurogenesis under certain conditions (3). Elevated glucocorticoid levels are associated with suppressed neurogenesis and gliogenesis, increased apoptosis, dendritic retraction, and reduced dendritic spines in the hippocampus (4–6). However, postmortem analyses of hippocampi from depressed patients did not reveal substantial neuronal and glial loss (7,8) but instead demonstrated decreased density of glial fibrillary acidic protein (GFAP)-immunoreactive astrocytes (9), increased neuronal and glial packing densities, and decreased neuronal size (10). These studies suggest that alterations in neuropil and glia may contribute more to volume reductions in depression than changes in neuronal number (10). However, most human hippocampal studies are based on relatively few sections from patients with histories of antidepressant exposure. In animal studies, antidepressant treatment prevents stress-induced glial reductions in mood-related brain regions (11,12), suggesting that antidepressant exposure in human subjects might prevent glial reductions in depression. Glial deficits have been reported in depressed patients in brain regions that share functional connectivity with the hippocampus, including the anterior cingulate (13,14) and prefrontal cortices (15), providing evidence for glial abnormalities in the neurocircuitry of depression.

Despite the greater prevalence of depression in women (16), little is known about the depressed female brain. Experimental studies of depression in females are critical because contributing mechanisms likely differ between sexes. Sex-dependent differences in behavioral and neurobiological responses to stress have been observed in both humans and rodents (17,18). Ovarian steroid-induced changes in hippocampal morphology have been described in rodents (19), and volume alterations in the anterior hippocampus are associated with menstrual cycle phase in women (20). Moreover, glial deficits associated with depression-like behavior in prenatally stressed mice are limited to the hippocampus of female and not male offspring (21), and estrogen protects against neuronal loss in the hippocampus of chronically stressed female rats (22). Notably, these reproductive system influences on rodent hippocampus are confined to the anterior region, that is, the portion of the hippocampus that is associated with emotional functioning (23). Thus, estrogen-influenced structural alterations in the hippocampus in response to stress may be central to the neurobiology of depression in females.

From the Neuroscience Graduate Program (SLW), Department of Neurobiology & Anatomy (DRR, MEF), and Department of Pathology (Comparative Medicine) (CAF), Wake Forest School of Medicine, Winston-Salem, North Carolina.

Address correspondence to Carol A. Shively, Ph.D., Wake Forest University Baptist Medical Center, Department of Pathology, Section on Comparative Medicine, Medical Center Boulevard, Winston-Salem, NC 27157-1040; E-mail: cshively@wakehealth.edu.

Received Oct 4, 2012; revised and accepted Mar 19, 2013.

0006-3223/$36.00
http://dx.doi.org/10.1016/j.biopsych.2013.03.013

To promote understanding of the depressed female primate brain, we have studied social stress-associated depressive behavior and accompanying physiological and neurobiological factors in adult female cynomolgus macaques (*Macaca fascicularis*) for 25 years (24,25). Briefly, depressive behavior in female cynomolgus monkeys is naturally occurring in captivity and not induced by experimental manipulation. It may be observed in relation to social environmental challenges such as social isolation and social subordination. Social stress-associated behavioral depression in adult female cynomolgus macaques is similar to human depression in physiological, neurobiological, and behavioral characteristics, including reduced body mass, hypothalamic-pituitary-adrenal (HPA) axis perturbations, autonomic dysfunction, increased cardiovascular disease risk, reduced hippocampal volume, altered serotonergic function, decreased activity levels, and increased mortality. In addition, behaviorally depressed monkeys also have low ovarian steroid concentrations, even though they continue to have menstrual cycles. The menstrual cycles of cynomolgus macaques are like those of women in length and hormonal fluctuations, and the macaque hippocampus more closely parallels the cellular organization and connectivity patterns of the human hippocampus than does that of the rat (26).

Previously, we reported reduced anterior hippocampal volume in antidepressant-naïve, behaviorally depressed female cynomolgus macaques (27). The goal of the present study was to elucidate the cellular components of anterior hippocampal volume reduction by systematic investigation of the entire hippocampus in depressed female primates, while controlling for ovarian steroids and eliminating the confounding influence of treatment. To accomplish this, design-based stereologic methods were used to estimate neuronal and glial numbers, neuronal soma size, and laminar volumes in all subregions of the anterior and posterior hippocampus, in tissue derived from a carefully matched sample of antidepressant-naïve, adult, female monkeys characterized for behavioral depression. Based on previous studies, we hypothesized that alterations in glia and neuropil would contribute more than neuronal alterations to differences in hippocampus size and that these effects would be limited to the anterior hippocampus.

Methods and Materials

Subjects

Twenty-eight reproductive-aged female cynomolgus macaques (*Macaca fascicularis*) were obtained from Institut Pertanian Bogor, Bogor, Indonesia, and housed for 24 months in stable social groups of four animals each. As part of a study investigating the comorbidity of depression and cardiovascular disease risk factors, the animals consumed a diet designed to mimic that typical in North America, containing .07 mg cholesterol/J (.28 mg cholesterol/cal) and 42% of calories as fat (28–30). All procedures involving primates were conducted in compliance with institutional (Institutional Animal Care and Use Committee #A99-102), state, and federal laws for the usage of primates in laboratory settings.

Behavior

Social status hierarchies, determined monthly by recording outcomes of agonistic interactions (28,31,32), were stable over time as in previous experiments (33). Behavioral depression is operationally defined as a slumped or collapsed body posture (head lower than shoulders), in which an animal's eyes are open but the animal lacks interest in or responsivity to environmental stimuli (28,31,32) (Figure S1 in Supplement 1). Behavioral depression in captive cynomolgus macaques appears spontaneously and is not induced by an experimental manipulation. Each monkey was observed for 15 minutes per week × 52 weeks per year × 2 years for a total of 26 hours per monkey. Percent time spent behaviorally depressed was calculated as (time spent in behavioral depression / total observation time) × 100. Interobserver reliability was ≥92%. Detailed characteristics of the behavioral and peripheral physiology of these animals have been described (28–30,34).

HPA Axis Function

One month before necropsy, a dexamethasone suppression test (DST) was used to assess the sensitivity of the HPA axis to glucocorticoid negative feedback (28,31,32). Morning blood samples were taken for baseline cortisol determinations, dexamethasone (130 μg/kg body weight, intramuscular) was administered later in the evening, and then another blood sample was taken the following morning for cortisol assay. Percent suppression of cortisol, calculated as [(baseline − second morning cortisol levels) / baseline cortisol] × 100, was used as an indicator of sensitivity to glucocorticoid negative feedback (35).

Steroid Hormone Assays

Estradiol and progesterone were assayed in blood collected at necropsy. Ovarian steroids and cortisol concentrations were determined using commercially available radioimmunoassays (Diagnostic Products Corporation, Los Angeles, California).

Subject Selection

Given that depressive behavior is variably expressed among individuals, the sample used in the present study roughly represented the upper and lower tertiles of the distribution of depressive behavior. From the parent population of 28 animals, 8 animals from the upper 32% of the distribution of time spent in the depressed posture were matched with 8 animals from the lower 39% of the distribution on body weight, age, social status, basal cortisol levels, cortisol response in the DST, percent suppression of cortisol, and estradiol and progesterone levels at the time of necropsy (Table 1), for a final sample of $n = 16$ animals. This matching was done to reduce variance in the relatively small sample, thus assuring that behavioral depression was the only statistically significant difference between the two groups with respect to characteristics known to affect hippocampal structure.

Tissue Preparation and Cell Labeling

Procedures for tissue collection and cell labeling are provided in Supplement 1 and summarized here. Hemisected brains were frozen and stored at −80°C. Temporal lobes were dissected, fixed, and cut coronally at 50 μm. To control for laterality, experimental groups were counterbalanced for hemisphere (27). Sections were processed in batches equally representing behavioral depression status. Pyramidal neurons were labeled by NeuN immunohistochemistry visualized using avidin-biotin-peroxidase and diaminobenzidine (Figure S2A in Supplement 1). Granule neurons and glia were labeled by histochemistry for Nissl substance (cresyl violet, Figure S2B–D in Supplement 1).

Quantitative Stereological Analyses

Total numbers of neurons and glia were estimated using the optical fractionator technique (36) and the Stereo Investigator