Neuroscience Letters 563 (2014) 1–5

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Altered expression of glial and synaptic markers in the anterior hippocampus of behaviorally depressed female monkeys

Stephanie L. Willard[a], Scott E. Hemby[b], Thomas C. Register[c], Scot McIntosh[b], Carol A. Shively[c,*]

[a] Neuroscience Graduate Program, Wake Forest School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157-1040, USA
[b] Department of Physiology & Pharmacology, Wake Forest School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157-1040, USA
[c] Department of Pathology (Comparative Medicine), Wake Forest School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157-1040, USA

HIGHLIGHTS

- Behaviorally depressed female primates have increased GFAP protein in the CA1.
- Depressed female primates have decreased spinophilin and PSD-95 protein in the CA1.
- Estrogen may modulate astrocyte-mediated impairments in synaptic plasticity.
- Behavioral depressed female cynomolgus monkeys are a useful model of depression.

ARTICLE INFO

Article history:
Received 10 October 2013
Received in revised form
17 December 2013
Accepted 7 January 2014

Keywords:
GFAP
PSD-95
Spinophilin
CA1
Macaca fascicularis

ABSTRACT

The anterior hippocampus is associated with emotional functioning and hippocampal volume is reduced in depression. We reported reduced neuropil volume and number of glia in the dentate gyrus (DG) and cornu ammonis (CA)1 of the anterior hippocampus in behaviorally depressed adult female cynomolgus macaques. To determine the biochemical correlates of morphometric and behavioral differences between behaviorally depressed and nondepressed adult female monkeys, glial and synaptic transcripts and protein levels were assessed in the DG, CA3 and CA1 of the anterior hippocampus. Glial fibrillary acidic protein (GFAP) was increased whereas spinophilin and postsynaptic density (PSD)-95 protein were decreased in the CA1 of depressed monkeys. GFAP was reciprocally related to spinophilin and PSD-95 protein in the CA1. Gene expression of GFAP paralleled the protein changes observed in the CA1 and was inversely related to serum estradiol levels in depressed monkeys. These results suggest that behavioral depression in female primates is accompanied by astrocytic and synaptic protein alterations in the CA1. Moreover, these findings indicate a potential role for estrogen in modulating astrocyte-mediated impairments in synaptic plasticity.

1. Introduction

Numerous studies support an association between structural and functional alterations of the hippocampus and the pathophysiology of depression. Hippocampal volume is consistently reduced in depressed patients [15]. Studies in the postmortem hippocampus of depressed patients reported limited evidence of neuron loss [5,17,20], while others found decreased density of glial fibrillary acidic protein (GFAP)-immunoreactive astrocytes [20], increased neuron packing densities, decreased neuron size [32], and decreased cell layer volume [5], suggesting that neuropil alterations may contribute to volume reductions in depression. Studies in male rodents also indicate neuropil alterations, with dendritic retraction and fewer synapses in the cornu ammonis (CA)3 of the hippocampus in response to stress or glucocorticoid exposure [18,35]. Similarly, stress decreased levels of the presynaptic marker synaptophysin (SYN) and the postsynaptic marker postsynaptic density (PSD)-95 in the CA3 of male rodents [6,23], suggesting that synaptic integrity may be compromised in the neuropil of these animals. However, no differences were reported between major depressive disorder (MDD) patients and controls in SYN immunoreactivity [20] or PSD-95 expression in the postmortem hippocampus [37]. Importantly, MDD patients

* Corresponding author. Tel.: +1 336 716 1500; fax: +1 336 716 1515.
E-mail addresses: willards@mail.med.upenn.edu (S.L. Willard), shemby@wakehealth.edu (S.E. Hemby), register@wakehealth.edu (T.C. Register), smcintos@wakehealth.edu (S. McIntosh), cshively@wakehealth.edu (C.A. Shively).

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http://dx.doi.org/10.1016/j.neulet.2014.01.012

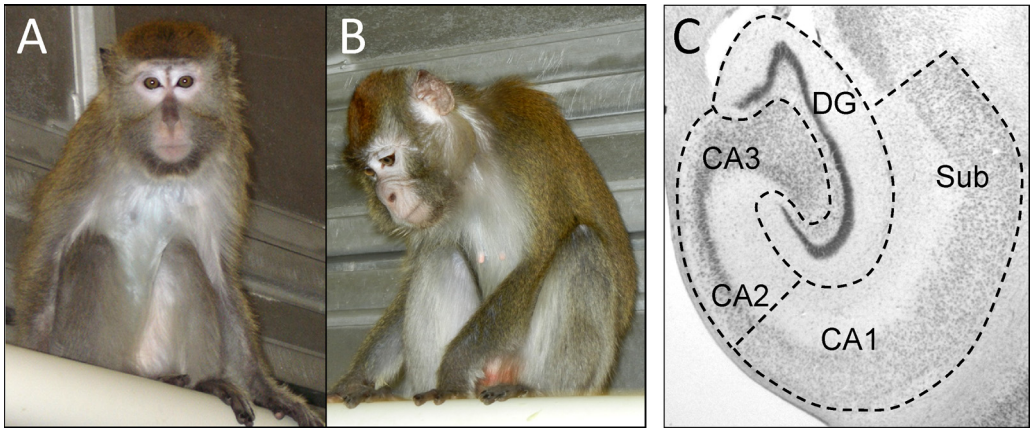

Fig. 1. A nondepressed monkey (A) compared to a behaviorally depressed (B) monkey. The nondepressed monkey (A) is alert and attentive to the photographer, whereas the behaviorally depressed monkey (B) remains in a slumped, collapsed body posture with eyes pointed downward, and is unresponsive to environmental events. Behavioral depression in captive cynomolgus macaques occurs spontaneously and is not induced by a specific experimental manipulation. (C) Cresyl violet stained section of the hippocampus in the coronal plane indicating subregional dissection boundaries.

are frequently medicated with antidepressants that reversed or prevented deficits in SYN, PSD-95 and GFAP-immunoreactive astrocytes in stressed animal models [7,23], which may account for a lack of observed differences in patients.

Despite the increased prevalence of depression in women, little is known about the depressed female hippocampus. Sex differences in behavioral and neurobiological responses to stress have been reported [11,36]. Glial deficits associated with depression-like behavior in prenatally stressed mice are limited to female offspring [4], and estrogen protects stressed female rodents from the dendritic retraction observed in the CA3 of males [10]. In the CA1 of the hippocampus, estrogen increases spine density in rodents [12], and immunoreactivity for spinophilin, a marker of dendritic spines, in monkeys [14]. As such, estrogen-dependent alterations in hippocampal neuropil, particularly in response to stress, may be central to the neurobiology of depression in females.

To increase understanding of the depressed female primate brain, we have studied social stress-associated depressive behavior and the accompanying physiology and neurobiology in adult female cynomolgus macaques (*Macaca fascicularis*) for over two decades [28,40]. Cynomolgus macaques have menstrual cycles like women in length and hormone fluctuations, and the macaque hippocampus closely resembles the organization and connectivity of the human hippocampus. Behaviorally depressed monkeys have poor ovarian function relative to their nondepressed counterparts [28,40]. Recently, we reported morphological deficits in the CA1 and dentate gyrus (DG) of the anterior hippocampus in antidepressant-naïve, behaviorally depressed females, including reductions in region and cell layer volumes, neuropil size, and glia number [39]. The present study was designed to elucidate the molecular correlates of anterior hippocampal volume reduction in depressed female primates, while eliminating the confound of treatment. Protein and gene expression of glial and synaptic markers were measured in subregions of the anterior hippocampus from the same matched set of adult, female monkeys characterized for behavioral depression used in the previous morphologic study [39]. We hypothesized that markers of glia and synaptic integrity would be dysregulated in the anterior CA1 and DG of behaviorally depressed female monkeys.

2. Materials and methods

2.1. Subjects

As previously described, 28 reproductive-aged female cynomolgus macaques (*M. fascicularis*) were housed in stable social groups ($N = 4$/group) under a 12/12 light/dark cycle for 24 months [25–27,39]. All procedures involving primates were conducted in compliance with institutional, state, and federal laws for the usage of primates in laboratory settings. As part of a larger study investigating the comorbidity of depression and cardiovascular disease risk, the animals consumed a Western diet, containing 0.28 mg cholesterol/Cal and 42% of calories as fat [27]. Characteristics of the behavior and physiology of this specific cohort of animals have been described in detail elsewhere [25–27]. Briefly, social status was determined monthly by recording the outcomes of agonistic interactions [24,27,29], and the resulting social status hierarchy within each social group was stable over time, as in previous experiments. Behavioral depression was operationally defined as a slumped or collapsed body posture (head lower than shoulders), in which an animal's eyes are open, yet the animal lacks interest or responsivity to environmental stimuli [24,27,29] (Fig. 1A–B). A dexamethasone suppression test (DST) was administered one month before necropsy to assess the sensitivity of the hypothalamic–pituitary–adrenal axis to glucocorticoid negative feedback [27]. Estradiol and progesterone were assayed in serum collected at necropsy [27].

2.2. Tissue preparation

At necropsy, brains were rapidly removed, hemisected, and frozen at −80 °C. From the initial population of 28 animals, eight animals from the upper tertile of the distribution of time spent in the depressed posture were matched with eight animals from the lower tertile as reported in Willard et al. [39]. Briefly, to reduce variance from characteristics known to affect hippocampal structure and function, animals were matched for body weight, age, social status, basal cortisol levels, cortisol response in the DST, % suppression of cortisol, and estradiol and progesterone levels at the time of necropsy. The anterior hippocampus was delineated from the posterior hippocampus by the presence of the uncus [38,39], and the anterior DG, CA1 and CA3 regions were dissected from one hemisphere and pulverized (Fig. 1C). To control for effects of laterality, the dissected hemisphere was counterbalanced within groups, as reported previously [38,39]. Dissections were pulverized in liquid nitrogen and stored at −80 °C for protein and gene expression analyses.

2.3. Western blotting

Dissected hippocampal regions were fractionated into membrane and cytosolic subcellular compartments using a modified