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Research article

Chronic corticosterone exposure reduces hippocampal astrocyte structural plasticity and induces hippocampal atrophy in mice

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HIGHLIGHTS

- Chronic CORT injection damages the astrocyte structural plasticity.
- Chronic CORT injection reduces hippocampal volume.
- Chronic CORT injection induces depression-like behavior in mice.

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ABSTRACT

Long-term exposure to stress or high glucocorticoid levels significantly reduces hippocampal volume and induces depression-like behavior in rodents; however, the cause of which remains unknown. Increasing evidence shows that astrocytes are the most abundant cells in the central nervous system (CNS). Astrocytes support, nourish, protect, and repair neurons, and these characteristics are closely linked to neuronal activities. This study focused on the effects of corticosterone (CORT) on astrocytes. We probed into the influence of chronic CORT exposure on structural plasticity of astrocytes in hippocampal tissues of male C57BL/6N mice. The number, somal volume, and protrusion length of glial fibrillary acidic protein-positive astrocytes and the hippocampal volume were assayed by immunocytochemistry and quantitative stereological techniques. Results showed that chronic CORT injection induced depression-like behavior in male mice and significantly decreased hippocampal volume, as well as the number of astroctyes, somal volume, and protrusion length in the hippocampus. The reduction in the hippocampal astrocyte structural plasticity may represent the mechanism by which chronic CORT treatment causes hippocampal atrophy and depression-like behavior in male mice.

1. Introduction

Depression is a highly complex mood disorder or an affective and widespread disorder, resulting in significant suffering of patients [1]. Chronic stress or psychological trauma is believed to be the major causal or exacerbating factor of depression [2]. Neuroendocrine activity increases upon exposure to strong and long-term physical and psychological stress stimuli, thereby leading to hypothalamic–pituitary–adrenocortical axis hyperactivity and elevated glucocorticoid levels. Cortisol is becoming valuable in valid biomarkers of stress-related and psychiatric disorders [3]. Increasing amounts of data suggest that cortisol levels in depressed patients are elevated, thus, resulting in cognitive dysfunction and mood disorders through hippocampal atrophy [4,5]. In severely depressed patients, bilateral hippocampal volume is remarkably reduced [6]. In rodents, long-term subcutaneous CORT injection can lead to hippocampal atrophy and induce depression-like behavior [7,8].

Studies have discussed the underlying mechanism of CORT-induced hippocampal atrophy from the perspective of neurons [9], but little is known about the effects of CORT on astrocytes in vivo. In a tree shrew model for depression related to psychosocial stress, Lucassen et al. [10] found that stress resulted in decreased cytogenesis and increased cell death in the hippocampus. Increased labeling for apoptosis after stress occurs mainly in glial cells, which

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suggests that these cells might be the target of early neuropathological changes. Astrocytes are the most abundant cells in CNS. These cells secrete neurotrophic and supportive factors that support, nourish, protect, and repair neurons. Astrocytes also effectively prompt the survival, development, and differentiation of neurons [11]. In addition to nourishing and protecting neurons, astrocytes regulate the uptake and clearance of transmitters across the synapse gaps through amino acid neurotransmitter transporters; thus, these cells are important for maintaining synaptic efficacy [12].

The structural plasticity of astrocytes refers to the remarkable flexibility of their characteristics, such as astrocyte phenotype, cell number, cell protrusion density, and nuclear morphology in response to internal and external stimuli. Such plasticity is mainly reflected by the morphological variation in cell protrusion, which is related to the number and activity of synapses. Astrocytic protrusions become part of the synapses and facilitate synaptogenesis because they are in close contact with synapses and have demonstrating important functions in synapse maturation and stability [13]. Astrocytes may inhibit or promote synaptogenesis via morphological changes. Given that astrocytes are abundant in the hippocampus and structural plasticity affects functional integration, the reduced astrocyte structural plasticity is hypothesized to be responsible for CORT-induced hippocampal atrophy and depression-like behavior.

2. Materials and methods

2.1. Experimental animals

Experimental procedures were conducted in accordance with the regulations set by the Committee on the Use of Live Animals in Research [certificate no. 0025330, permit no. SCXK (Su), 2012-0004], Laboratory Animal Center of Nanjing Medical University. Adult male C57BL/6N mice (18–20 g, aged 4–5 weeks, housed 5/cage) were kept in a room on standard 12 h light/dark cycle at fixed temperature of 25 ± 1 °C and provided with food and water ad libitum. Mice were allowed 1 week to adapt to the laboratory environment prior to actual experiments. The mice were treated in accordance with the Guidelines of Accommodation and Care for Animals by the Chinese Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.

2.2. CORT administration

The mice were divided into two groups (n = 10/group), namely, control and CORT. CORT injections were administered as described by Zhao et al. [7]. The mice in the CORT group received subcutaneous CORT injections (20 mg/kg) once per day (5 ml/kg) at random times during light phase. The control mice received subcutaneous injections of the vehicle alone. All behavioral tests were performed during the day to avoid bias of dark-cycle locomotor activity changes. After administration for 4 weeks, depression-like behavior of mice was followed by forced swim test (FST) and tail suspension test (TST). The mice were anesthetized with phenobarbital sodium, and cardiac blood was collected after CORT injection at day 29. Plasma was separated via centrifugation at 3000 rpm and stored at −20 °C until needed for CORT concentration assay. Brains were used for immunohistochemical analysis.

2.3. Forced swim and tail suspension tests

Behavioral tests were conducted in JLBehv-FSG-4 sound insulation boxes controlled by a Dig Behav animal behavior video analysis system. This system can automatically record and analyze animal movements to provide total immobility times during FST and TST. The tests were conducted in a 5000 ml glass beaker swim tank, filled with 15 cm-deep water at a temperature of 25 ± 1 °C. Each mouse was placed separately and forced into a glass beaker for 6 min. Durations of immobility were videotaped during the last 4 min. After 6 min, the mouse was removed from the glass beaker. At 24 h after FST, the mice were individually suspended by securely taping the tail 1 cm from the tip and secured to a shelf 25 cm above the bottom of the sound insulation box. Each mouse was hung for 6 min, and the duration of immobility was videotaped during the last 4 min.

2.4. CORT assays

Plasma CORT was measured using a commercial AssayMax CORT ELISA kit (Assaypro, catalog no. EC3001-1). Each sample serum (25 μl) and standard was loaded into microtiter plates, and then 25 μl of biotinylated CORT was then added. The plates were cleansed after 2 h incubation. Streptavidin–peroxidase (50 μl) was added and incubated for 30 min at room temperature. The optical density was identified (absorbance at 450 nm) on a microplate reader (Molecular Devices, US). The standard curve suggested a direct relationship between optical density and serum sample concentration.

2.5. Perfusion and brain tissue preparation

After cardiac blood collection, the mice were perfused with saline solution followed by 4% paraformaldehyde in phosphate-buffered saline (PBS) through the left ventricle. The brains were immediately removed, and postfixed overnight at 4 °C in the same fixation solution. After cryoprotection with 0.1 M PBS containing 30% sucrose at 4 °C, 1.5–5.0 mm behind the brain tissue of the Bregma was embedded at an optimal cutting temperature and sectioned into 60 μm thick slices made along the entire hippocampus based on a mouse brain atlas. The brains were used for immunohistochemical analysis.

2.6. Glial fibrillary acidic protein immunohistochemistry

Slices were washed in 0.1 M PBS and incubated in 3% H_2O_2 for 15 min. After rinsing, nonspecific antibody binding was blocked by incubating the slices for 30 min in 5% normal goat serum (Sigma, USA) at room temperature. The slices were incubated with primary antibodies at 4 °C overnight. The primary antibodies used were anti-glial fibrillary acidic protein (GFAP) (1:1000; Santa Cruz, USA) antibodies. The next day, the slices were rinsed, incubated in a biotinylated mouse secondary antibody (1:100; Vector Laboratories, Burlingame, CA, USA) for 1 h at room temperature, developed for 5 min in DAB, thoroughly rinsed, and then covered with a coverslip.

2.7. Stereological estimates of hippocampal volume, astrocytic number, size, and protrusion length

The stereological estimates were similar to those described by Czeh and Lucassen [14]. Hippocampal volume was determined using GFAP immunohistochemical method for stereological measurements. Each mouse was cut with 24 consecutive hippocampal slices at intervals of four to split into four groups, and each group can be restored to full hippocampus. Each parameter was set on the basis of stereological counting principles, One of the four groups was randomly selected. A Zeiss III RS microscope (Carl Zeiss, Oberkochen, Germany) was used for hippocampus side accurate stereological standardization with 10 × 4 image magnification. 2D information in each slice was selected to reconstruct a 3D hippocampus by using Stereo-Investigator version 4.04 software automatic program to accurately and quantitatively analyze