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Research report

Chronic traumatic stress impairs memory in mice: Potential roles of acetylcholine, neuroinflammation and corticotropin releasing factor expression in the hippocampus

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ABSTRACT

Chronic stress in humans can result in multiple adverse psychiatric and neurobiological outcomes, including memory deficits. These adverse outcomes can be more severe if each episode of stress is very traumatic. When compared to acute or short term stress relatively little is known about the effects of chronic traumatic stress on memory and molecular changes in hippocampus, a brain area involved in memory processing. Here we studied the effects of chronic traumatic stress in mice by exposing them to adult Long Evan rats for 28 consecutive days and subsequently analyzing behavioral outcomes and the changes in the hippocampus. Results show that stressed mice developed memory deficits when assayed with radial arm maze tasks. However, chronic traumatic stress did not induce anxiety, locomotor hyperactivity or anhedonia. In the hippocampus of stressed mice interleukin-1β protein expression was increased along with decreased corticotropin releasing hormone (CRH) gene expression. Furthermore, there was a reduction in acetylcholine levels in the hippocampus of stressed mice. There were no changes in brain derived neurotrophic factor (BDNF) or nerve growth factor (NGF) levels in the hippocampus of stressed mice. Gene expression of immediate early genes (Zif268, Arc, C-Fos) as well as glucocorticoid and mineralocorticoid receptors were also not affected by chronic stress. These data demonstrate that chronic traumatic stress followed by a recovery period might lead to development of resilience resulting in the development of selected, most vulnerable behavioral alterations and molecular changes in the hippocampus.

1. Introduction

In humans exposure to stressful conditions can result in debilitating mental disorders with altered memory performances [1–3]. Response to stress is complex and includes avoidance of contexts and cues associated with the stressor. This avoidance behavior is mainly due to development of fear memory. In addition to the development of fear memory, which is often strong and persistent, there could be impairment of other forms of memories, along with the development of anxiety and depression as well as physiological changes. While acute stress and its neurological effects were the focus of several studies, chronic stress can also disrupt memory performance and change hippocampal cell structure and function [4,5]. Interestingly chronic stress results in remarkable variability in memory performances [6–8] indicating multiple factors may be involved in modulation of memory with chronic stress. However, little is known about these factors and especially their relationship with molecular and physiological processes that modulate memory.

Strength of the stress is a major factor that is positively correlated to the stress responses. Traumatic stress such as exposure to predators can result in strong adverse psychobiological effects in rodents. Furthermore, exposure to predators [9] better mimic the life threatening stress that is often associated with human stress related psychiatric conditions such as PTSD. Especially ethological relevance and potency of predator exposure is believed to be a highly relevant approach for developing an intense, psychological response in rodents [9]. Chronic traumatic stress also has high human health relevance as many stressors like childhood abuse, sexual abuse or domestic violence can often be continual in nature. Despite this relevance preclinical research on chronic traumatic stress is scant and the propensity of chronic predator stress to develop defective memory performance is not well studied.

The hippocampus located in the medial temporal lobe of the brain is crucial for formation of memories [10,11]. Patients with stress disorders tend to have lower hippocampal volume and impaired hippocampus dependent memories [12–15]. In addition to neurotransmitter receptors, such as cholinergic receptors, that are involved in the molecular mechanisms of memory encoding, the neurons in the

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hippocampus also express receptors for stress hormones and inflammatory mediators [16–19]. The inflammatory and stress responses mediated by these receptors are often disruptive to memory. Elevated inflammatory and immunological responses in the hippocampus are also associated with certain stress disorders [20–24]. Furthermore, increased levels of stress hormones and inflammatory mediators are also known to cause cell death in the hippocampus and other brain regions [25–30]. These findings indicate that stress hormones and inflammatory mediators in the hippocampus may play a crucial role in stress induced memory deficits. In addition to increased inflammatory responses, alterations of neurotrophins are also strongly correlated with the pathophysiology of stress [31–33]. The stress related and inflammation associated cellular processes associated with decreased memory are not fully understood. Therefore, in this present study we utilized the rat-mouse predator-prey relationship [34] in order to analyze the chronic traumatic stress exposure induced behavioral and molecular changes in mice.

2. Materials and methods

2.1. Animals and stress procedure

Male C57BL/B6 mice (1 month old) and old (> 1 year) male Long Evans rats were housed in standard housing conditions (12 h dark; 12 h light) with free access to food and water. Rats and mice were housed in separate rooms. Mice were randomly divided into control and predator stress groups. All procedures involving live animals were in accordance with National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and with the animal use protocol approved by Institutional Animal Care and Use Committee.

Chronic traumatic stress in mice was induced by exposure to the Long Evan rats [34]. Briefly, a mouse was placed inside a clear plastic hamster ball (Super Pet, Elk Grove Village, IL; material # 100079348) and then the hamster ball was placed inside the home cage of an aggressive rat for 30 min for 28 consecutive days (Fig. 1). This type of stress allows visual and olfactory exposure to the predator without direct physical contacts that might lead to injury or death to the mice. An individual mouse was not exposed to the same rat for two consecutive days.

2.2. Behavioral procedures

Open field activity was studied by placing each mouse at the center of a rectangular open field area (34 cm X 34 cm) and allowing them to explore for 10 min. Elevated plus maze test was performed as previously described [35] and the time spent in open and closed arms were measured. Sucrose preference test was performed by providing mice with a choice between two identical bottles, one filled with tap water and the other with 2% sucrose solution, in their home cages for 48 h.

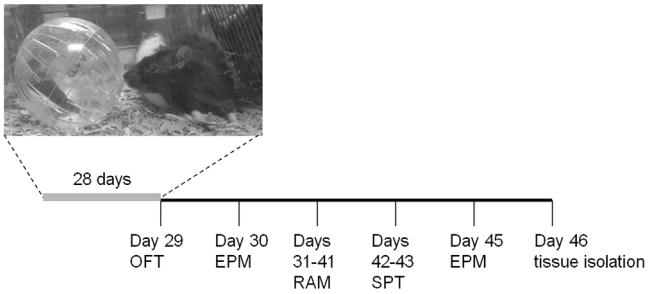

Fig 1. Design and time line for chronic predator stress exposure in mice. Each stress episode consisted of placing a mouse in a transparent plastic ball and placing the ball inside the home cage of a rat. Control and stressed mice underwent a battery of behavioral evaluations that contained open field test (OFT), elevated plus maze (EPM), radial arm maze test (RAM), and sucrose preference test (SPT).

The bottles were switched after 24 h to avoid any side preference [34,36]. Amount of sucrose solution consumed was measured.

Radial arm maze (RAM) experiments were performed as previously described [37] with some minor modifications. Prior to radial arm maze experiments mice were diet-restricted to reduce and maintain a body weight of ~85.0% of their *ad libitum* food body weight. During this period mice were introduced to a food reward (Reese's peanut butter chips). Mice were pre-trained for 3 days to associate the maze with the experience of obtaining the food reward by allowing each animal free access to all eight arms until food reward from each arm is consumed. Radial arm maze tests consisted of two phases, each lasting for 5 consecutive days and there was one trial per day. Prior to each trial each mouse was placed in the central chamber of the maze for a two minute acclimation period. During the first test phase (one test/day for 5 days) mice freely explored all eight arms with each arm baited with the food reward. For each mouse the trial ended after either collecting the last food reward or 15 min of elapsed time. In the second phase (one pair of test/day for 5 days) each mouse was tested twice with 3 min interval between tests. In the first test 4 arms were closed and the remaining 4 arms were baited with the food reward. Mice were individually allowed to explore the open arms and consume the food reward. This first test ended after the last food reward was consumed or 15 min of elapsed time and the mouse was taken out and placed in its home cage for the inter-test interval. In the second test all 8 arms were open but only four arms that were previously closed were baited with the food reward. The test ended after either the last food reward was consumed or after 15 min of elapsed time. In between trials the behavioral apparatus were wiped with 70% ethanol to eliminate odor cues.

2.3. Western blot

Western blot experiments were performed by following standard protocols that included separation of equal quantities of total proteins in separate wells of a SDS-PAGE gel and subsequent transfer of proteins to PVDF membranes. Membranes were probed overnight at 4 °C with the following primary antibodies: anti-interleukin-1β (IL-β; Cell Signaling Technology, Danvers, MA), anti-nerve growth factor (NGF; Santa Cruz Biotechnology, Dallas, TX), anti-brain derived neurotrophic factor (BDNF; Santa Cruz) and β-actin (Cell Signaling). Membranes were then probed with an appropriate horseradish peroxidase conjugated secondary antibody, incubated with SuperSignal® West Pico Chemiluminescent Substrate (Thermo Scientific, Rockford, IL, USA) and the bands were developed in a ChemiDoc MP system (Bio-Rad, Hercules, California, USA). Band intensities of each membrane were normalized with corresponding β-actin band intensities using ImageJ (National Institutes of Health, Bethesda, MD) program.

2.4. Quantitative RT-PCR

Relative gene expression in the hippocampus was determined by using a two-step quantitative reverse transcription PCR process combined with Livak's ($2^{-\Delta\Delta Ct}$) method. Total RNA was extracted and purified using the Trizol® Plus PureLink™ RNA Mini Kit (Invitrogen, Carlsbad, CA, USA). RNA concentration and purity was determined by using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). An equal amount of starting RNA was used for subsequent cDNA synthesis by using the qScript™ cDNA SuperMix (Quanta Biosciences, Gaithersburg, MD, USA). The qPCR analysis was performed using PerfeCTa® SYBR® Green FastMix (Quanta Biosciences, Gaithersburg, MD, USA) and the Rotor-Gene® Q HRM System (Quiagen, Hilden, Germany). Validated *Mus musculus* primer sets (Table 1) were obtained from a database [38–40] and 18s rRNA was used as the endogenous control for relative gene expression analysis. The 20 µl qPCR reaction mix contained 70 ng cDNA, 1 x PerfeCTa® SYBR® Green FastMix, and 0.25 µM of each forward and reverse primers.