Behavioural Brain Research 267 (2014) 111–118

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Female-dependent impaired fear memory of adult rats induced by maternal separation, and screening of possible related genes in the hippocampal CA1

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HIGHLIGHTS

- Rats experienced MS exhibit impaired fear memory in female in adulthood.
- Nine up-regulated and one down-regulated genes were founded in the hippocampal CA1 area.
- TorsinA, MACF1 and Nd1-L gene may be the predisposing genes of PTSD.

ARTICLE INFO

Article history:
Received 3 January 2014
Received in revised form 14 March 2014
Accepted 15 March 2014
Available online 22 March 2014

Keywords:
Maternal separation
PTSD
Contextual fear conditioning
Hippocampus
CA1
SSH

ABSTRACT

Early life stress is one of the major susceptible factors for stress-related pathologies like posttraumatic stress disorder (PTSD). Recent studies in rats suggest that rather than being overall unfavorable, early life stress may prepare the organism to perform optimally to stressful environments later in life. In this study, severely adverse early life stress was conducted by six consecutive hours of maternal separation (MS), from PND1 to PND21, and contextual fear conditioning model was used on PND90 to mimic the second stress in adulthood and the re-experiencing symptom of PTSD. It was observed that in this investigation pups experienced MS showed decreased sensibility to contextual fear conditioning in adulthood, and there sex plays an important role. For example, female rats suffered MS had much lower freezing than males and controls. Meanwhile, Morris water maze test indicated that MS did not impair rat's performance of spatial learning and memory. Furthermore, suppression subtractive hybridization (SSH) was used to screen the related genes of fear memory, by examining the changes of mRNA expression in CA1 area between female MS and control rats after contextual fear conditioning. Finally, nine up-regulated and one down-regulated genes, including β2-MG, MAF, Nd1-L, TorsinA and MACF1 gene were found in this study. It is assumed that the TorsinA, MACF1 and Nd1-L gene may contribute to the decreased sensitivity of PTSD induced by MS.

1. Introduction

Posttraumatic stress disorder (PTSD) is a highly disabling condition observed in people after exposure to severe emotional or physically life-threatening traumatic events. According to DSM-IV, traumatic event is defined as follows: (a) the person experienced, witnessed, or was confronted with an event that involved actual or perceived threat to life or physical integrity; and (b) the person's emotional response to this event included horror, helplessness, or intense fear. Children who are directly experienced the traumatic event or witnessed the event occurred to others especially primary caregivers, may have more difficult to deal with this event, this might lead them to be more vulnerable to develop PTSD few months or years later. Thus in DSM-V, there is a specific description of PTSD for children under the age of 6 years old.

Maternal separation (MS), childhood abuse and neglect, are the most common traumatic events in early life, which may alter the normal pattern of brain development, and cause various short- and long-term disturbances in cognitive, learning, emotional and other behavioral performance [1–3], and change adult responses to stress [4]. Clinical researchers have already reported that victims of child abuse and neglect are at an increased risk for developing

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http://dx.doi.org/10.1016/j.bbr.2014.03.022
0166-4328/

depression [5] and PTSD [6]. However, the exact mechanisms are poorly investigated.

Over the past decades, numerous studies focused on identifying the pathophysiology of PTSD. It has been suggested that the structural brain abnormalities associated with PTSD mainly involve the amygdala, hippocampus, and medial prefrontal cortex. The hippocampus, in particular, is thought to be involved because of its critical role in learning and memory [7,8], as well as its regulation of the hypothalamic–pituitary–adrenocortical axis [9]. Our previous work and several other studies have reported that patients suffering from PTSD or depression exhibited hippocampal volumetric loss [10,11]. Sustained stress, both in early life and adulthood, could adversely affect hippocampal structure and function [12,13]. Molecular biology evidence has shown that hippocampal cell presents apoptosis and atrophy after prolonged exposure to stress [14]. While environmental factors such as trauma exposure alone may be associated with hippocampal volume deficits, Gilbertson et al. [15] and Gurvits et al. [16] showed that small premorbid hippocampus may predict pathologic vulnerability to PTSD. In a study of monozygotic and dizygotic twins in combat, Sullivan et al. found out that only 40% of the variance in hippocampal volume is attributed to direct genetic influence. This result showed that both genetic and environmental factors may ultimately interact to determine hippocampal volume and PTSD [17]. There may be an interaction between early life stress and hippocampal changes on vulnerability of PTSD. One opinion is that early life stress may modulate the expression of molecules involved in cellular plasticity within the hippocampus, and thereby contributes to permanent alterations of brain structure and function, which might ultimately lead to an increased vulnerability to PTSD [18]. However, the underlying molecular mechanisms are largely unknown, even the predisposing genes of PTSD are still unclear. Our previous work suggested that maternal deprivation in early life has harmful effects on neurobehavioral development, and causes the down-regulation of reelin mRNA by further DNA methylation in postnatal hippocampus [19], and repeated MS could cause hippocampal reelin expression alteration [20]. Other related genes in previous studies includes D2 dopamine receptor (DRD2) gene [21], serotonin transporter promoter gene [22], GABA(A) receptor subunit gene [23,24], BDNF gene [25], glucocorticoid receptor [26], etc.

Moreover, it is well known that different subfields of hippocampus may have respective roles. In the studies of fear conditioning and extinction, which are usually used as animal paradigm of PTSD, both hippocampal CA1 and CA3 contribute to the acquisition of context-dependent extinction, but only CA1 is required for contextual memory retrieval [27].

Early MS paradigms established animal models to induce early life stress had been extensively investigated to study the long-term consequences of stress experience [28]. As early as the prenatal or neonatal period, stress can alter the rate of cognitive decline and neurodegenerative changes in the brain in a stressor dependent manner, with prenatal restraint and maternal separation usually causing damage to the brain [29]. Long repeated MS of the intact litter were thought to increase animals' sensibility to stress [28], such as reduced stress resistance, increased anxiety, and impaired spatial navigation learning. Interestingly, recent studies suggested that severe early life stress may improve the hippocampal synaptic plasticity and emotional learning under high-stress conditions in adulthood, rather than being overall unfavorable [30]. These results may be mediated by differential regulations of gene expression which are important for neuronal development or plasticity [31].

To further exploring the related genes of PTSD, a suppression-subtractive hybridization (SSH) library potentially enriched for transcripts was constructed. And the MS model was used as early life stress and potential influence factor of PTSD, while contextual fear conditioning paradigm mimicked the second stress in adult and re-experiencing symptom of PTSD in this study. In addition, since sexual differences involved in the lasting early-life stress can induce alterations in neurogenesis in rodents [18], the sex was also considered in this study.

2. Experimental procedures

2.1. Animals and maternal separation

Wistar rats were purchased and housed individually in standard polypropylene cages containing 2.5 cm of wood chip bedding material. They were kept at a constant temperature room ($23 \pm 1\,^{\circ}C$) and maintained on a 12/12 h light/dark cycle (lights on at 8:00). Food and water were available at will.

Birth was designated as postnatal day 1(PND 1). On PND1, whole pups were randomly assigned to MS group ($n = 27$) and control group ($n = 29$). According to the previously described method, MS mission was performed daily for six consecutive hours (from 13:00 to 19:00), rat pups were removed from their home cage and kept together in other room in temperature-controlled cages at $28 \pm 1\,^{\circ}C$, where bedding was renewed every day. This procedure was applied between PND 1 and PND 21.

All the rats were weaned on PND22. All experimental protocols described in this study were approved by the Institutional Animal Care and Use Committee of the Zhongshan School of Medicine, Sun Yat-Sen University, in accordance with the US National Institutes of Health Guide for the Care and Use of Laboratory Animals. These experiments were designed to minimize the number, and discomfort, of the animals used. Body weight (BW) of the pups was measured on PND8, 14, 22.

2.2. Contextual fear conditioning

A fear-conditioning chamber ($28\,cm \times 21\,cm \times 22\,cm$; Coulbourn Instruments, Allentown, PA, USA) was used, the top and two opposite sides of the chamber were made of aluminum panels, the other two sides were made of transparent Plexiglas (rear wall and front door). The chamber was equipped with a floor composed of 18 steel rods connected to precision-regulated shocker (Coulbourn Instruments) delivering electric footshock stimuli. The apparatus was enclosed in a ventilated and sound-attenuated box. All stimuli were controlled by a computer software package (Graphic State, Coulbourn Instruments, Allentown, PA, USA). The ventilation fan and the number, duration and intervals between electric shocks were controlled by a computer.

The context fear conditioning procedure was performed on PND 90. As previously reported, Freezing was defined as the complete absence of movement besides respiration, was used as a measure of learning and memory. The rats were placed into the chamber (conditioned stimulus, CS), and a 2 s, 0.8 mA shock for three times with an interval of 60 s were given after 180 s. Rats were left in the conditioning chamber for another 60 s and returned to their home cage. After 1 h of the contextual fear acquisition, the rats were returned to the chamber for 5 min in the absence of shock to test for short-term contextual fear. Another 5 min test session was conducted after 24 h of conditioning to test for long-term contextual fear.

2.3. Morris water maze

To explore whether or not MS on fear memory can affect the spatial learning and memory, a half of both MS ($n = 13$) and control ($n = 13$) rats were assigned to Morris water maze on PND 90. For spatial orientation, rats were given four trials per day for four consecutive days. For each trial, rats were allowed to search for the hidden platform within 90 s, which was hidden 1 cm below the