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Research Paper

Prenatal stress-induced impairments of cognitive flexibility and bidirectional synaptic plasticity are possibly associated with autophagy in adolescent male-offspring

Hui Zhang[1], Yingchun Shang[1], Xi Xiao, Mei Yu, Tao Zhang*

College of Life Sciences, Nankai University, 300071 Tianjin, PR China
Key Laboratory of Bioactive Materials Ministry of Education, Nankai University, 300071 Tianjin, PR China

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ABSTRACT

Prenatal stress (PS) brings numerous outcomes on offspring, including anxiety, depression-like behavior and other cognitive disorder. In this study, a rat model of PS was established by using restraint stress for 45 min three times per day from the 15th to 21st day of pregnancy. Behavioral tests, including open field test (OPT), elevated plus-maze (EPM) and Morris water-maze (MWM), were performed in adolescent male-offspring. The bidirectional synaptic plasticity, including long-term potential (LTP) and depotentiation (DEP), from the hippocampal *Schaffer collaterals* to CA1 region was subsequently measured. Furthermore, Western blot assay, immunofluorescence staining and hematoxylin-eosin (HE) staining were employed. The MWM test showed that the cognitive flexibility was remarkably damaged in PS offspring. Meanwhile, PS considerably aggravated the anxiety and depression-like behavior in OPT and EPM. Both LTP and DEP were significantly inhibited by PS. Furthermore, PS considerably altered the expression of synaptic-related proteins NR2A, NR2B and PSD-95 in adolescent male-offspring. Interestingly, PS significantly elevated the autophagy level in the hippocampus of male-offspring. In order to investigate the role of autophagy on the negative impacts of PS in adolescent male-offspring, both *in vitro* and *in vivo* studies were performed. It was found that autophagy inhibitors significantly eliminated the alterations in gene expression induced by corticosterone. The results suggest that regulating autophagy may become a new targeted therapy to relieve the damage induced by PS in adolescent male-offspring.

1. Introduction

Prenatal stress (PS) has numerous detrimental effects on development and function of CNS (central nervous system) in humans and animals. Several clinical studies reported that prenatal exposure to maternal stress was associated with the abnormal motor, cognitive and linguistic function of children (Cao et al., 2014; King and Laplante, 2005; Laplante et al., 2007). In animal studies, the effects of PS on the development of neurons and synapses in several brain components such as hypothalamus, hippocampus, cortex and amygdala have been revealed (Fujioka et al., 1999; Kraszpulski et al., 2006; Mychasiuk et al., 2012). Moreover, it has been proved that there are long-term impacts of PS on offspring's cognitive and psychosocial functions (Weinstock, 2008).

Adolescence is a critical stage in the development of brain (Andersen, 2003). It was well known that the susceptibility of stress-related psychological disorders, such as anxiety and depression, was remarkably increased during adolescence (Romeo and McEwen, 2006). The hypothalamic-pituitary-adrenal (HPA) axis, an endocrine axis which mediates the stress response, is significantly changed in adolescent development period (Romeo, 2010). Investigations in rodents showed that adolescence animals exhibit enhanced vulnerability to chronic stress as well as prolonged HPA axis activity compared to that of adult animals (Jankord et al., 2010). PS-induced HPA-axis dysfunction was found in offspring animals. Interestingly, the effect of PS on HPA-axis reactivity was especially obvious in young offspring animals (Weinstock, 2005, 2008). Meanwhile, a previous study reported that the impairment of hippocampal synaptic plasticity, which was induced by PS in young offspring rats, did not persist into adulthood (Yeh et al., 2012). Moreover, intervention by environmental enrichment during adolescence stage could reverse the effect of PS on HPA-axis response to stress in adult offspring rats (Morley-Fletcher et al., 2003). It proposes

* Corresponding author at: College of Life Sciences, Nankai University, 300071 Tianjin, PR China.
E-mail address: zhangtao@nankai.edu.cn (T. Zhang).
[1] These two authors contribute equally in this work.

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that adolescence may be an important stage to explore the underlying mechanisms of cognitive deficits and synaptic plasticity impairments induced by PS.

Flexibility of memory is critical to learning ability and environmental adaptation (Brito and Barr, 2012; Eslinger and Grattan, 1993). A growing body of evidences showed that hippocampal-dependent spatial learning and memory abilities were altered by PS. However, there are few *in vivo* studies in which the effect of PS on memory flexibility and its underlying mechanisms have been performed. The synaptic plasticity in the hippocampal CA1 region plays an essential role in learning and memory. A previous study reported that PS impaired the long-term potentiation (LTP) but facilitated long-term depression (LTD) in the hippocampal slices of young offspring rats (Yang et al., 2006). Comparable observations *in vitro* can be found (Yaka et al., 2007; Yeh et al., 2012). Although it was proved in adult offspring rats that the synaptic plasticity *in vivo* was altered by PS, but the results from those studies were inconsistent. Depotentiation (DEP), the long-term reduction of synaptic strength after LTP inducement, is different from LTD in both sensitivity and molecular mechanism (Jouvenceau et al., 2003; Zhuo et al., 1999). As a form of synaptic plasticity, DEP is probably involved in memory process and flexibility (Zhang and Wang, 2013). Accordingly, it is necessary to get *in vivo* data about the effect of PS on the bidirectional synaptic plasticity, including LTP and DEP, of adolescent offspring rats.

The majority of studies on attempting to reveal the mechanism of PS effects on offspring behavior and synaptic function has been performed in HPA-axis and glucosteroids. However, there are few studies related to the downstream mechanism underlying HPA-axis. It was found that PS altered the hippocampal expression of the synapse proteins, which could contribute to LTP impairment (Yaka et al., 2007). Nevertheless, the cause of these alterations needs to be investigated. Autophagy is an evolutionarily conserved process which mediates the degradation of proteins and organelles. Dysfunction of autophagy is associated with the imbalance of protein synthesis and degradation (Klionsky and Emr, 2000). Meanwhile, autophagy plays a key role in synaptic pruning, a developmental procedure during adolescence which is critical for the maturation of neural circuits (Tang et al., 2014). These findings have implied a possibility that autophagy may participate in the alterations and impairments of synapse protein expression and synaptic plasticity in adolescent PS offspring rats.

Consequently, our major aim in present study was to investigate the effect of PS on the memory flexibility and bidirectional *in vivo* synaptic plasticity in adolescent offspring rats and explore the underlying mechanism. This was done by establishing a rat model of PS and performing Morris water-maze (MWM) test, open field test and elevated plus-maze test in the PS offspring. Afterwards, paired-pulse ratio (PPR), LTP and DEP in the hippocampus *Schaffer collaterals*-CA1 pathway were recorded *in vivo*. Furthermore, synapse-related protein expression and autophagy level were measured by Western blot assay in the hippocampus of adolescent male-offspring. We further hypothesized that autophagy possibly played an important role in the impairment of bidirectional synaptic plasticity and cognitive flexibility induced by PS in adolescent male-offspring rats. Since PS-induced excess maternal corticosterone mediates the behavioral alterations in the offspring (Barbazanges et al., 1996; Zagron and Weinstock, 2007), both *in vitro* and *in vivo* experiments have been performed in both corticosterone-treated PC12 cells and Wistar rats to assess the possible role of autophagy in the PS-induced alterations of synapse proteins.

2. Materials and methods

2.1. Animals

Six pregnant Wistar rats and twelve male Wistar rats were purchased from the Laboratory Animal Center of the Academy of Military Medical Science of People's Liberation Army. And then they were housed in individual cages with a 12 h light/dark cycle (light on at 7:00 to 19:00) and a room temperature of 25 ± 2 °C. Food and water were given *ad libitum*. All procedures were carried out according to the NIH Guide for the Care and Use of Laboratory Animals and approved by the Ethical Commission at Nankai University. All efforts were made to minimize the animals' suffering and the number of animals.

2.2. PS procedure

From day 15 to 21 of gestation, only three pregnant rats were used for establishing a rat model of PS, in which the animals were restraint stressed in a transparent plastic cylinder (64 mm in diameter) for 3 times daily, 45 min each time at random interval (at least 3 h). The other three pregnant rats served as control. After birth, all the offspring were kept together with their biological mothers. No more than 2 pups from same litter were used. On day 21, only male pups were used after all offspring were weaned. In the present study, two groups were named: control group (Control, $n = 6$) and prenatal stress group (PS, $n = 6$).

2.3. Corticosterone and chloroquine injections

Adolescent male Wistar rats were treated with corticosterone (Cort group, $n = 4$, 40 mg/kg per day, s.c.) or corticosterone + chloroquine (Cort + CQ group, $n = 4$, 20 mg/kg per day, i.p.) from postnatal day 28 to 48. Corticosterone was suspended in sesame oil for 33.3 mg/mL. Chloroquine (CQ) was dissolved in physiological saline solution at 5 mg/mL. Normal rats were injected with same doses of vehicles (Control group, $n = 4$).

2.4. Body weight and sucrose preference test

Body weight of adolescent offspring rats in both the Control group and the PS group was measured on postnatal day 35. Sucrose preference test (SPT) was performed as previously described (Yu et al., 2016). Animals were habituated to drink 1% sucrose solution for 48 h before SPT. Afterwards, food and water was deprived for 24 h. On the test day, both 1% sucrose solution and water were provided to each rat for 1 h. Bottles containing sucrose solution and water were weighed before and after test in order to calculate the sucrose and water consumption. The percentage of sucrose consumption was calculated as sucrose solution consumption (g) / total fluid consumption (g) × 100%.

2.5. Open field test

Open field test (OPT) was carried out as previously described with slight modifications (Shang et al., 2016a). Briefly, the test was performed in an enclosed square box (60 × 60 × 40 cm) with an open top and a dark floor. The bottom of box was divided into 16 equal squares. Central area was defined as 4 middle squares and peripheral area was defined as 12 around squares. Rats were placed individually on the center of the test zone at the beginning of the test and allowed to explore it freely. The box was cleaned using 75% ethanol and completely dried before the next animal was tested. During the test period (5 min), the track of rat was captured by a CCD camera which connected to a computer. The total number of crossing and the time spent in the central were automatically calculated by the recording software. The total number of rearing was counted manually.

2.6. Elevated plus-maze

The protocol for elevated plus-maze (EPM) test was complying with a pervious guideline (Walf and Frye, 2007). To begin the test, an animal was placed at the center of apparatus and facing the open arm. The test session is 5 min in duration. The maze was cleaned using 75% ethanol and completely dried before the next rat was tested. The number of