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Exposure of mother rats to chronic unpredictable stress before pregnancy alters the metabolism of gamma-aminobutyric acid and glutamate in the right hippocampus of offspring in early adolescence in a sexually dimorphic manner

Yuejun Huang[a,b], Zhiwei Shen[c], Liu Hu[b], Fang Xia[b], Yuewa Li[b], Jingwen Zhuang[b,*], Peishan Chen[d,*], Qingjun Huang[e,*]

[a] Department of Pediatrics, First Affiliated Hospital of Jinan University, West Huangpu Rd, Guangzhou 510632, Guangdong, China
[b] Department of Pediatrics, Second Affiliated Hospital of Medical College of Shantou University, North Dongxia Rd, Shantou 515041, Guangdong, China
[c] Department of Imageology, Second Affiliated Hospital of Medical College of Shantou University, North Dongxia Rd, Shantou 515041, Guangdong, China
[d] Department of Obstetrics, Second Affiliated Hospital of Medical College of Shantou University, North Dongxia Rd, Shantou 515041, Guangdong, China
[e] Joint Lab of Biological Psychiatry, Mental Health Center of Shantou University, Taishan Rd, Shantou 515041, Guangdong, China

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ABSTRACT

There is increasing evidence that mothers' exposure to stress before or during pregnancy is linked to an incidence of psychiatric disorders in offspring. However, a few studies have estimated the role of sex in the detrimental effects of pre-gestational stress on the offspring rats at early adolescence. Sex differences regarding the metabolism of gamma-aminobutyric acid and glutamate in the right hippocampus were investigated by MRS when the offspring rats reached 30 days. Additionally, the impact of pre-gestational stress exposed on an additional short-term acute stressor, such as forced swim, was examined in the male and female offspring rats. Our findings showed female offspring rats were more vulnerable to stressful conditions for either pre-gestational stress or acute stress in early adolescence, and had decreased GABA/Cr+PCr and Glu/Cr+PCr in the right hippocampus. Interestingly, in response to forced swim, male offspring rats whose mothers were exposed to pre-gestational stress were more affected by the short-term acute stressor and this was manifested by change of Glu/GABA and Glu/Gln in the right hippocampus. These data indicated that although female offspring rats were more vulnerable to pre-gestational stress from their mothers than males, in response to an additional acute stressor they showed better response. Therefore, both sexually dimorphic manner and combination of stressful procedures should be carefully considered in the study of stress-related psychiatric disorders in early adolescence.

1. Introduction

Many studies have shown that exposure to the chronic stress before or during pregnancy is harmful to the brain development of offspring (Baquedano et al., 2011). In our preliminary study (Huang et al., 2013, 2010, 2012), we used a chronic unpredictable stress (CUS) (Katz and Schmaltz, 1980; Willner, 1997) animal model to examine the effects of pre-gestational stress on offspring rats. And we found that the pre-gestational CUS could result in a decreased coping ability of offspring rats in the forced swimming test as well as increased corticotropin-releasing hormone (CRH) and corticosterone (COR) release in the serum of these offspring rats following exposure to stressful stimuli. These neurochemical changes might alter the programing of the hypothalamic–pituitary–adrenal (HPA) axis function, resulting in the decreased ability of an organism to respond to, cope with, and adapt to stressful stimuli (Baquedano et al., 2011; Kapoor et al., 2009). Many studies have found a sexually dimorphic connection to the HPA axis response to stress (McCormick et al., 2002; Rhodes and Rubin, 1999). Moreover, sex differences in behavior have been linked with indices of HPA axis activity that are differently modulated in male and female rats following either short-term (Koch et al., 2014; Toledo-Rodriguez and Sandi, 2011) or long-term stressful procedures (Kuipers et al., 2013; Naninck et al., 2015). HPA axis function is modulated by a complex feedback involving local hypothalamic inhibitory and excitatory systems as well as projections from stressor-sensitive brain regions such as the hippocampus and amygdala (Herman et al., 2002a; Jankord and

* Corresponding authors.
E-mail addresses: 344202589@qq.com (J. Zhuang), f2fckcps@163.com (P. Chen), huangqj@stumhc.cn (Q. Huang).

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Herman, 2008). Some studies found that stress had influenced the hippocampus predominantly, altering the excitatory neurotransmitter glutamate (Glu) and the inhibitory neurotransmitter gamma-amino-butyric acid (GABA) (Herman et al., 2002b; Radley and Sawchenko, 2011).

Glu, glutamine (Gln), and GABA are the central components of metabolism in the brain (Peng et al., 1993). Glu, a major excitatory neurotransmitter in the mammalian central nervous system (CNS), participates in the integration of brain function and in the synaptic plasticity, memory, and learning processes (Raudensky and Yamamoto, 2007). Glu is virtually confined to neurons (Sheng and Kim, 2002), while Gln is mainly distributed in the glia (Kanamori et al., 1995). Once synthesized in astrocytes, most Glu is converted to Gln by Gln synthase which is the astrocyte-specific enzyme. And then, Gln is subsequently transferred to the neuronal compartment and metabolized in the Glu–Gln cycle (Wu et al., 2015). This cycle is a major pathway to maintaining synaptic glutamate. In addition, production of GABA, a major inhibitory neurotransmitter derived from Glu, also depends on the cycle (Liang et al., 2006). While this neurotransmitter plays a major role in the modulation of virtually all cognitive and behavioral processes, many studies have particularly highlighted the role of the GABA-related system in the central modulation for stress responses (Hasler et al., 2009; Herman et al., 2004). Adolescence is a critical stage for the development of emotional maturity and diverse forms of psychopathology (Cunningham et al., 2002). In rats, the rapid phase of hippocampal development occurs in the first 4 weeks after birth. Therefore, metabolic disturbances of GABA and Glu in the hippocampus in adolescence may increase the risk for psychiatric disorders. (Almeida-Suhett et al., 2014; Lebhardt et al., 2016; Li et al., 2015; Northoff and Sibille, 2014; Pezze et al., 2014; Stan et al., 2014). Moreover, there is sexually dimorphic manner in the endocrine and neuroanatomical structure in early adolescence (Del Cerro et al., 2015; Uzturk et al., 2015), so sex difference should be carefully considered in the study of stress-related disorders in this special time.

Despite the availability of several relevant reports (Huang et al., 2010; Li et al., 2010), there are no studies that have estimated the role of sex in the detrimental effects of pre-gestational CUS on the metabolism of GABA and Glu in the hippocampus of offspring rats in early adolescence. In the present study, the metabolism of GABA and Glu in the right hippocampus was investigated by magnetic resonance spectroscopy (MRS) when the offspring rats reached 30 days. Additionally, the impact of mother rats exposed to pre-gestational stress of an additional short-term stressful procedure, such as forced swim, was examined in both sex offspring rats. In our preliminary study (Huang et al., 2013), we found forced swim induced a number of sex-specific changes in behavior and in dopaminergic activity of offspring rats whose mothers were exposed to CUS before pregnancy, and the alteration of dopaminergic activity in the brain occurred in the right hemisphere. Therefore, we focused on the metabolism of GABA and Glu in the right hippocampus of offspring rats in this study. In the present study, we hypothesized that the application of forced swim 24 h prior to MRS measurement in the offspring rats who were already disturbed by exposure to pre-gestational CUS, would give us additional information on sex differentiations in the metabolism of GABA and Glu in the right hippocampus of offspring rats in early adolescence.

2. Methods

2.1. Animals

Adult male (n=7) and female (n=14) SD rats (approximately 8 weeks old; 250–300 g and 200–250 g, respectively) were provided by the Animal Centre of Shantou University. Female rats were used for mother rats. Before the beginning of any stress procedures, mother rats were divided into two groups for treatment: control (n=6) and chronic unpredictable stress (CUS) (n=8), and were housed singly, in non-transparent cages (60 cm×40 cm×25 cm) in separate rooms under controlled 12-h light/12-h dark conditions (lights on at 8:00 a.m.) and temperature (24 °C). All rats had free access to food and water throughout the experiments, unless specified by the experimental procedure. Mother rats exhibited a normal oestrous cycle (4–5 days of oestrous) before the start of the experiment, with equal distribution of different stages of the oestrous cycle. The oestrous cycle was reconfirmed during the last week of CUS with the use of vaginal smears. All animal experiments were reviewed and approved by the Medical Animals Care and Welfare Committee of Shantou University Medical College (Shantou, China). All studies were carried out in accordance with the US National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications no. 80–23 revised 1996). Every effort was made to minimize the number of animals used and to reduce suffering. The flow chart of experiment design is shown in Fig. 1..

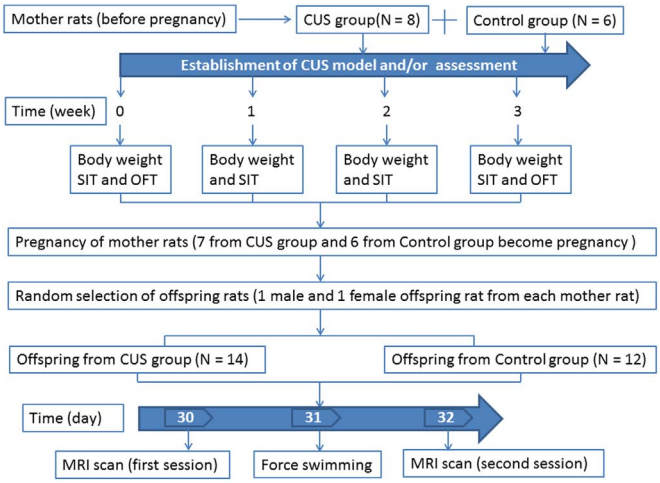

Fig. 1. The flow chart of experiment design. Before the beginning of any stress procedures, mother rats were divided into control (n=6) and chronic unpredictable stress (CUS) (n=8) groups. The schedule of CUS procedure is shown in Table 1. Body weight and SIT were measured before CUS (W0) procedure and once a week (W1, W2, W3) during CUS for 3 weeks. OFT was performed before (W0) and after (W3) the procedure of CUS. When the CUS procedure was finished, all mother rats were housed in pairs with a male rat for 1 week for mating. One female offspring and one male offspring were selected from each mother rat for MRI scan and forced swimming.

2.2. CUS procedure

Body weight of mother rats was measured before the start of CUS (W0) and once a week (W1, W2, W3) during CUS for 3 weeks. Open field test (OFT) and sucrose intake test (SIT) were used to assess the effectiveness of CUS model in mother rats. The OFT is used to measure the activity and curiosity of rats in the novel environment (Willner, 1997). The SIT is an additional, objective indicator to evaluate the rewarding value of sucrose solution in rodent chronic stress models (Willner et al., 1992). The CUS procedure, OFT, and SIT were performed as we previously described (Huang et al., 2013, 2012). The schedule is shown in Table 1.

2.2.1. SIT

The mother (n=14) rats experienced a 2-week adaptive period for consumption of 1% sucrose solution in water. The adaptive period consisted of four one-bottle-sucrose test sessions. Each session lasted 1 h (between 8:00 a.m. and 9:00 a.m.), and performed with an interval of 3 days following a period of food and water deprivation for 24 h. Sucrose consumption was calculated by weighing the bottle before and after the test. The last session of the adaptive period was measured before the start of CUS (W0) and was used as the baseline of sucrose consumption. Once a week (W1, W2, W3) during CUS, a one-bottle-