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PRENATAL CHRONIC MILD STRESS INDUCES DEPRESSION-LIKE BEHAVIOR AND SEX-SPECIFIC CHANGES IN REGIONAL GLUTAMATE RECEPTOR EXPRESSION PATTERNS IN ADULT RATS

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Abstract—Chronic stress during critical periods of human fetal brain development is associated with cognitive, behavioral, and mood disorders in later life. Altered glutamate receptor (GluR) expression has been implicated in the pathogenesis of stress-dependent disorders. To test whether prenatal chronic mild stress (PCMS) enhances offspring's vulnerability to stress-induced behavioral and neurobiological abnormalities and if this enhanced vulnerability is sex-dependent, we measured depression-like behavior in the forced swimming test (FST) and regional changes in GluR subunit expression in PCMS-exposed adult male and female rats. Both male and female PCMS-exposed rats exhibited stronger depression-like behavior than controls. Males and females exhibited unique regional changes in GluR expression in response to PCMS alone, FST alone (CON-FST), and PCMS with FST (PCMS-FST). In females, PCMS alone did not alter N-methyl-D-aspartate receptor (NMDAR) or metabotropic glutamate receptor (mGluR) expression, while in PCMS males, higher mGluR2/3, mGluR5, and NR1 expression levels were observed in the prefrontal cortex. In addition, PCMS altered the change in GluR expression induced by acute stress (the FST test), and this too was sex-specific. Male PCMS-FST rats expressed significantly lower mGluR5 levels in the hippocampus, lower mGluR5, NR1, postsynaptic density protein (PSD)95, and higher mGluR2/3 in the prefrontal cortex, and higher mGluR5 and PSD95 in the amygdala than male CON-FST rats. Female PCMS-FST rats expressed lower NR1 in the hippocampus, lower NR2B and PSD95 in the prefrontal cortex, lower mGluR2/3 in the amygdala, and higher PSD95 in the amygdala than female CON-FST rats. PCMS may increase the offspring's vulnerability to depression by altering sex-specific stress-induced changes in glutamatergic signaling. © **2015 Published by Elsevier Ltd. on behalf of IBRO.**

Key words: prenatal stress, depression, sex difference, glutamate receptors.

INTRODUCTION

The mammalian brain is highly vulnerable to stressors during fetal development. Recent studies have demonstrated an association between exposure to prenatal stress and adverse neurodevelopmental outcomes in human offspring later in life, including atten tion-deficit/hyperactivity disorder, autism, schizophrenia, anxiety, and depressive disorders (reviewed in Linnet et al., 2003; Glover, 2011). It has been suggested that a disturbance in the glutamatergic system of the central nervous system (CNS) may be involved in the pathophysiology of stress and stress-related psychiatric disorders (Levine et al., 2000; Frye et al., 2007); for instance, a number of studies have provided evidence for the role of glutamate and its receptors in the pathogenesis of stress-related disorders such as depression as well as in antidepressant responses (Musazzi et al., 2013).

The N-methyl-D-aspartate (NMDA) subclass of ionotropic glutamate receptors (iGluRs) is critical for induction of experience-dependent neuroplasticity, thereby conferring individual differences in the stress response. Activation of NMDA receptors requires concomitant binding of glycine/D-serine to the NR1 subunit and glutamate to NR2 (Clements and Westbrook, 1991; Matsui et al., 1995). Stress and stress hormones alter the expression levels of NR1 and NR2B subunits (Bartanusz et al., 1995). In addition, the NR1 co-agonist D-serine produces antidepressant-like effects in rodent behavioral despair models (Malkesman et al., 2012), while a NR2B receptor antagonist was effective

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Abbreviations: AD, amygdala; ANOVA, analysis of variance; CMS, chronic mild stress; FST, forced swimming test; GD, gestational day; GluR, glutamate receptor; HIP, hippocampus; iGluRs, ionotropic glutamate receptors; mGluRs, metabotropic glutamate receptors; NMDA, N-methyl-D-aspartate; PCMS, prenatal chronic mild stress; PFC, prefrontal cortex; PND, postnatal day; PSD, postsynaptic density protein.

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for treatment-resistant depression patients (Preskorn et al., 2008).

All three subgroups of G-protein-coupled glutamate receptors (metabotropic glutamate receptors (mGluRs)s I, II, and III) (Harvey and Shahid, 2012) have also been implicated in stress responses. Numerous studies have shown increased group I receptor (mGluR1 and mGluR5) protein levels in the hippocampus after stress, suggesting possible involvement in depression and neuroendocrine sensitization to stress (Wieronska et al., 2001; Inoue et al., 2013). Indeed, group I receptor antagonists have antidepressant-like activities in a variety of experimental paradigms in laboratory animals (Paul and Skolnick, 2003; Legutko et al., 2006; Belozertseva et al., 2007). The group II mGluRs (mGluR2/3) are largely localized to presynaptic membranes where they act as autoreceptors to decrease excitatory glutamatergic transmission (Pinheiro and Mulle, 2008; Sanacora et al., 2008). Stam et al. (2007) revealed increased expression of mGluR3 in the basolateral nucleus of the amygdala and increased mGluR2 expression in the agranular insular cortex of preshocked (stressed) rats compared to controls, which could be an endogenous mechanism to counteract stress-induced neuronal sensitization. However, different animal studies have produced conflicting results, showing antidepressant-like activities of both selective mGlu2/3 receptor agonists and antagonists (Kawashima et al., 2005; Palucha-Poniewiera et al., 2010; Koike et al., 2011; Iijima et al., 2013).

Prenatal stress impairs both iGluR- and mGluR-dependent synaptic plasticity, consistent with the often reported deleterious effects of prenatal stress on learning and memory as well as sensitized behavior responses to stress (Berger et al., 2002; Owen and Matthews, 2007; Jia et al., 2010; Matrisciano et al., 2012; Burt et al., 2013; Holloway et al., 2013; Sun et al., 2013; Tavassoli et al., 2013). Much of the animal research on the effects of prenatal stress on anxiety- and depression-like behaviors has focused on male offspring, mainly to obviate the hormonal effects of estrous. Studies on sex differences on the effects of prenatal stress have generated mixed results. For example, prenatal restraint stress appeared to have no effect on the immobility time of male offspring in the forced swimming test (FST), but did increase immobility time in females (Alonso et al., 1991, 2000; Frye and Wawrzycki, 2003; Van den Hove et al., 2005). Conversely, several studies reported increased immobility time in the FST only in prenatally stressed male offspring (Mueller and Bale, 2008; Van den Hove et al., 2014). There is a paucity of research examining these sex differences at the neurobiological and mechanistic levels. Prenatal stress may affect GluR expression in the hippocampus, prefrontal cortex, and amygdala in male offspring (Zuena et al., 2008; Fumagalli et al., 2009; Biala et al., 2011; Morley-Fletcher et al., 2011; Laloux et al., 2012; Chutabhakdikul and Surakul, 2013; Sun et al., 2013), but there have been few studies comparing expression patterns between sexes. Biala et al. (2011) found that prenatal stress reduced levels of NR1 in the hippocampus of male offspring, with no alterations in female offspring. Zuena et al. (2008) reported that prenatal stress reduced the levels of mGluR5 in the hippocampus of male offspring, while increased hippocampal mGluR5 expression was found in female offspring. Male offspring exposed to prenatal stress showed increased levels of NR2B in the prefrontal cortex, while females exhibited decreased levels (Fumagalli et al., 2009).

Most of these studies have focused on changes in glutamatergic transmission and plasticity in the hippocampus rather than the prefrontal cortex or amygdala, regions critical for behavioral stress responses. Furthermore, many of these studies used restraint stress, a severe acute stressor that does not mimic the usual types of psychosocial stressors that may affect women during pregnancy. Moreover, restraint stress may cause trauma to the fetus through physical pressure (Yum et al., 2012). Chronic mild stress (CMS) paradigms have been developed in an attempt to establish animal models of neuropsychiatric disorders that better reflect the usual mild stressors encountered by pregnant women.

In the present study, we examined the effects of prenatal chronic mild stress (PCMS) on (i) depression-like behavior, (ii) basal GluR subunit expression in the hippocampus, prefrontal cortex, and amygdala, and (iii) sex-specific changes in regional GluR expression patterns following forced-swim stress. We demonstrate that PCMS alters the changes in glutamate subunit expression patterns associated with FST, suggesting altered stress responsivity and enhanced vulnerability to stress-induced behavioral and neurological abnormalities.

EXPERIMENTAL PROCEDURES

Animals

Virgin female Sprague–Dawley rats, 3−4 months old and weighing 250−270 g, were obtained from the Laboratory Animal Center of the China Medical University, Shenyang, Liaoning, China, and group-housed four per cage. All animals were treated in accordance with the guidelines outlined in the Care and Use of Laboratory Animals (NIH publication, 85-23, revised 1996). All experiments were approved by the Institutional Animal Care and Use Committee of the China Medical University. For one week after arrival, females were placed with male rats for mating (1:1), and the vaginal smear was examined the following morning. The day on which the smear was sperm-positive was regarded as gestational day 0 (GD0). Pregnant rats were then housed separately and randomly assigned to either the PCMS group (n = 26) or the control group (n = 26).

Procedure of PCMS

The PCMS procedure was conducted according to a standard protocol from GD7 to GD20. This procedure included only environmental and social stressors with no nociceptive events: space restriction by cage tilt (angle of 45°), placing the rats in a mouse cage, housing in an empty cage with no sawdust, housing in a cage with wet bedding, cage crowding, food and water deprivation,