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Sex-specific effects of prenatal chronic mild stress on adult spatial learning capacity and regional glutamate receptor expression profiles

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ABSTRACT

Both animal experiments and clinical studies have demonstrated that prenatal stress can cause cognitive disorders in offspring. To explore the scope of these deficits and identify potential underlying mechanisms, we examined the spatial learning and memory performance and glutamate receptor (GluR) expression patterns of adult rats exposed to prenatal chronic mild stress (PCMS). Principal component analysis (PCA) was employed to reveal the interrelationships among spatial learning indices and GluR expression changes. Female PCMS-exposed offspring exhibited markedly impaired spatial learning and memory in the Morris water maze (MWM) task compared to control females, while PCMS-exposed males showed better initial spatial learning in the MWM compared to control males. PCMS also altered basal and post-MWM glutamate receptor expression patterns, but these effects differed markedly between sexes. Male PCMS-exposed offspring exhibited elevated basal expression of NR1, mGluR5, and mGluR2/3 in the prefrontal cortex (PFC), whereas females showed no basal expression changes. Following MWM training, PCMS-exposed males expressed higher NR1 in the PFC and mammillary body (MB), higher mGluR2/3 in PFC, and lower NR2B in the hippocampus (HIP), PFC, and MB compared to unstressed MWM-trained males. Female PCMS-exposed offspring showed strongly reduced NR1 in MB and NR2B in the HIP, PFC, and MB, and increased mGluR2/3 in PFC compared to unstressed MWM-trained females. This is the first report suggesting that NMDA subunits in the MB are involved in spatial learning. Additionally, PCA further suggests that the NR1–NR2B form is the most important for spatial memory formation. These results reveal long-term sex-specific effects of PCMS on spatial learning and memory performance in adulthood and implicate GluR expression changes within HIP, PFC, and MB as possible molecular mechanisms underlying cognitive dysfunction in offspring exposed to prenatal stress.

1. Introduction

Exposure to stress during pregnancy (prenatal stress, PS) can have long-term deleterious effects on the neurodevelopment and behavior of offspring, which may lead to increased incidence of neuropsychiatric disorders (Charil et al., 2010; Lupien et al., 2009; Rice et al., 2007), such as autism, attention deficit hyperactivity disorder and schizophrenia (Lee et al., 2015; Moore and Susser, 2011; Ronald et al., 2010). A hallmark of these disorders is cognitive impairment (Bergman et al., 2007; Paris and Frye, 2011; Richetto and Riva, 2014). Evidence indicates that PS affects cognitive function by disrupting synaptic plasticity at glutamatergic synapses (Rangon et al., 2007; Zhao et al., 2013). For instance, PS diminishes hippocampal NMDA receptor (NMDAR)-dependent long-term potentiation (LTP) and enhances long-term depression (LTD) (Jia et al., 2010; Son et al., 2006; Yaka et al., 2007; Yang et al., 2006), both of which are considered major neurocellular mechanisms underlying learning and memory.

Glutamate receptors are heteromultimers formed from various combinations of obligatory and regulatory subunits, and the large number of isoforms within each receptor subunit family confers substantial functional heterogeneity. Thus, changes in regional subunit expression patterns by PS could have a myriad of disruptive effects on

Abbreviations: CON, control; DAG, diacylglycerol; GD, gestational day; HIP, hippocampus; iGluRs, ionotropic glutamate receptors; LTD, long-term depression; LTP, long-term potentiation; MB, mammillary body; mGluRs, metabotropic glutamate receptors; MWM, Morris water maze; NMDAR, *N*-methyl-D-aspartate receptor; PCMS, prenatal chronic mild stress; PFC, prefrontal cortex; PND, postnatal day; PCA, principal component analysis; PKA, protein kinase A; PKC, protein kinase C; PRS, prenatal restraint stress; PSD, postsynaptic density protein.

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neurodevelopment, synaptic plasticity, and behavior. NMDARs belong to the ionotropic glutamate receptor (iGluR) family. Activation of NMDAR is a prerequisite for the induction of several forms of LTP and LTD (Ghosh, 2002). The metabotropic glutamate receptors (mGluRs) are divided into three groups. Group I mGluRs (mGluR1 and mGluR5) are mainly postsynaptic and regulate neuronal excitability and neurotransmitter response efficacy (De Blasi et al., 2001), whereas group II and III mGluRs are largely presynaptic and are believed to regulate neurotransmitter release (Sanacora et al., 2008). Glutamate receptors are the predominant mediators of excitatory synaptic transmission in the mature central nervous system (CNS) and are also critical for neural development (Michaelis, 1998). Dysregulation of glutamate release and receptor expression is thus a major pathogenic factor in CNS diseases such as epilepsy, anxiety, depression, schizophrenia, and both acute and chronic neurodegenerative disorders (Spooren et al., 2003), suggesting that PS may enhance the risks of these diseases in later life.

Prenatal stress influences the morphological and functional development of brain regions involved in motorcontrol, executive function, cognition, and emotional regulation, such as the hippocampus (HIP), prefrontal cortex (PFC), hypothalamus, and amygdala (Charil et al., 2010). In an effort to understand the mechanisms underlying impaired cognitive function induced by PS, most investigators have focused on the HIP, given the well known contribution of HIP synaptic plasticity to associative learning and long-term declarative memory. Recent studies have also demonstrated altered neurotransmission in the PFC of prenatally stressed animals (Kolb et al., 2012; Mychasiuk et al., 2011). In addition, as part of an "extended hippocampal system", the mammillary bodies (MBs) have been implicated in memory processes by relaying hippocampal inputs to the anterior thalamic nuclei and from there to the cingulate cortex or the PFC (Gaffan, 2001; Vann, 2010). However, at present, it is still unclear if the MB is vulnerable to PS or whether PS-induced changes to the MB contribute to the observed deficits in adult function.

Recent studies have shown that the aberrant effects of PS on neural function and behavior are sex-specific, implicating hormonal dysregulation in these changes. Even so, there are no recognized loci for memory impairment and, hence, no agreed mechanisms to explain these sex-specific effects of PS. For example, adult male offspring exposed to prenatal restraint stress (PRS) showed reduced spatial memory in the Morris water maze (MWM) (Lemaire et al., 2000). Conversely, PRS improved learning in female rats with no impact on males (Zuena et al., 2008). Previous study implicated that PS reduced the levels of NR1 in the HIP of male offspring, thus providing a plausible mechanism for reduced adult MWM performance, while having no effect on female offspring (Biala et al., 2011). However, Sun and his colleagues found that PS reduced NR1 expression in the HIP with no sex difference (Sun et al., 2013). Therefore, it is still unclear whether PS causes long-lasting sex-specific changes in regional glutamate receptor expression patterns and if such changes ultimately contribute to higher incidence of cognitive and sex-specific neuropsychiatric disorders. Undoubtedly, differences in PS stimuli have contributed to these discordant results. For instance, restraint stress does not replicate the daily mild stressors encountered by pregnant women. On the other hand, chronic mild stress is presumed to mimic typical socio-environmental stressors and so may be a better model to evaluate the consequences of PS on offspring neurodevelopment, cognition, and behavior.

These findings lead to the following hypothesis: prenatal chronic mild stress (PCMS) induces sex-specific changes in cognitive performance by altering glutamate receptor expression in the HIP, PFC, and MB of adult offspring. To test this hypothesis, we exposed rats to PCMS and measured adult spatial learning in the MWM and analyzed the relationships between MWM performance and glutamate receptor expression in the HIP, PFC, and MB. In addition, principal component analysis (PCA) was employed to test for underlying trends to reveal the interrelationships among spatial learning indices and regional GluR expression changes.

2. Materials and methods

2.1. Animals

Nulliparous female Sprague-Dawley rats at 3–4 months (250–270 g) were obtained from the Experimental Animal Center of China Medical University (Shenyang, Liaoning, China). Females were group housed (4 per cage) for at least one week before mating and then were individually housed with a male rat (280–300 g) for mating. A sperm-positive vaginal smear was defined as "day 0" of gestation (GD0). All rats were housed in a room maintained at 22 °C and 50% humidity under a 12 h light/dark cycle and had free access to food and water. All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. PCMS paradigm

Beginning on day 7 and continuing through day 20 of gestation, pregnant dams were randomly assigned to either the PCMS group or the control group. PCMS group rats were exposed to an unpredictable chronic mild stress paradigm in which various relatively mild stressors were presented on a random schedule (Hougaard et al., 2005). Briefly, the stresses used in this paradigm were: (1) space restriction by cage tilt (angle of 45°), (2) space restriction by placing the rat in a mouse cage, (3) housing in a cage without bedding (sawdust), (4) housing in a cage with wet bedding, (5) food and water deprivation, (6) flashing light during the dark phase, (7) reversed light/dark cycle, and (8) social stress induced by overcrowded housing conditions during the dark (active) phase of the cycle. One or two of these stressors were administered per day in a randomized order with respect to the light and dark phases of the animals. After termination of exposures at GD20, the females were singly housed. Control dams were left undisturbed throughout the experiment. All time-mated dams gave birth on GD 21. At weaning (postnatal day 21), male and female offspring were housed in same-sex, like-treated groups of four. Pups were exposed to normal animal husbandry procedures from that point forward until experimental use. Only one or two animals per sex/treatment group were included from each litter, as is appropriate for studies of prenatal treatment effects.

2.3. Morris water maze (MWM) test

This test was performed as described previously (Vorhees and Williams, 2006), and half of the experimental rats were tested in the MWM. The MWM setting (150 cm in diameter; 60 cm in height) was used in combination with a platform that had a diameter of 12 cm. The MWM comprises a circular pool filled with water at 20 ± 1 °C. It relies on distal cues to navigate from start locations around the perimeter of an open swimming arena to locate a submerged escape platform. The escape platform was placed at a fixed position in the center of one quadrant and hidden 2 cm beneath the water surface. Curtains were drawn around the pool with distinctive visual marks serving as distal cues, and the experimental room was kept invariable. A video camera was set above the center of the pool and connected to a video tracker system (EthoVision; Noldus, The Netherlands). An automated video tracking program was used to record the swimming length, velocity, escape latency in finding the hidden platform, first latency to platform, and the time spent in each quadrant.

Most MWM protocols use four start locations: N, S, E and W. One concern about the cardinal start position is that they are not equidistant from the goal, creating short and long paths to the goal. A partial solution that we have used is to use only distal start locations (Vorhees and Williams, 2006). By this, we choose the goal location of SW, then we use start locations of N, W, NW and SE. Table 1 illustrates the set of semi-randomly selected distal start positions for basic acquisition training, with the platform being located in the SW quadrant. These are designed so that the animal is not able to learn a specific order of right