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Research report

Involvement of NR1, NR2A different expression in brain regions in anxiety-like behavior of prenatally stressed offspring

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HIGHLIGHTS

- Prenatal stress caused behavioral change in OFT and EPM of 3-month rat offspring.
- Prenatal stress changed NR1 and NR2A expression in offspring brain.
- NR1 and NR2A might be involved in offspring anxiety-like behavior.

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ABSTRACT

Prenatal stress (PS) has been shown to be associated with anxiety. However, the underlying neurological mechanisms are not well understood. To determine the effects of PS on anxiety-like behavior in the adult offspring, we evaluated anxiety-like behavior using open field test (OFT) and elevated plus maze (EPM) in the 3-month offspring. Both male and female offspring showed a significant reduction of crossing counts in the center, total crossing counts, rearing counts and time spent in the center in the OFT, and only male offspring showed a decreased percentage of open-arm entries and open-arm time in open arms in the EPM. Additionally, expression of NR1 and NR2A subunit of N-methyl-D-aspartate receptor (NMDAR) in the hippocampus (HIP), prefrontal cortex (PFC) and striatum (STR) was studied. Our results showed that PS reduced NR1 and NR2A expression in the HIP, NR2A expression in the PFC and STR in the offspring. The altered NR1 and NR2A could have potential impact on anxiety-like behavior in the adult offspring exposed to PS.

1. Introduction

A considerable number of epidemiological surveys and experimental studies have established connections between prenatal maternal stress and anxiety [1,2]. Negative impact from PS may persist during adulthood and are commonly associated with anxiety [3], but the underlying mechanism is still unclear. A disturbance of glutamatergic transmission in the central nervous system (CNS) has been suggested to contribute to the pathophysiology of anxiety [4]. NMDARs are among of the major classes of ionotropic Glu receptors that play a central role in both emotion and cognition. The NMDARs are tetrameric complexes composed of obligatory NR1 subunits co-assembled with varying expression of NR2 family of subunits, *i.e.* NR2 (A–D) and less commonly NR3 (A–B) subunits [5]. NR1/NR2A receptors are mainly present at synaptic location and mediate fast neurotransmission [6]. PS markedly elevated the Glu level in the HIP, and the decreased protein level of the NR1 in the HIP of juvenile female offspring rats [7]. In the research of Son and colleagues, the reduction in NR1 receptor in the post-synaptic density in offspring of prenatally stressed mice was correlated to a reduced LTP in response to tetanic stimulation [8]. In male prepubertal offspring of maternally stressed Wistar rats, only the NR2B subunit was decreased in synaptic fractions of the CA1 region of the hippocampus, whereas protein levels of the NR1 and NR2A receptor subunits remained stable [9]. Another study provided that exposure to PS increased NR1 expression only in females as a compensatory [10]. In one study it was concluded that NR2A receptors are required for hippocampal LTP, but not LTD [11]. The NMDAR competitive antagonist, AP5, has been shown anxiolytic-like effects in several rodent studies with unconditioned anxiety [12].

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Exposure to stress may alter glutamatergic neurotransmission in brain areas related to anxiety, such as the HIP, PFC and STR [13–15]. The HIP is a crucial area in the brain related to anxiety. The effect for the HIP in innate anxiety is supported by the decreased fear and avoidant behavior in rodents following HIP lesion [16]. NMDARs in the HIP may be the key locus for the anxiolytic effects [17]. A connective region to the HIP related to anxiety is the PFC that represents the largest division of the frontal cortex. The density of NMDA receptors was decreased in the PFC or HIP in the depressive patients [18]. Previous behavioral assays have revealed that the PFC also plays a critical role in the modulation of anxiety-like behaviors [19]. In addition, Glu is the most important excitatory neurotransmitter in the STR, the principal region of the basal ganglia [20]. A study showed that NR2A-containing NMDARs regulated glutamatergic synaptic transmission in the STR [21]. Previous studies showed that animals with high anxiety had lower ventral striatal tissue levels of 5-HT [22–24]. Schneier and his colleagues suggested that generalized social anxiety disorder and trait detachment are associated with CNS dopamine dysfunction, particularly in the striatum [25]. In addition, a recent study explored the role of striatal areas related to rat analog of the Iowa Gambling Task performance using c-fos immunohistochemistry following the last training-session, found that the ventral striatum might play a pivotal role in decision-making related to anxiety in rats [26]. To the best of our knowledge, there was little research reported involvement of glutamatergic neurotransmission in striatum related to anxiety-like behavior. Therefore, all of three regions (HIP, PFC and STR) of the limbic circuit were tested in the present study due to their possible roles in anxiety-like behavior in the offspring exposed to PS.

The present study was to analyze the effect of PS on anxiety-like behavior in the 3-month offspring. We also determined whether the behavior and protein level changes were different between the male and female in various brain regions of offspring rats. Furthermore, we tested if repeated exposure to PS may result in anxiety-like behavior related to NR1 and NR2A expression in the HIP, PFC and STR.

2. Materials and methods

2.1. Animals

Sprague-Dawley rats were maintained at constant temperature (22 °C) and humidity (60%) on a 12 h light/dark cycle (light on 08:00–20:00 h), freely accessing to food and water throughout the experiment. All procedures were carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animals Care and Use Committee at Xi'an Jiaotong University. Every effort was made to optimize comfort and to minimize the use of animals. Nulliparous female rats weighing 230–250 g were housed with a sexually experienced male rat (280–350 g) for mating (3:1), and the vaginal smear was examined on the following morning. The day on which the vaginal smear was positive was defined as day 1 of gestation. Each pregnant rat was then housed individually. The pregnant rats were randomly allocated to either PS group (PS) or control group (CON).

2.2. Procedure of PS

The PS model we used is restraint stress model [27]. The device was a transparent cylinder (6.8 cm in diameter) and the length could be adjusted to accommodate the size of the animals. Air holes of the cylinder were for breathing. The mothers of the PS were stressed for 3 times/day (The interval should not be <2 h) for 45 min under bright light from gestational days 14–21. Mothers of the CON were undisturbed in their home cages during the same time period. To avoid animals from being habituated to the daily program, phases were randomly shifted within certain time periods (08:00–11:00, 11:00–14:00 and 16:00–19:00). On the day of parturition litter characteristics were recorded. All offspring rats were placed in the same plastic cage, and kept together with their natural mothers until weaning. The litters containing 8–14 pups with a suitable number of males and females were used for the experiment. After weaning, male and female pups were separated and housed four in each cage. On postnatal day (PND) 90, one or two female and male pups were taken from each litter for the behavioral measures.

2.3. Behavioral measures

2.3.1. OFT

Both sexes were tested using a modified version of the OFT [28]. During a 5-min observation period, rats were exposed to an open field (150 cm × 150 cm × 49.5 cm; black acrylic walls, green floor) divided into a 5 × 5 grid of equally sized squares using white tape. The central region of the box (3 × 3 = 9 squares) was subdivided into a large center and a small center of 8 and 1 squares respectively. The test started by placing the rat in the same side of the small center. During the test, the time spent in the center was recorded. In addition, the frequency of the following behaviors was quantified, crossing counts (the number of squares crossed with the four paws), rearing counts (standing on hind legs, with or without contact with the sides of the arena), grooming counts (using paws or tongue to clean/scratch body). The open field was cleaned with 5% ethanol solution between each test.

2.3.2. EPM

Both sexes were tested using a modified version of the EPM [29]. The apparatus consisted of two open arms (10 cm × 50 cm) and two enclosed arms (10 cm × 50 cm × 40 cm high walls) extending from a central platform (10 cm × 10 cm). The whole apparatus was elevated 50 cm above the floor. Each rat was placed on the center platform, facing an open arm, the animals were observed during the 5-min test period using a real-time video system and the behavior was assessed using a computer-aided system (PM-200, Microsoft Office 2000 System, China). The total number of four-paw entries and the total time spent in the two arms were blindly measured. The maze was cleaned with 5% ethanol solution between each test.

2.4. Western blotting

Ten male and ten female offsprings from different dams were used for each group. The rats were anesthetized with 20% ethylcarbamate (1.2–1.5 mL/100 g, i.p.), and decapitated within 30 min after behavior tests ended. The HIP, PFC and STR tissues were dissected on the petridish filled with ice and immediately frozen in liquid nitrogen, and then kept in a −80 °C freezer until analysis. HIP, PFC and STR sample were homogenized in 1000 μL ice-cold Tris–HCl buffer (10 mM, pH 7.4), containing 1% SDS, 0.1 mM EDTA, 0.1 mM EGTA, 0.1 mM PMSF (Sigma, USA) (B1), Samples were centrifuged at 1000 × *g* for 10 min at 4 °C to remove nuclei and large debris (P1). The supernatant (S1) was then transferred to another tube and centrifuged again at 18,000 × *g* for 30 min at 4 °C to obtain a clarified fraction of cytosolic and light membrane fraction (S2) and a pellet corresponding to the crude synaptosomal fraction (P2), which was then resuspended in the same buffer and centrifuged at 25,000 × *g* to obtain a synaptosomal membrane fraction (LP1). Finally, the pellet was resuspended in 100 μL the same buffer (B1) added with 320 mM sucrose. Protein concentrations were determined using a Nano Drop ND-1000 spectrophotometer (Nano Drop,