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The persistent effects of maternal infection on the offspring's cognitive performance and rates of hippocampal neurogenesis

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ABSTRACT

Accumulating evidence indicates that perinatal infection is a major cause of neonatal neurologic morbidity. Here we explored the effects of maternal infection on the offspring's cognitive performance and hippocampal neurogenesis. Pregnant rats were treated with *Escherichia coli* suspension and allowed to deliver. Proliferating cells in the hippocampus were examined at postnatal (P) 3, 7, 14, and 28 days and neuronal survival/differentiation was assessed at P28. Additionally, we examined the expressions of BDNF, TrkB and Akt. The cognitive performance of the offspring was assessed by the Morris water maze test. We found that maternal infection significantly impaired the offspring's spatial learning ability and spatial memory, thus could delay the cognitive performance development. Maternal infection significantly increased the number of proliferating cells in the offspring's hippocampus at postnatal 3, 7 and 14 days, accompanied by significantly increased expressions of BDNF, TrkB and p-Akt at postnatal 3 and 7 days. On postnatal 28 days, maternal infection did not significantly affect the neuronal and glial differentiation, nor any significant changes in the expression levels of BDNF and TrkB in the hippocampus. Our result suggests that the hippocampal neurogenesis level may increase during early postnatal period after maternal infection. Increase of BDNF/TrkB expression and Akt activity may be the contributing molecular mechanism.

1. Introduction

Intrauterine infection/inflammation has been identified as the most common cause of preterm delivery and neonatal complications (Romero et al., 2003). When microorganisms or their metabolic products gain access to the fetus, they stimulate the production of cytokines with a systemic response. Intrauterine infection may lead to activation of the cytokine network, which in turn can cause white matter brain damage and preterm delivery, as well as future onset of cerebral palsy (CP) (Back et al., 2007; Buser et al., 2012). This white matter insult is identified clinically as periventricular leucomalacia (PVL), which is related to various impaired neurological outcomes including CP. Our previous studies demonstrated that oligodendrocyte loss and axonal degeneration occurred in periventricular white matter after intrauterine *Escherichia coli* infection and reactive astrogliosis was a characteristic response of the astrocytes to inflammation and damage (Yu et al., 2004). Although studies about hypoxia–ischemia and cognitive dysfunction have been investigated extensively, no studies to date have examined the long-term effects of maternal infection on the offspring's cognitive performance.

Neurogenesis occurs in the adult brain throughout the lives of all mammals. The dentate gyrus (DG) of the hippocampus and the subventricular zone (SVZ) of the lateral ventricles have been established as the primary sites of adult neurogenesis, and recent studies have shown that inflammation has a modulating effect on adult neurogenesis. The inflammatory factors have multiple functions and can change the microenvironment of the brain tissue to regulate neurogenesis. Although cellular composition, proliferation, migration and differentiation in the SVZ and DG have been investigated extensively in normal animals and in a broad range of pathological conditions (Fatemi et al., 2002; Gould et al., 1999a; Guo et al., 2009; Kempermann et al., 1997; Kuhn et al., 1996; Magavi et al., 2000; Parent and Silverstein, 2007; Segovia et al., 2006; van Praag et al., 1999), data on neurogenesis in response to intrauterine infection/inflammation are limited. To date, neurogenesis has not yet been studied in the intrauterine *E. coli* infection model in rats.

Abbreviations: Akt, serine/threonine kinase; BrdU, bromodeoxyuridine; BDNF, brain-derived neurotrophic factor; CP, cerebral palsy; CNS, central nervous system; DG, dentate gyrus; DGC, dentate granule cell; E, embryonic day; *E. coli*, *Escherichia coli*; GFAP, glial fibrillary acidic protein; MWM, Morris water maze; NeuN, neuron-specific nuclear protein; NSC/NPC, neural stem/progenitor cell; PBS, phosphate-buffered saline; P, postnatal day; PI3K, phosphatidylinositol 3′-kinase; PVDF, polyvinylidene fluoride; PVL, periventricular leucomalacia; SGZ, subgranular zone; SVZ, subventricular zone; TrkB, tyrosine kinase receptor B.

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The phosphatidylinositol 3′-kinase (PI-3K)-Akt pathway is a critical transducer for several major survival signals in the central nervous system (CNS), and is most commonly associated with cell survival by inhibiting the activation of proapoptotic proteins and transcription factors (Le Belle et al., 2011; Zhang et al., 2007). BDNF is known to play central roles in neuronal growth, development, plasticity, survival, neuroprotection and repair (Kuzumaki et al., 2011; Li et al., 2008). Luikart et al. (2008) found that BDNF carried out these functions through a complex array of intracellular signaling cascade, including the PI3K–Akt pathway. Some studies show that the acute activation of Akt is neuroprotective after cerebral ischemia, traumatic brain injury and cell death following spinal cord injury (Paterniti et al., 2011). To date, however, the potential mechanisms that PI3K–Akt signaling pathway subserves the beneficial effects of inflammation-induced neurogenesis remain largely unknown.

Our group has established and characterized the *E. coli*-infected animal model (Jiang et al., 2012; Shen et al., 2007; Yuan et al., 2005). This study aims to investigate whether maternal infection delayed the offspring's cognitive development. The evaluation of neurogenesis in a well-established *E. coli*-infected animal model may provide crucial information in relation to the capacity of the brain for self-repair in inflammatory conditions. In addition, understanding the pathophysiological features such as the neuronal regeneration after the induction of inflammation may help to find therapeutic alternatives for delayed cognitive development induced by intrauterine *E. coli* infection.

2. Methods

2.1. Animals

Rats used in the present study were obtained from the Experimental Animal Center of Zhejiang Medical Academy of Science at 12 days of gestation, and allowed to acclimate to the animal facility prior to experimental manipulation. The animals were maintained on a standard feed, with drinking water ad libitum. All animal experiments were approved by the Animal Care Committee of Zhejiang University in accordance with the Principles of Laboratory Animal Care (NIH publication 80-23, revised 1996).

2.2. Experimental groups

E. coli (ATCC-25922) was supplied by the Bacteriology Laboratory of Children's Hospital, Zhejiang University School of Medicine. The pregnant Sprague–Dawley rats at embryonic day 15 (E15) were anesthetized with an intraperitoneal dose of 40 mg/kg body weight of 2% sodium pentobarbital, followed by an endocervical injection of either 0.4 mL of *E. coli* suspension (*E. coli*-treated pregnant rats: n = 10) or the same volume of saline (saline-treated pregnant rats: n = 10). Identification of intrauterine infection was done as previously described (Jiang et al., 2012).

Experiment 1. After delivery, the number of newborn pups per litter was culled to 10 to minimize the effect of litter size on nutrition and body weight. The female pups were removed at weaning. 20 male pups at postnatal 28 days (P28) were randomly divided into two groups (*E. coli* group: n = 10; control group: n = 10) to carry out the Morris water maze test.

Experiment 2. 90 male pups were randomly divided into two groups (*E. coli* group: n = 45; control group: n = 45) and were used for neurogenesis analysis. Five pups from different litters in each group were killed at P3, 7, 14, and 28 respectively, and hippocampal tissues were used for cell proliferation analysis. Five pups from different litters in each group were killed at P28 and their hippocampal tissues were used for neuronal survival and differentiation analysis. Other 5 pups from different litters in each group were killed at P3, 7, 14, and 28 respectively, and hippocampal tissues were then immediately dissected and frozen in liquid nitrogen and stored at −80 °C for future examinations.

2.3. Morris water maze test

The Morris water maze pool is round, with a diameter of 150 cm and a height of 60 cm. The platform has a diameter of 12 cm, with the height ranges from 20 to 35 cm. The pool is divided into four equal quadrants (A, B, C and D). The platform was placed 2 cm below the surface of water in the same location for every experiment. The water maze acquisition devices and data processing software were purchased from Huaibei Biological Equipment Co., Ltd., China. When the rat stayed on the platform for more than 3 s, the camera over the pool would automatically stop recording. The reference outside the frame remained the same during training in the maze, and the water temperature was maintained at 22 °C.

2.3.1. Navigation test

The model was designed to test the learning ability of rats by observing the duration of escape latency to find the platform in training rats. The whole training process lasted for 5 days. On the first day, the rats were placed on the platform for 1 min to adapt, then they were left to swim away and find the platform again. If the platform was found after 120 s, the rats would be guided to the platform by the experimenter. From the second day, the rats were gently placed into the water facing the wall of the maze at a fixed point. They were trained four times a day to direct navigation to find the platform from 4 quadrants with a resting period of 10 s on the platform. Each animal was subjected to four consecutive trials on each day with a gap of 5 min. If the platform was not found within 120 s, the rats were led to the platform by the experimenter, and in such case, the escape latency was recorded as 120 s. The average escape latency is calculated from the duration to find the platform from 4 quadrants. The training procedure was repeated over the next 4 days.

2.3.2. Orientation test

The platform was removed 6 days after the training had ended. The duration of swimming time in the target quadrant, and the frequency of passing through the area where the platform had previously been, were recorded.

2.4. Bromodeoxyuridine (BrdU) treatment

Neurogenesis was examined by incorporation of BrdU (Sigma Aldrich, Inc., St. Louis, MO), a marker of proliferating cells and their progeny. BrdU (10 μg/μL in 0.9% NaCl) is incorporated into DNA during the S phase of cell cycle, as well as during DNA repair. Since DNA repair primarily occurs within hours, and cell proliferation primarily within days, intraperitoneal injections (i.p.) of BrdU (50 mg/kg) were given twice daily (at 12-h intervals) for 3 consecutive days (Arvidsson et al., 2002). Rats were killed at P3, P7, P14 and P28 after receiving BrdU at P1–3, P5–7, P12–14 and P26–28, respectively. To evaluate neuronal survival and differentiation, same dose of BrdU was cumulatively delivered at P1–7 for 7 consecutive days and rats were killed at P28. $BrdU^+/NeuN^+$ cells were identified as newly differentiated neurons and $BrdU^+/GFAP^+$ cells were identified as newly differentiated astrocytes.

2.5. Tissue fixation and immunohistochemistry

Under anesthesia with sodium pentobarbital (40 mg/kg, i.p.), rats were perfused transcardially with 0.9% saline, followed by 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) [pH 7.4]. The brains were removed and fixed in 4% paraformaldehyde for 2 days at room temperature. The immunohistochemical staining for BrdU was

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