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

Peifang Jiang a, Tao Zhu b, Wenting Zhao c, Jue Shen a, Yonglin Yu a, Jialu Xu a, Xi Chen d, Huimin Yu c,*

a Department of Neurology, Children’s Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, China
b Department of Critical Care Medicine, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou 310016, China
c Department of Neonatology, Children’s Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, China
d Central Laboratory, Children’s Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, China

A R T I C L E   I N F O

Article history:
Received 13 December 2012
Received in revised form 27 February 2013
Accepted 26 March 2013
Available online 4 April 2013

Keywords:
Cognitive performance
Intrauterine infection
Neurogenesis
PI3K–Akt signaling pathway

A B S T R A C T

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.

© 2013 Elsevier Inc. All rights reserved.

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., 1999; 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.
The phosphatidylinositol 3′-kinase (PI3K)-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 acclimatize 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 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
performed on formalin-fixed, paraffin-embedded brain tissue. Five pup brains from each group were examined at different time points (P3, P7, P14 or P28). In each case, 5-μm thick serial paraffin sections were obtained on silanized slides. After deparaffinization and hydration with xylene and graded alcohols, the antigens were unmasked by pressurized heating for 2 min in a 0.01 mol/L citrate buffer [pH 6.0]. The endogenous peroxidase activity was inhibited by hydrogen peroxide. The slides were incubated overnight at 4 °C in humidified atmosphere with primary antibody anti-BrdU (1:50; Santa Cruz Biotechnology, Santa Cruz, CA). Then polymer horseradish peroxidase immunohistochemistry (HRP IHC) kit (DAKO, Glostrup, Denmark) was used to detect immunopositive cells following the manufacturer’s instructions. The sections were stained with 3,3′-diaminobenzidine, and then counterstained with hematoxylin for 1 min. The BrdU-labeled cells in the hippocampal DG were examined and counted, using the MCID system.

2.6. Immunofluorescent staining

For immunofluorescent labeling of BrdU combined with markers of glial cells (glial fibrillary acidic protein, GFAP) or of mature neurons (neuron-specific nuclear protein, NeuN), sections were treated in order to denaturate DNA (2.4 N HCl for 20 min at 37 °C) and were incubated overnight in anti-NeuN (1:100; Millipore), anti-GFAP (1:60; DAKO, Glostrup, Denmark) and anti-BrdU (1:40; Abcam) in PBST containing 5% normal goat serum. FITC or TRITC conjugated secondary antibodies (1:100; Zymed) were added, and the sections were held at 37 °C for 40 min, followed by counterstaining with DAPI for 1 min. Tissue sections were mounted with Vectashield mounting medium (Vector laboratories, Burlingame, CA). Fluorescently labeled sections were analyzed by a confocal laser scanning microscope (Zeiss LSM 510 Meta; Carl Zeiss MicroImaging, Inc., Jena, Germany) equipped with four lasers (Diode 405, Argon 488, HeNe 543, and HeNe 633), images were acquired using Zeiss LSM software.

2.7. Cell counting and quantification

Quantification was conducted in a blind manner. Stereological quantification of BrdU-labeled nuclei was conducted in the left and right DG as described previously (Brueel-Jungerman et al., 2005). Every sixth section throughout the hippocampus was processed for BrdU immunohistochemistry. To avoid oversampling errors, nuclei intersecting the uppermost plane were excluded. Absolute numbers of BrdU-labeled cells were obtained by multiplying BrdU-labeled cell density by the reference volume. For double-labeling, percentages of BrdU-labeled nuclei co-expressing NeuN or GFAP were determined by analyzing 100 randomly selected BrdU-labeled nuclei throughout the dentate granule cell (DGC) layer and the subgranular zone (SGZ) of dorsal DG using a Zeiss confocal microscope. Absolute numbers of new neurons (BrdU–NeuN) or new astrocytes (BrdU–GFAP) were estimated by multiplying the absolute numbers of BrdU cells by the percent of co-localization for those two markers. BrdU-labeled nuclei were analyzed (63 × oil objective) in their entire z-axis (0.5 μm steps) and were rotated in orthogonal planes (x-y) to verify double-labeling and exclude false double-labeling caused by overlay of signals from different cells. Analyses were performed in sequential scanning mode to rule out cross-bleeding between detection channels.

2.8. Western blot analysis

Hippocampal tissues were homogenized in RIPA buffer with standard protease inhibitors and phosphorylase inhibitors. Samples were centrifuged at 12,000 ×g for 5 min at 4 °C. Protein concentration was measured using the BCA method. Fifty micrograms of protein was resolved on 8–10% SDS polyacrylamide gels and electrotransferred onto polyvinylidene fluoride (PVDF) membranes (Amersham) using a BioRad gel blotting apparatus. Membranes were incubated overnight at 4 °C with the primary antibodies (anti-BDNF 1:1000 and anti-TrkB 1:1000, Abcam, UK; anti-p-Akt-Ser473 1:2000, anti-Akt 1:1000, Cell Signaling Technology, USA). To confirm equivalent loading of samples, the same membranes were incubated with anti-β-actin (Beyotime, China). Protein bands were visualized by enhanced chemiluminescence (ECL Plus Detection Kit, Beyotime, China) and exposure to ECL films (Amersham, UK) for appropriate durations. The bands were quantitated by densitometry and normalized with β-actin using Image Pro Plus.

2.9. Statistical analysis

All data are presented as the means ± SEM. Two-factor multi-level analysis of repeated measures was used for the comparison of navigation test, cell proliferation and Western blot. For analysis of orientation test and neurogenesis, independent sample t-test was performed to detect the difference between E. coli and control group. Statistical significance was set at p < 0.05.

3. Results

After inoculation with E. coli, none of the dams died. The E. coli-treated pregnant rats had higher body temperature (Table 1) and reduction in food intake (Table 2) compared with the control rats. The maternal uterus and placentas of the E. coli-treated pregnant rats showed signs of diffuse neutrophil infiltration and there was no evidence of inflammation in the control group. Few fetal deaths and pregnant losses after intrauterine E. coli infection occurred. There were no significant differences in survival/completion of pregnancy

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>1 h</th>
<th>3 h</th>
<th>6 h</th>
<th>12 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.08 ± 0.06</td>
<td>0.21 ± 0.06</td>
<td>0.01 ± 0.06</td>
<td>0.06 ± 0.06</td>
<td>0.04 ± 0.06</td>
<td>0.03 ± 0.06</td>
<td>0.06 ± 0.06</td>
</tr>
<tr>
<td>E. coli</td>
<td>0.90 ± 0.06</td>
<td>1.42 ± 0.06</td>
<td>1.03 ± 0.06</td>
<td>0.33 ± 0.06</td>
<td>0.22 ± 0.06</td>
<td>0.05 ± 0.06</td>
<td>0.03 ± 0.06</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM. *p < 0.05 vs. the control group.

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>E15 (1 day)</th>
<th>E16 (2 days)</th>
<th>E17 (3 days)</th>
<th>E18 (4 days)</th>
<th>E19 (5 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>26.31 ± 1.10</td>
<td>30.82 ± 1.10</td>
<td>35.06 ± 1.10</td>
<td>32.40 ± 1.10</td>
<td>31.59 ± 1.10</td>
</tr>
<tr>
<td>E. coli</td>
<td>10</td>
<td>8.57 ± 1.10</td>
<td>16.15 ± 1.10</td>
<td>23.42 ± 1.10</td>
<td>31.30 ± 1.10</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM. *p < 0.05 vs. the control group.
and litter size between the E. coli-treated and saline-treated groups. In both groups, the pup’s body weight increased progressively with age. The pup’s body weights of E. coli group decreased at early postnatal days compared with the controls. There was no significant difference in body weight after P7 between the two groups (Table 3).

3.1. Effect of maternal infection on the offspring’s cognitive performance

In the Morris water maze navigation task, all rats were able to swim to locate the platform in the training process, and the average latency of finding the platform was shorter after training (Overall repeated ANOVA analysis: group: F(1,18) = 37.23, p < 0.01; day: F(4,72) = 217.84, p < 0.01; day × group: F(4,72) = 9.51, p < 0.01). Compared with the control group, the average incubation period of seeking the platform was significantly longer in the E. coli group (p < 0.05). This implies that the maternal infection significantly impaired the spatial learning ability (Table 4) (Fig. 1). In the orientation task, the control group had significantly longer dwelling times in the former platform quadrant and more frequent cross-platform movement than the E. coli group, even after removal of the platform (p < 0.05). This indicates that rats in the E. coli group had worse spatial memory of the original platform location (Table 5).

3.2. Effect of maternal infection on hippocampal neurogenesis

3.2.1. Cell proliferation

The subgranular zone (SGZ), the border between the granule cell layer and hilus, has been shown to contain the progenitor cells that divide and migrate into the granule cell layer where they mature into neurons or astrocytes (Cameron et al., 1993). Newborn cells in the developing dentate gyrus (DG) can be quantified by immunostaining of BrdU incorporated into the nuclei of dividing cells (Kuhn et al., 1996). The typical morphology of BrdU-labeled nuclei is dark and round in shape, and frequently with granular nuclei. Significant increase of clustered BrdU-labeled nuclei was found in the E. coli group (Fig. 2A–B). We quantified the number of BrdU-labeled cells 2 h following the last injection of BrdU. Quantitative analysis revealed that there was significant increase of BrdU-labeled cells in the E. coli group (Overall repeated ANOVA analysis: group: F(1,8) = 92.09, p < 0.01; day: F(3,24) = 49.83, p < 0.01; day × group: F(3,24) = 7.40, p < 0.01). And on P28, there was no significant differences in the rate of BrdU-labeling between the E. coli and the control groups (p > 0.05) (Table 6) (Fig. 2C). These results indicate that intrauterine E. coli infection may promote cell proliferation.

3.2.2. Neuronal survival and differentiation

Newborn cells in the hippocampus can either die, or survive and differentiate into mature neurons or astrocytes. To examine the influence of intrauterine infection on cell fate, the number and phenotype of the BrdU-labeled cells was determined 28 d after BrdU administration, a time point when the cells have matured.

At this time the mature cells were found throughout the DG, had normal granular morphology, and appeared ovoid or round with uniform BrdU staining throughout the nuclei. No clusters of mature cells were found. There were no significant differences of BrdU-labeled cells between the E. coli and control groups (Fig. 3A–C, control: 2700.00 ± 148.32, E. coli: 3000.00 ± 158.11, t = 1.38, p = 0.20). This indicates that the proliferating cells were still surviving 28 days

### Table 3
Comparison of pup’s body weight in two groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>P1</th>
<th>P3</th>
<th>P5</th>
<th>P7</th>
<th>P14</th>
<th>P21</th>
<th>P28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.24 ± 0.25</td>
<td>9.84 ± 0.23</td>
<td>12.61 ± 0.31</td>
<td>17.86 ± 0.31</td>
<td>31.36 ± 2.31</td>
<td>51.62 ± 1.80</td>
<td>72.73 ± 2.50</td>
</tr>
<tr>
<td>E. coli</td>
<td>5.96 ± 0.25</td>
<td>8.36 ± 0.23</td>
<td>11.01 ± 0.31</td>
<td>16.99 ± 0.31</td>
<td>31.18 ± 2.31</td>
<td>49.29 ± 1.80</td>
<td>71.55 ± 2.50</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM.
* p < 0.05 vs. the control group.

### Table 4
The escape latency of pups in different groups (cm/s).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>67.46 ± 3.80</td>
<td>31.42 ± 4.13</td>
<td>19.13 ± 4.05</td>
<td>12.03 ± 2.72</td>
<td>8.13 ± 1.95</td>
</tr>
<tr>
<td>E. coli</td>
<td>10</td>
<td>97.20 ± 3.80</td>
<td>67.13 ± 4.13</td>
<td>37.55 ± 4.05</td>
<td>26.52 ± 2.72</td>
<td>14.29 ± 1.95</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM.
* p < 0.05 vs. the control group.

Fig. 1. Maternal infection prolongs the escape latency of the offspring. The rats from the E. coli group and the control group were placed in the swimming pool to locate the platform. The escape latency was significantly longer in the E. coli group compared with the control group. * p < 0.05, n = 10.

### Table 5
The pups’ residence time and target crossings in the target quadrant.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Residence time</th>
<th>Target crossings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>48.36 ± 4.22 (%)</td>
<td>6.60 ± 0.46</td>
</tr>
<tr>
<td>E. coli</td>
<td>10</td>
<td>36.31 ± 3.33 (%)*</td>
<td>4.30 ± 0.52</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM.
* p < 0.05 vs. the control group.
later. But the absolute number of BrdU-labeled cells at P28 was lower relative to the P7 and P14, in agreement with previous studies demonstrating a decline in total cell number 2 weeks after BrdU administration (Gould et al., 1999b).

The phenotype of BrdU-labeled cells was examined by immunofluorescent double-labeling for BrdU and NeuN, a neuron-specific marker (Fig. 4A–C) or GFAP, a astrocyte-specific marker (Fig. 5A–C). Confocal microscopy was used to count the number of double- and single-labeled BrdU-labeled cells in the DG. In the two experimental groups, colocalization of BrdU with NeuN showed that the majority of BrdU-labeled cells expressed a neuronal phenotype. Despite the huge increase in the total number of BrdU-labeled cells in E. coli group at P7, no change was found in the percentage of newborn cells expressing a neuronal phenotype at P28 (control: 27.27 ± 1.98%; E. coli: 29.22 ± 1.65%; t = 0.76, p = 0.47). Calculation of the absolute number of cells co-expressing BrdU and NeuN in two groups showed similar results (Fig. 4D, control: 727.22 ± 32.00; E. coli: 878.65 ± 73.36; t = 1.89, p = 0.10).

![Image of Figure 2](image_url)

**Table 6**
The number of BrdU-labeled cells in different groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>P3</th>
<th>P7</th>
<th>P14</th>
<th>P28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>2060.00 ± 197.48</td>
<td>4060.00 ± 255.15</td>
<td>2890.00 ± 259.23</td>
<td>2720.00 ± 180.00</td>
</tr>
<tr>
<td>E. coli</td>
<td>5</td>
<td>3210.00 ± 197.48*</td>
<td>4340.00 ± 255.15</td>
<td>3750.00 ± 259.23</td>
<td>3280.00 ± 180.00</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM.
* p < 0.05 vs. the control group.
Similarly, intrauterine infection had the same effect on glial differentiation as neuronal differentiation. We found no evidence supporting that intrauterine infection affected the proportion of BrdU–GFAP co-labeled cells (control: 40.83 ± 0.78%; E. coli: 41.24 ± 1.30%; t = 0.27, p = 0.79). Furthermore, no significant reduction of the absolute number of BrdU–GFAP co-labeled cells was found in the E. coli group (Fig. 5D, control: 1104.69 ± 75.59; E. coli: 1239.74 ± 83.89; t = 1.20, p = 0.27). Thus, intrauterine infection did not affect neuronal commitment of newly generated dentate granule cells (DGCs).

3.3. Effect of maternal infection on the activation of the PI3K–Akt signaling pathway

To assess if there was Akt activation following intrauterine infection, Western blots were performed to evaluate the protein levels of both p-Akt and Akt, an index of Akt activation. Firstly, there was no significant difference in total Akt (group: F(1,8) = 2.30, p > 0.05; day: F(3,24) = 1.38, p > 0.05; day×group: F(3,24) = 1.9, p > 0.05).

Secondly, Akt was hyperphosphorylated at serine site in the E. coli group. ANOVA analyses showed there were significant differences from P3 to P28 between the control and E. coli groups (F(1,8) = 293.50, p < 0.01; day: F(3,24) = 61.34, p < 0.01; day×group: F(3,24) = 62.22, p < 0.01). Data suggest that there was significant increase of p-Akt during postnatal 3–7 days in the E. coli group (Fig. 6A). To understand the regulation of Akt, several of its upstream or downstream targets including BDNF and TrkB were examined. As observed for p-Akt, similar increases were observed in BDNF expression during postnatal 3–14 days (Fig. 6B) and in TrkB during postnatal 3–7 days (Fig. 6C). These data suggest that intrauterine E. coli infection induced activation of Akt at serine phosphorylation site through BDNF and TrkB to benefit cell survival.

4. Discussion

The perinatal period is critical for brain growth and development, and infection in this stage can cause serious developmental defects. In
this study, we found that in addition to the direct effects of infection on the in utero development, there were many sickness behavior changes in the pregnant rats (changes in body temperature, reduction in food intake) that could have contributed to the inflammatory status and thereby affected the brain development of the offspring. Previous studies have revealed that white matter changes characterized by blood–brain barrier disruption, glial activation and oligodendrocyte loss, which is associated with impaired memory (Ihara and Tomimoto, 2011). Due to the associated memory decline, the Morris water maze test was employed in the current study to assess the persistent effect of maternal infection on the offspring's cognitive performance. The results demonstrated that during the navigation test, the escape latency in the *E. coli* group was considerably longer than that of the control group, and the search time during the first four days was about twice longer. In the spatial probe test, rats from the control group spent significantly more time in the former platform quadrant and made more frequent cross-platform movements than those from the *E. coli* group, even after removal of the platform. These results indicate that maternal infection can delay the offspring's cognitive performance development.

Although the pathophysiology of intrauterine inflammation and cognitive dysfunction remains unclear and various mechanisms have been proposed, it is well established that apoptosis contributes to the immunopathogenesis of systemic inflammation. Indeed, increased apoptotic death of neurons has been shown in various brain regions in response to systemic inflammation (Kafa et al., 2010; Messaris et al., 2010; Semmler et al., 2005). Many central nervous system insults lead
to increased proliferation of progenitors (Goldman and Luskin, 1998; Temple and Alvarez-Buylla, 1999), and the generation of new cortical pyramidal neurons in adulthood in response to apoptosis of resident neurons has already been established (Magavi et al., 2000). Therefore, the increased proliferation observed in the intrauterine *E. coli* infection rat brains in the present study may be an outcome of increased apoptosis in various regions of the brain. Since our results also suggest that most of the BrdU-labeled cells are still surviving at 28-day, we propose that cellular proliferation may in fact be triggered by accompanying apoptotic cell death observed in various brain regions after intrauterine *E. coli* infection. Evaluation of the phenotype of the surviving cells showed that *E. coli* infected and control rats had a similar proportion of neuronal and glial differentiation, which is in agreement with the previous findings that the percentage of cell differentiation of progenitor cells did not significantly differ in hypoxic animals (Fagel et al., 2006; Felling et al., 2006; Yang and Levison, 2006). These data indicate that although *E. coli* infection enhanced neural stem/progenitor cell (NSC/NPC) proliferation at early postnatal period, some of them either died or failed to differentiate into mature neurons and astrocytes. The number of radial glia cells may change dynamically in order to adapt to the changes in neuron production. However, further work is required to elucidate the nature and fate of differentiated cells and their possible roles in the balanced replacement of different neuronal subtypes.

There are extensive studies investigating how the proliferation, maturation and migration of neural stem cells are modulated in hypoxia-ischemia mammalian brains. However, the potential mechanisms
Fig. 6. Akt, p-Akt, BDNF and TrkB protein expression in the hippocampus shown by Western blot. (A)–(C), a representative autoradiograph of p-Akt/Akt, BDNF and TrkB expression is shown. Intensities of p-Akt/Akt (A1), BDNF (B1) and TrkB (C1) protein bands were quantified by densitometry, respectively. Data represent mean ± SEM. *p < 0.05, n = 5.
that subserve the beneficial effects of inflammation-induced neurogenesis remain largely unknown. Certain regulators of proliferation and survival of newborn cells have been identified, that include growth factors and morphogens, hormones, certain neurotransmitters, intracellular signaling molecules and transcription factors (Gould, 2007). In this study, we investigated the potential role of BDNF–PI3K/Akt signaling pathway in intrauterine infection-induced hippocampal neurogenesis. Data showed that there was significant increase of BDNF, TrkB and p-Akt levels during postnatal 7 days in the E. coli group. Thus, we hypothesize that endogenous neurogenesis may occur in neonatal rats after intrauterine E. coli infection, and the PI3K/Akt pathway may be involved in the regulation of neurogenesis. Activation of PI3K/Akt signaling pathway is a hallmark of cell survival/proliferation and plays a protective role in many neuropathological conditions, including stroke, spinal cord injury, and epilepsy (Chavali et al., 2011; Song et al., 2010). In intrauterine infection-induced brain injury, phosphorylation of Akt followed by increased BDNF level might regulate the expression of antiapoptotic proteins through transcription factors such as survivin (Jiang et al., 2012). Akt phosphorylation might be related to neurogenesis, and nuclear translocation of Akt might be required for cell cycle progression following the activation of TrkB (Brazil et al., 2002; Parcellier et al., 2008). In various animal models of neurodegeneration, treatment with PI3K/Akt enhancer has been shown to prevent neuronal apoptosis, providing therapeutic potential (Brunet et al., 2001; Chan et al., 2011). Optimizing trophic support and cell survival of newly integrating cells may therefore activate this intrinsic mechanism to sustain more cells for functional regeneration. Our result about the activation of PI3K/Akt pathway after intrauterine infection being beneficial to neurogenesis may lend additional evidence to support the therapeutic importance of Akt enhancers. It raises the possibility that modulation of the survival parameters of neuronal progenitor cells may bring a significant increase in the number of new neurons available for regeneration.

5. Conclusions

The data we presented here suggest that maternal infection delays the offspring’s cognitive performance development. Intrauterine infection may promote cell proliferation and induce hippocampal neurogenesis in the developing brain. The potential relationship between PI3K/Akt signaling pathway and endogenous neurogenesis is of great interest. Our results suggest that the BDNF–PI3K/Akt signaling pathway may play an important role in neuronal protection and repair in immature brain after intrauterine infection. In this field, much remains to be investigated with regard to the link between cognitive development and neurogenesis. Further investigation is needed to demonstrate the association of PI3K with neurogenesis, and to elucidate the exact molecular mechanisms of the PI3K/Akt pathway, and will provide evidence toward novel treatment strategies for delayed cognitive development induced by maternal infection.

Acknowledgments

The work of the authors is supported by grants from National Natural Science Foundation of China (81201511), Health Bureau of Zhejiang Province (2010KYB064) and Education Bureau of Zhejiang Province (Y201225805) and Family Planning Commission of Zhejiang Province (JSW2012-A011). We sincerely thank Guifeng Xiao for the assistance with the confocal imaging.

References


Segovia G, Yague AG, Garcia-Verdugo JM, Mora F. Environmental enrichment promotes neurogenesis and changes the extracellular concentrations of glutamate and GABA in the hippocampus of aged rats. Brain Res Bull 2006;70:8–14.


