Prenatal Inflammation Dampens Neurogenesis and Enhances Serotonin Transporter Expression in the Hippocampus of Adult Female Rats

Abdeslam Mouihate  Samah Kalakh  Rawan AlMutairi  Abdelrahman Alashqar
Department of Physiology, Faculty of Medicine, Kuwait University, Kuwait City, Kuwait

Significance of the Study

- Selective serotonin reuptake inhibitors (SSRI) are prescribed to manage depression and anxiety. We provide experimental evidence that prenatal exposure to lipopolysaccharide enhances hippocampal serotonin transporter levels in adult females. The increase in this SSRI target was associated with decreased hippocampal neurogenesis. These data suggest that pharmacological management of psychiatric disorders should take the history of early-life immune challenges into consideration.

Keywords
Dentate gyrus · Neurogenesis · Serotonin transporter · Elevated plus maze · Anxiety-like behavior

Abstract

Background/Aims: Prenatal exposure to lipopolysaccharide (LPS) dampens hippocampal neurogenesis. This effect is associated with increased anxiety-like behavior in adult offspring. Furthermore, blocking serotonin transporters (SERT) promotes adult neurogenesis. Previous studies were performed largely in males. Therefore, we explored the impact of prenatal LPS on neurogenesis, SERT expression in the hippocampus, and anxiety-like behavior in female rats during prepubertal and adulthood stages. Materials and Methods: Timed pregnant rats were injected with either saline or LPS (100 µg/kg, i.p.) on gestational days 15, 17, and 19. Newly born neurons were monitored by immunohistochemistry, and anxiety-like behavior was monitored using the elevated plus maze and open-field test. SERT expression in the hippocampus was assessed by Western blot and immunofluorescence. Results: Prenatal LPS led to reduced hippocampal neurogenesis in adult but not in prepubertal female offspring. This reduced neurogenesis was associated with enhanced hippocampal expression of SERT protein. However, there was no significant impact of prenatal LPS on anxiety-like behavior. Conclusions: Prenatal LPS-induced reduction in neurogenesis was dissociated from anxiety-like behavior in adult female rats. Furthermore, the long-lasting impact of prenatal LPS on neurogenesis in female offspring was age-dependent.

Introduction

Early-life immune and behavioral stresses have a long-lasting impact on brain development and function later in life [1]. Experimentally, exposing rodents to viral mimetics or bacterial active ingredients, such as lipopolysaccharide, can alter neurogenesis and behavioral outcomes. This is of particular interest in the field of psychiatry, as depression and anxiety disorders are associated with altered neurogenesis and decreased expression of serotonin transporters [2]. The current study aimed to investigate the impact of prenatal lipopolysaccharide (LPS) exposure on neurogenesis, serotonin transporter expression, and anxiety-like behavior in female rats.
The Impact of Prenatal LPS on Neurogenesis and SERT Expression

charide (LPS), during critical periods of brain development results in enhanced anxiety- and depression-like behaviors possibly through re-programming of the hypothalamic-pituitary-adrenal axis [2, 3]. These prenatal immune challenges have been associated with altered brain plasticity during adulthood such as reduction in naturally occurring neurogenesis [4, 5]. Interestingly, anxiety-like behavior is also associated with altered hippocampal neurogenesis [6–8]. Furthermore, the administration of anxiolytic drugs, such as blockers of serotonin transporter (SERT), results in increased hippocampal neurogenesis [9]. It is not known yet whether prenatal LPS-induced alteration in hippocampal neurogenesis is associated with SERT expression.

In addition to hippocampal neurogenesis, adult rodents show extensive birth of new neurons in the subventricular zone (SVZ). These neurons migrate through the rostral migratory stream (RMS), reach the olfactory bulb, and differentiate into GABAergic neurons involved in sharpening olfactory sensory information, a major sensory modality used in rodents [10]. Data regarding the impact of prenatal LPS on SVZ neurogenesis are scarce.

The exploration of the long-lasting impact of prenatal immune challenge on adult brain function has been performed largely in males. Relatively few studies have addressed the effect of prenatal immune challenge on female offspring despite the increased prevalence of depression- and anxiety-like behaviors in females [11, 12]. For this reason, we asked the following questions: Does prenatal LPS affect newly born neurons in the hippocampus and the SVZ of female rats? Would the alteration in neurogenesis be associated with anxiety-like behavior and SERT expression? To account for the potential development-dependent effect of prenatal LPS, these questions were addressed in prepubertal and sexually mature female rats.

**Methods**

**Animal Breeding and Prenatal Intervention**

Sprague Dawley rats were housed in a room with an ambient temperature set at ~22 °C and kept under a 12-h light-dark cycle (light on from 7:00 a.m. to 7:00 p.m.). The rats had access to pelleted chow and water ad libitum.

Female rats (2.5–3 months old) were housed with proven male breeders in the same cage. Vaginal smears were performed daily to detect sperm. The day of sperm detection was considered as gestational day 0 (GD0). Subsequently, pregnant rats were housed individually and received an injection of either LPS (100 µg/kg, *Escherichia coli*; serotype 026: B6; Sigma-Aldrich, St. Louis, MO, USA) or an equivalent volume of pyrogen-free saline on GD15, GD17, and GD19. Once born, the pups were kept with their dams until weaned on postnatal day 21. Female offspring were housed 4 per cage until they reached the age of 2 months, after which they were housed 2 per cage. Offspring of dams given either saline (pSal) or LPS (pLPS) during pregnancy were randomly selected for behavioral testing and protein detection using immunohistochemical and Western blot techniques. Each experimental group contains rats born to different dams to minimize the potential litter effect. Prenatal LPS injection affected neither the litter size nor the body weight of the offspring (see online suppl. data 1; for all online suppl. material, see www.karger.com/doi/10.1159/000499658).

**Immunofluorescent Detection of Newly Born Neurons**

Immunofluorescent staining was performed as previously described [13]. Briefly, paraffin-embedded brains of prepubertal (29–30 days old) and adult rat offspring (70 days old) of dams given either LPS or pyrogen-free saline were serially cut at the rostral, medial, and caudal parts of the SVZ at interaural locations of ~10.5, 9.5, and 8.5, respectively, according to the rat stereotaxic coordinates [14] and mounted on super-frost plus slides (VWR International, IL, USA). Brains were also serially cut through rostral, medial, and caudal parts of the dentate gyrus (DG) at interaural locations of ~6.5, 5.5, and 4.5, respectively. Brain sections were incubated with doublecortin (DCX) antibody made in goat (1:1,000; Santa Cruz Biotechnology, CA, USA) followed by Alexa Fluor 555 bound secondary antibody (donkey anti-goat IgG at 1:1,000; Life Technologies, CA, USA). DCX expression was used to monitor ongoing neurogenesis [15]. To assess for the localization of SERT in the neurogenic zone, a rabbit antibody anti-SERT (1:1,000; Millipore, MA, USA) was co-applied with a goat antibody anti-DCX to brain sections as described above. Alexa Fluor 488 bound secondary antibody (donkey anti-rabbit IgG, 1:1,000; Life Technologies) and Alexa Fluor 555 bound secondary antibody (donkey anti-goat IgG, 1:1,000; Life Technologies) were used to co-detect SERT and DCX, respectively. Three sections at each of the rostral, medial, and caudal regions of either the SVZ (total of 9 sections per rat) or the DG of the hippocampus (total of 9 sections per rat) were viewed using 20× (cell counts) or 40× (illustrative images of DCX and SERT) objectives of a confocal laser scanning microscope (Zeiss LSM 700 META microscope; Carl Zeiss, Göttingen, Germany). The DCX+ cells in both the left and right of SVZ and DG areas of the brain were counted by an experimenter blind to the rat’s treatments. To allow for a three-dimension viewing of the potential interaction between SERT and DCX, a set of brain sections was cut at a thickness of 20 µm. A z-stack of images of brain sections labeled for both DCX (red channel) and SERT (green channel) were acquired using z steps of 0.5 µm. Three-dimensional images were built using ImageJ software [16] and are shown in online supplementary data 2.

**Western Blot**

Rats were transcardially perfused with ice-cold phosphate-buffered saline solution, their hippocampi were collected, and SERT protein (rabbit polyclonal antibody anti-SERT, 1:10,000; Millipore) was detected using Western blot as previously described [13]. Protein bands were detected by applying a chemiluminescence substrate (prime ECL kit; GE Healthcare, UK) and exposure to Kodak X-Omat film (Eastman Kodak, NY, USA). The nitrocellulose membranes were subsequently exposed to β-mercaptoethanol (Sigma-Aldrich) to remove the primary antibodies and

The Impact of Prenatal LPS on Neurogenesis and SERT Expression

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re-exposed to a rabbit polyclonal anti-actin antibody followed by a HRP-conjugated secondary antibody. The actin band was detected as described above.

ImageJ software was used to determine the optical density for each protein band as previously described [17]. The area under the curve of each optical density profile was determined and used as a semi-quantitative indicator of protein levels. The ratios of the area under the curve of the protein of interest to that of actin were determined and expressed as a multiple of the values in the control animal group (pSal rat group).

The Elevated Plus Maze

The elevated plus maze (EPM) was used to monitor the anxiety-like behavior in prepubertal and adult female offspring born to dams given either LPS or saline during pregnancy. All tests were performed between 10.00 and 14.00 h under a constant illumination of 40 lux. The EPM apparatus consisted of 2 open arms (50 × 10 cm) and 2 closed arms (50 × 10 cm). These open and close arms are leveled and mounted on a support at 50 cm above the floor level. A video camera was hanged on the wall above the EPM to record rats’ movement. The rats were brought to the test room 1

**Fig. 1.** Prenatal LPS reduces neurogenesis in the DG of the hippocampus of adult rats, but not in prepubertal female rats. Doublecortin (DCX) was detected in the DG of the hippocampus of pSal and pLPS prepubertal (upper micrograph panel in a) and adult offspring (lower micrograph panels in a). The lowermost micrograph panels in a show the magnified area delimited by the dashed rectangle in the micrograph of adult offspring. b There was no significant effect of prenatal LPS on DCX count in prepubertal female rats. However, prenatal LPS led to a significant reduction in DCX count in adult offspring. Scale bar = 50 µm. LPS, lipopolysaccharide; DG, dentate gyrus; DCX, doublecortin. *p < 0.05.
day before the EPM testing. On the testing day, each rat was placed at the center of the platform where the open and close arms are crossing. Each group contains rats born to different dams to minimize the litter effect. The movements of the rat were recorded for 5 min, and the time spent in the open and closed arms was monitored. The surface of the EPM was cleaned with 70% alcohol before each test. Videos were analyzed using SynoQuant 3.26 software [18]. Using this software, the static features of the rat (e.g., white rat in a dark background) were extracted and its dynamic features (change of location over time) were monitored to track the movement of the animal.

The Open-Field Test

Anxiety-like behavior was also assessed in a different cohort of animals, using an automated open-field test apparatus (VersaMax Animal Activity Monitoring System; AccuScan Instruments, Inc., OH, USA). Prepubertal and adult female offspring born to dams given either saline or LPS were brought to the test room 1 day before behavioral assessment. All behavioral tests were performed between 10.00 and 14.00 h. The open field consisted of a square area (42 x 42 cm) surrounded by 4 walls (30 cm high). Rats were placed at the center of the arena and allowed to move freely. The time spent at the center and on the margin of the open field was monitored, and the fraction of time spent on the margin was used as an index of anxiety-like behavior [19]. The arena was cleaned with 70% ethanol before each behavioral testing. Rat behaviors were recorded for 10 min.

Statistical Analysis

Data were compared using unpaired t test. A p value of less than 0.05 was considered statistically significant. All data are presented as mean ± standard error of the mean.

Results

Impact of Prenatal LPS on Neurogenesis in Prepubertal and Adult Female Offspring

The effect of prenatal immune challenge on neurogenesis was assessed at prepubertal age and during adulthood. As shown in Figure 1, administration of LPS during pregnancy did not have a significant effect on newly born neurons in the DG of the hippocampus of prepubertal offspring (pSal \( n = 3 \) vs. pLPS \( n = 3 \); \( p > 0.05 \)) (Fig. 1a, prepubertal; Fig. 1b, left graph bar). However, newly born neurons in the DG of the hippocampus were reduced in adult pLPS female offspring when compared to those seen in pSal adult female offspring (pSal \( n = 5 \) vs. pLPS \( n = 3 \); \( p < 0.05 \)) (Fig. 1a, prepubertal; Fig. 1b, right graph bar). Moreover, this long-lasting impact of prenatal LPS on neurogenesis was specific to the hippocampus, as this reduced neurogenesis was not seen in the SVZ (pSal \( n = 4 \) vs. pLPS \( n = 3 \); \( p > 0.05 \)) or the RMS (pSal \( n = 4 \) vs. pLPS \( n = 3 \); \( p > 0.05 \)) of adult female offspring (Fig. 2).

Impact of Prenatal LPS on Anxiety in Prepubertal and Adult Offspring

Prenatal LPS did not have a significant effect on the behavior of the offspring in the EPM (Fig. 3, left and middle bar graphs in panels a and b). Prepubertal and adult rats spent more time in the closed arm. By using a tracking software, we mapped the spatial location of
these animals over time (5 min). We noticed that prenatal LPS led to a significant decrease in the motor activity of adult (pSal \( n = 5 \) vs. pLPS \( n = 5 \); \( p < 0.01 \)) (Fig. 3b, right bar graph) but not that of prepubertal rat offspring, when compared to their corresponding pSal rat groups (pSal \( n = 5 \) vs. pLPS \( n = 5 \); \( p > 0.05 \)) (Fig. 3a, right bar graph). Prenatal LPS did not have a significant effect on anxiety-like behavior in prepubertal (Fig. 3c) and adult
**Fig. 4.** Prenatal LPS enhances the expression of SERT in the hippocampus of adult female rats. The protein expression of SERT in the hippocampus of prepubertal (a) and adult rats (b) born to dams given either saline (pSal) or LPS (pLPS) during pregnancy was detected using Western blot. Prenatal LPS led to significantly increased levels of SERT in adult, but not in prepubertal offspring. Upper micrographs in panel c show double immunofluorescent detection of SERT and DCX in the hippocampus of pSal and pLPS adult female rats. A higher magnification of the area delimited by the dashed rectangle is shown in the lower micrograph panels. Note the close localization of SERT and the base of DCX immunoreactive cells (double arrowheads). Some SERT immunoreactive fibers are in close contact with DCX immunoreactive fibers (arrows in the right panel). Scale bar = 50 µm. LPS, lipopolysaccharide; SERT, serotonin transporter; DCX, doublecortin. ***p < 0.001.
offspring (Fig. 3d) (pSal \( n = 5 \) vs. pLPS \( n = 5 \); \( p > 0.05 \)), nor did it affect motor activity of these offspring (data not shown).

**Long-Lasting Effect of LPS on the Expression of SERT in the Hippocampus of Adult Offspring**

Since SERT plays an important role in neurogenesis and is the prime target of anxiolytic drugs, we assessed whether prenatal LPS has a long-lasting effect on the expression of SERT protein in the hippocampus during adulthood. Western blot data illustrated in Figure 4 show that prenatal LPS led to an increased level of SERT protein expression during adulthood (pSal \( n = 3 \) vs. pLPS \( n = 3 \); \( p < 0.001 \)) (Fig. 4b). This effect was age-specific as there was no alteration in the expression of SERT in prepubertal animals (pSal \( n = 3 \) vs. pLPS \( n = 3 \); \( p > 0.05 \)) (Fig. 4a). Since the increase in SERT levels was seen in adult offspring, we performed a double immunofluorescent staining of DCX and SERT within the hippocampus of adult offspring to qualitatively assess the potential interaction between serotonin innervation and DCX cells. SERT immunoreactivity was seen at the base of DCX+ cells as well as along some of the DCX+ branches (Fig. 4c). A much clearer view of this SERT/DCX double immunostaining is presented as a z-stack in online supplementary data 2.

**Discussion**

A large body of evidence supports the long-lasting impact of prenatal immune challenge on brain development and the consequent alteration of behavior during adulthood. Experimentally, rodents born to immune-challenged dams during pregnancy showed signs of behavioral alterations assimilated to several brain ailments such as schizophrenia, anxiety, and depression [1, 20, 21]. In the present study, we explored the long-lasting impact of prenatal immune challenge on brain plasticity and behavior in female offspring. We showed that prenatal LPS dampens neurogenesis regardless of the sex of the offspring. It appears that the reduced neurogenesis in these female offspring is dissociated from anxiety-like behavior. The dissociation between anxiety-like behavior and hippocampal neurogenesis is in conflict with previous studies [6–8]. This apparent discrepancy could be due to differences in animal species (rats vs. mice), as there is evidence to suggest that neurogenesis in rats and mice has a different behavioral significance [22]. It is noteworthy that previous studies have shown that exposure of rats to LPS during the neonatal period had no significant effect on anxiety-like behavior during adulthood [23].

In addition to hippocampal neurogenesis, rodents show extensive birth of new neurons in the SVZ. These neurons migrate through the RMS, reach the olfactory bulb, and differentiate into GABAergic neurons involved in sharpening olfactory sensory information, a major sensory modality used in rodents [10]. We and others have previously reported on the selective impact of prenatal LPS on hippocampal neurogenesis with no significant effect on neurogenesis in the SVZ of male offspring [4, 5]. In the present study, we showed that neurogenesis in the SVZ of adult females was not affected by prenatal LPS. The mechanism underlying the differential effect of prenatal LPS on neurogenesis in SVZ and DG is not clear yet. It has been noted that the renewal of SVZ progenitor cells is much larger than that of DG [24], likely due to the high level of “stemness” in the SVZ. On the other hand, the DG of the hippocampus contains restricted progenitor cells [25]. The potential consequence of such differential effect suggests that olfaction-driven behaviors are less likely to be affected by early-life immune challenges.

**Prenatal LPS Dampens Neurogenesis in the DG during Adulthood**

It is well accepted that postnatal neurogenesis occurs in the DG of the hippocampus. These newly born neurons are believed to be involved in acquiring new memories and play an important role in affective states such as anxiety and depression [6–8]. We and others have shown that prenatal LPS reduces hippocampal neurogenesis in adult males [4, 5]. In the present study, we showed that exposure to LPS during pregnancy leads to reduced hippocampal neurogenesis in adult female offspring. These data complement previous observations and suggest that prenatal LPS alter neurogenesis regardless of the sex of the offspring. It appears that the reduced neurogenesis in these female offspring is dissociated from anxiety-like behavior. The dissociation between anxiety-like behavior and hippocampal neurogenesis is in conflict with previous studies [6–8]. This apparent discrepancy could be due to differences in animal species (rats vs. mice), as there is evidence to suggest that neurogenesis in rats and mice has a different behavioral significance [22]. It is noteworthy that previous studies have shown that exposure of rats to LPS during the neonatal period had no significant effect on anxiety-like behavior during adulthood [23].

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**Prenatal LPS Enhances SERT Expression in the Hippocampus during Adulthood**

We further explored the impact of prenatal LPS on SERT, a good marker of serotonergic innervation, as this serotonergic innervation has been implicated in
hippocampal neurogenesis [26–29]. We noticed that prenatal LPS led to increased expression of SERT in the hippocampus of adult female offspring. We also noted that serotonergic fibers were intertwined with newly born neurons (Fig. 4c). While this observation is qualitative, it suggests a potential interaction between serotonergic innervation and the birth of new neurons. The physiological significance of this increased SERT expression is unclear. LPS-induced enhanced SERT expression in the hippocampus could underlie the reduced hippocampal neurogenesis seen during adulthood. It is possible that the increased SERT expression might lead to increased presynaptic reuptake of serotonin, a neurotransmitter known to promote neurogenesis [27]. It is noteworthy that experimental studies exploring the role of SERT in behavioral and cognitive functions relied mainly on the use of SERT knockout rodents. For example, there is a consensus that rodents lacking the functional SLC6A4 gene, which encodes for SERT protein, show enhanced anxiety-like behavior [30]. There are no available experimental data on the impact of increased expression of SERT on anxiety-like behavior. Our data are the first to show that the increased SERT expression is dissociated from anxiety-like behavior. Interestingly, this long-lasting impact of LPS on SERT is apparent only after sexual maturation. There is a potential interaction between early-life environment (prenatal immune challenge) and factors associated with sexual maturation (potentially ovarian hormones) leading to enhanced expression of SERT. The mechanisms underlying these interactions and the functional consequence of increased expression of SERT, while beyond the scope of the present paper, warrant further in-depth exploration.

Conclusion

Exposure to bacterial LPS during pregnancy leads to reduced neurogenesis in the DG of the hippocampus but not in the SVZ of adult female offspring. This reduced neurogenesis was associated with increased expression of SERT. The increased expression of SERT enhances the sensitivity of the brain to selective serotonin reuptake inhibitors. This experimental study paves the way for clinical exploration into the importance of early-life immune challenge in the pharmacological management of psychiatric disorders.

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Statement of Ethics

All experimental procedures were approved by the Animal Research Ethics Committee of the Health Sciences Centre, Kuwait University.

Disclosure Statement

The authors have no conflicts of interest to declare.

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