Life Sciences 149 (2016) 129–137

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Prenatal lipopolysaccharide exposure affects sexual dimorphism in different germlines of mice with a depressive phenotype

Thiago M. Reis-Silva [a,b], Daniel W.H. Cohn [b,c], Thaísa M. Sandini [c], Mariana S.B. Udo [c], Elizabeth Teodorov [d], Maria Martha Bernardi [d,e,*]

[a] Department of Neuroscience and Behavior, Psychology Institute, University of São Paulo, Brazil
[b] Neuroimmunomodulation Research Group, School of Veterinary Medicine, University of São Paulo, Brazil
[c] Department of Pathology, School of Veterinary Medicine, University of São Paulo, Brazil
[d] Mathematic, Computation and Cognition Center, Federal University of ABC, Brazil
[e] Post-Graduate Program of Environmental and Experimental Pathology and Post-Graduate Program of Dentistry, Paulista University, Brazil

ARTICLE INFO

Article history:
Received 7 August 2015
Received in revised form 14 February 2016
Accepted 16 February 2016
Available online 17 February 2016

Keywords:
Endotoxin
Lipopolysaccharide
Tail suspension test
Prenatal
Generations

ABSTRACT

The objective of the present study was to investigate whether prenatal lipopolysaccharide (LPS) administration modifies the expression of depressive and non-depressive-like behavior in male and female mice across two generations. The sexual dimorphism of these mice was also examined in the open-field test. Male and female mice of the parental (F0) generation were selected for depressive- or non-depressive-like behavioral profiles using the tail suspension test (TST). Animals with similar profiles were matched for further mating. On gestation day (GD) 15, pregnant F0 mice received LPS (100 μg/kg, i.p.) and were allowed to nurture their offspring freely. Adult male and female of the F1 generation were then selected according to behavioral profiles and observed in the open field. Male and female mice of the two behavioral profiles were then mated to obtain the F2 generation. Adults from the F2 generation were also behaviorally phenotyped, and open field behavior was assessed. Male mice that were selected for depressive- and non-depressive-like behaviors and treated or not with LPS in the parental generation exhibited similar proportions of behavioral profiles in both filial lines, but LPS exposure increased the number of depressive-like behavior. An effect of gender was observed in the F1 and F2 generations, in which male mice were more sensitive to the intergenerational effects of LPS in the TST. These data indicate that prenatal LPS exposure on GD15 in the F0 generation influenced the transmission of depressive- and non-depressive-like behavior across filial lines, with sexual dimorphism between phenotypes.

1. Introduction

Major Depressive Disorder (MDD) is a worldwide disease that affects over 340 million people and generates a high burden to society. It is characterized by a wide range of symptoms, such as feelings of worthlessness and tiredness, sleep disturbances, thoughts of death, and in many cases suicide [7]. The World Health Organization estimated that depression will rank second in the next decade as the disease that is most responsible for premature life lost among all ages and sexes [43].

Environmental stressors and lipopolysaccharide (LPS) can trigger nonspecific immune events [13,16]. Lipopolysaccharide, also known as lipoglycans and endotoxin, is a large molecule that consists of a lipid and a polysaccharide that is composed of *O*-antigen and outer and inner cores that are joined by a covalent bond. Lipopolysaccharide is found in the outer membrane of Gram-negative bacteria and elicits strong immune responses in animals. By activating the immune system similarly to infections, LPS can cause several behavioral changes (e.g., an increase in slow-wave sleep, anorexia, and depressive-like behavior) that may be associated with MDD and are referred to as sickness behavior [8,18]. From an immune-neuroendocrine perspective, LPS triggers the secretion of proinflammatory cytokines by the innate immune system and also increases the activity of the hypothalamic-adrenal-pituitary (HPA) axis, an important system that is known to be responsive to environmental stressors and infections [25,29,31,52] and has been reported to be related to MDD [40,59,61].

Depressive phenotypes can be studied in mice using different tests. Although they cannot precisely reproduce human psychopathology, much can be clarified using such tools [18,38]. Different MDD symptoms can be assessed by the learned helplessness paradigm and different tests that employ uncontrollable and inescapable stress, such as behavioral despair in the tail suspension test (TST; [6,27]). Strain differences have been observed in the TST. High levels of immobility can be selected among animals, thus providing mice that are more sensitive for different studies. The results of the TST are easily reproducible, in contrast to other models that employ acute inescapable stress, such as the forced swim test (FST; [47]. Additionally, the TST avoids the possible water-

* Corresponding author at: Rua Dr. Bacelar, 1212, Vila Clementino, São Paulo, SP 04026-002, Brazil.
E-mail address: marthabernardi@gmail.com (M.M. Bernardi).

http://dx.doi.org/10.1016/j.lfs.2016.02.068
0024-3205/

induced hypothermic conditions that are associated with the FST [9]. Because of its ability to detect numerous antidepressant-like effects, the TST has become popular for the rapid screening of antidepressant drugs [34].

Previous studies from our laboratory reported the intergenerational effects of maternal LPS exposure [22–24,45,51]. In the present study, we selected mice with different behavioral profiles (i.e., depressive- and non-depressive-like behavior) in the TST by considering the following factors: (1) Major Depressive Disorder may be related to the release of inflammatory mediators, (2) the TST is an adequate tool for assessing depressive-like behavior, and (3) maternal LPS exposure impacts subsequent generations. The objective of the present study was to investigate whether a single prenatal LPS administration in the parental generation affects these behaviors in subsequent generations of mice in the TST. The mice were also evaluated in the open field test (OFT) because LPS may also induce alterations in the sexual dimorphism of open field behavior that was reported previously [3]. The dose of LPS used here was shown to induce sickness behavior [3], which is related to depressive illness [12].

2. Methods

2.1. Ethics statement

All of the animals that were used in the present study were maintained in accordance with the Guide for the Care and Use of Laboratory Animals, National Research Council, USA. The protocols for the experimental studies were approved by Laboratory Animal Resources, School of Veterinary Medicine, and University of São Paulo, Brazil (protocol no. 2653/2012, FMVZ-USP). These guidelines are similar to those of the United States National Institutes of Health. The experiments were performed in accordance with good laboratory practice protocols and quality assurance methods. All efforts were made to minimize the suffering of the animals.

2.2. Animals

A total of 40 male and 50 female Swiss mice, 6 weeks of age and weighing 25–30 g, were obtained from the Department of Pathology, School of the Veterinary Medicine, University of São Paulo, Brazil. Seven days before beginning the experiments, the mice were housed in groups of five in polypropylene cages (28 × 17 × 12 cm) under controlled room temperature (20–25 °C) and humidity (55–65%) with a 12 h/12 h light/dark cycle (lights on at 7:00 AM). The mice received standard rodent chow and water ad libitum.

2.3. Lipopolysaccharide

Lipopolysaccharide (from *Escherichia coli*; Sigma, St. Louis, MO, USA; serotype 0127:B8) was dissolved in sterile saline (50 μg/ml LPS in 0.9% NaCl solution), and a single 100 μg/kg dose was administered intraperitoneally exclusively to pregnant dams of the parental generation (F0) on gestational day 15 (GD15). This dose was chosen because it elicits sickness behavior, induces endocrine alterations in dams, and increases cytokines at the placental level [21]. The control dams were treated with 0.1 ml/100 g sterile saline solution (0.9% NaCl) according to the same treatment schedule as the LPS animals.

2.4. Behavioral testing

2.4.1. Tail suspension test

The TST was performed as described previously [6,53]. Briefly, the mice were suspended by the tail using tape that was attached to a hook that was connected to a strain gauge. Immobility time, defined as the mouse not struggling, was recorded in a single 6 min trial using a video camera that was positioned in front of the apparatus.

2.4.2. General activity in the open field test

The OFT was performed as described previously [63]. The open field device consisted of a round wooden arena (40 cm diameter with 25.5 cm high walls) that was painted black with an acrylic washable covering. For the observations, each mouse was individually placed in the center of the apparatus, and total locomotor activity (i.e., distance travelled, in centimeters) and mean velocity were automatically measured over a 5 min period. A video camera that was mounted 100 cm above the arena was used to collect the data, which were analyzed using Ethovision 2.3 software (Noldus Information Technology, Leesburg, VA, USA) that was installed on an IBM-compatible computer in an adjacent room. The time spent grooming, rearing frequency, freezing time, and freezing frequency were manually scored by an experimenter who was unaware of the pharmacological treatments. The device was washed with a 5% alcohol/water solution before each animal was placed in it to obviate possible bias that may be caused by odor cues that were left by previous animals. The control and experimental mice were intermixed for observations that were performed from 8:00 AM to 12:00 PM.

2.5. Experimental design

2.5.1. F0 generation groups: treatment and mating

Male and female mice were tested in the TST at 7 weeks age to assign them to different phenotypes. Higher immobility time (≥60 s) resulted in the selection of depressive-like behavior. Lower immobility time (≤30 s) resulted in the selection of non-depressive-like behavior. These cut-off points were chosen because they highlighted differences between both behaviors in the present study. After evaluation, the male and female mice were separated into a depressive-like group (DG; $n = 12$ males, $n = 18$ females) and non-depressive-like group (NDG; $n = 12$ males, $n = 18$ females). During evaluation, an intermediate group (IG) was also identified (immobility time > 30 and <60) and excluded from the experiment ($n = 16$ males, $n = 14$ females). At 8 weeks of age, female and male mice that presented the same behavior were mated. In this first step in order to avoid litter effects the animals used were originally from different mothers. Pregnancy was detected by the presence of semen in vaginal smears, thus defining GD0. The females were then further divided into four groups: LPSD (depressive-like behavior + LPS), SALD (depressive-like behavior + saline), LPSND (non-depressive-like behavior + LPS), and SALND (non-depressive-like behavior + saline). On GD15, females in the experimental groups received 100 μg/kg LPS (i.p.), and females in the control groups received 0.1 ml/100 g sterile saline solution. The GD15 time point was chosen because it is a critical period for central nervous system development.

2.5.2. F1 generation groups

Female mice of the F0 generation were allowed to give birth and nurture their offspring (F1 generation) normally. At birth, the F1 generation was culled to eight pups per litter when possible (four males and four females), yielding a total of 97 male and 87 female mice. At 3 weeks of age, all of the pups were weaned and allocated to the same conditions as their parents and kept undisturbed until 7 weeks age for the behavioral analyses in the TST and OFT.

At 7 weeks of age, immobility time was tested in 97 males (LPSD $n = 31$, SALD $n = 19$, LPSND $n = 23$, SALND $n = 24$) and 87 females (LPSD $n = 27$, SALD $n = 24$, LPSND $n = 18$, and SALND $n = 18$) of the F1 generation divided into the four experimental groups according to the behavior in the previous generation. Similar to observations in the F0 generation, each group presented percentages of mice that exhibited different behavior. After the TST, animals that presented the highest immobility time in the LPSD group ($n = 10$ males, $n = 10$ females) and SALD group ($n = 10$ males, $n = 10$ females) and animals that presented the lowest immobility time in the LPSND group ($n = 9$