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

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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-

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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 ($n = 9$

males, $n = 10$ females) and SALND group ($n = 8$ males, $n = 10$ females) were selected for the evaluation of general activity in the OFT.

2.5.3. F2 generation groups

To obtain the F2 generation, males and females of the F1 generation that exhibited the same behavioral profile were mated. The animals selected were the same ones that were previously evaluated in the OFT. No LPS treatment was given during the gestational period in the F2 generation. The same procedure as in the F1 generation was employed in the F2 generation, yielding a total of 78 males and 110 females. Special care was taken to prevent mating between siblings in the F1 generation. However, although siblings were not crossed to obtain the F2 generation, siblings were used as part of the *n* to achieve the appropriate number of animals in each group. Even though, no more than one male and one female offspring from any given litter were added in our analyses to minimize the contribution of litter effects to the data [64]. Specifically, each group had two siblings to complete the *n*.

At 7 weeks of age, immobility time was tested in 78 males (LPSD $n = 20$, SALD $n = 22$, LPSND $n = 17$, SALND $n = 19$) and 110 females (LPSD $n = 28$, SALD $n = 34$, LPSND $n = 23$, SALND $n = 25$) of the F2 generation divided into the four experimental groups, according to the behavior in the previous generation. Animals that presented the highest immobility time in the LPSD group ($n = 10$ males, $n = 10$ females), SALD group ($n = 10$ males, $n = 10$ females), LPSND group ($n = 10$ males, $n = 10$ females), and SALND group ($n = 7$ males, $n = 10$ females) were selected for the evaluation of general activity in the OFT. The overall experimental design is shown in Fig. 1.

2.6. Statistical analysis

The results are expressed as mean ± SEM. Homoscedasticity was verified using Bartlett's test. Immobility time in the TST and behavior in the OFT were analyzed using three-way analysis of variance (ANOVA), with generation, behavior, and treatment as factors. When no interaction was found between factors, two-way ANOVA followed by the Holm-Sidak *post hoc* test was applied for data from experiments that consisted of four groups. Data from experiments that consisted of three groups were analyzed with two-way ANOVA followed by Tukey's *post hoc* test. The proportion of phenotypes was analyzed using Fisher's exact test. The minimum probability level of 5% was considered able to show significant differences between groups. Prism 6 software (GraphPad, La Jolla, CA, USA) was used to analyze the data.

3. Results

3.1. F0 generation

3.1.1. Immobility time in males and females

Three different behaviors were identified among male and female mice. To determine differences in behavioral parameters and differences between sexes, we performed two-way ANOVAs. Fig. 2 shows significant differences between behaviors ($F_{2,62} = 114.7$, $p < 0.0001$) but no differences between males and females ($F_{2,62} = 0.2429$, $p = 0.6238$) and no interaction between factors ($F_{2,62} = 0.2551$, $p = 0.7756$). We then performed Tukey's multiple-comparison test and detected a higher immobility time between the DG and NDG ($p < 0.0001$),

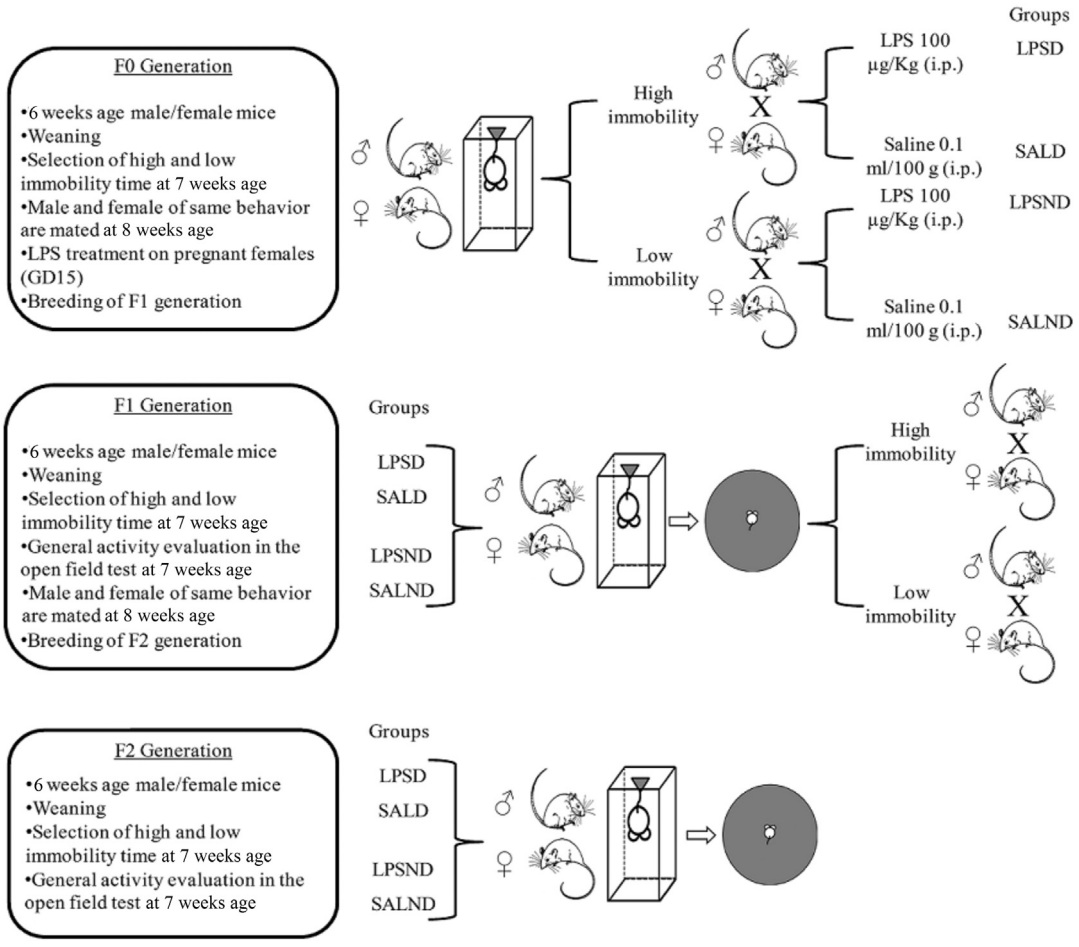

Fig. 1. Experimental design for the different germ lines. LPSD, LPS-treated mice with depressive-like behavior; SALD, saline-treated mice with depressive-like behavior; LPSND, LPS-treated mice with non-depressive-like behavior; SALND, saline-treated mice with non-depressive-like behavior.

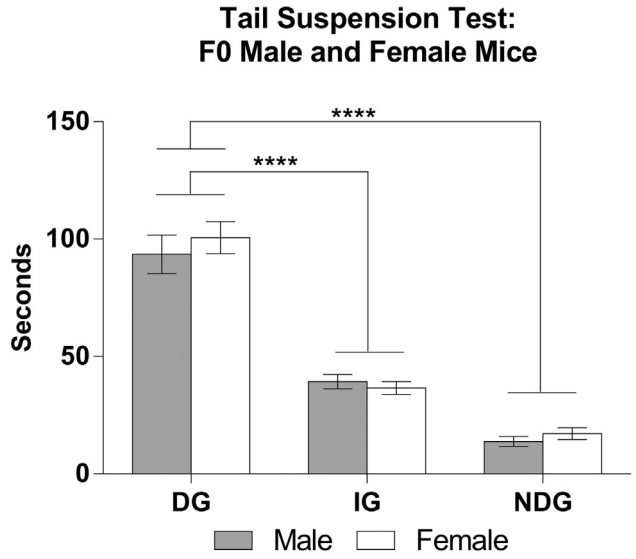

Fig. 2. Immobility time (in seconds) in male F0 mice (DG, $n = 12$; IG, $n = 16$, ND, $n = 12$) and female F0 mice (DG, $n = 18$; IG, $n = 14$; NDG, $n = 18$) in the tail suspension test. The data are expressed as mean ± SEM. $^{****}p < 0.001$ (Kruskal-Wallis one-way analysis of variance on ranks with Dunn's all pairwise multiple-comparison test).

between the DG and IG ($p < 0.0001$), and between the IG and NDG ($p < 0.05$) for both genders.

3.2. Effects of lipopolysaccharide treatment in different germ lines

3.2.1. Immobility time in F1 and F2 males and females in the TST

Our initial hypothesis was that prenatal LPS exposure would promote behavioral differences that would be detectable through subsequent generations. This effect was also hypothesized to differentially affect males and females. To test this hypothesis, we conducted separate three-way ANOVAs in males and females, with generation, behavior, and treatment as factors. Considering the results of the TST, the three-way ANOVA did not reveal interactions between generation, the depressive-like behavior and the LPS treatment ($F_{1,167} = 0.006$, $p = 0.939$) or influence of the different generations over the LPS treatment ($F_{1,167} = 1.124$, $p = 0.291$) and the depressive-like behavior ($F_{1,167} = 1.160$, $p = 0.689$). Treatment did not affect the occurrence of depressive- and non-depressive-like behavior ($F_{1,167} = 1.996$, $p = 0.160$), but significant differences were found between depressive- and non-depressive-like animals ($F_{1,167} = 57.50$, $p < 0.001$).

Once the generation factor was not significant in this test, we analyzed males of the F1 and F2 generations separately. With regard to behavior and treatment in F1 males, Fig. 3A shows significant differences in the immobility time between depressive- and non-depressive-like animals ($F_{1,93} = 26.92$, $p < 0.0001$) but no effect of the LPS treatment ($F_{1,93} = 0.008$, $p = 0.927$) and no interaction between factors ($F_{1,93} = 1.164$, $p = 0.283$). The Holm-Sidak *post hoc* test revealed significant differences in the immobility time between the LPSD and LPSND groups ($p < 0.0001$) and between the SALD and SALND groups ($p < 0.05$).

Fig. 3B shows the results of the two-way ANOVA for F2 males. The analysis revealed significant differences in the immobility time between depressive- and non-depressive-like animals ($F_{1,74} = 31.50$, $p < 0.001$) but no effect of the LPS treatment ($F_{1,74} = 1.965$, $p = 0.165$) and no interaction between factors ($F_{1,74} = 0.876$, $p = 0.352$). The Holm-Sidak *post hoc* test revealed differences in the immobility time between the LPSD and LPSND groups ($p < 0.001$) and between the SALD and SALND groups ($p < 0.01$).

With regard to behavior and treatment in females of the F1 generation, Fig. 4A shows significant differences in the immobility time between depressive- and non-depressive-like animals ($F_{1,83} = 10.67$, $p = 0.001$) but no effect of the LPS treatment ($F_{1,83} = 0.141$, $p = 0.707$) and no interaction between factors ($F_{1,83} = 0.348$, $p = 0.556$). However, the Holm-Sidak *post hoc* test did not reveal immobility time differences between groups. In F2 females, as shown in Fig. 4B, a significant interaction between depressive-like behavior and the LPS treatment was observed ($F_{1,106} = 4.934$, $p = 0.028$). A significant difference in the immobility time was found between depressive- and non-depressive-like females ($F_{1,106} = 22.98$, $p < 0.0001$), with no effect of the LPS treatment ($F_{1,106} = 1.428$, $p = 0.234$). The Holm-Sidak *post hoc* test revealed significant differences in the immobility time between the LPSD and LPSND groups ($p < 0.0001$) and between the LPSD and SALD groups ($p < 0.5$).

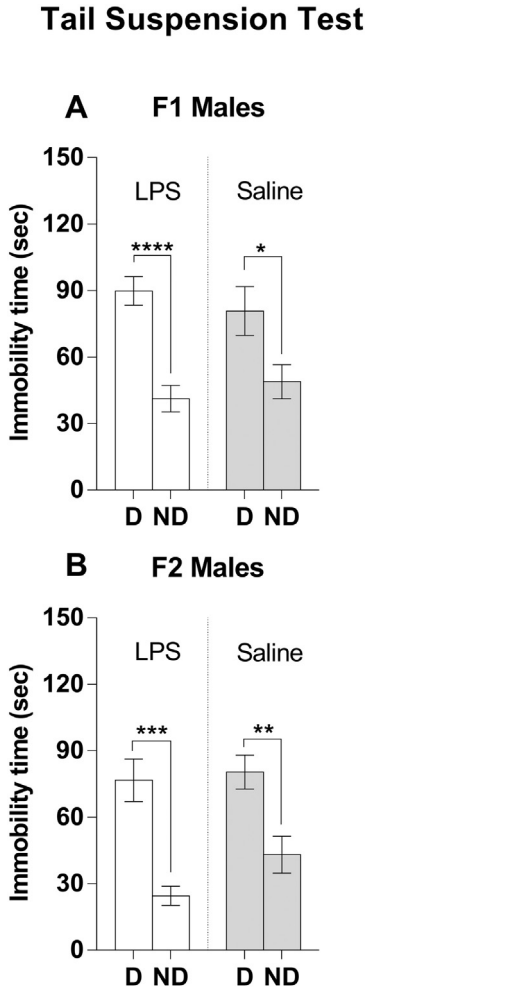

Fig. 3. Immobility time in the TST (seconds) in F1 males (A) and F2 males (B) whose F0 dams received 100 μg/kg LPS on GD15. The data are expressed as mean ± SEM. $^{****}p < 0.0001$, $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.5$ (two-way ANOVA followed by Holm-Sidak *post hoc* test).

3.2.2. Behavioral trend in male and female offspring

We performed Fisher's exact test in F0 and F1 offspring to identify trends in the transmission of behavioral phenotypes. Fig. 5A shows a positive correlation in LPS-treated male offspring of the F0 generation ($^{***}p < 0.0001$) and positive correlations both saline- and LPS-treated male offspring of the F1 generation (saline: $^{**}p = 0.0014$; LPS: $^{***}p = 0.0002$). These data indicate that depressive- and non-depressive-like behavior was transmitted to the male filial germ lines.

Fisher's exact test that was applied for F0 and F1 female offspring revealed a positive correlation in the saline-treated offspring of the F0 generation ($^{*}p = 0.0383$) and positive correlations in both saline- and LPS-treated offspring of the F1 generation (saline: $^{*}p = 0.0394$; LPS:

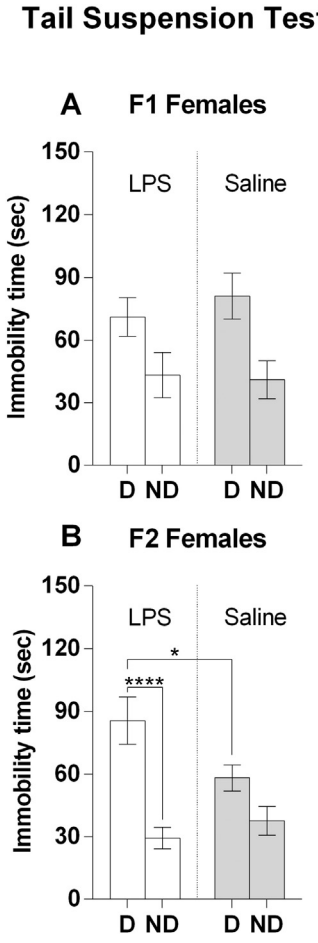

Fig. 4. Immobility time (in seconds) in the TST in F1 females (A) and F2 females (B) whose F0 dams received 100 μg/kg LPS on GD15. The data are expressed as mean ± SEM. $^{****}p < 0.0001$, $^{*}p < 0.5$ (two-way ANOVA followed by Tukey *post hoc* test).

$^{**}p = 0.0018$; Fig. 5B). These data indicate that depressive- and non-depressive like behavior was transmitted to the female filial germ lines, similar to males.

3.2.3. General activity in males and females of the F1 and F2 generations

We performed a three-way ANOVA of different parameters in the OFT for the selected depressive- and non-depressive-like male mice. The results indicated a significant interaction between generation, behavior, and treatment for grooming frequency ($F_{1,66} = 4.033$, $p = 0.049$). Significant differences were found in mean velocity ($F_{1,66} = 43.26$, $p < 0.001$), rearing frequency ($F_{1,66} = 20.67$, $p < 0.001$), and distance travelled ($F_{1,66} = 40.25$, $p < 0.001$) between F1 and F2 males. Differences were also found in immobility frequency ($F_{1,66} = 12.81$, $p < 0.001$) and immobility time ($F_{1,66} = 16.91$, $p < 0.001$) between generations, with significant differences in the effects of LPS treatment on both parameters ($F_{1,66} = 19.84$, $p < 0.001$, and $F_{1,66} = 12.22$, $p < 0.001$, respectively; Table 1).

In F1 females, the three-way ANOVA of the different parameters in the OFT in depressive- and non-depressive-like females revealed differences in mean velocity ($F_{1,70} = 37.12$, $p < 0.001$) and distance travelled ($F_{1,70} = 34.87$, $p < 0.001$) between the F1 and F2 generations and significant interactions between generation and treatment for grooming frequency ($F_{1,70} = 7.576$, $p = 0.008$), rearing frequency ($F_{1,70} = 17.59$, $p < 0.001$), and immobility frequency ($F_{1,70} = 15.48$, $p < 0.001$). Significant interactions between generation and behavior were also found for immobility frequency and immobility time in the OFT ($F_{1,70} = 7.956$, $p = 0.006$, and $F_{1,70} = 6.991$, $p = 0.010$, respectively; Table 2).

3.3. Sexual dimorphism in the TST and OF among males and females

In the F1 generation, the three-way ANOVA revealed no differences in immobility time between males and females in the TST ($F_{1,177} = 0.784$, $p = 0.377$; Fig. 4A). However, different parameters in the OFT indicated differences between males and females (Table 3). Significant differences in immobility time were found between depressive- and non-depressive-like males and females ($F_{1,67} = 7.487$, $p = 0.008$). A significant interaction was found between gender, behavior, and treatment for immobility frequency ($F_{1,67} = 50.69$, $p < 0.001$).

Similar to the F1 generation, the three-way ANOVA in the F2 generation did not reveal significant differences in immobility time between males and females in the TST ($F_{1,180} = 0.370$, $p = 0.544$; Fig. 4B). In the OFT, significant differences were observed between males and females (Table 4). Significant differences in rearing frequency ($F_{1,69} = 8.984$, $p = 0.004$), immobility time ($F_{1,69} = 5.503$, $p = 0.022$), and immobility frequency ($F_{1,69} = 6.198$, $p = 0.015$) were observed between males and females mice among the treatment (Table 4).

4. Discussion

The TST is commonly used to screen the antidepressant effects of drugs or assess depressive-like behavior. It is also known as a test of behavioral despair that consists of inescapable stress. We employed the TST because of its relatively easy applicability and advantages with regard to avoiding stress that is induced by water exposure and hypothermia, which differentiates it from the FST (i.e., another commonly used test that evaluates behavioral despair) [9].

In the present study, we found that males and females could be selected in different generations based on different immobility responses in the TST. High immobility times reflects depressive-like behavior. Low immobility times reflect non-depressive-like behavior. In addition to these two main phenotypes, we also identified an intermediate group, in which the mice did not present sufficient immobility time to be considered depressed or non-depressed.

Depressive-like females from the F0 generation, when mated with males of the same generation that exhibited the same behavior, had a higher percentage of offspring (i.e., the F1 generation) that also exhibited this characteristic. This phenotype transmission was also evident in the F2 generation when the same mating procedure was applied to F1 mice. Similar phenotype transmission was also evident among non-depressive-like mice that were subjected to the same mating procedures. Male mice maintained the initial proportion of these behaviors through two generations (74% for non-depressive-like behavior and 82% for depressive-like behavior). Female mice also maintained a similar proportion through two generations, despite being slightly less than males (71% for non-depressive-like behavior and 72% for depressive-like behavior), suggesting that genetic factors might be involved in this selection and its manifestation. Although these phenotype results were significant, the present study has limitations that need to be considered. For example, although siblings were not crossed with each other to create the F2 generation, they were used as part of the *n* of the F1 generation groups.

In a 20-year-long longitudinal study, Weissman et al. [60] followed three generations of patients with either a history or no history of MDD. These authors reported that the rates of psychopathology were the highest among grandchildren whose relatives had MDD. Gene-environment interactions are fundamental to this issue because relatives can provide information about both genetics and environmental risk. However, establishing the specific influences of genetics and the environment on the development of disease is difficult because both might modulate the response to the other. According to Caspi et al. [5], a functional polymorphism that is located in the promoter region of the serotonin transporter gene can act as a modulator of stressful events over an individual's lifespan, which can lead to high, low, or no expression of depressive symptoms. This may help explain why people

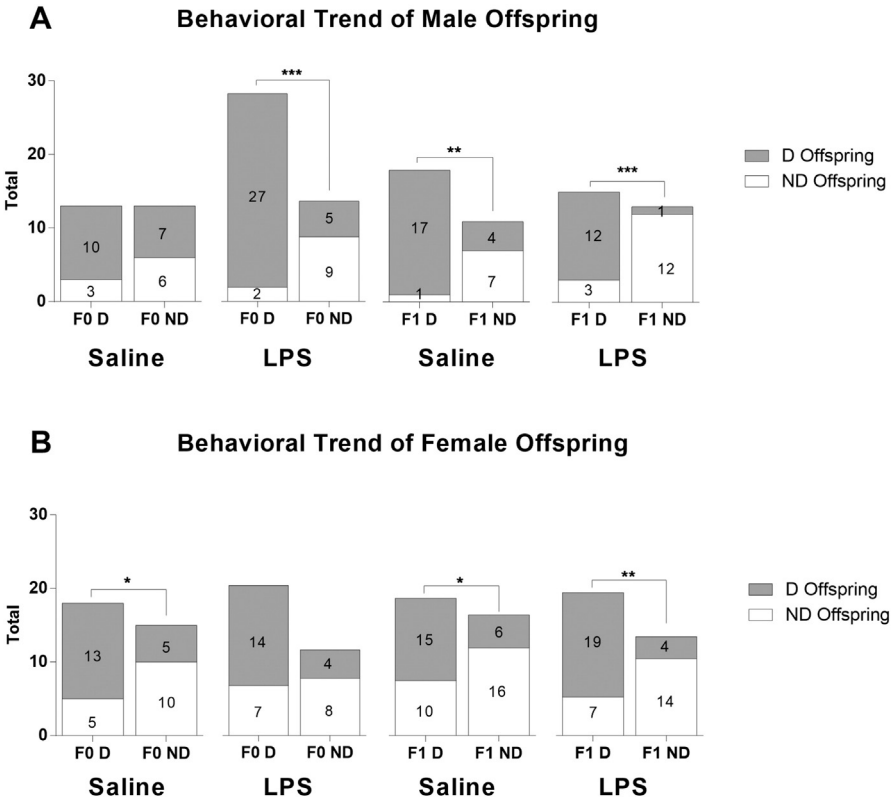

Fig. 5. Behavioral phenotype transmission through depressive- and non-depressive germlines. (A) Male germline. The results show a Fisher's exact test of F0 offspring (Saline: F0 D: D $n = 10$, ND $n = 3$ and F0 ND: D $n = 7$, ND $n = 6$; LPS: F0 D: D $n = 27$, ND $n = 2$ and F0 ND: D $n = 5$, ND $n = 9$) and F1 males offspring (Saline: F1 D: D $n = 17$, ND $n = 1$ and F1 ND: D $n = 4$, ND $n = 7$; LPS: F1 D: D $n = 12$, ND $n = 3$ and F1 ND: D $n = 1$, ND $n = 12$), F0 LPS $^{***}p < 0.0001$, F1 Saline: $^{**}p = 0.0014$; F1 LPS: $^{***}p = 0.0002$. (B) Female germline. The results show a Fisher's exact test of F0 offspring (Saline: F0 D: D $n = 13$, ND $n = 5$ and F0 ND: D $n = 5$, ND $n = 10$; LPS: F0 D: D $n = 14$, ND $n = 7$ and F0 ND: D $n = 4$, ND $n = 8$) and F1 female offspring (Saline: F1 D: D $n = 15$, ND $n = 10$ and F1 ND: D $n = 6$, ND $n = 16$; LPS: F1 D: D $n = 19$, ND $n = 7$ and F1 ND: D $n = 4$, ND $n = 14$), F0 Saline $^{*}p = 0.0383$, F1 Saline: $^{*}p = 0.0394$; F1 LPS: $^{**}p = 0.0018$.

respond differently to the same stressors but not how the stressor might modulate gene expression through generations. Stressful environmental events might also produce epigenetic modifications, such as DNA methylation or histone alterations. These epigenetic alterations may affect the way in which the organism responds to MDD or may even alter a predetermined behavior-environment response [14].

To understand the biological mechanisms that underlie various disorders, valid animal models are indispensable, particularly in neuropsychiatric research. Mice that are genetically or selectively bred to model specific key symptoms of human depression can be successfully used to discover neurobiological endophenotypes that may bridge the gap between behavioral phenotypes and genotypes [10,19,20,55]. As an alternative to transgenic mice that carry specific genetic mutations, selective breeding has been proven to be a powerful strategy for unraveling the genetic basis of disorders, providing unique information about pleiotropy, epistasis, and gene-environment interactions [55].

With regard to gender differences, we found that the incidence of a depressive-like phenotype was more robust in male mice than in female mice of the F1 generation that were born from dams that were treated with saline solution or LPS (i.e., there were more depressive-like males than females of the F1 generation, independent of treatment).

Depression is a mental disease that affects complex cognitive and emotional functions. Depression has been reported to be twice more prevalent in women than in men [41,42,48]. The incidence of depression in women also varies by life stage, with many interacting factors (e.g., childbearing and cyclic hormonal changes). This phenomenon may influence the response to various antidepressant therapies, and these differences are still underestimated in clinical treatment.

Table 1
Analysis of parameters in the open field test in male F1 vs. F2 mice. The F1 generation was prenatally exposed to LPS or saline, in which F0 dams received 100 μg/kg LPS or 0.9% NaCl solution on GD15. The F2 generation was not directly exposed to LPS or saline. The data were analyzed using three-way ANOVA followed by the Holm-Sidak *post hoc* test.

Source of variation of male F1 vs. F2 mice	Velocity			Rearing			Grooming			Freezing time			Freezing frequency			Distance		
	df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
Generation	1	43.265	<0.001***	1	20.675	<0.001***	1	0.239	0.626	1	16.913	<0.001***	1	12.811	<0.001***	1	40.255	<0.001***
Behavior	1	0.859	0.357	1	3.199	0.078	1	4.271	0.043*	1	1.174	0.282	1	7.068	0.01	1	0.845	0.361
Treatment	1	1.301	0.258	1	0.016	0.9	1	0.385	0.537	1	12.229	<0.001***	1	19.847	<0.001***	1	1.156	0.286
Generation × behavior	1	0.585	0.447	1	0.945	0.335	1	1.03	0.314	1	0.18	0.673	1	0.62	0.434	1	0.431	0.514
Generation × treatment	1	0.409	0.525	1	2.507	0.118	1	0.186	0.668	1	1.981	0.164	1	0.351	0.555	1	0.348	0.557
Behavior × treatment	1	1.09	0.3	1	1.105	0.297	1	0.915	0.342	1	1.691	0.198	1	8.689	0.004***	1	1.271	0.264
Generation × behavior × treatment	1	0.0169	0.897	1	1.098	0.298	1	4.033	0.049*	1	1.181	0.281	1	0.0002	0.989	1	0.003	0.957
Residual	66			66			66			66			66			66		
Total	73			73			73			73			73			73		

df, degrees of freedom; *F*, *F* ratio; *p*, probability.

Table 2
Analyses of parameters observed in the open field test in female F1 vs. F2 mice. The F1 generation was prenatally exposed to LPS or saline, in which F0 dams received 100 μg/kg LPS or 0.9% NaCl solution on GD15. The F2 generation was not directly exposed to LPS or saline. The data were analyzed using three-way ANOVA followed by the Holm-Sidak *post hoc* test.

Source of variation of female F1 vs. F2 mice	Velocity			Rearing			Grooming			Freezing time			Freezing frequency			Distance		
	df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
Generation	1	37.126	<0.001***	1	1.54	0.219	1	5.345	0.024*	1	2.371	0.128	1	0.447	0.506	1	34.872	<0.001***
Behavior	1	1.693	0.197	1	1.224	0.272	1	0.594	0.443	1	0.533	0.468	1	0.269	0.606	1	1.949	0.167
Treatment	1	0.00527	0.942	1	2.214	0.141	1	0.594	0.443	1	0.134	0.716	1	1.384	0.243	1	0.0002	0.988
Generation × behavior	1	0.767	0.384	1	0.508	0.478	1	0.0121	0.913	1	6.991	0.01**	1	7.956	0.006***	1	0.579	0.449
Generation × treatment	1	3.156	0.08	1	17.592	<0.001***	1	7.576	0.008***	1	2.389	0.127	1	15.488	<0.001***	1	3.314	0.073
Behavior × treatment	1	0.451	0.504	1	2.248	0.138	1	0.303	0.584	1	0.214	0.645	1	3.426	0.068	1	0.556	0.459
Generation × behavior × treatment	1	0.243	0.624	1	0.0026	0.96	1	2.048	0.157	1	0.122	0.728	1	2.073	0.154	1	0.237	0.628
Residual	70			70			70			70			70			70		
Total	77			77			77			77			77			77		

df, degrees of freedom; *F*, *F* ratio; *p*, probability.

Data from animal models of depression have been contradictory. Previous studies that utilized the TST or FST found higher depressive-like behavior in females than in males [11,33], lower depressive-like behavior in females than in males [44], or no differences between genders [4,46].

Both the TST and FST are based on rodents' innate behavior to develop an immobile posture after initial escape-oriented movements when placed in an inescapable stressful environment. In the TST, the stressful situation involves hemodynamic stress, in which the mouse is suspended by its tail in an uncontrollable fashion. In the FST, the mouse is placed in a cylinder that is filled with water [54]. The presence of corticosterone has been suggested to be crucial for the incorporation of the learned response after swim stress. Therefore, corticosterone is implicated in helpless behavior in the FST and likely in the TST. Kokras et al. [26] found that manipulating corticosterone levels effectively reversed the effects of adrenalectomy in male rats but not in female rats. These authors proposed the existence of two sex-oriented mechanisms of the stress response. The predominantly male-type response is associated with peripheral endogenous corticosterone. The female-type response is less dependent on this influence of corticosterone. Importantly, both depressive- and non-depressive mice were obtained by mating female and male mice that presented the same phenotype, suggesting a genetic influence on our results. Thus, differences between male and female mice that were caused by the genetic selection of depressive-like behavior, with consequently high corticosterone levels and responsiveness to stress, resulted in more vulnerability to depression in male mice in the D group compared with female mice in the same group.

Previous studies reported that the estrous cycle does not significantly modulate depressive- or anxiety-like behavior [1,49]. However, other studies reported a role for female gonadal hormones in the expression of both anxiety- and depressive-like behavior [28,32]. In the present study, we did not determine the estrous phase in female mice but attempted to reduce estrous phase variations by housing female mice and their offspring in neighboring cages after weaning [37].

Prenatal exposure to LPS promoted significant differences between both phenotypes in male mice compared with saline-treated mice of the F1 and F2 generations. Prenatal LPS exposure increased the outcome of depressive-like behavior in male mice. The significant differences were greater in LPS-treated male mice compared with saline-treated male mice. Similar results were not observed among male F1 mice with non-depressive-like behavior. These results might indicate sensitization to LPS that is caused by parental exposure in male mice. Such differences may be associated with the actions of various stress-related hormones, such as corticosterone, and epigenetic modifications that can contribute to sex-specific brain development and the susceptibility to environmental stress [17].

Stressful environmental situations, including psychological trauma during the early stages of neonatal development and prenatal exposure to various viral and bacterial infections, can cause short- and long-term changes in behavior and central nervous system activity [39,52]. Prenatal infection that is mimicked by LPS exposure causes profound changes in fetal brain development, affecting such areas as the HPA axis and increasing the levels of glucocorticoids and proinflammatory cytokines within the maternal circulation and fetal brain [2,39,56].

Walker et al. [58] reported that neonatal LPS exposure increased anxiety-like behavior in mice in adulthood, and the anxiety-like phenotype was transmitted to filial embryo lines when stressed females were bred with non-manipulated males. Neonatal stress can induce alterations that vary from impairments in maternal care to phenotype transmission across filial lines. Neonatal and prenatal stress also affects behavior. Penteado et al. [45] reported that prenatal LPS exposure increased maternal care in the parental generation and promoted tolerance to an LPS challenge during infancy in the F1 generation, leading to the hypothesis that maternal inflammation disrupts behavioral and immune responses.

Table 3
Analyses of parameters in the open field test in male vs. female F1 mice. The F1 generation was prenatally exposed to LPS or saline, in which F0 dams received 100 μg/kg LPS or 0.9% NaCl solution on GD15. The F2 generation was not directly exposed to LPS or saline. The data were analyzed using three-way ANOVA followed by the Holm-Sidak *post hoc* test.

Source of variation of male vs. female F1 mice	Velocity			Rearing			Grooming			Freezing time			Freezing frequency			Distance		
	df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
Generation	1	0.0101	0.92	1	0.173	0.679	1	1.948	0.167	1	3.693	0.059	1	49.718	<0.001***	1	0.000618	0.98
Behavior	1	1.437	0.235	1	2.65	0.108	1	2.608	0.111	1	3.636	0.061	1	5.074	0.028*	1	1.572	0.214
Treatment	1	4.493	0.038*	1	4.392	0.04*	1	0.825	0.367	1	1.923	0.17	1	110.08	<0.001***	1	4.448	0.039*
Generation × behavior	1	0.381	0.539	1	1.534	0.22	1	0.916	0.342	1	7.487	0.008***	1	57.221	<0.001***	1	0.159	0.692
Generation × treatment	1	0.0437	0.835	1	0.34	0.562	1	1.137	0.29	1	0.0561	0.813	1	20.59	<0.001***	1	0.132	0.717
Behavior × treatment	1	0.136	0.714	1	3.265	0.075	1	4.948	0.029*	1	0.25	0.619	1	6.526	0.013	1	0.199	0.657
Generation × behavior × treatment	1	0.376	0.542	1	0.139	0.71	1	0.0624	0.803	1	0.381	0.539	1	50.697	<0.001***	1	0.63	0.43
Residual	67			67			67			67			67			67		
Total	74			74			74			74			74			74		

df, degrees of freedom; *F*, *F* ratio; *p*, probability.

Table 4
Analyses of parameters in the open field test in male vs. female F2 mice. The F2 generation was not directly exposed to LPS or saline treatment. The data were analyzed using three-way ANOVA followed by the Holm-Sidak *post hoc* test.

Source of variation of male vs. female F2 mice	Velocity			Rearing			Grooming			Freezing time			Freezing frequency			Distance		
	df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
Generation	1	0.917	0.342	1	8.984	0.004***	1	2.416	0.125	1	2.451	0.122	1	1.202	0.277	1	0.84	0.363
Behavior	1	1.214	0.274	1	1.664	0.201	1	1.074	0.304	1	1.741	0.191	1	2.287	0.135	1	1.305	0.257
Treatment	1	0.484	0.489	1	12.987	<0.001***	1	6.71	0.012*	1	0.000893	0.976	1	0.00266	0.959	1	0.477	0.492
Generation × behavior	1	0.961	0.33	1	0.215	0.644	1	1.82E−32	1	1	1	0.321	1	0.37	0.545	1	0.892	0.348
Generation × treatment	1	1.15	0.287	1	3.514	0.065	1	2.416	0.125	1	5.503	0.022*	1	6.198	0.015*	1	1.115	0.295
Behavior × treatment	1	0.0135	0.908	1	0.56	0.457	1	1.074	0.304	1	0.26	0.612	1	0.27	0.605	1	0.0369	0.848
Generation × behavior × treatment	1	0.995	0.322	1	0.556	0.459	1	0	1	1	0.418	0.52	1	0.806	0.372	1	1.001	0.321
Residual	69			69			69			69			69			69		
Total	76			76			76			76			76			76		

df, degrees of freedom; *F*, *F* ratio; *p*, probability.

Elevated levels of proinflammatory cytokines, such as interleukin-1, interleukin-6, and tumor necrosis factor-α, have been reported in patients with MDD [35,36,50]. In fact, many of the symptoms of MDD can be understood as sickness behavior [30,50,62]. However, cytokines likely act in concert with other systems, such as monoamines (particularly norepinephrine and serotonin), to produce the characteristic symptoms of depression [57].

Lipopolysaccharide exposure affects HPA axis activation and increases cytokine and brain norepinephrine and serotonin levels [15]. Specific phenotypes that are initially caused by LPS-induced inflammation can also lead to transmission along generations [58].

Interestingly, no differences in depressive- and non-depressive-like behavior, regardless of LPS treatment, were found in female mice of the F1 generation, indicating possible sexual dimorphism. In the F2 generation, female mice in the LPS group exhibited an increase in depressive-like behavior not only compared with non-depressive female mice in the LPS group but also compared with depressive-like female mice in the saline group.

Prenatal LPS exposure in the parental generation can readily reprogram the HPA and immune axes in F2 females in adulthood. LPS exposure in the parental generation did not modify the proportion of depressive- and non-depressive-like phenotypes in the F1 generation, reinforcing the genetic nature of this phenomenon. However, the proportion of mice with the depressive-like phenotype was affected in the F2 generation by parental exposure to LPS. The reasons why these effects were not observed in the F1 generation remain to be explored.

5. Conclusion

Male mice that were selected for depressive- and non-depressive-like behavior exhibited the same behavioral profile in the F1 and F2 generations. Lipopolysaccharide exposure augmented these differences, suggesting that LPS reprograms the HPA and immune axes in both generations. Female mice of the F1 generation did not present significant differences in the incidence of either the depressive- or non-depressive-like phenotype, suggesting sexual dimorphism. However, female F2 mice from the parental (F0) generation that was treated with LPS exhibited an increase in the incidence of depressive-like behavior compared with non-depressive-like behavior. These results suggest that maternal exposure to LPS affects subsequent generations in a sexually dimorphic manner.

We cannot state that prenatal inflammation did not promote other aspects of behavior in the filial lines. Lipopolysaccharide exposure could have promoted tolerance to its effects, thus masking possible changes. More studies will be necessary to elucidate other possible alterations, including neurochemical and genetic effects that are caused by LPS exposure.

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