Research Article

# Early-Life Immune Activation by Lipopolysaccharides and Its Impact on Cognitive and Negative Signs in a Schizophrenia Rat Model

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**Abstract** | Disruption of central nervous system development during critical periods, particularly due to adverse events like early infections, can profoundly impact brain structure and function. These disturbances may contribute to the onset of adult neuropsychiatric disorders, including schizophrenia. In this study, we focused on the critical phases of postnatal brain development, evaluating the long-term effects of repeated early postnatal exposure to lipopolysaccharides (LPS), a bacterial endotoxin, on cognitive and negative symptoms associated with schizophrenia in male and female Wistar rats. The rats were randomized into two groups: the group that received intraperitoneal injections (IP) of LPS on postnatal days (PND) 0, 4, 9, and 14 and the control group that received IP injections of saline on the same PND. After two months, the rats were subjected to neurobehavioral assessment, biochemical and histological analysis. The behavioral consequences studied following exposure to LPS early in life reflect the cognitive and negative signs of schizophrenia. Rats subjected to repeated LPS administration showed increased anxiety and depression, reduced social interaction, and cognitive decline in comparison with control groups. This difference was significantly greater in females than in males. In addition, groups receiving LPS treatment also showed significantly higher levels of oxidative stress in the prefrontal cortex and a reduction in the number of neurons in the CA3 region of the hippocampus (HPC). Overall, these findings demonstrate that repeated postnatal administration of LPS may result in the cognitive and negative impairments of schizophrenia, highlighting the sex-dependent nature of the effects of LPS.

**Keywords** | Lipopolysaccharide, Schizophrenia, Hippocampus, Mammalian brain, Oxidative stress, Sexual dimorphism

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## INTRODUCTION

In the mammalian brain, sensitive and critical periods represent specific timeframes where an organism's development is most likely altered by extrinsic and intrinsic factors, leading to lasting changes at various levels, from morphology to physiological and behavioral properties (Meredith, 2015). Accumulating epidemiological evidence suggests that adverse early-life events during nervous system development, such as early postnatal infection at specific stages with various pathogens such as bacteria, viruses, or parasites, may elevate the likelihood of developing psychopathology and neuropsychiatric disorders in adulthood, exerting enduring impacts on brain function (Guerrin *et al.*, 2023), such as schizophrenia (Chaplin *et al.*, 2022).

Schizophrenia is a psychiatric, neurodevelopment complex

disorder (Allgäuer *et al*., 2023), affecting 0.5-1% of the population worldwide. It commonly starts when synapses are pruned during the late adolescence or early adulthood stages (Morales-Medina *et al*., 2021). Schizophrenia in etiopathology involves the interaction of environmental or immunological alongside genetic risk predispositions (Allgäuer *et al*., 2023). This interaction during specific periods is marked by a combination of negative (the inability to derive pleasure), positive (hallucinations, delusions, thought disorders), and cognitive signs (attention and working memory). The intensity of these impairments may vary over time depending on the disease stage (Allgäuer *et al*., 2023). Numerous reports indicate that activating the immune system during both prenatal and postnatal development can target various brain cells, potentially contributing to the onset of schizophrenia (Esshili *et al*., 2020). In addition, substantial evidence supports that the emergence of schizophrenia may be linked to abnormal postnatal neurogenesis due to the late onset of the pathology (Iannitelli *et al*., 2017).

A comprehensive understanding of this disorder requires integrating epigenetics, genetics, neuroimaging, molecular biology, environmental factors, clinic research and animal models (Kelly *et al*., 2021). Despite their limitations, studies using animal models are crucial for obtaining information about the pathophysiology of brain development and the contributory role of inflammation in fostering vulnerability to psychiatric disorders like schizophrenia. In this regard, using lipopolysaccharide an endotoxin sourced from the outermost membrane of gram-negative bacteria, the primary objective of this experimental research is to investigate, for the first time, the effects of repeated LPS administration during early postnatal development (PND 0–PND 14) on cognitive and negative impairments associated with schizophrenia, as well as its impact on key biochemical markers in the prefrontal cortex (PFC) and hippocampus (HPC), including lipid peroxidation, nitric oxide and catalase in adulthood. The novelty of this research lies in the use of a multiple-exposure LPS model during a critical developmental window, allowing us to examine how early immune activation alters brain function and induces schizophrenia-like symptoms. Notably, to our knowledge, no previous study has investigated the effects of LPS injections at these specific developmental time points using the same dosing regimen. Furthermore, by including both male and female rats, this study provides insight into potential sex differences in response to early-life immune challenges.

## MATERIALS AND METHODS

### Experimental animals

For this experiment, we utilized 40 two-month-old Wistar rats, consisting of 20 females and 20 males. Female Wistar in a state of pregnancy were individually accommodated in polypropylene cages under controlled conditions of humidity (50–60%), temperature (24 °C), and a 12:12 hour light-dark cycle, with unrestricted access to food and water. These rats were obtained from the animal housing facility located within the Department of Biology at Ibn Tofail University in Kenitra, Morocco. The cages were regularly cleaned by replacing the bedding with wood shavings. After parturition, the females gave birth to 40 rats, with the day of birth designated as postnatal day (PND) 0.

### Experimental design

On PND 0, neonatal rats of both genders were separated into two groups and given either a repeated intraperitoneal injection of 250 µg/kg LPS (Berkiks *et al*., 2018) (LPS, *Escherichia coli*, serotype 026: B6, L-3755, Sigma, St. Louis) (at PND 0, 4, 9, and 14) or the same dose of PBS (phosphate-buffered saline). The selected time points for LPS or PBS administration correspond to a critical period in CNS development, marked by active synaptogenesis and synaptic pruning. These neurodevelopmental processes render the neonatal brain particularly vulnerable to inflammatory insults (Morimoto and Nakajima, 2019).

All injections were performed at 10:30 am. Subsequently, after PND 21, the pups were weaned and kept until they reached two months of age, at which point all behavioral tests were completed. During the entire experiment, the rats had free access to water and food (provided by Alf Sahel, a company specializing in compound animal feeds). The rats were euthanized at the end of the tests. Subsequently, their brains were extracted to isolate the PFC and HPC regions, and homogenates were prepared for biochemical marker assays (Ibouzine-Dine *et al*. 2024a). All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Ibn Tofail University. The study was conducted in full compliance with the ARRIVE guidelines and the NIH guidelines for the care and use of laboratory animals.

### Neurobehavioral tests

At PND 60, affective and cognitive behaviors were assessed through a series of behavioral tests: open field (OF), elevated plus maze (EPM), forced swimming (FS), Object Recognition Test (ORT), Y Maze, and Social Interaction Test (SI). Before testing, each animal was given ample opportunity to acclimate to the testing arena. Tests were conducted under high illumination (600 LUX) conditions. Daily behavioral assessments were performed from 09:00 to 15:00. The behavior of the rats was recorded using a video camera and analyzed with the ANY-maze software to ensure objective and automated assessment.

### Measurement of anxiety-like behavior using the open field test (OFT)

The OFT was employed to provide a quantitative and qualitative measure of locomotor activity and anxiety-related behaviors in rodents. It typically consists of a wooden apparatus with a 40 cm high wall. The test area is divided into 25 equal squares, consisting of 16 peripheral squares and 9 central squares (Azirar *et al*., 2025). The animals were placed in the center of the apparatus and allowed to explore for 10 minutes. Time spent in the central area (TCA), entries into peripheral and central areas (NRC), and the total number of returns to the central squares (NTS), were employed as indicators of anxiety-like behaviors or locomotion.

A rat was considered in the center when all four paws were within the central squares, and time spent in this area was recorded in seconds. Between tests, the apparatus was cleaned using 70% ethyl alcohol.

### Measurement of anxiety-like behavior using the elevated plus maze (EPM)

The EPM is a well-established method for evaluating anxiety levels in rats. The setup used in this study was constructed in the laboratory. The apparatus comprises closed arms (10 cm × 50 cm) with walls 40 cm high, intersecting orthogonally with two opposing open arms of identical dimensions, except at the central junction where the arms intersect. The entire EPM is positioned 50 cm above the ground (Brikat *et al*., 2025). It has been established as a method for detecting emotional reactions to both anxiolytic and anxiogenic drugs, based on the natural fear of exposed and open spaces and the exploratory drive of the rat. Each animal was started on the test by being positioned in the central arena of the maze, facing one of the open arms, and given five minutes to freely explore the arms freely. The assessment of anxiety-related parameters involved documenting the duration and the frequency of the animals' visits to both the closed and open arms, offering a comprehensive evaluation of anxiety-like behavior. Accordingly, a decrease in anxiety-related behavior was indicated by an increase in exploration of the open arms. The maze was cleaned between each animal using ethanol 70% to remove any residual olfactory cues.

### Measurement of depression using forced swimming test (FST)

This test involves placing rats individually into glass cylinders submerged in water (23±2 ˚C), with no opportunity to escape (El-Brouzi *et al*., 2021). The animals were placed in the test apparatus, and all activity was captured on video for further examination. The total immobility period (TI) and episodes of immobility (EI) were recorded over a five-minute period. Immobility was defined as when a rat ceased swimming, jumping, and struggling, and instead floated while making minimal movements to keep its nose above water. Increased depressive-like response is considered to result in a higher percentage of time spent floating.

### Cognitive measurement

### Novel object recognition test (ORT)

To assess recognition memory in rodents, the novel object recognition test is utilized. Rats naturally display a preference for unfamiliar objects over familiar ones. This experiment consists of three sessions conducted over three days and is conducted in an open field (OF) box. On the first day, during the familiarization session, each rat is placed in the box without any objects present and is permitted to explore freely for 5 minutes. On the second training day, two identical objects are placed approximately 30 cm apart from each other in the box. The rats are given five minutes to explore the objects, and the duration of time spent investigating each object is recorded. After being kept in their individual cages for 24 hours, the rats were subjected to a final test session on the last day. During this session, the rats were once more introduced in the test box where they encountered both a novel object and a familiar one. The rats were exposed to these objects for 5 minutes and the time spent examining each object was recorded. Memory preference was assessed using a recognition index (RI), calculated as follows: RI = (TN / (TN + TF)) * 100, where TN represents the time spent on the unfamiliar object and TF the time spent on the familiar object (Nassiri *et al*., 2024).

### Y-maze test

The Y-maze test was used to evaluate spontaneous alternation, a measure of spatial working memory (Sierksma *et al*., 2014). The apparatus consists of three similar arms (A, B, and C; 35*12*61 $cm^3$), intersecting at 120˚ angles. The animals were positioned at the center area of the maze and given eight minutes to explore the arena under moderate lighting conditions. The number of times a rat visited each arm of the maze was recorded (i.e. ACBCABCAABCAABAC, etc). A 'triplet' was considered when the rat visited all three arms in sequence. Rat working memory was determined by measuring the proportion of alternations, calculated using the formula: [Number of alternations/ (total arm entries – 2)] * 100 (Harifi *et al*., 2024). Rats that scored markedly more than 50% alternations are considered to have functional working memory.

### Social preference test

To assess the animals' social memory and interaction, a social preference test was conducted, adapted from Matthew with minor adjustments (Rein et al., 2020). This test is capitalizes on rat's inherent inclination to investigate

unfamiliar conspecifics over familiar ones. The test apparatus consists of a three-chamber box with an opening allowing rats to move from one chamber to another. Prior to the social interaction test, all rats underwent a 5-minute habituation period in the three-chamber apparatus. During this time, the animals were allowed to freely explore all three chambers without any stimuli present. During the first session of the test, the animals were initially introduced to a new rat that was sex- and age-matched for 5 minutes. After this, they were placed individually for 30 minutes. Subsequently, they were once again exposed to the initial familiar animal and a second new animal for another 5 minutes during the second session. The activity of the animals was monitored throughout this period, and a social discrimination score was computed using the following formula: (novel investigation time/ (novel + familiar investigation time)) × 100.

## Biochemical analyses

Once the rats had been sacrificed, their brains were immediately removed. The HPC and PFC of each hemisphere were isolated from the surrounding brain before being homogenized in phosphate lysis buffer (PB). The homogenates were then centrifuged for 30 minutes at 3000 rpm and used to extract supernatants, which were then utilized for assessing nitric oxide content, lipid peroxidation levels, and catalase activity (Benmhammed *et al*., 2024).

## Nitrite oxide assay

Griess reagent was used to measure NO in the HPC and PFC regions. 50 µl of sample was mixed with 50 ml of Griess reagent and incubated for 30 minutes at room temperature (20 ± 2 °C). The NO level was measured at a wavelength of 540 nm using an optic ivymen system microplate reader 2100C, and its levels in the tissue were determined in µmol/g of tissue (Ibouzine-Dine *et al*., 2024a).

## Lipid peroxidation assay

Using Buege's procedure, the estimation of lipid peroxidation (LPO) in the HPC and PFC was performed. Using 150 µl of supernatant was added to an equal volume of trichloroacetic acid (TCA) in a 1.5 ml microfuge tube. The mixture was then centrifuged for 10 minutes at 3000 rpm at room temperature, and then 150 µl of thiobarbituric acid (TBA) was added to the obtained supernatant. The sample was mixed and subjected to a boiling water bath for fifteen minutes, then 200 µl were pipetted into an ELISA plate, and subsequently, the optical density was determined at a wavelength of 531 nm using an optic ivymen system microplate reader 2100C (Nassiri *et al*., 2024).

## Catalase activity assay

The activity of CAT was analyzed according to the procedures outlined in Aebi's protocol (Aebi, 1984), which involves the conversion of $H_2O_2$ into $H_2O$ and $O_2$. Catalase activity was determined by calculating the decrease in $H_2O_2$ concentration at a wavelength of 240 nm using spectrophotometer UV-2005. The action of the antioxidant enzyme is expressed in U/g tissue.

The experimental methodology, detailing all procedural steps, is depicted in Figure 1.

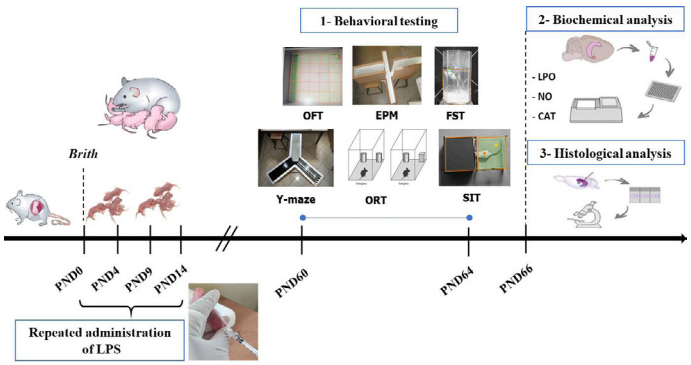


**Figure 1:** Experimental design. Neonatal rats received intraperitoneal injections of lipopolysaccharides (LPS) on postnatal days (PND) 0, 4, 9, and 14 days. (1) After 2 months, the behavioral test assessment through open field (OF) elevated plus maze (EPM), forced swimming (FS), Object Recognition Test (ORT), Y-Maze, and Social Interaction Test (SI) were performed. The animals were euthanized. Thereafter, (2) oxidative stress markers (lipid peroxidation (LPO), nitric oxide (NO) levels, and catalase (CAT) activities), and (3) histology of the hippocampus and prefrontal cortex were evaluated.

## Histology analysis

Brain tissue sections were prepared and cut to a thickness of 30 µm using a laboratory vibratome. These sections were mounted on gelatin-coated glass slides and air-dried (Hicham *et al*., 2018). For histological analysis, Cresyl violet staining was performed to visualize Nissl bodies within neuronal cell bodies (Lamtai *et al*., 2021). The staining protocol included immersion in xylene, followed by rehydration through a graded ethanol series (100% to 70%). After rinsing with distilled water, sections were treated with 0.5% Cresyl violet to ensure effective staining of the neuronal soma. Finally, the sections were dehydrated, cleared with xylene, and sealed with a coverslip. Using Image J software 1.45 with Java pre-installed, images of slices were analyzed and cell counts were performed over a 180,000 µm$^2$ (600 × 300 µm) region (National Institute of Health, free online).

## Statistical assessment

In this study, the data analysis was carried out utilizing the SPSS software version 22 (IBM Corp., Armonk, NY, United States). The student's t-test was employed to determine the variance between the control group and

the treated group (El-Brouzi *et al*., 2021), followed by the Bonferroni test for multiple comparisons, with statistical significance set at $p < 0.05$. Data were expressed as mean ± SEM.

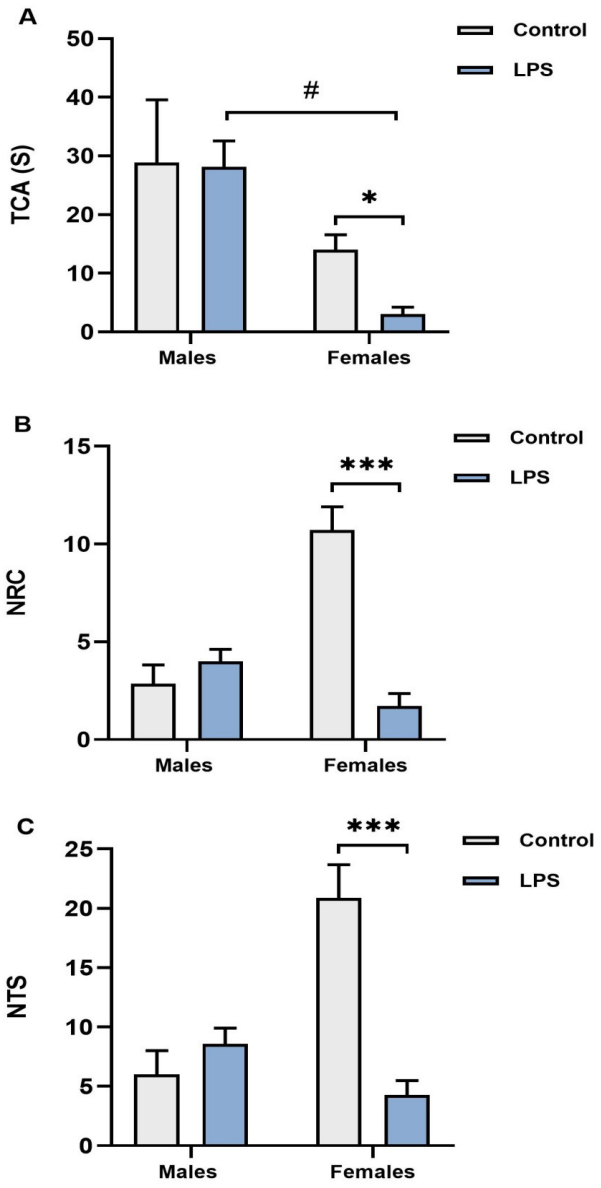


**Figure 2:** Effects of repeated LPS administration on anxiety-related behavior of female and male rats using to the open field test. TCA: the time spent in the center of the area; NRC: the number of returns to the center; NTS: the number of total squares crossed. The data are presented as means ± SEM (n = 10). *p < 0.05 vs. control group; and #p < 0.05 vs. gender counterpart.

## RESULTS

### Anxiety assessment

#### LPS impact on anxiety-like behavior as assessed in the OFT

As shown in Figures 2A and 2B, the repeated LPS administration significantly reduced the TCA and NRC in female rats compared to control rats ($p = 0.025$ and $p < 0.0001$, respectively), indicating heightened anxiety-like behavior. Additionally, the NTS was significantly lower in LPS-treated females than in controls ($p < 0.0001$) (Figure 2C). In contrast, male rats did not exhibit significant changes in TCA, NRC, or NTS following LPS treatment ($p > 0.05$). Furthermore, a significant sex-dependent effect was observed, as LPS-treated females showed lower TCA ($p = 0.036$), NRC ($p > 0.05$), and NTS ($p > 0.05$) compared to LPS-treated males (Figure 2).

#### LPS impact on anxiety-like behavior as evaluated in the EPM

As shown in Figures 3A, B, repeated postnatal exposure to LPS significantly reduced the Time spent in open arms (TOA) in female rats compared to the control group ($p < 0.001$). However, the EOA was not significantly affected ($p > 0.05$). In contrast, LPS exposure did not induce significant changes in TOA or EOA in male rats compared to controls ($p = 0.77$). Furthermore, the TEA remained unchanged in both female and male rats following repeated LPS administration ($p > 0.05$) (Figure 3C). Additionally, no significant sex-dependent differences were observed between LPS-treated males and females ($p = 0.99$) (Figure 3).

#### Effects of LPS on depressive-like behavior

Statistical analysis revealed that repeated LPS administration significantly increased TI in both male and female rats compared to their respective control groups ($p > 0.05$ and $p=0.019$, respectively) (Figure 4A), indicating heightened depression-like behavior. Similarly, LPS-treated females exhibited a significant increase in EI ($p = 0.008$), while males also showed a significant but less pronounced increase ($p = 0.034$) compared to controls (Figure 4B). However, no significant sex-dependent differences were observed between LPS-treated males and females ($p > 0.05$) (Figure 4).

#### LPS impact on memory function

##### Y-maze

In this test, statistical analysis revealed that LPS treatment did not significantly affect the percentage of alternation in male rats ($p > 0.05$), indicating no impairment in working memory. Similarly, female rats exposed to LPS did not show a significant reduction in alternation compared to the control group ($p > 0.05$). Furthermore, no significant sex-dependent differences were observed ($p > 0.05$) (Figure 5).

#### Object recognition test

In both male and female rats subjected to repeated LPS administration, a strong preference for the familiar object was observed, consistent with the impairment in discrimination index ($p = 0.0058$ and $p = 0.0096$, respectively) (Figure 6A).

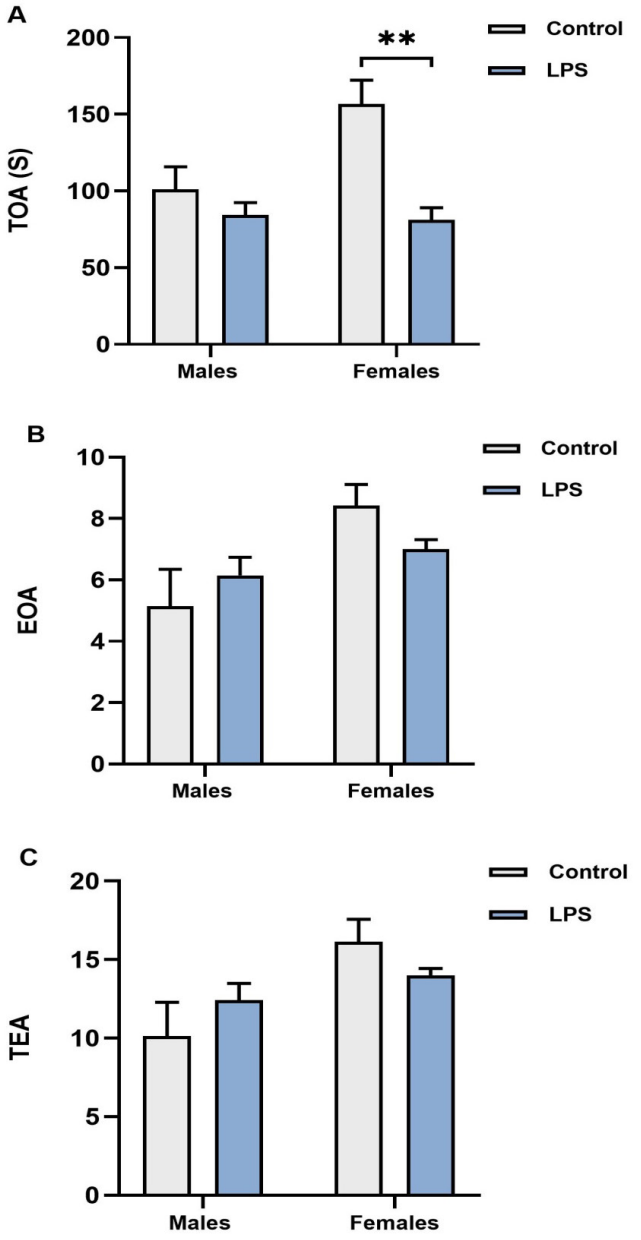


**Figure 3:** Effects of repeated LPS administration on anxiety-related behavior of female and male rats subjected to the elevated plus maze. TOA: the time spent on the open arms; EOA: the entries into open arms; TEA: The number of full entries into the arms. The data are presented as means ± SEM (n = 10). *p < 0.05 vs. control group; and #p < 0.05 vs. gender counterpart.

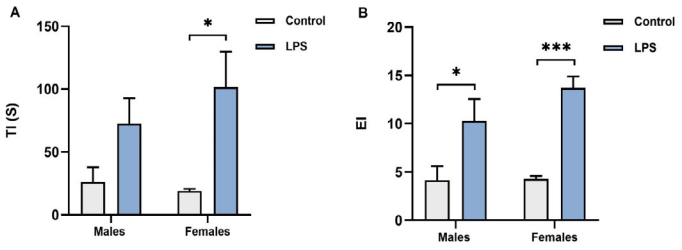


**Figure 4:** Effects of repeated LPS administration on depression-related behavior of female and male rats subjected to the forced swimming test. TI: time of immobility (Sec). EI: episode of immobility. Mean ± S.E.M (n = 10). #p < 0.05 vs. gender and *p < 0.05 vs. control group counterparts are the presentation formats for the results.

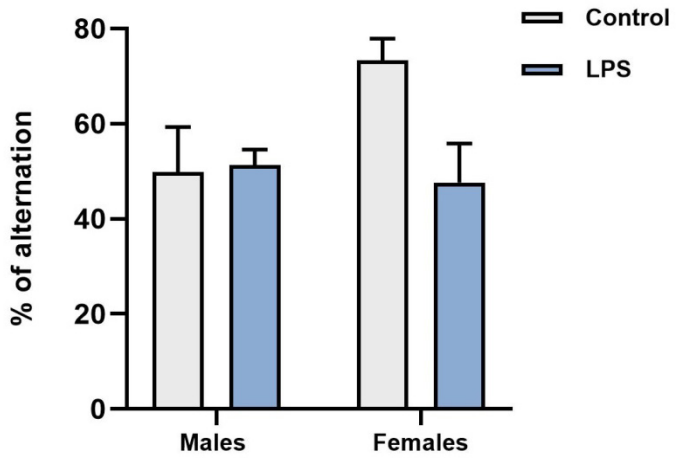


**Figure 5:** Effects of repeated LPS administration on % of alternation of female and male rats exposed to the Y-maze. The data are presented as means ± SEM (n = 10). *p < 0.05 vs. control group; and #p < 0.05 vs. gender counterpart.

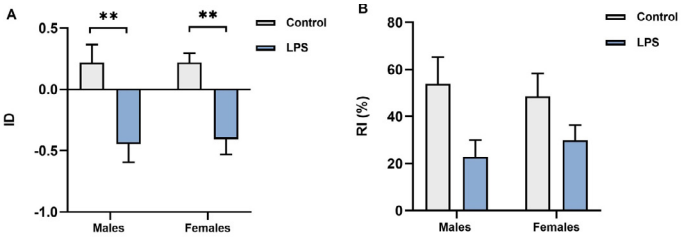


**Figure 6:** Effects of repeated LPS administration on recognition memory of male and female rats subjected to the object recognition test. ID: Index of discrimination. RI: recognition index. The data are presented as means ± SEM (n = 10). *p < 0.05 vs. control group; and #p < 0.05 vs. gender counterpart.

This finding was further supported by a RI of less than 50% in both sexes; however, this reduction did not reach statistical significance (Figure 6B). Additionally, no significant sex-dependent differences were detected (p > 0.05).

## Social preference test

Statistical analysis of the social interaction test revealed a decrease in the percentage of social discrimination in both male and female rats following LPS administration compared to their respective controls. This reduction was particularly significant in females (p < 0.0088). However, no significant sex-dependent effect was observed (p > 0.05) (Figure 7).

## Effects of LPS on oxidative stress parameters

As shown in Figure 8A, LPS administration resulted in a significant increase in thiobarbituric acid reactive substances (TBARS) a key marker of LPO, in the PFC of both male and female rats, with statistical significance observed in females (p = 0.023). However, no significant changes in TBARS levels were detected in the HPC of either sex (p > 0.05) (Figure 8B). Additionally, when comparing males and females, no significant sex-dependent differences were observed in either brain region (p > 0.05).

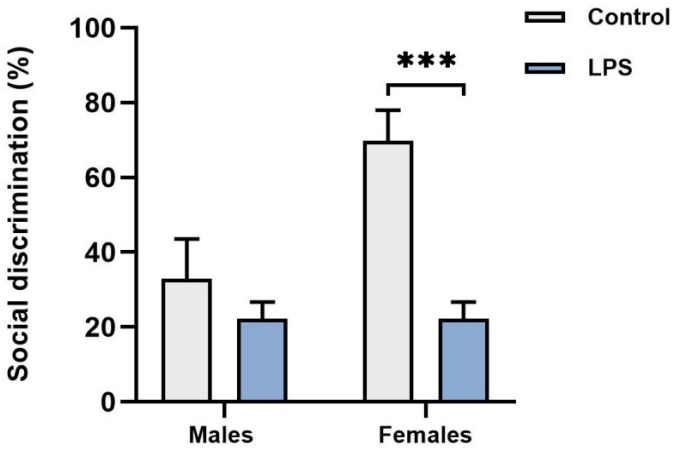


**Figure 7:** Effects of repeated LPS administration on social interaction of male and female rats subjected to the social preference test. The data are presented as means ± SEM (n = 10). *p < 0.05 vs. control group; and #p < 0.05 vs. gender counterpart.

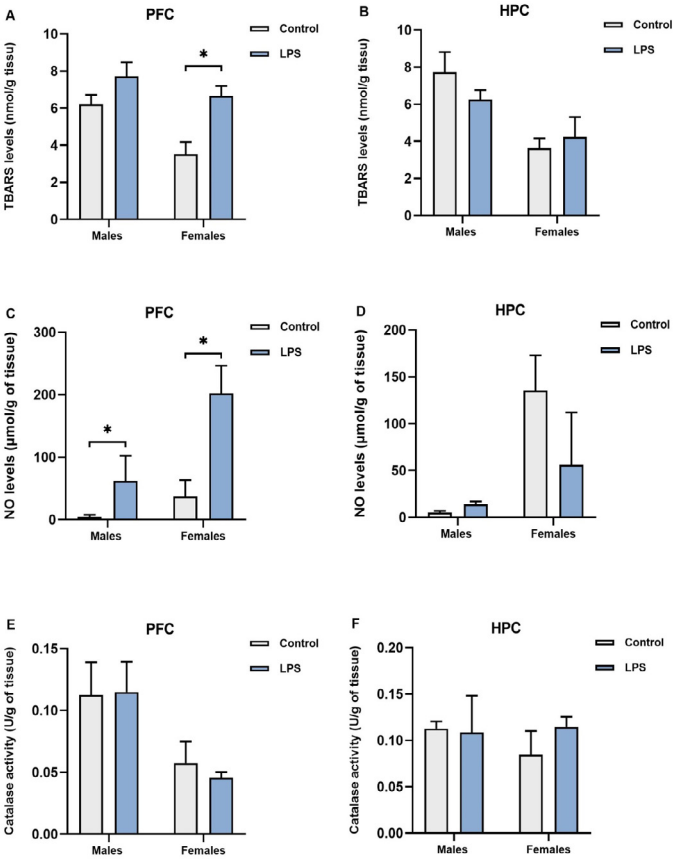


**Figure 8:** Effects of repeated LPS administration on levels of Thiobarbituric Acid reactive substances (TBARS) in (A) prefrontal cortex (PFC) and (B) hippocampus (HPC), and nitric oxide (NO) in (C) prefrontal cortex and (D) hippocampus, and catalase (CAT) activities in (E) prefrontal cortex and (F) hippocampus in both female and male rats. The data are presented as means ± SEM (n = 10). *p < 0.05 vs. control group; and #p < 0.05 vs. gender counterpart.

Furthermore, repeated LPS injections led to a significant increase in NO concentration in the PFC of both sexes compared to controls (p = 0.041 and p = 0.25, respectively) (Figure 8C), while NO levels remained unaffected in the HPC (p > 0.05) (Figure 8D), with no sex-specific differences detected.

Regarding CAT activity, LPS treatment did not induce any statistically significant alterations in either the PFC or HPC compared to the control group (p > 0.05) (Figure 8E, F), and no significant gender-related effects were observed (p > 0.05).

## Effects of LPS on brain histology

Histological analysis revealed a significant reduction in neuronal density following LPS treatment compared to control groups. Specifically, LPS-exposed rats exhibited a pronounced decrease in neuron density within the HPC, particularly in the CA3 region (Figures 9 and 10). Furthermore, female rats treated with LPS showed a more pronounced reduction in neuronal count within the CA3 area compared to their male counterparts.

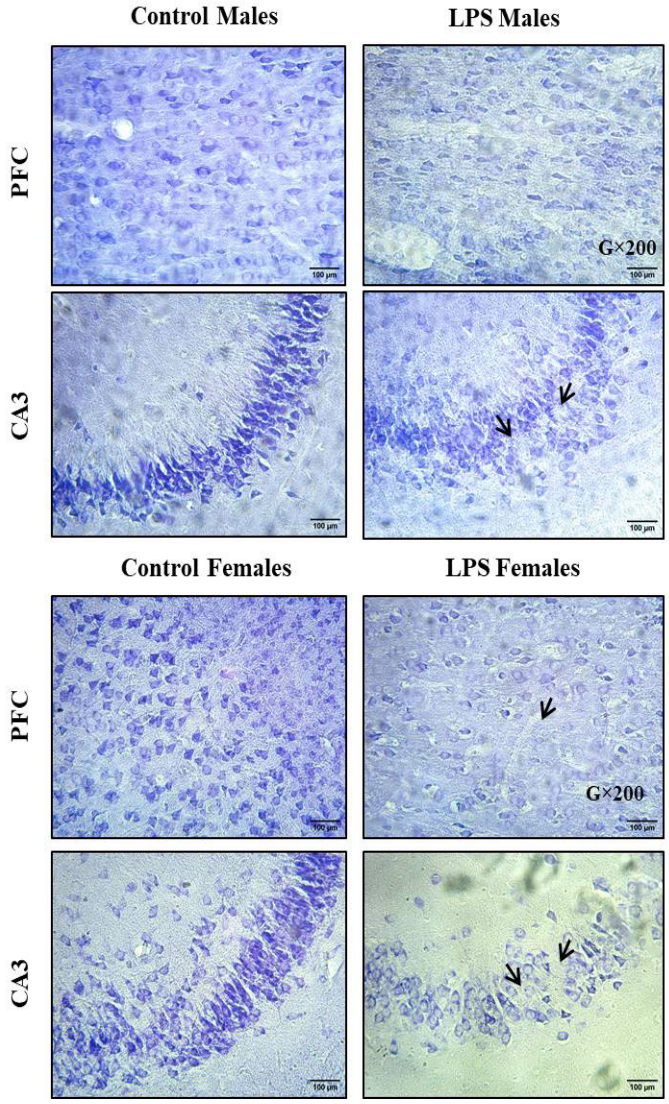


**Figure 9:** Light micrographs of prefrontal cortex (PFC) and hippocampus (CA3) with cresyl violet staining in the control and LPS groups of both genders. Black arrows indicate the intact neurons. Bar represents 100 µm.

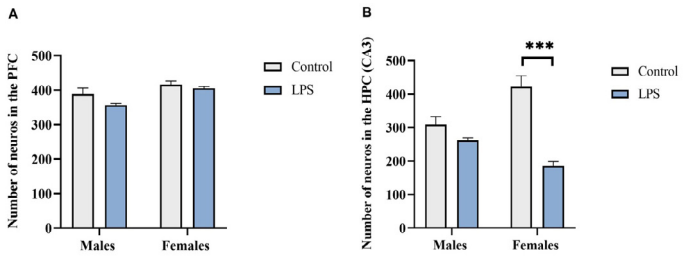


**Figure 10:** Neuron Count in the prefrontal cortex (PFC) (A) and the hippocampus (HPC-CA3) (B) Regions. The data are presented as means ± SEM (n = 10). *p < 0.05 vs. control group; and #p < 0.05 vs. gender counterpart.

## DISCUSSION

This study aims to investigate the impact of repeated administration of LPS during specific periods in rats of both genders (PND 0, 4, 9, and 14) on the signs (cognitive and negative) of schizophrenia and its effect on biochemical and histological parameters in the HPC and PFC in adulthood.

Considering that anxiety occurs in up to 65% of schizophrenia patients (Custódio *et al*., 2018), it is believed that the behavioral consequences studied following early-life exposure to LPS reflect one of the negative signs of schizophrenia. When assessing anxiety measures based on the results of the OF and EPM tests, our findings indicate that repeated i.p. injections of LPS significantly increased anxiety-like behaviors. In addition, the results of the FST indicated that rats subjected to repeated injections of LPS tended to display depressive-type behavior. Our findings are in line with the study of Custódio *et al*. (2018) which showed that injection of LPS at PND 5 and 7 induces depressive and anxious behaviors during adolescence and adulthood in rats. Similar effects were also noted in the study by Benmhammed *et al*. (2019). Moreover, LPS injection (0.05 mg/kg) at PND 3 and 5 has been shown to induce anxiety-like behavior in adulthood (Tishkina *et al*., 2016). In contrast, higher doses of LPS (2 mg/kg) administered from PND 5 to PND 9, as well as intraperitoneal injections of LPS (0.2 mg/kg) at PND 0, 4, 6, and 8, did not result in anxiety-like behavioral alterations (Vojtechova *et al*., 2018). Additionally, we observed a decline in locomotor activity in the LPS-treated group, consistent with previous findings in adult rats exposed to LPS at PND 14, 16, and 18 (Zubareva *et al*., 2020). However, conflicting results have been reported following LPS administration at PND 3 (Réus *et al*., 2017), and from PND 5 to PND 9 (Vojtechova *et al*., 2018). These discrepancies may be attributed to differences in LPS dosage, route of administration, timing, and treatment intervals. To our knowledge, this is the first study to evaluate the effects of repeated LPS administration (250 µg/kg) at PND 0, 4, 9, and 14.

As known, patients with schizophrenia experience severe cognitive deficits, including impairments in learning and memory (Wischhof *et al*., 2015). In this context, our findings revealed a reduction in the alternation coefficient in LPS-treated rats, suggesting potential alterations in spatial working memory. This observation is consistent with findings by Custódio *et al*. (2018), who reported a decreased alternation coefficient in rats treated with LPS at PND 5 and PND 7 during adolescence (Custódio *et al*., 2018). Also, our results indicate that LPS-exposed rats exhibited impaired recognition memory. Despite these findings, the neurobiological mechanisms underlying LPS-induced cognitive dysfunction remain unclear.

Moreover, our findings revealed a reduction in social interaction in rats treated to repeated LPS injections. Reduced social interaction represents one of the main signs of schizophrenia (Peng *et al*., 2019). In line with our results, several studies have demonstrated that both postnatal intrahippocampal and intraperitoneal LPS injections at PND 14 induce social deficits in adulthood (Doenni *et al*., 2016). However, conflicting results have been reported by Peng *et al*., who found that LPS administration between PND 5 and PND 9 did not affect social behavior (Peng *et al*., 2019). These discrepancies may be explained by variations in the experimental setting or the LPS application protocol.

Many lines of research have indicated a potential association between oxidative stress (OS) and the onset of psychiatric disorders (Benmhammed *et al*., 2019, 2024). In this sense, the behavioral disturbances observed in our experiment in response to repeated administration of LPS were also marked by elevated indicators of OS, particularly in the PFC, a region central to memory functions and involved in emotion regulation. This was evidenced by increased levels of NO and TBARS. In addition, it should be noted that there were no notable variations in CAT activities observed between the treatment and control groups. The stable CAT activity may result from compensatory mechanisms by other antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase, may compensate for its activity by neutralizing reactive oxygen species (ROS) (Benmhammed *et al*., 2024). Future studies should incorporate a more complete panel of OS markers to better elucidate the pathways involved. Similarly, our previous findings demonstrated that exposure to LPS during PND 1 led to an increase in behaviors resembling anxiety and depression in adulthood. These behaviors were linked to increased levels of OS in the HPC and PFC, as revealed by increased levels of NO and LPO. As known, NO contributes to various facets of brain plasticity, including synapse formation and elimination (Ibouzine-Dine *et al*., 2024a), and elevated NO levels have been associated with altered behavior (Hoeijmakers *et al*., 2016). However, if

NO production surpasses the compensatory mechanisms of intrinsic antioxidants, it can significantly impair brain function (Ibouzine-Dine *et al*., 2024a), acting as a powerful oxidizing agent capable of raising levels of LPO (Ibouzine-Dine *et al*., 2024b). Following the induction of LPO, the breakdown of cell membranes occurs, resulting in modifications in membrane permeability and fluidity, which adversely affects neuronal function (Benmhammed *et al*., 2019). These effects may, in part, explain an aspect of schizophrenia signs observed in the group exposed to LPS.

Investigating hippocampal and PFC tissues is essential for understanding the effect of LPS on neurobehavioral and cognitive functions. Early-life infections have been shown to reduce synaptic density and cause neuronal loss in hippocampal regions (Wischhof *et al*., 2015). In this regard, our study revealed that repeated LPS administration led to neuronal loss in the CA3 sub-region of the HPC, a critical area involved in neurotransmitter metabolism and essential for memory and mood regulation (Zhu *et al*., 2017). These findings are consistent with those reported by Pulido *et al*. (2019). Growing experimental evidence highlights the crucial role of OS mechanisms in neuronal loss (Lamtai *et al*., 2021). In this context, the LPS-induced OS observed in our study may be a key contributor to hippocampal neuronal damage. Such neurotoxic effects can disrupt neurotransmission and neuronal function, potentially exacerbating cognitive and negative symptoms associated with schizophrenia.

Another important aspect to consider is the sex-specific impact of perinatal LPS exposure. In our study, we observed that the effects on emotional behavior, social preference, and OS were more pronounced in females than in males. This difference may be attributed to variations in gonadal hormones, as well as other environmental and neurodevelopmental factors (Cuskelly *et al*., 2022). The estrous cycle phase in female rats may influence behavioral outcomes, as hormonal fluctuations throughout the cycle are known to modulate behavior. Elevated estrogen levels, for instance, have been associated with reduced anxiety-like behavior, while progesterone-dominant phases may exacerbate it (Yoo and Lee, 2016). Moreover, the pro-inflammatory response elicited by LPS administration can interfere with the production of key reproductive hormones such as follicle-stimulating hormone and luteinizing hormone, both of which play essential roles in regulating the estrous cycle. In addition, the sexual dimorphism noted in our investigation may also be linked to endotoxin tolerance in males. Generally, endotoxin tolerance is a mechanism that suppresses the levels of expression of pro-inflammatory cytokines, consequently leading to desensitization of the hypothalamic-pituitary-adrenal axis following subsequent LPS injections. Bernardi *et al*. (2014) reported that male corticosterone and pro-inflammatory cytokine levels were not altered, suggesting that endotoxin tolerance prevented an increase in these concentrations (Bernardi *et al*., 2014). However, the precise mechanisms underlying this sexual dimorphism remain to be elucidated.

## CONCLUSIONS AND RECOMMENDATIONS

In summary, our study demonstrates that repeated postnatal exposure to LPS induces cognitive and negative signs associated with schizophrenia, with pronounced OS in the PFC. Notably; our findings reveal a sex-dependent susceptibility, with females exhibiting greater vulnerability to LPS-induced damage. Given these findings, future research should further explore the underlying mechanisms linking neuroinflammation, OS, and schizophrenia-like behaviors. Additionally, sex-specific therapeutic strategies should be considered to address differential vulnerabilities. Targeting OS through antioxidant-based interventions may offer a potential avenue for mitigating cognitive and behavioral impairments. Finally, preventive measures, such as maternal infection monitoring and immune-modulating strategies during pregnancy and early development, may help reduce the risk of neurodevelopmental disorders associated with early immune activation.

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## NOVELTY STATEMENT

Our study uniquely demonstrates that early exposure to immune challenges during critical neonatal periods of nervous system development leads to long-term behavioral and biochemical changes reminiscent of schizophrenia symptoms. By correlating the observed behavioral modifications with the differential kinetics of OS levels in the brains of male and female subjects, this research provides new insights into the mechanisms underlying psychiatric disorders such as schizophrenia. Our findings enhance the understanding of the pathophysiology of these conditions, highlighting the importance of early immune challenges in shaping long-term mental health outcomes.

## AUTHOR'S CONTRIBUTION

Zineb El-Marzouki and Laila Ibouzine-Dine conducted the experiments, analyzed the data and authored the paper. Mouloud Lamtai and El-Hassan Ouanouche participated in behavioral analysis and statistical significance.

Abdelhalem Mesfioui reviewed and provided comments on the content and interpretation of the manuscript. Aboubaker El-Hessni supervised the work, revised and approved the manuscript.

### Conflict of interest

The authors have declared no conflict of interest.

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