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Prenatal Lipopolysaccharide Reduces Social Behavior in Male Offspring

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Key Words
Lipopolysaccharide · Prenatal infection · Development · Play behavior · Social interaction · Plus maze · Brain morphology

Abstract
Objective: This study investigated the relationship between maternal sickness behavior during pregnancy and offspring development and behavior. Methods: Pregnant Wistar rats were administered with lipopolysaccharide (LPS, 100 μg/kg, i.p.) on gestation day (GD) 9.5. Dams’ sickness behavior was analyzed, and at birth, offspring number and weight were evaluated. Male offspring was evaluated through physical development, play behavior, adult social interaction, plus maze studies and morphological analysis of the brain. Results: Results, with respect to the control group, showed that: (1) LPS decreased general activity, food intake, and weight gain in dams, but no pyrexia was observed following treatment; (2) LPS reduced litter size, but no alterations in physical development were observed; (3) LPS reduced play behavior parameters in baby rats; (4) LPS decreased adult social interaction; (5) no alterations were observed between groups on plus maze studies; (6) no differences were observed between groups on morphological analyses of the brain. Conclusion: These data reveal that LPS administered on GD 9.5 impaired male offspring’s social behavior in infancy and adulthood. These results may be related to an alteration in motivational states or/and increased anxiety.

Introduction
Lipopolysaccharide (LPS), an endotoxin that originates from the cell wall of gram-negative bacteria, activates the immune system to release proinflammatory cytokines (interleukin-1, interleukin-6, tumor necrosis factor-α, and some others) and stimulates monocytes, neutrophils, blood platelets, endothelial cells, and primarily macrophage activities. Several authors reported that LPS also affects central nervous system activity, leading to sickness behavior in many species [1–3]. The innate immune system is responsible for many of the acute sickness symptoms related to systemic inflammation or infection [4, 5]. LPS-induced sickness behavior is generally accompanied by a decrease in exploratory activity, social behavior, ingestive behavior, sexual behavior and induced anhedonia, and poor learning and cognitive functions [6].

Prenatal LPS exposure causes reproductive, behavioral and neurochemical injuries both to the mother and the
pups [7–16]. It has been suggested that the effects of maternal LPS exposure on the developing fetal brain are mediated by cytokine induction within the maternal circulation or placenta [17]. In this respect, several research efforts have attempted to model the long-term consequences of maternal infection during pregnancy on subsequent offspring behavior. These models include exposure to LPS [18, 19], infection with human influenza virus, and exposure to polyriboinosinic-polyribocytidilic acid that mimics viral RNA [20, 21].

Meyer et al. [22] integrate both epidemiological and experimental findings supporting the hypothesis that infection-associated immunological events in early fetal life may have a stronger neurodevelopmental impact compared to late-pregnancy infections. This is because infection in early gestation may not interfere with fundamental neurodevelopmental events such as cell proliferation and differentiation, but it may also predispose the developing nervous system to additional failures in subsequent cell migration, target selection, and synapse maturation, eventually leading to multiple brain and behavioral abnormalities in adult offspring. Several data indicate that early/middle infection (gestation day 9, GD 9) induces the most relevant long-term neuropathological consequences in neural substrates [20, 22, 23]. Thus, GD 9.5 was chosen to study the effects of prenatal infection induced by LPS administration. Our objective was to verify if a prenatal maternal infection (100 μg/kg LPS on GD 9.5) impairs the social behavior of male pups during infancy and adulthood. Moreover, the physical development, stress/anxiety levels and brain morphology were analyzed. Besides that, maternal sickness behavior and reproductive performance were investigated to confirm our LPS dose effects as previously reported in the literature.

Methods

Animals

Pregnant Wistar rats from our own colony, weighing 216–263 g each, were used (GD 0 = spermatozoa in the vaginal smear). Dams were individually housed in polypropylene cages (38 × 32 × 16 cm³) at controlled room temperature (22 ± 2°C), humidity (65–70%), and artificial lighting (12-hour light/12-hour dark cycle, lights on at 6.00 a.m.) with free access to Nuvilab® rodent chow (Nuvital Co., São Paulo, Brazil) and filtered water. Sterilized and residue-free wood shavings were used as animal bedding. The dams were randomly distributed into control and experimental groups. The animals used in this study were maintained in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources of the School of Veterinary Medicine, University of São Paulo, Brazil (protocol No. 925/2006, FMVZ – USP). These guidelines are similar to those of the National Research Council, USA.

Treatment

LPS (from Escherichia coli, Sigma®, serotype 0127: B8) was dissolved in saline (50 μg/ml of LPS in 0.9% NaCl solution) and was administered intraperitoneally (i.p.) to pregnant rats at a dose of 100 μg/kg on GD 9.5. This dose was chosen because it has been shown to induce behavioral and endocrine alterations and to increase cytokines at the placental level [13, 24]. GD 9.5 was selected, since in rats it coincides with the period of cerebral organogenesis, particularly neural-plate formation [25, 26]. The control group consisted of pregnant rats submitted to the same treatment schedule with saline. Each dam was administered 0.1 ml/100 g saline or LPS solution.

Maternal Sickness Behavior

Experiments were carried out in accordance with the GLP protocols and quality assurance methods. Maternal sickness behavior was analyzed in pregnant female rats treated with both LPS and control solution on GD 9.5 (n = 8 for both groups). One hour after LPS or 0.9% NaCl treatment, dams’ general activity was observed in an open field. The device used consisted of a round wooden arena with acrylic washable covering (90 cm in diameter, walls 28 cm in height) painted gray and subdivided into 25 parts [27]. For the observations, each dam was individually placed in the center of the apparatus and the following parameters were measured over a period of 5 min: locomotion frequency (number of floor units entered with both feet), rearing frequency (number of times the rodents stood on their hind legs), and self-grooming time (seconds of licking or biting of the fur, limbs, or genital region). Hand-operated counters and stopwatches were employed to count these behaviors. To minimize the influence of possible circadian changes on the open-field behavior, the rodents were observed at the same time of the day (2.00–4.00 p.m.) during each session. Also, the device was washed with a 5% alcohol/water solution before placing the animals, to obviate possible biasing effects owing to odor clues left by earlier rats.

Body temperature of experimental dams was measured after 1, 24 and 48 h of LPS or control solution treatments using an infrared thermometer (Techline® TS-201) placed on their ear. This method was used to avoid interference with pregnancy since other methods such as biotelemetry (including surgery) or rectal temperature (cervix stimulus) measurements are stressful management.

The animals’ food consumption was also evaluated 24, 48 and 72 h after the treatment.

Reproductive Parameters and Offspring Studies

Pregnant rats were assigned to control and LPS-treated groups to study maternal reproductive parameters. These rats (n = 10 for both groups) were allowed to give birth and nurture their offspring normally. No cross-fostering procedure was used. The day of birth was considered as postnatal day (PND) 1. Parturition day was observed to analyze premature deliveries.

Pregnant rats were weighed on GD 9.5 and PND 2 to estimate their weight gain. No handling was done on PND 1, while on PND 2, the number of rat pups and the total offspring weight were measured. Immediately after weighing, 8 offspring (4 males and 4 females) were randomly selected (by anogenital difference, greater
in males). Litters smaller than 8 pups were culled. One male offspring from each litter (n = 10 for both groups) was subcutaneously marked with China ink in the right hind paw on PND 2. These animals were used to study physical development. The second and third offspring of control and experimental litters were employed for play behavior studies, whereas the fourth and the last male offspring of these litters were used for morphological analyses of the brain. Different pregnant rats treated or not with LPS were obtained and their offspring were assigned for adult social interaction and plus maze studies (n = 7 for both groups). Female rat pups were kept apart to be used in another experiment. These procedures are summarized in figure 1.

To assess physical development, the following parameters were observed on the rat pups prenatally treated with LPS or the control solution: pinna detachment [the first day that the point of a pinna (earflap) was detached from a rat pup’s head, beginning on PND 3], coat appearance [rat pups were examined for the appearance of hair (bristles) on the dorsal surface, beginning on PND 5], eruption of teeth (rat pups were examined for the appearance of either upper or lower incisor eruption, beginning on PND 8), opening of ears (determined by visualization of the open auditory meatus, beginning on PND 11), opening of eyes (determined by the visualization of a longitudinal eyelid fissure, beginning on PND 14) and adult gait (when the rat pups walk without propelling their ventral portion on the floor, beginning on PND 13). The animals were weighed individually on PND 2, PND 21, and PND 60. The baby rats were observed daily between 8.00 and 10.00 a.m.

**Behavioral Tests**

On PND 21, 40 different male rat pups of the control and LPS-treated groups were assigned to one of the following two conditions for play behavior analysis: individual housing (isolated-control rat pups: n = 10; isolated-treated rat pups: n = 10) and group housing, comprising 2 rat pups per cage (control-grouped rat pups: n = 10; treated-grouped rat pups: n = 10). The rationale for socially depriving some of the experimental rats is to increase the motivation to initiate play behavior [28]. The animals were left undisturbed in polypropylene cages (38 × 32 × 16 cm³) until PND 30. The play behavior was observed both on PND 30 and 31, respectively, as this period was found to be the peak time for its occurrence [29].

On PND 30 (9.00–11.00 a.m.), 1 experimental or control-isolated rat pup was confronted with 1 control or treated-grouped rat pup, respectively (the difference in weight was less than 10 g). Thus, prenatal LPS-treated rat pups either kept in groups or isolated were always observed for play behavior with the nontreated rat pups (isolated or grouped) to avoid treatment interferences (e.g., decrease in social stimulus by one partner may affect the behavior of the other). Observations (filmed procedure) were made for 10 min in the cage of the isolated pup (between 8.00 and 10.00 a.m.). A period of 5 min was allowed for the animals to adapt to the test room before matching. After the trial, rats were returned to their previous housing conditions (isolated or grouped); on the subsequent day (PND 31), the same matches and studies were made for an additional 10 min. The rationale for 2-day analysis is based on the observation that the subject intermittently continues to solicit play until the lack of social reinforcement results in the extinction of play solicitation behavior. This extinction phenomenon may have played a role in our treated-untreated mixed pairs, since the pattern of responding to play solicitations changed over the 2-day testing period [29].

On each observation day, the following parameters were measured: pinning frequency (the frequency of play behavior, i.e., the number of times an animal lied on its back with its partner standing over it; this behavior was counted for both animals, since both are actively involved in it), sniffing the partner (time, s), frequency of crawls over/under the other, frequency of partner mounting, following the partner (time, s), and locomotion (time, s) [29, 30]. All play behavioral parameters, except pinning, were observed individually.

An ethogram of the play behavior test administered to male rat pups included these parameters. It also included: hiding under wooden shavings, body shaking (to remove wood shaving), rearing, rearing plus sniffing at walls/roof, biting the partner, running without interactions, pinning plus sniffing the partner, running at the partner, pinning attempts (pinning did not occur), sniffing at wood shaving, self-grooming, scratching and spinning around each other (at least 360°).

On PND 54, 28 different male pups of the control and LPS-treated groups were assigned to one of the following two conditions for adult social interaction analysis: individual housing (isolated-control rat pups: n = 7; isolated-treated rat pups: n = 7) and group housing, comprising 2 rat pups per cage (control-grouped rat pups: n = 7; treated-grouped rat pups: n = 7). As observed, some rat pups were isolated to increase their social interaction [31]. The animals were left undisturbed in polypropylene cages (38 × 32 × 16 cm³) until PND 60.

On PND 60, 1 experimental or control-isolated rat pup was confronted with 1 control or experimental-grouped rat pup, re-
were analyzed on an optical microscope.

Histological sections were made (5 μm) and stained using hematoxylin eosin. Slides were analyzed on an optical microscope.

The rationale for socially depriving part of the experimental rats is the same as that for the play behavior test. Thus, prenatal LPS-treated rat pups kept in groups or isolated were always observed for social interaction with the nontreated rat pups (isolated or grouped) to avoid treatment Interferences. Observations (filmed procedure) were made for 5 min in a new polypropylene cage (between 8.00 and 10.00 a.m.). A period of 5 min was allowed for the animals to adapt to the test room before the matching. The following parameters were measured: sniffing at the partner (time, s), frequency of crawls over/under the other, frequency of partner mounting, following the partner (time, s), and locomotion (time, s) [29, 30]. All parameters were observed individually.

The plus maze behaviors of offspring were measured on PND 90–95. This test was applied to 14 male rat pups divided into two equal groups (one experimental group and one control group). The device consisted of two opposite open arms (50 cm long × 10 cm wide) and two opposite closed arms (50 cm long × 10 cm wide × 40 cm high) arranged at 90° angles. The floor of the maze was made of wood, painted gray (with acrylic washable covering) and located 50 cm above the floor. The center of the maze was open and the walls of the closed arms started 2 cm from the center of the maze. Each rat was observed using a video camera mounted above the arena to record the behavioral data. Data were collected and analyzed by an Ethovision System software (Noldus Information Technology, Leesburg, Va., USA) installed on an IBM-compatible computer placed in an adjacent room.

For the observations, each animal was individually placed in the center of the maze with the head facing one of the open arms, and the following parameters were measured over a period of 5 min: number of entries into the open arms, number of entries into the closed arms, time spent in the open arms, time spent in the closed arms. The measures that reflect stress/anxiety levels in this test are the percentage of entries into open arms versus closed arms and the percentage of time spent in the open arms versus closed arms [32]. The following formulas were employed for calculation: % open arm entries = [open-arm entries/open-arm entries + closed-arm entries] × 100; % time in the open arms = [time in the open arms/time in the open arms + time in the closed arms] × 100.

To minimize the influence of possible circadian changes on plus maze behaviors, control and experimental animals were alternated. The device was washed with a 5% alcohol/water solution before placing the animals on it to obviate possible biasing effects due to odor clues left by previous rats. Observations were made between 2.00 and 5.00 p.m.

**Morphological Analyses of the Brain**

On PND 60 (between 2.00 and 4.00 p.m.), 5 male pups from each group, obtained from different litters, were deeply anesthetized with xylazine and ketamine and immediately perfused intracardially with saline solution containing EDTA 0.01 M for 10 min, followed by paraformaldehyde 10% for another 10 min. The brains were removed and postfixed in paraformaldehyde for 24–48 h. Sagittal-medial slides were done and submitted to dehydration, diaphanization and paraffin inclusion. Histological sections were made (5 μm) and stained using hematoxylin eosin. Slides were analyzed on an optical microscope.

### Statistical Analysis

Results were expressed as mean ± SEM. One male rat pup from each dam (used as unit) was considered in the offspring studies to avoid litter effects. Homoscedasticity was verified through the F test. Normality was verified through Kolmogorov-Smirnov test. Thus, the Student’s t test (unpaired, two-tailed) was used to compare group data that were parametric. For nonparametric data, the Mann-Whitney U test was employed. On the play behavior test, three-way ANOVA was used to analyze the data of control and experimental groups, with housing conditions and observation days as the factors. In this case, ANOVA, followed by the Holm-Sidak post-hoc test, was also used to compare the homoscedastic data presenting interaction. In all cases, results were considered significant if p < 0.05.

### Results

**Maternal Sickness Behavior**

Figure 2 shows that in comparison with the control group, LPS-treated pregnant rats presented decreased locomotion (p = 0.0035), rearing (p = 0.0077), and self-grooming (p = 0.0379) activities in an open field. As depicted in figure 3A, compared with the data of the control group, LPS treatment was not capable of modifying pregnant rats’ body temperature, both 1 h (p = 0.9999) and 24 h (p = 0.3741) after the treatment; however, 48 h after LPS treatment, the dams showed increased body temperature with respect to that measured in the control animals (p = 0.0158). Figure 3B shows that food intake was reduced in the experimental group at approximately 24 h (p < 0.0001), 48 h (p = 0.0003), and 72 h (p = 0.0116) after LPS treatment with respect to the data of the control group.

**Reproductive Parameters and Offspring Studies**

Table 1 shows reproductive parameters of rats exposed to LPS (100 μg/kg) treatment and control rats on GD 9.5. Thus, prenatal exposure to LPS reduced both maternal weight (p = 0.0116) and offspring number (p = 0.0004). However, this treatment did not modify the parturition day (p = 0.3553), the total body weight of rat pups on PND 2 (p = 0.1530), and individual body weight of the offspring on PND 2 (p = 0.6184), PND 21 (p = 0.8922), and PND 60 (p = 0.4159).

No differences were observed between the control and the experimental male offspring data on physical evaluation (data not shown).

**Behavioral Tests**

Figure 4A–F illustrates the data of play behavior of animals exposed to LPS (100 μg/kg) and control animals
on GD 9.5. In comparison with the control group, pinning (Fig. 4A) was reduced in the experimental group [F (36/39) = 9.54, p = 0.004]. However, no differences were detected between the two observation days [F (36/39) = 0.06, p = 0.814], and between the treatments and the observation days [F (36/39) = 0.09, p = 0.762]. The multiple comparisons test showed that the animals in the experimental group had lower values in pinning than those in the control group (p = 0.004).

Three-way ANOVA showed differences in the sniffing parameter (Fig. 4B) between treatments [F (72/79) = 36.44, p < 0.001], housing conditions [F (72/79) = 1,080.10, p < 0.001], both treatments and housing conditions [F (72/79) = 11.88, p < 0.001], and housing conditions and observation days [F (72/79) = 7.44, p = 0.008]. However, no differences were detected between the two observa-
tion days [F (72/79) = 1.00, p = 0.320], the treatments and the observation days [F (72/79) = 0.05, p = 0.820], and the three factors: treatments, conditioning, and observation days [F (72/79) = 0.61, p = 0.439]. The multiple comparisons test showed that the rats of the experimental group presented lower values of sniffing than those in the control group (p < 0.001), and this effect was present in isolated animals (p < 0.001) but not in grouped animals (p = 0.071). Furthermore, it was also observed that the isolated animals presented larger values on the conditioning parameter than the grouped ones, both on days 30 (p < 0.001) and 31 (p < 0.001).

Three-way ANOVA showed differences in the crawls over/under parameter (fig. 4C) between treatments [F (72/79) = 10.91, p = 0.001], housing conditions [F (72/79) = 161.34, p < 0.001], and treatments and housing conditions [F (72/79) = 9.19, p = 0.003]. No differences were found between the observation days [F (72/79) = 0.61, p = 0.436], treatments and observation days [F (72/79) = 0.26, p = 0.611], housing conditions and observation days [F (72/79) = 0.61, p = 0.436], and the three factors together: treatments, house conditioning, and observation days [F (72/79) = 0.001, p = 0.973]. The multiple comparisons test showed that rats prenatally treated with LPS presented lower values in crawls over/under parameter than the control group (p = 0.848). With respect to the housing conditions factor, it was also observed that the isolated animals presented larger values of the crawls over/under parameter than the grouped ones, both on days 30 (p < 0.001) and 31 (p < 0.001).

As shown in figure 4D, some differences in the mounting parameter were detected between treatments [F (72/79) = 5.26, p = 0.025], housing conditions [F (72/79) = 48.14, p < 0.001], and both treatments and housing conditions [F (72/79) = 5.52, p = 0.021] (three-way ANOVA). However, no differences were detected between observation days [F (72/79) = 0.67, p = 0.414], treatments and observation days [F (72/79) = 0.58, p = 0.447], conditioning and observation days [F (72/79) = 0.98, p = 0.325], and the three factors together: treatments, housing conditions, and observation days [F (72/79) = 0.67, p = 0.414]. Application of the multiple comparisons test showed that the rats prenatally treated with LPS presented lower values in mounting than control rats (p = 0.025), and this effect was observed in isolated (p = 0.002) but not grouped animals (p = 0.968). The same observation was made with respect to the housing conditions factor: isolated animals presented larger values of mounting than grouped ones, both on days 30 (p < 0.001) and 31 (p < 0.001).

| Table 1. Effects of LPS (100 µg/kg on GD 9.5) on parturition day, weight gain of dams, number and total weight of the offspring, and individual offspring body weights (n = 10 for both groups) |
|-----------------|-----------------|-----------------|
|                  | Groups          | LPS-treated     |
| Parturition day  | 22.20 ± 0.13    | 22.40 ± 0.16    |
| Maternal weight during pregnancy, g | 16.63 ± 2.92 | 6.62 ± 2.05* |
| Pups, n          | 10.80 ± 0.29    | 7.60 ± 0.68*** |
| Total weight of the offspring, g | 70.64 ± 4.92 | 59.00 ± 6.06 |
| Offspring individual body weight, g |              |                  |
| PND2             | 7.61 ± 0.26     | 7.80 ± 0.27     |
| PND21            | 44.22 ± 0.97    | 44.47 ± 1.54    |
| PND60            | 276.10 ± 6.41   | 267.84 ± 7.57   |

Values are represented as means ± SEM. * p < 0.05, *** p < 0.001, compared with the control group (Student’s t test).

Finally, three-way ANOVA showed differences in the locomotion parameter (fig. 4F) between housing conditions [F (72/79) = 678.91, p < 0.001]. However, no differences were observed between treatments [F (72/79) = 0.41, p = 0.524], observation days [F (72/79) = 3.69, p = 0.059], treatments and housing conditions [F (72/79) = 0.026, p = 0.872], treatments and observation days [F (72/79) = 0.15, p = 0.701], housing conditions and observation days [F (72/79) = 0.19, p = 0.660], and the three factors together: treatments, house conditioning, and observation days [F (72/79) = 0.01, p = 0.915]. The multiple comparisons test showed that the housing condition factor of the isolated animals was higher than that of the grouped ones (p < 0.001).
The other parameters mentioned on the ethogram of the play behavior test did not show any differences between treatments (data not shown).

As shown in figure 5, adult social interaction was reduced in prenatal LPS-treated isolated rats in the following parameters: crawls over/under (p = 0.0264) and mounting (p = 0.0471) with respect to the data of the control group, but was not significantly different on sniffing (p = 0.1714), following (p = 0.4174) and locomotion (p = 0.3889). Among the grouped animals, no dif-

**Fig. 4.** Effects of prenatal LPS exposure (100 μg/kg on GD 9.5) on the play behavior of rats. Clear bars = Control group; grey bars = experimental group. Values are represented as mean ± SEM. Litters: n = 10 for both groups. Means followed by different letters are statistically different (three-way ANOVA, p < 0.05).
ferences were observed between LPS-treated and control animals in all parameters that were analyzed (data not shown).

As shown in figure 5, no differences were observed between the control and the experimental male offspring data for plus maze studies: percent open arm entries \( (p = 0.9256) \), percent time in the open arms \( (p = 0.3541) \).

**Morphological Analyses of the Brain**

Morphological analyses of the brain (optical microscope) did not show differences between LPS-treated animals and those of the control group in any specific part of the brain (data not shown).

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**Fig. 5.** Effects of prenatal LPS exposure \( (100 \, \mu g/kg \text{ on GD 9.5}) \) on social interaction and plus maze studies of male rat pups on PND 60. Clear bars = Control group; grey bars = experimental group. Sn = Sniffing the partner; Fo = following the partner; Lo = locomotion; Cr = crawling over/under the partner; Mo = mounting the partner. \( * \, p < 0.05 \) (Student’s t test) compared with the control group. Values are represented as means ± SEM. Litters: \( n = 7 \) for both groups.

**Discussion**

For more than 23 years, it has been documented that infections and inflammation cause sickness behavior. LPS is a potent sickness behavior inductor [33–35]. The present data partially agree with the sickness behavior literature. We demonstrated that LPS-treated female rats exhibit a decrease in general activity and in the amount of food ingested. However, our treated female rats showed no fever 1 and 24 h after the treatment. In this regard, a significant difference in body temperature between the animals of both groups was observed 48 h after treatment. Even so, the normal body temperature of the rats varied from 35.9 to 37.5°C [36], and hence, our results
obtained at 48 h do not represent fever. In fact, it has been documented that rats have an attenuated febrile response to LPS administered during pregnancy [37].

Offspring viability was also impaired by LPS. LPS was reported to induce spontaneous abortions [38] and intrauterine fetal death or embryonic resorption [14, 39]. Thus, our observation of a reduced number of stillborn offspring might be due to fetal death or fetus resorption as a consequence of prenatal exposure to LPS.

With respect to offspring, no differences were found among rat pups’ birth weight. Shi et al. [20] also showed that prenatal maternal infections on GD 9.5 do not alter pups’ body weight in mice. However, Collins et al. [7] observed biphasic effects after LPS administration on GD 8 in pregnant hamsters: an increased body weight in litters prenatally treated with LPS (3 μg/kg) and a decreased body weight when the dose was around 100 μg/kg were observed. The lack of LPS effects on the offspring body weight might be related to the strain differences in LPS sensitivity or, most probably, owing to the small number of fetuses to be nourished as a consequence of embryonic resorption. However, possible maternal compensatory mechanisms cannot be ignored.

Despite LPS-induced maternal injuries, rat pups’ physical development was not modified by prenatal exposure to LPS on GD 9.5. Thus, physical impairments were not responsible for the prenatal LPS effects on subsequent behavioral tests shown here.

The prenatal administration of LPS on GD 9.5 decreased the offspring’s play behavior on PND 30. In fact, not only pinning, but also soliciting behaviors (mounting, sniffing, and crawls over/under) were decreased due to LPS administration. No interference with motor/exploratory parameters (such as locomotion) was detected. In addition, abnormal play solicitations were only observed among isolated rat pups prenatally treated with LPS on GD 9.5.

Only male rat pups were evaluated in this study because male rats (and males of many other mammals) spend more time on social interaction processes, particularly on play behavior, as compared with females [40, 41].

Social play, one of the earliest forms of non-mother-directed social behavior observed in mammals, has been shown to contain a behavioral pattern that correlates with the social, sexual, and aggressive behaviors displayed by the mammal later in life in an exaggerated or out-of-context fashion [42]. Play solicitation or play signaling is a set of behaviors inviting social play or signaling readiness to engage in social play. Solicitation behaviors have been suggested to reflect an appetitive phase of social play (play motivation). When observed in pairs, one rat is usually isolated prior to testing, as it is already known that isolated rats initiate more play than group-housed ones [29]. Play solicitation in isolated rats also occurs irrespective of the grouped rats’ willingness or their capacity to become involved in play [42]. Accordingly, we found that the control and experimental isolated rat pups exhibited significantly more play solicitation than the grouped ones. However, exposure to LPS on GD 9.5 reduced play solicitations only in isolated animals. The lack of differences in socially grouped pairs reveals that social isolation was necessary to show injuries caused by prenatal LPS treatment. Thus, social isolation might not be a challenge in socially grouped rats.

In our design, we crossed treated animals with non-treated ones to separately estimate the effects of treatment infection on the behavior of isolated and grouped rats [29] and to prevent that the partner’s reduction in socialization could impair the interaction per se. Thus, LPS-treated rats’ unwillingness to engage in play was not due to the lack of play solicitation behavior by their partners. Finally, pinning behavior did not change over the 2-day testing period, suggesting that preexposure to the partner was incapable of modifying this behavior. However, crawling over/under, mounting and following related to soliciting behaviors were attenuated.

Abnormal play behavior might indicate a decrease in the pup’s motivation, induced by prenatal LPS exposure. In this regard, play behavior increases after an isolation period [43, 44], and a satiation process was observed when this behavior was performed [45].

Interestingly, isolated pups initiated the pinning behavior. Such an event is attributed to the higher motivation in isolated rats to play and initiate socialization [43–45].

In addition, some parameters such as pinning and following measured with the play behavior test could be interpreted as agonistic behaviors or play-fighting [46]. Play-fighting is involved in hierarchy establishment patterns and sexual behavior [47, 48]. This way, even if prenatal LPS treatment did not change some agonistic behaviors, such as biting and pouncing, the treatment impaired pinning and following and might prejudice the social hierarchy of these animals.

Similar to the play behavior test, male isolated offspring exposed to prenatal LPS (100 μg/kg on GD 9.5) presented a decrease in adult social interaction when compared with the control group. This experiment was
carried out to evaluate whether abnormal social interactions during infancy are maintained or reverted in adulthood. Thus, the design was essentially the same as that applied on the play behavior test.

Our result that a single LPS prenatal exposure on GD 9.5 impairs rat pups’ play behavior and adult social interaction suggests that maternal inflammation has long-term effects on rat pups.

We believe that all behavioral impairment detected in rat offspring in this study results from LPS treatment, released cytokines and induced maternal sickness behavior.

Maternal immune stimulation affects brain development and causes an altered HHA axis upon reaching adulthood [49]. Moreover, social interaction is a suitable model to evaluate anxiety [50, 51]. Thus, the decreased social interaction observed here might be a consequence of an anxiety stress induced by prenatal LPS treatment. However, social anxiety and anxiety caused by a novel environment, as measured in the plus maze, may have a neurobiologically different basis. Hence, our observations did not disconfirm this hypothesis even though the behavioral data of the plus maze test were negative for anxiety.

In addition, prenatal LPS exposure could impair maternal care and be responsible, at least in part, for the deleterious effects on the pup’s behavior. We do not believe that LPS treatment interfered with maternal care, since immunological and behavioral parameters return to normal in most of the rats 72 h after LPS i.p. treatment (100 µg/kg) [52]. So, dams are recovered on delivery. Moreover, an experiment recently performed in our laboratory did not show impairment in maternal behavior of dams that received this treatment (data not published).

Since play behavior is commonly used to study the relationship between injuries produced by infection during pregnancy and in autism [20, 29, 53, 54], we speculate that maternal sickness behavior induced by LPS administered on GD 9.5 might be used as an animal model for this mental illness. To reinforce this hypothesis, many studies associate prenatal maternal infection and cytokine release with autism [55–58].

However, the brain morphology of male rat pups exposed to prenatal LPS was not modified when compared with the control group, particularly that of the cerebellum – one of the most affected brain areas in autism. These findings do not rule out morphological alterations that are analyzed with other specific methodologies.

As previously reported, LPS induces sickness behavior in animals, including the decrease in social behavior [6]. This decrease is considered a behavioral strategy to prevent spread of infectious disease (in case of contagious infection). It is also a way to reduce energy expenditure in order to prioritize disease recovery [33].

Considering this, the impairment of social interaction in pups prenatally treated with LPS found in this study may have evolutionary implications. Eventually, these pups might be more prepared to cope with an environment with higher incidence of contagious pathogens [59]. To better understand this issue, genetic and epigenetic studies are planned to be performed.

In conclusion, prenatal LPS administration (100 µg/kg on GD 9.5) impairs the male offspring’s social behavior in infancy and in adulthood through decreased motivational state and/or increased anxiety. While these data may be useful as an animal model for autism, stereotyped behavior and communication impairments must be studied further to better diagnose this neuropsychiatric disorder. This study confirms that behavioral impairment may be attributed to prenatal maternal infection.

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