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Single early prenatal lipopolysaccharide exposure impairs striatal monoamines and maternal care in female rats

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ABSTRACT

Aims: Environmental information received by a mother can induce a phenotype change in her offspring, commonly known as a maternal effect (trans-generational effect). The present work verified the effects of lipopolysaccharide (LPS), which mimics bacterial infection, on maternal care and on the activity of related brain areas in F1 offspring, i.e., female rats that were prenatally exposed to LPS.
Main methods: Pregnant rats received 100 μg/kg of LPS intraperitoneally on gestational day (GD) 9.5. Female offspring of the F1 generation were mated to naïve males and were evaluated during their lactation period for open field, maternal and aggressive behaviors. Striatal and hypothalamic dopamine and serotonin levels and turnover were also evaluated. Furthermore, astrocyte protein expression in the nucleus accumbens (NA) was analyzed in F1 females to assess LPS-induced neuroinflammation.
Key findings: Prenatal LPS did not change open field behavior but impaired both maternal and maternal aggressive behaviors in the F1 generation. LPS exposure also reduced both striatal levels of dopamine and serotonin and its metabolites, but induced no changes in NA astrocyte expression.
Significance: We suggested that the observed impairments in the F1 females were a consequence of a motivational change induced by prenatal LPS, as (1) no changes in motor activity were observed, (2) prenatal LPS-exposure was reported by our group to induce motivational impairments in males, and (3) the existence of a strong connection between striatal dopaminergic activity and motivation-oriented activities. The present findings strongly indicate a maternal effect for prenatal LPS, at least for the F1 generation.

Introduction

Lipopolysaccharide (LPS), an endotoxin that originates from the cell wall of gram-negative bacteria, which mimics bacterial infection, activates the immune system to release proinflammatory cytokines (Avitsur et al., 1997; Saluk-Juszczak and Wachowicz, 2005). Viral and bacterial infections, including those caused by prenatal LPS exposure, induce short and long-term changes in behavior and central nervous system activity (Boksa, 2010; Golan et al., 2005; Meyer et al., 2009). Previous investigations by our group have shown that prenatal LPS (100 μg/kg, given intraperitoneally on gestational day [GD] 9.5) reduces the social behavior of F1 males both in infancy and in adulthood and decreases their striatal dopamine (DA) and DA metabolite levels in the absence of signs indicative of neuroinflammation (Kirsten et al., 2010a, 2010b, 2011). Interestingly, our model also showed that parental maternal behavior was slightly improved in pregnant rats treated with LPS on GD 9.5 (Kirsten et al., 2011), whereas treatment on GD 21 decreased this behavior (Bernardi et al., 2010).

It has been suggested that the effects of maternal LPS exposure on the developing fetal brain are not directly mediated by LPS, but are instead indirectly induced via increases in proinflammatory cytokines and glucocorticoid levels within the maternal circulation, placenta and fetal brain (Ashdown et al., 2006; Cai et al., 2000; Gayle et al., 2004; Urakubo et al., 2001). Infections associated with immunological events in the early/middle fetal stages (e.g., GD 8–10 in rats and mice) might have a stronger impact on neurodevelopment than late-stage pregnancy infections. Immune activation during the early/middle stages of pregnancy was shown to modify fetal cell proliferation and differentiation, cell migration, target selection, and synapse maturation (Ghiani et al., 2011; Meyer et al., 2006, 2007; Samuelsson et al., 2006; Shi et al., 2003). Multiple brain injuries and behavioral abnormalities persisting through adulthood, were also reported after early/middle stage pregnancy infections (Meyer et al., 2007).

Environmental information received by a mother can induce a phenotypic change in her offspring, commonly known as a maternal or trans-generational effect (Agrawal et al., 1999; Curno et al., 2009).

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Certain cues in the maternal environment, e.g., the prevalence of predators, or maternal infection can lead to behavioral, morphological and immunological changes in the following generation (Agrawal et al., 1999; Grindstaff et al., 2006).

The present experiment was designed to analyze possible LPS-induced effects (transgenerational effect) on the maternal care of the F1 generation, i.e., in adult female rats prenatally exposed to LPS (100 μg/kg LPS on GD 9.5). We determined the behavioral, neurochemical and neuroinflammatory outcomes related to maternal care in the F1 generation. Specifically, the following parameters were analyzed: maternal, aggressive and open field behaviors, striatal and hypothalamic DA and serotonin (5-HT) levels, levels of DA and 5-HT metabolites and turnover, and astrocyte expression in the nucleus accumbens (NA). Astrocytes that increase in number and become activated following an infection or an immunological challenge, such as an acute administration of LPS were considered indicative of neuroinflammation (Pang et al., 2010).

Material and methods

Animals

Forty-eight pregnant Wistar rats (parental generation) between 12 and 13 weeks of age and weighing 216–263 g were used to generate the F1 offspring (GD 0 was defined as the day when spermatozoa were detected in a vaginal smear). Pregnant dams were individually housed in polypropylene cages (38 × 32 × 16 cm) at a controlled temperature (22 ± 2 °C) and humidity (65–70%) with artificial lighting (12-hour light/12-hour dark cycle, lights on at 6:00 AM). The animals had free access to Nuvilab® rodent chow (Nuvital Co., Sao Paulo, SP, Brazil) and filtered water. Sterilized, residue-free wood shavings were used as bedding. The animals were divided into control (saline-treated) and experimental (LPS-treated) groups (n = 24 dams/group). The dams were allowed to give birth and nurture their offspring with no interference. The day of birth was recorded as postnatal day (PND) 1. No handling was performed on PND 1, but on PND 2, 8 pups (4 males and 4 females) from each litter were randomly selected. No cross-fostering procedures were used. Litters with fewer than 8 pups were culled. The 8 randomly selected pups remained with each dam until weaning (PND 21). On PND 21, littermates were separated and housed by sex under the same conditions as their parents. One adult female from each litter (F1 generation) was used for each experiments, using different animals in each experiment. Male pups were kept apart for use elsewhere (Kirsten et al., 2010a, 2010b).

As depicted in Fig. 1, on PND 90, adult female rats of the F1 generation were mated with experienced males from our colony. These female rats were not used in any other study. The gestational and neonatal procedures were the same as those described above for their parents. On lactation days (LD) 5 and 6 of the F1 generation, the following experiments were performed: open field behavior, maternal and aggressive behavior, neurochemistry and immunohistochemistry. The animals used in these experiments were kept in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources of Paulista University, Brazil (protocol No. 014/09, CEUA-UNIP). These guidelines are similar to those of the National Institutes of Health, Bethesda, MD. Experiments were carried out in accordance with good laboratory practice protocols and with quality assurance methods.

Treatment

LPS (from *Escherichia coli*, Sigma®, Saint Louis, MO, USA, serotype 0127: B8) was dissolved in sterile saline (50 μg/ml LPS in a 0.9% NaCl solution) and administered intraperitoneally to pregnant dams (parental generation) at a dose of 100 μg/kg on GD 9.5. This dose was chosen because it has been shown to (1) elicit sickness behavior, (2) induce endocrine alterations in dams, (3) increase cytokines at the placental level, (4) impair the offspring birth rate and (5) reduce the social behavior of male offspring during infancy and adulthood (Kirsten et al., 2010b; Spencer et al., 2007; Wang et al., 2006). The control group consisted of pregnant rats that received only sterile saline (0.9% NaCl) on the same treatment schedule as the LPS animals. Each control dam was treated with 0.1 ml/100 g saline solution.

General activity in the open field

The general activity test was performed on LD 5 of the F1 generation, i.e., in adult female rats prenatally exposed to LPS (n = 10 dams/group). The open field device consisted of a round wooden arena (90 cm in diameter with 28 cm high walls) that was painted gray and had a washable, acrylic covering (Broadhurst, 1960). For the observations, each rat was individually placed in the center of the apparatus and the following parameters were automatically measured using Ethovision software (Ethovision; Noldus Information Technology, Leesburg, VA) over a period of 5 min: total locomotor activity (traveled distance [cm]), time spent in locomotor activity (time movement [s]), mean velocity (cm/s), and rearing and grooming time (s) and frequencies. A video camera mounted 100 cm above the arena was used to collect the data that were analyzed by the Ethovision System® software which was installed on an IBM-compatible computer placed in an adjacent room. The arena was washed with a 5% alcohol/water solution before placement of the animals to obviate possible biasing effects from odor clues left by the previously tested rats. Control and experimental (F1) rats were intermixed for observations.

Maternal behavior

Maternal behavior testing was performed on LD 6 of the F1 generation, i.e., in adult female rats prenatally exposed to LPS (n = 10 dams/group). Pups were removed from their dams and placed in a different

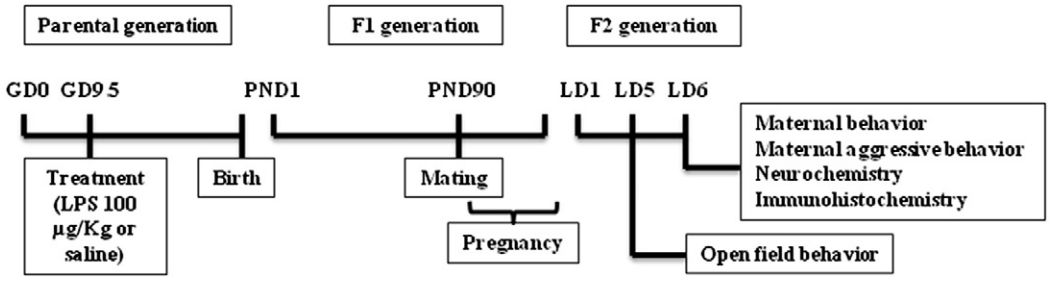

Fig. 1. Experimental design diagram. Gestational day: GD (parental generation). Postnatal day: PND (F1 generation). Lactation day: LD (F2 generation). All the behavioral and neurological tests were performed on the F1 generation.

cage, away from their mother. Immediately following separation, the presence of a nest in the home cage was evaluated. Sixty minutes following maternal separation, all pups were returned to the home cage, and maternal behavior testing began. The retrieval of the first pup (time, s and %), the retrieval of all the pups (s and %), grouping (%), full maternal behavior (s), crouching (%), self grooming (s) and pup maternal grooming (s) were recorded (Bernardi et al., 2010). Dams were scored as displaying full maternal behavior if they retrieved all of the pups to the nest and if they displayed nursing behavior (back arched over the pups) for 3 consecutive min. If animals did not display full maternal behavior following 30 min of continuous observation, they were observed every 15 min for 60 min and then hourly thereafter until full maternal behavior was observed. Events observed following the first 30 min of continuous observation were recorded at the time of the first observation (e.g., if full maternal behavior was first observed at 60 min, the full maternal behavior latency was scored as 60 min). The same criterion was used for all maternal responses.

Maternal aggressive behavior

Maternal aggressive behavior testing was performed on LD 6 of the F1 generation, i.e., in adult female rats prenatally exposed to LPS (n = 12 dams/group). These rats were subjected to a 10 min maternal defense test (Svare et al., 1981). A male Wistar rat, the intruder, was introduced into the home cages of each dam and offspring. Intruder rats were only used once. Behaviors against the intruder were recorded via a video camera mounted 100 cm above the arena and were later analyzed for offensive behaviors exhibited by the dam. Offensive behaviors analyzed included latency (s) to first attack, attack frequency, total time (s) of attacks, boxing frequency, and time (s) of boxing. Furthermore, maternal behavior in the presence of the intruder was analyzed. Maternal behaviors analyzed included frequencies of carrying and hiding pups, percentage of pups that remained in the nest at the end of the test, and frequency of the intruder sniffing the pups.

Monoamine levels and turnover in the striatum and hypothalamus

Monoamine and monoamine metabolite levels in the striatum and hypothalamus were measured by high-pressure liquid chromatography (HPLC) on LD 6 of the F1 generation, i.e., in adult female rats prenatally exposed to LPS (n = 10 dams/group). The rats were decapitated and their brains were rapidly dissected on dry ice and prepared as described previously (Felicio et al., 1996). Briefly, both the striatum and hypothalamus were dissected, weighed, stored at −80 °C and used for further analyses. Following sample collection, tissue was placed in a perchloric acid solution and homogenized by sonication for the immediate determination of neurotransmitter and metabolite levels. DA and its metabolites (3,4-dihydroxyphenylacetic acid [DOPAC], and homovanillic acid [HVA]), and 5-HT and its metabolite (5-hydroxyindolacetic acid [5HIAA]) were measured by HPLC (Shimadzu, model 6A, Kyoto, Japan) using a C-18 column (Shimpak, ODS, Kyoto, Japan), an electrochemical detector (model 6A, Shimadzu, Kyoto, Japan), a sample injector (15 and 20 Al valve), and an integrator (Chromatopac, Shimatzu, Kyoto, Japan). DA and 5-HT turnovers (metabolite/neurotransmitter ratio) were also evaluated. Each sample was run for 18 min. The detection limit for DA, DOPAC, HVA, 5-HT, and 5HIAA was 2 pg. The coefficients of variation were less than 15%, and the curve linearities were greater than 0.9. As described by Causon (1997), the absolute recuperation was measured by comparing the responses of extracted samples at medium spiked matrix concentrations (30.6 ng/mL, 33.6 ng/mL, 36.4 ng/mL, 35.2 ng/mL, and 38.2 ng/mL for DA, DOPAC, HVA, 5-HT, and 5HIAA, respectively) in six replicates with those of non-extracted standards (response of a pure standard) which represent 100% recovery. The extraction yields were 90%, 87%, 86%, 85%, and 90% for DA, DOPAC, HVA, 5-HT, and 5HIAA, respectively.

Astrocyte immunohistochemistry in the nucleus accumbens

The expression of the astrocyte marker protein GFAP was qualitatively analyzed using immunohistochemistry in the nucleus accumbens on LD 6 of the F1 generation, i.e., in adult female rats prenatally exposed to LPS (n = 7 dams/group). These rats were euthanized and their brains were removed and fixed for 3 days in buffered 10% formaldehyde solution. Sections from the nucleus accumbens (coordinates +11.52 to +9.96 mm from interaural line and +2.52 to +0.96 mm from bregma (Paxinos and Watson, 1998)) were subjected to immunohistochemical analysis and the method used for analysis of astrocyte GFAP expression was the avidin–biotin peroxidase complex (ABC method, following Bondan et al., 2003). Briefly, the sections were deparaffinized in xylene and rehydrated in alcohol. Endogenous peroxidase was blocked at room temperature with a specific blocker and sections were incubated in 5% BSA in PBS to block unspecific proteins. Two washes with PBS were carried out between all incubations. The material was visualized with diaminobenzidine (DAB) and counterstained with 1:2 Harris hematoxylin. An enzymatic process of antigenic reactivation was used to demonstrate astrocytes. The process used pronase (S2013, Dako Corporation, Carpinteria, CA, USA), followed by the application of rabbit anti-cow GFAP antibody (1:1000, ZO334, Dako Corporation, Carpinteria, CA, USA). Secondary goat anti-rabbit antibody (1:100, E0433, Dako Corporation, Carpinteria, CA, USA) was used as a binding antibody. Astrocytic evaluation was performed using a computerized image analysis system (Image-Pro-Plus 4.5, Media Cybernetics, Silver Spring, USA); colorimetry measured the area stained brown in a total area of 302952.5 μm^2, using both the "core" and the "shell" of the nucleus accumbens in GFAP immunolabelled sections.

Statistical analysis

In the behavioral and neurochemical experiments, Student's *t*-tests were used to compare means of parametric data. Data expressed as percentages were analyzed by Fisher's Exact Test. The analysis of astrocytic GFAP expression in the NA used the Shapiro–Wilks test, followed by the qq-plot. GFAP immunoreactivity was analyzed by the Kruskal–Wallis and Mann–Whitney tests. In all cases, the results were considered significant if $p < 0.05$.

Results

The present experiment was designed to analyze possible LPS-induced effects on the maternal care of the F1 generation (transgenerational effect), i.e., in adult female rats prenatally exposed to LPS (100 μg/kg LPS on GD 9.5). This is because environmental information received by a mother, such as maternal infection can induce a phenotypic change in her offspring (Curno et al., 2009; Grindstaff et al., 2006).

No differences were observed in open field general activity between control and rats treated prenatally with LPS, suggesting no impairments in motor activity (Supplementary Table 1).

Maternal behavior was evaluated when the pups were returned to the cage after being separated from the mother for 1 h. Compared to the control group, the dams of the F1 generation prenatally treated with LPS presented increased time to display full maternal behavior, i.e., the behavior of the dams retrieving all pups to the nest and displaying nursing behavior with their back arched over the pups for minimal 3 consecutive min were delayed in the LPS group, revealing an impairment in this reflexive maternal response (Table 1). Moreover, F1 animals from the LPS group presented a significantly increased time to retrieve all pups as well as increased latencies to retrieve the 4th, 5th, 6th, 7th, and 8th pups in comparison to the control group, revealing impairments also in dam's motivation (Table 1 and Fig. 2). Specifically in relation to the increased latencies to retrieve the pups, a higher dispersion was observed in the data of F1-LPS treated females, compared to

Table 1
The effects of prenatal LPS exposure (100 μg/kg on GD 9.5) on maternal behavior on LD 6 of the adult F1 generation, i.e., females whose mothers received LPS during gestation (n = 10 for both groups; data presented as the means ± SEMs or percentage).

Parameters	Control group	LPS group	*p*
Pup retrieval			
1st pup (s)	9.75 ± 3.91	11.77 ± 4.35	0.414
1st pup (%)	100	100	–
All pups (s)	146.19 ± 29.23	384.40 ± 116.01*	0.042
All pups (%)	100	71.43	0.461
Grouping (%)	100	75	0.467
Full maternal behavior (s)	762.00 ± 171.00	1656.60 ± 48.00**	0.006
% Full maternal behavior	100	75	0.467
% Crouching	100	75	0.467
Self grooming (s)	10.29 ± 3.97	16.50 ± 2.71	0.216
Pups grooming (s)	34.86 ± 6.13	33.643 ± 4.11	0.850

Data in seconds were analyzed by Student's *t*-test and data in percentages by Fisher's Exact Test (crude values).
* $p < 0.05$ versus the control group.
** $p < 0.01$ versus the control group.

Table 2
The effects of prenatal LPS exposure (100 μg/kg on GD 9.5) on maternal aggressive behavior on LD 6 of the adult F1 generation, i.e., females whose mothers received LPS during gestation (n = 12 for both groups; data presented as the means ± SEMs or percentage).

Parameters	Control group	LPS group	*p*
Latency to 1st attack (s)	80.75 ± 12.24	178.67 ± 23.71***	0.001
Attack frequency	15.00 ± 2.70	4.60 ± 0.59***	0.001
Total time of attacks (s)	12.00 ± 2.50	2.70 ± 0.81**	0.003
Boxing frequency	7.20 ± 1.60	5.00 ± 0.87	0.244
Time of boxing (s)	2.00 ± 0.47	0.97 ± 0.16*	0.049
Pups carrying frequency	3.90 ± 1.20	3.20 ± 0.74	0.609
Frequency of hiding the pups	4.20 ± 1.00	2.10 ± 0.58	0.091
% of pups in the nest at the end of the test	82	54***	0.0001
Frequency of the intruder to sniff the pups	0.91 ± 0.34	2.20 ± 0.63	0.091

Data in seconds and in frequencies were analyzed by Student's *t*-test and data in percentages by Fisher's Exact Test (crude values).
* $p < 0.05$ versus the control group.
** $p < 0.01$ versus the control group.
*** $p < 0.001$ versus the control group.

control group. This dispersion was due to some dams which presented higher latencies to collect their pups; despite in all F1-LPS animals this parameter was increased. Probably, individual differences to prenatal LPS sensitivity were responsible for this dispersion. Interestingly, rats prenatally treated with LPS continued digging in the wood chips and building nests instead of retrieving pups, resulting in longer latencies to pup retrieval; this behavior was not observed in animals of the control group. All evaluated cages of control and prenatally LPS-treated groups presented a nest. The other parameters of maternal behavior were similar between the control and F1 LPS groups (Table 1).

Maternal aggressive behavior test measured the reaction of the mother to an unknown intruder introduced in the cage. As depicted in Table 2, prenatal exposure to LPS impaired the maternal aggressive behavior in the F1 generation. In relation to the control group, latency to the first attack increased and the frequency of attack, total time of attacks, time of boxing and the percentage of pups that remained in the nest at the end of the test decreased in the F1 LPS group. No differences were observed in the remaining parameters.

There are several studies relating striatum and the dopamine system with maternal behavior (Giordano et al., 1990; Numan, 2007; Robinson et al., 2011; Rosengarten and Friedhoff, 1979; Saltzman and Maestripieri, 2011; Zhao and Li, 2012). For example, dopamine deficiencies induced by either 6-OHDA lesions or antagonists in striatum give rise to deficits in maternal motivation (Hansen, 1994). We studied the brain areas related to the maternal care, i.e., monoamine (DA and 5-HT) levels, metabolites and turnover rates in the striatum and hypothalamus (Numan, 2007; Saltzman and Maestripieri, 2011). We also performed the neurochemical studies, because we had previous data of striatal DA and metabolite levels decrease in males prenatally exposed to LPS (Kirsten et al., 2010a), although there may be gender differences (Grimm and Frieder, 1985; Kirsten et al., 2012). Monoamine and monoamine metabolite levels and turnover rates in the striatum and hypothalamus of the F1 LPS and control groups are shown in Table 3. Adult female rats prenatally treated with LPS (F1 generation) presented a reduction in striatal DA and DA metabolite (DOPAC and HVA) levels with respect to the control group. Likewise, striatal 5-HT and 5HIAA levels as well as the 5HIAA/5-HT ratio were reduced in animals of LPS group. However, no differences in hypothalamic DA and 5-HT were observed in the F1 LPS-treated group when compared with controls.

For a long time, it is known that nucleus accumbens are intimately related to reproduction events, including maternal behavior (Brake et al., 1997; Liu et al., 1998; Numan, 2007; Stern and Lonstein, 2001; Zhao and Li, 2012). For example, rats sustaining lesions of the nucleus accumbens failed to show a maternal experience effect (Lee et al., 1999). Thus, for maternal behavior studies, it is important to study the

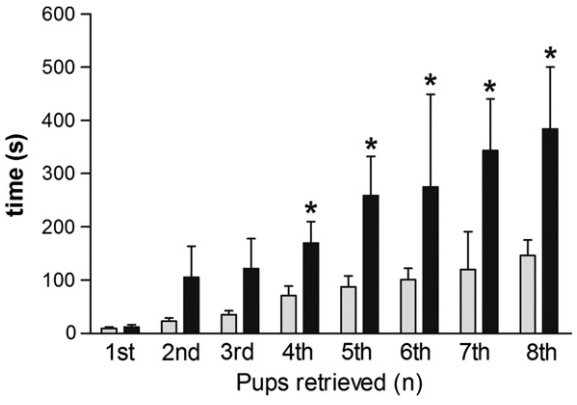

Fig. 2. The effects of prenatal LPS exposure (100 μg/kg on GD 9.5) on latencies (s) to retrieve pups on LD 6 in the F1 generation, i.e., females whose mothers received LPS during gestation; gray bar = control (saline) group; black bar = LPS group; n = 10 for both groups. *$p < 0.05$ (Student's *t*-test) compared with the control group. The values are given as the means ± SEM.

Table 3
The effects of prenatal LPS exposure (100 μg/kg on GD 9.5) on striatal and hypothalamic monoamine and metabolite levels (ng/g/wet weight tissue) and turnover rates on LD 6 of the adult F1 generation, i.e., females whose mothers received LPS during gestation (n = 10 for both groups; data presented as the means ± SEMs).

Parameters	Control group	LPS group	*p*
Striatum			
DA	9620.20 ± 414.12	7660.40 ± 368.29	0.001
DOPAC	864.87 ± 32.75	674.92 ± 42.00	0.001
HVA	981.88 ± 53.38	749.74 ± 64.48	0.006
DOPAC/DA	0.091 ± 0.003	0.088 ± 0.003	0.301
HVA/DA	0.103 ± 0.005	0.097 ± 0.006	0.278
5-HT	2563.20 ± 126.23	2210.83 ± 155.38	0.003
5HIAA	1091.74 ± 43.66	874.47 ± 54.17	0.005
5HIAA/5-HT	0.429 ± 0.011	0.399 ± 0.011	0.003
Hypothalamus			
DA	76.20 ± 7.69	80.60 ± 6.91	0.338
DOPAC	20.00 ± 2.30	21.70 ± 1.83	0.298
HVA	103.60 ± 11.69	103.00 ± 6.36	0.482
DOPAC/DA	0.264 ± 0.021	0.272 ± 0.011	0.369
HVA/DA	1.353 ± 0.112	1.306 ± 0.050	0.353
5-HT	1159.18 ± 503.49	1336.78 ± 611.26	0.413
5HIAA	329.73 ± 124.15	357.90 ± 148.84	0.443
5HIAA/5-HT	0.289 ± 0.039	0.282 ± 0.121	0.478

One-tail Student's *t*-test.

nucleus accumbens. GFAP-positive cells were identified via light microscopy by the intense brown staining in their cytoplasm and were considered as astrocytes. No difference was observed in GFAP expression by astrocytes in the nucleus accumbens between F1 LPS and control groups. The results are presented as median and interquartile interval (because of the non-normal distribution). Median areas (in μm^2) of GFAP expression in this nucleus for the animals from the F1 LPS and control groups were, respectively, 6673.43 (12543.22) and 7113.31 (13425.30), with no significant difference ($p > 0.05$) between them (Supplementary Fig. 1).

Discussion

The present findings clearly show that prenatal LPS (100 μg/kg on GD 9.5) exposure impaired maternal behavior and maternal aggressive behavior in the F1 generation. Moreover, prenatal LPS reduced striatal DA and DA metabolite levels and the striatal serotoninergic metabolite level in the F1 animals, but induced no changes in open field behavior and NA astrocyte protein expression.

The impairments in maternal care induced by prenatal LPS exposure might not be a consequence of a decreased motor activity, as the open field data showed no significant differences between control and F1 LPS groups. It is important to discard motor interference because pup retrieval behavior and other maternal care behaviors involve a chain of motor responses elicited by a variety of stimuli emanating from the female and/or pups, which promote orientation, attention and arousal (Stern, 1990). The impairments in maternal care presently observed in the F1 generation might be thought of as being a consequence of motivational changes in maternal care, as pup retrieval and other maternal care behaviors are motivated behaviors (Pedersen et al., 2006; Stern, 1990; Stern and Protomastro, 2000). The remarkable ability of postpartum females to successfully care for their developing infants is served by a distributed neural network. The motivational circuitry carries out efficient and dynamic processing of complex, constantly changing incoming environmental and pup-related stimuli and ultimately allows the appropriate expression of maternal responsiveness throughout the postpartum period (Pereira and Morrell, 2011). The present data showing impairment in maternal behavior suggests that prenatal LPS exposure reduced motivation in the F1 generation.

One important component of maternal care is the protection of vulnerable offspring from potential aggressors, i.e., the presence of a maternal aggressive behavior (Gaffori and Le Moal, 1979; Keer and Stern, 1999; Weil et al., 2006). This behavior has been described in numerous species, ranging from domesticated cattle to laboratory mice. This so-called maternal aggression or 'nest defense' likely serves to protect the pups from infanticide (Gaffori and Le Moal, 1979; Keer and Stern, 1999). The defensive behaviors appear to be the result of an evolutionary trade-off among the risks of injury to the mother and infants, as well as prior investment in the litter (Weil et al., 2006). Maternal aggressive behavior is also a motivated behavior. Agrati et al. (2011) have shown that postpartum females exhibit a range of sexual and maternal aggressive responses toward male intruders in their home cage. Female rats can optimally express two opposite and independently regulated motivations when the male is perceived as an ambivalent stimulus. The present data showing reduction in maternal aggressive behavior in the LPS F1 generation reinforces the notion that prenatal LPS exposure reduces motivationally driven behavior of the F1 generation towards their offspring.

This reduced behavioral motivation agrees with our previous data conducted with males from the same F1 generation (Kirsten et al., 2010a, 2010b). The male offspring presented impairments in social behavior in infancy and adulthood, but no changes in motor activities such as reflexological development, open field behavior in infancy and adulthood, haloperidol-induced catalepsy and apomorphine-induced stereotypy (Kirsten et al., 2010a, 2010b).

DA systems are also involved with motivation (Ikemoto, 2007). The present data showing decreased striatal DA and DA metabolite levels might be consistent with observed motivational impairments in rats prenatally exposed to LPS. In accordance with the present data, a decrease in DA and DA metabolite levels in the striatum of male rats prenatally exposed to LPS was already reported by our group (Kirsten et al., 2010a). We also showed that tyrosine hydroxylase levels were reduced in the striatum, although the expression of DA receptors was unchanged (Kirsten et al., 2012). In fact, studies have shown that DA is involved in both the onset and maintenance of maternal care (Numan and Stolzenberg, 2009; Robinson et al., 2011; Stern and Protomastro, 2000; Stolzenberg et al., 2010).

Neurochemical analysis of the striatal serotoninergic system showed a decrease in serotonin and in their metabolite levels as well as in the metabolite/neurotransmitter ratio in females of the F1 generation, suggesting a reduction in striatal 5-HT activity. Variable rates of maternal rejection in infancy were reported to affect development of the serotonergic system, and variation in serotonergic function may, in turn, contribute to the expression of maternal rejection toward one's own offspring later in life (Maestripieri et al., 2007; Saltzman and Maestripieri, 2011). Thus, prenatal LPS exposure, through 5-HT impairment, might have contributed to the maternal changes reported here in females of the F1 generation.

LPS administration is known to interfere with an animal's motivation. LPS causes sickness behavior in many species, a recuperative behavior that is controlled by motivation (Aubert et al., 1997; Aubert, 1999; Larson and Dunn, 2001). If this is the case, then the present data suggest that animals exposed prenatally to LPS remain an "open system", i.e., they are able to differentially respond to environmental stimuli until adulthood. The motivational impairment presented here in female adult rats prenatally exposed to LPS and the direct effect of LPS exposure to induce sickness behavior (Aubert, 1999; Kirsten et al., 2010b) indicates a trans-generational effect, as information in the environment received by the mother (the maternal infection) changed the phenotype of the offspring (Agrawal et al., 1999; Curno et al., 2009; Grindstaff et al., 2006). In fact, our results showed that both pregnant rats treated with LPS (Bernardi et al., 2010) and their adult offspring displayed impairments in maternal behavior.

In our study, the exposure to LPS was prenatal, and no changes occurred in astrocytes (GFAP) of the adult F1 female rats, indicating apparently a lack of permanent neuroinflammatory processes in the NA of adult females. Likewise, our previous studies, conducted in the striatum and in the olfactory bulb of F1 males prenatally exposed to LPS (brothers of present subjects) revealed no permanent neuroinflammatory processes, i.e., no changes in astrocytes and in the microglia (Kirsten et al., 2011, 2012). Thus, the behavioral impairments presented here are unlikely to be a consequence of an adult neuroinflammatory processes, but instead represent a prenatally induced reversible neuroinflammation. In this sense, acute administration of LPS increases the number of astrocytes (Bjorklung and Lindvall, 1984; Pang et al., 2010). Exposure to intrauterine LPS (GD 15 or 18.5) has been shown to evoke fetal brain injury, as evidenced by alterations in cytokine expression and neuronal injury (Elovitz et al., 2011). To confirm the lack of permanent neuroinflammation in the F1 rats, other studies might be performed to possibly reveal other evidences besides the astrocytes data. For example, it would be interesting to analyze cytokine levels in the cerebral spinal fluid of the F1 animals.

Conclusions

In summary, prenatal LPS exposure (100 μg/kg on GD 9.5) impaired maternal behavior and maternal aggressive behavior in the F1 generation. This result was most likely a consequence of changes in motivation, as (1) our present and previous data showed no changes in motor activity (Kirsten et al., 2010a), (2) maternal care is a motivated behavior (Pedersen et al., 2006; Stern, 1990), (3) LPS induced motivational

impairments in males prenatally treated with LPS (Kirsten et al., 2010b), (4) LPS interferes with the motivational state of adult animals (Aubert, 1999), and (5) LPS decreased striatal DA and DA metabolite levels, and DA is involved in motivation (Ikemoto, 2007). Finally, the present findings indicate a maternal effect of LPS, at least in the F1 offspring.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.lfs.2013.03.003.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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