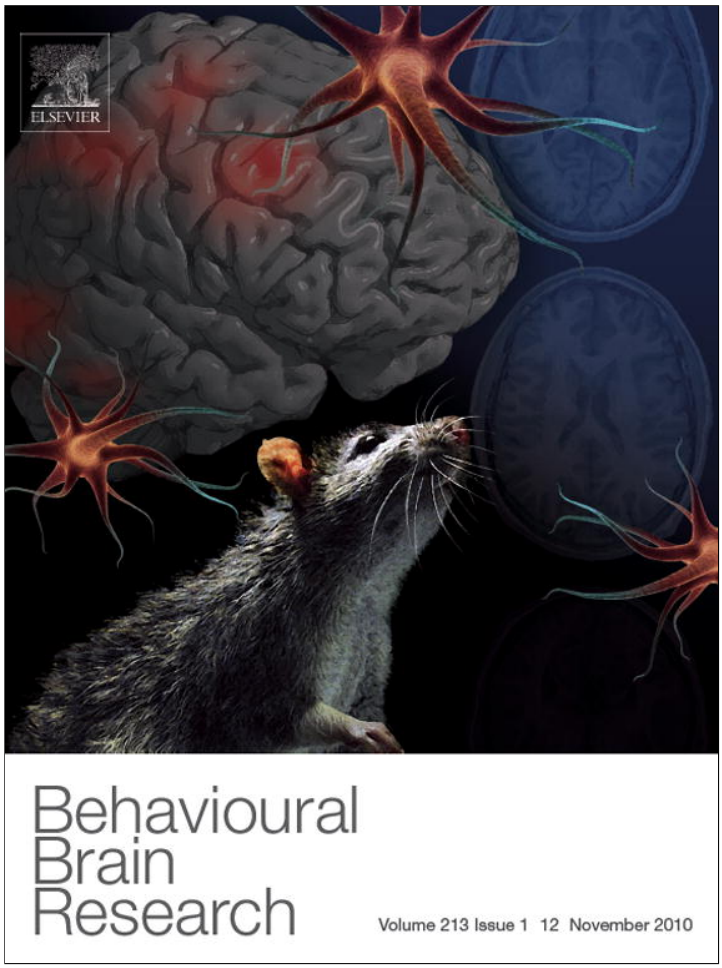
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Research report

Evaluating the interaction between early postnatal inflammation and maternal care in the programming of adult anxiety and depression-related behaviors

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ABSTRACT

The perinatal development of the nervous system is influenced by different external and internal stimuli. Previous data show that maternal care and perinatal inflammation can induce long-term changes in anxiety- and depression-related behavior. Our hypothesis is that both maternal care and perinatal inflammation act through interacting biological pathways to program adult behavior. To evaluate this interaction, we combined a protocol of maternal care variation in mice (C57BL/6J × BALB/c reciprocal F1 offspring) with the administration of bacterial wall lipopolysaccharide (LPS) at a previously reported sensitive development age (postnatal day 3, P3). The analysis of maternal behavior revealed that pups from C57BL/6J dams received more maternal attention than those taken care by BALB/c dams. Pups receiving LPS at P3 showed an acute corticosterone response, and a dose-dependent desensitization of this hormonal response when challenged with LPS at adulthood. We analyzed adult behavior on 6 highly validated tests and found an interaction between maternal care and early postnatal LPS on 7 anxiety-related behaviors in 4 different tests. In particular, early postnatal LPS treatment resulted in higher anxiety-related behavior when administered to females receiving more maternal care (C57 pedigree), but reduced depression-related behavior in males of the same pedigree. These results suggest that specific coping strategies are sensitive to maternal care and/or postnatal inflammation programming of adult anxiety- and depression-related behaviors, suggesting that both divergent and convergent mechanisms participate in this programming.

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1. Introduction

Epidemiological studies linking the prevalence of different common adult disorders with the weight at birth have initially led to the concept of fetal or developmental origins of disease. Moreover, it has been shown that early experiences can have profound long-term effects on the development of behavior. This has led to the concept of perinatal programming of adult behavior [1].

Exposure to early life stressors, such as childhood maltreatment and/or neglect, is one of the main environmental factors associated with the risk to develop mood and anxiety disorders [2]. Adverse early events can affect the development of systems involved in the response to stress, which may in turn contribute to mood disorders [3–5]. In rodents, changes in maternal care not only affect behavior (e.g. [6]), but also adult hippocampal gene expression [6] and alterations in other brain regions such as the prefrontal cortex [7], the amygdala [8] and the nucleus ceruleus [9] have been reported.

Mammals deal with stressful situations by a coordinated response of their nervous, immune and endocrine systems. Therefore, it is not surprising that early inflammatory stimuli can affect brain function. Maternal inflammation has been shown to result in alterations in the behavior of adult offspring mice, showing increased anxiety-related behavior [10,11]. Many alterations in brain function have been reported, including changes in adult neurogenesis, gliosis and neurotransmitter levels (reviewed in [12]). These effects appear to be at least partly dependent on the time of prenatal immune challenge [13] and involve different brain regions (e.g. hippocampus, amygdala, cortex, striatum and nucleus accumbens). Less is known about the long-term effects of postnatal inflammatory stimuli. Nevertheless, some reports have shown a detrimental effect of postnatal inflammation or infection on adult anxiety-related behavior [14,15].

There is previous evidence showing that both maternal care and perinatal inflammation can both act on specific, overlapping systems. For example, maternal care levels determine the adult hypothalamic–pituitary–adrenal (HPA) responses to stress [16] and

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postnatal inflammation can also affect the adult HPA response [1,17]. Moreover, as mentioned before, maternal care and prenatal inflammation can affect hippocampal, amygdalar and cortical function. However, because these effects were observed in different species, strains and experimental paradigms, it is not clear whether there are overlapping effects of these prenatal treatments and which, if any, is relevant to behavioral programming.

The main aim of this work is to study the long-term effects of an early postnatal inflammatory stimulus and that of maternal care on anxiety- and depression-related behaviors, and their interactions. Our hypothesis is that these stimuli act at least in part on the same developing system, affecting the programming of adult behavior.

C57BL/6 dams spend more time grooming and suckling their pups than the BALB/c dams [18], and these differences in maternal care have an impact on emotional reactivity of adult offspring [16,19]. Using two reciprocal F1 hybrids obtained by crossing C57BL/6 and BALB/c strains, Calatayud and Belzung [20] showed that mother type had a great influence on emotional reactivity of adult offspring. We chose C57BL/6J × BALB/c reciprocal F1s to alter the level of natural maternal care received by genetically similar pups. Although previous work has used different strategies to modify the levels of maternal care, we reasoned that the F1 hybrids had the advantage of avoiding the confounding effects of stress involved in artificial rearing conditions (e.g. using handling or maternal separation protocols [21]).

Immunological challenge in the early postnatal period, in the form of acute interleukin-1 (IL-1) or endotoxin exposure, is capable of producing long lasting changes in neuroendocrine function [22–25]. These immunological challenges induce classical stress-like responses in the neonate, including elevation of the corticotropin-releasing hormone (CRH), adrenocorticotropin-releasing hormone (ACTH), corticosterone, and glucose [24,26,27]. Moreover, in the rat, exposure to LPS from *Salmonella enteriditis* at postnatal days 3 and 5 resulted in an increase in anxiety-related behavior in the elevated plus maze at 80 days of age [15]. We thus chose an inflammatory stimulus (lipopolysaccharide, LPS) administered at postnatal day 3 (P3) in F1 mice to assess the long-term effects of postnatal inflammation on adult behavior.

The neural mechanisms driving the changes in anxiety- and depression-related behaviors observed after maternal care alterations or immune challenges have only been suggested. Because these stimuli can affect various brain regions, along with endocrine and immunological systems, more information about the interactions between different postnatal stimuli are highly valuable. Our approach aims to reveal possible convergence of maternal care and early postnatal inflammation to program adult behavior. We expect by this means to narrow the search for molecular and cellular mechanisms underlying this programming. Moreover, anxiety and depression represent a major mental health burden [28,29] and both maternal or postnatal infection [30] and childhood trauma [2] are risk factors for these disorders. Therefore, understanding the early contributions of environmental factors to these disorders could have a profound impact on the prevention and treatment of these disorders.

This study validates a method to study how the interaction between postnatal stimuli determines adult behavior. We show here that although programming of some specific behaviors appear to be modulated by both maternal care and early postnatal inflammation, in general these manipulations appear to act on different components of anxiety and depression-related behaviors.

2. Materials and methods

2.1. Animal subjects

BALB/cAnNCr1 (BALB/c, Charles River, Willminton, MA, USA) and C57BL/6J (C57, The Jackson Laboratory, Bar Harbor, Maine, USA) strains were bred for several generations in the laboratory. To obtain F1 hybrid offspring, BALB/c females were mated with C57 males (F1 BALB/c pedigree) and C57 females were mated with BALB/c males (F1 C57 pedigree) at 8–12 weeks of age.

For mating, each male mouse was mated with two nulliparous females. Female mice were then controlled daily and placed in a single cage when pregnancy was evident. The day of parturition was defined as postnatal day 0 (P0). The litters were culled to 10 pups. Pups were weaned and housed four to six per cage at P21. Mice from different litters were caged together at weaning to reduce cage effect. F1 hybrid mice from a total of 19 litters (9 C57 pedigree and 10 BALB/c pedigree) were used for behavioral characterization.

To measure corticosterone levels, F1 hybrid mice obtained from 12 independent litters (7 C57 and 5 BALB/c pedigree) were used. Mice were sacrificed at P3 or P60, 2 h after saline or LPS injection, and trunk blood collected. We did not use behaviorally tested animals to avoid effects of behavioral testing on corticosterone levels.

All animals had water and food *ad libitum* and were housed on a 12:12 light:dark cycle with lights on at 08:00 h. All animal procedures were performed according to the rules and standards of German animal law and the regulations for the use of laboratory animals of the National Institute of Health, USA. The Ethical Committee of the Institute Leloir Foundation approved these animal experiments.

2.2. Maternal behavior testing

Quantification of maternal behavior was performed as previously described [31]. Briefly, maternal observations (60 observations per hour) were performed for 2 h during the light period (11:30–12:30 and 16:30–17:30) each day from P1 to P7. Two behavioral measures were collected: time spent in arched-back posture on the pups (ABN) and time spent grooming and licking pups (LG).

2.3. Developmental markers

The normal development of the offspring was evaluated through the observation of some developmental markers: ears separation, appearance of dorsal fur, and eye opening. Animals were weighed weekly until the 8th week of life. Weight gain was also evaluated 24 h after the LPS or saline injection, as an indirect measure of prolonged sickness behavior and/or maternal neglect.

2.4. Postnatal inflammation

All pups within a litter were injected intraperitoneally on P3 using a 30 G needle. In each litter half of the pups were injected with LPS (25 μg/kg or 50 μg/kg of *Escherichia coli* LPS, serotype 0111:B4, Sigma) and half with sterile saline solution. As a result, 1–3 mice of the same sex received the same early postnatal treatment within each litter. We reasoned that this design would minimize the litter effect. Animals were injected at 12:30 p.m., after morning maternal behavior observations. First, the dam was removed and placed in a novel cage. The whole litter was injected in less than 3 min and the mother was then returned to the cage.

2.5. Corticosterone radioimmunoassay

Trunk blood was collected 2 h after LPS or saline injection between 11:00 and 12:00 am. RadioImmunoAssay (RIA) was performed on serum samples from P3 pups, whereas plasma samples were used for adult mice. To reach the appropriate amount of serum needed for the assay, sera from 2 pups were pooled and 3 of these pools per treatment were used for RIA (i.e., a total of 6 pups per treatment). Adult plasma samples were analyzed individually.

Samples were centrifuged and plasma or serum was removed and stored at −20 °C until RIA was performed. Serum was inactivated at 70 °C for 90 min. Corticosterone was extracted twice from serum or plasma, using 0.5 ml or 0.3 ml of ether, respectively and resuspended in 0.1 ml of buffer B (0.05 M Tris pH 8, 0.1 M NaCl, 0.1% sodium azide and 0.1% BSA).

The assay was carried out following the RIA protocol provided by the anti-corticosterone antibody manufacturer (C8784, Sigma) using ^{3}H-corticosterone (1,2,6,7-^{3}H(N)-corticosterone, PerkinElmer). The lower limit of detection of the assay was 12.5 pg corticosterone.

2.6. Anxiety- and depression-related behavior testing

All behavioral testing was performed during the light period (between 10:00 and 16:00 h) under dim light illumination. Mice were 8–10 weeks of age at the beginning of testing and behavioral testing were separated by 1-week intervals to reduce inter-test interactions. The tests were performed in the order listed below. Mice were tested in the holding room. After changing illumination, mice were habituated to it for 45 min prior to the test. After testing, each mouse was identified and placed in a holding cage until all animals in a cage had been tested. Each apparatus was cleaned with 20% ethanol and dried between sessions.

We used 156 mice in the following behavioral testing, randomly distributed to the following experimental groups. BALB/c pedigree: 38 male (18 with saline, 10 with 25 μg/kg LPS, 10 with 50 μg/kg LPS) and 38 female (16 with saline, 14 with 25 μg/kg LPS, 8 with 50 μg/kg LPS). C57 pedigree: 34 male (18 with saline, 10 with 25 μg/kg LPS, 6 with 50 μg/kg LPS) and 46 female (23 with saline, 15 with 25 μg/kg

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LPS, 8 with 50 μg/kg LPS). A list of the behavioral parameters recorded in all tests is provided in Table S1.

2.6.1. Elevated plus maze

Elevated plus maze (EPM) was performed as previously described [31]. Apparatus consisted of two open and two closed arms (open arms: 30 × 5 cm, 105 lx, surrounded by a 0.5 cm high border; closed arms: 30 × 5 cm, 43 lx, surrounded by 19 cm high walls). The walls were made of black PVC and the floor of grey PVC. The apparatus was elevated 50 cm above the floor.

Mice were placed into the central platform (5 × 5 cm, 102 lx) of the maze facing towards an open arm and allowed to explore the maze for 5 min. Locomotion data were collected by a video-tracking system (ANY-maze, Stoelting). Measured locomotor parameters were: time in open arms, time in closed arms, time in central platform, % entries into open arms, total distance, and total arm entries. Ethological parameters were scored manually during each session: time rearing, time grooming, and number of head dippings.

2.6.2. Open field

Open field (OF) test was performed as previously described [31]. Mice were placed in an arena (floor: 45 cm × 45 cm of grey PVC; walls: 30 cm high of black formic; 30 lx) for 5 min. Animals were initially placed along one side of the arena, and the center region was defined as the central 23 cm × 23 cm area. Locomotion data were collected by a video-tracking system (ANY-maze). Measured behavioral parameters were: time spent in the center, entries into the center, % distance in the center, latency to enter the center, total distance. Time grooming and time rearing were scored manually during each session.

2.6.3. Light/dark box

A 45 cm × 45 cm arena was divided at half with an inverted black box (lit side: 35 lx; dark side: 1 lx). Animals could cross from one compartment into the other through a small hole in the wall (12 cm height, 8 cm width). Each mouse was placed under the hole facing the illuminated side of the box and observed for 5 min. Behavioral parameters analyzed were: time spent in lit compartment and Entries into lit compartment.

2.6.4. Novelty suppressed feeding

Eighteen hours prior to testing, all food was removed from the home cage and the animals were weighed. Only animals that lost more than 5% of their body weight were considered in the analysis. At the time of testing, a single pellet of food (regular chow) was placed in the center of an open field arena. Each animal was placed along one side of the maze and was observed for up to 5 min. Behavioral parameters analyzed were: latencies to smell, bite and eat (to sit on its haunches and bit with the use of forepaws) the food pellet.

2.6.5. Tail suspension

Animals were suspended in the air using adhesive tape wrapped around the subject's tail (about 4/5 from the base) and fixed to a wire at 25 cm of height from a wooden surface. Behavioral parameters analyzed during 5 min were: Latency to the first bout of immobility and % immobility time. 14 animals were removed from this analysis because they learned how to climb their own tails during the test.

2.6.6. Forced swimming

Animals were gently placed in a beaker glass (15 cm in diameter and 25 cm in height), filled with 14 cm of water at room temperature (25 °C). Behavioral parameters analyzed during 5 min were: latency to the first bout of immobility, and % immobility time. At the end of the test, animals were dried with a paper towel and placed in a holding cage with normal bedding. Mice were dry after 20 min.

2.7. Statistical analysis

The effects of pedigree and day on maternal behavior variables were analyzed by repeated measure analysis of variance (ANOVA). To analyze the acute effect of LPS on corticosterone serum levels and weight gain of pups ANOVA was used. We compared the long-term effects on corticosterone induction by Student's *t* tests, using Welch's correction when the variances were not equal.

Behavioral parameters recorded in each test (Table S1) were first analyzed by multivariate analysis of variance (MANOVA), with *sex*, *pedigree* and *early postnatal LPS* as independent variables. MANOVA was followed by univariate ANOVA and, in cases of significance ($p < 0.05$), post hoc comparisons using Duncan's test.

To uncover common behavioral strategies that our mice used in the different tests, we performed the principal component analysis (PCA). Due to the nature of the PCA that limits the number of variables included in the analysis to the number of mice tested, we performed correlation analysis (Pearson coefficient) for the behavioral parameters recorded in each test. Only variables that were correlated less than 0.70 were included in the following PCA (Table 1). This procedure also helped uncovering correlations existing among different behavioral tests. Finally to compare the different experimental groups, factorial scores obtained from each mouse were analyzed by MANOVA, followed by ANOVA and, in cases of significance ($p < 0.05$), post hoc comparisons using Duncan's test. In all cases, data are presented as mean ± SEM. All analyses were performed using the Statistica (version 99, Statsoft) and SPSS (version 10, SPSS Inc.) software.

Table 1
Factors and factor loadings[a] obtained by PCA of selected variables.

Variables	Factor 1	Factor 2	Factor 3
Total distance—EPM	**0.614**	0.387	−0.262
Time grooming—EPM	0.132	−0.034	−0.138
Time rearing—EPM	**0.662**	0.150	0.058
Number of head dippings—EPM	−0.296	**0.615**	0.098
Time in central platform—EPM	−0.178	**0.430**	0.200
Time in open arms—EPM	**−0.763**	0.166	0.037
% Entries into open arms—EPM	**−0.759**	0.207	0.033
Total distance—OF	0.383	**0.766**	−0.065
Time grooming—OF	0.360	0.037	−0.161
Time rearing—OF	**0.432**	0.332	0.343
Entries into the center—OF	0.128	**0.718**	−0.265
Time spent in lit compartment—LD	−0.027	0.100	**0.758**
Entries into the lit compartment—LD	0.191	0.357	**−0.68**2
Latency to bite—NSF	−0.019	−0.337	0.310
% Immobility time—FS	−0.176	−0.068	**0.412**
% Immobility time—TS	0.046	0.013	0.235

[a] Factor loadings > 0.4 are shown in bold.

3. Results

3.1. Early effects of pedigree and early postnatal LPS on pups

To verify that BALB/c and C57 dams in our experiment provide different amount of care to their F1 pups, we measured their maternal behavior during the first postnatal week. The analysis of the maternal behavior recorded revealed a significant main effect of *strain* on the time spent arched-back nursing or licking and grooming the pups (Repeated measures ANOVA, ABN-LG: $F_{1,17} = 8.12$, $p = 0.0111$), with C57 dams spending more time arched-back nursing and licking and grooming their pups than BALB/c dams. This difference was mainly due to an increase in the time the C57 dams spent licking and grooming the pups (repeated measures ANOVA, main effect of *strain*, $F_{1,17} = 215.95$, $p < 0.0001$; *interaction between strain and postnatal day*: $F_{6,102} = 3.40$, $p = 0.0043$; Fig. 1A). We analyzed whether the amount of licking and grooming received during the first week of life would be a better discriminating factor than pedigree by performing a κ-mean cluster analysis [32]. Because of the strong differences in maternal care showed by BALB/c and C57 dams (see Supplementary Figure 1), clustering of the mothers was identical to the strain classification. Therefore, we used pedigree as the independent variable denoting maternal care.

To determine the ability of LPS injection to activate the hypothalamic–pituitary–adrenal (HPA) axis in P3 pups, we performed a corticosterone radioimmunoassay (RIA) on serum collected from mice injected with saline, 25 μg/kg LPS or 50 μg/kg LPS. This analysis showed an increase in the levels of serum corticosterone (ANOVA, $F_{2,6} = 17.41$, $p = 0.0032$) in a dose-dependent manner (Fig. 1B). We also evaluated whether LPS affected the weight gain of pups on the day of the injection. LPS-injected animals showed an increase of weight similar to their control littermates (ANOVA, $F_{2,33} = 2.520$, $p = 0.0958$; Fig. 1C). This result confirms that LPS has an acute effect on pups but it does not induce persistent sickness behavior and/or maternal neglect.

We also checked whether LPS and/or maternal care affected the development of pups. In 16 out of the 19 litters dorsal fur appeared at P3, and ears detached completely from the cranium at P4 as normal. Only three litters showed a delay of one day in both of these developmental markers. Interestingly, these litters were bigger at the moment of birth (11, 12 and 15 animals), and were culled to 10 at P0. All pups opened their eyes at P12. This result showed that neither postnatal LPS nor maternal care had effects on these mouse developmental markers.

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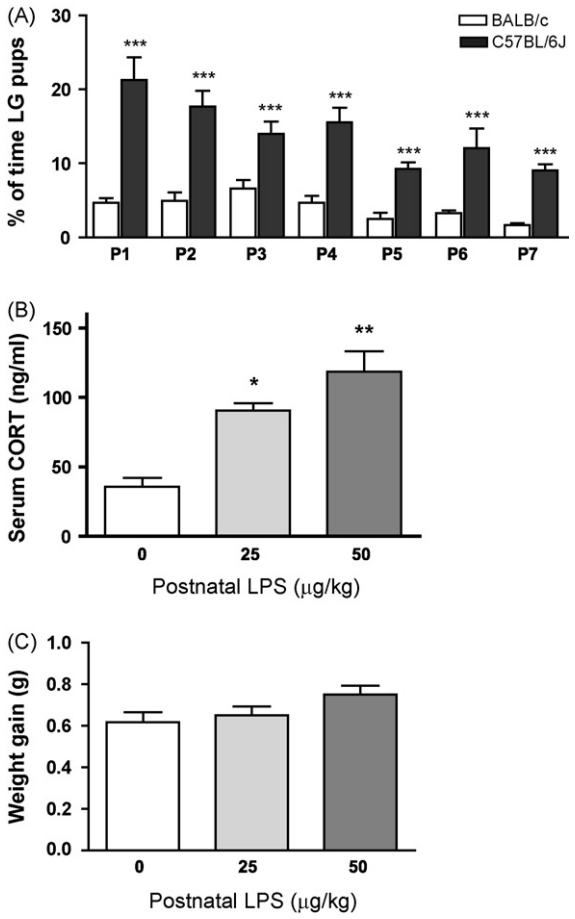


Fig. 1. Short-term effects of maternal care and early postnatal LPS. (A) Percentage of observations of licking and grooming the F1 pups (LG) by BALB/c and C57 dams, from P1 to P7. Mean ± SEM. Unpaired Student's *t* test, $^{***}p < 0.001$. $n = 10$ BALB/c dams, $n = 9$ C57 dams. (B) Serum corticosterone (CORT) levels 2 h after saline or LPS injection. Mean ± SEM. Dunnet's post hoc test, $^{*}p < 0.05$, $^{**}p < 0.01$. $n = 3$ pooled serum per group. (C) Weight gain 24 h after saline or LPS injection. Mean ± SEM. $n = 12$ pups per group.

3.2. Long-term effects of pedigree and early postnatal LPS on pups

To evaluate if early postnatal LPS injection and/or maternal care have long-term effects on body weight, this parameter was checked at weaning and adulthood (P60). No effect of *pedigree*, *early postnatal LPS*, *sex* or interaction between these factors was observed on weight at weaning. An effect of *sex* on body weight was instead observed at beginning of the behavioral testing (P60; ANOVA, $F_{1,112} = 246.67$, $p < 0.0001$).

We also checked whether early postnatal LPS injection (P3) had long-term effects on the response of HPA axis to a new inflammatory challenge in adulthood. To this aim, animals of each of the six experimental groups (*pedigree* × *early postnatal LPS*) were injected at P60 with saline or LPS (25 μg/kg), and their corticosterone response was evaluated 2 h after the injection by RIA (Fig. 2). The analysis showed an increase in the plasma corticosterone levels in animals injected postnatally either with saline (Balb/c pedigree: $t_3 = 3.96$, $p = 0.0289$, C57 pedigree: $t_6 = 3.30$, $p = 0.0164$) or the lowest dose of LPS (Balb/c pedigree: $t_9 = 3.20$, $p = 0.0108$, C57 pedigree: $t_4 = 3.67$, $p = 0.0214$). However, this increase was not observed in animals injected with the highest dose of LPS at P3 (Balb/c pedigree: $t_9 = 2.01$, $p = 0.0754$, C57 pedigree: $t_6 = 0.18$, $p = 0.8687$). This dependence on dosage was observed in both pedigrees, showing that it is only an effect of *early postnatal LPS*.

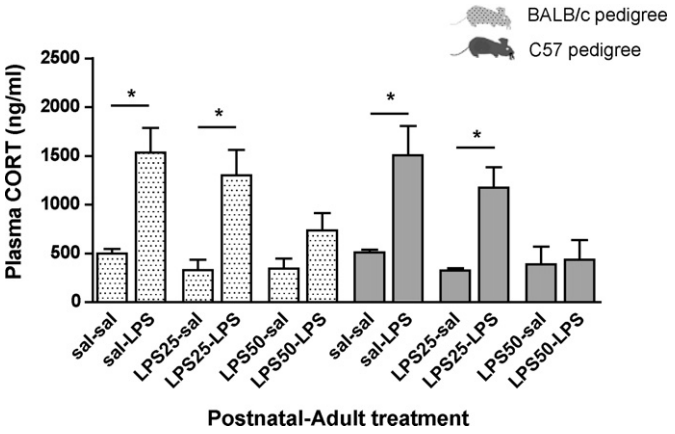


Fig. 2. Long-term effects of early postnatal LPS on HPA response in adulthood. Plasma corticosterone levels were measured 2 h after saline or LPS injection in animals injected postnatally with saline (sal), 25 μg/kg of LPS (LPS25) or 50 μg/kg of LPS (LPS50). Mean ± SEM. Unpaired *t* test, $^{*}p < 0.05$, $^{**}p < 0.01$. $n = 3$–6 per group.

3.3. Analysis of adult anxiety-related behaviors

MANOVA of anxiety-related behaviors in the EPM revealed a significant main effect of *pedigree* ($\lambda = 0.8647$, $F_{11,134} = 1.91$, $p = 0.0438$). Univariate ANOVAs showed a significant effect of: *pedigree* on time spent in closed arms ($F_{1,144} = 5.5900$, $p = 0.0194$) and open arms ($F_{1,144} = 4.0468$, $p = 0.0461$); *early postnatal LPS* on time spent in central platform ($F_{2,144} = 4.1687$, $p = 0.0174$); *interaction between early postnatal LPS and pedigree* on time spent in central platform ($F_{2,144} = 3.3741$, $p = 0.0370$); and *interaction between early postnatal LPS, pedigree and sex* on time spent grooming ($F_{2,144} = 3.3247$, $p = 0.0387$). In this test all mice of C57 pedigree spent less time in open arms and more time in closed arms than mice of Balb/c pedigree (Fig. 3A and B). The *early postnatal LPS* injection had an effect on time spent in central platform, with all mice receiving the highest dose of LPS spending the lowest amount of time in this area of the apparatus (Fig. 3C). In addition, this variable showed a dose effect of *early postnatal LPS* only in mice of the BALB/c pedigree (*interaction between pedigree and early postnatal LPS*, Fig. 3C). Finally, the significant *interaction between early postnatal LPS, pedigree and sex* on time grooming was due to the high amount of grooming showed by males of the C57 pedigree postnatally injected with the highest dose of LPS (Fig. 3D).

MANOVA of anxiety-related behaviors in the OF revealed a significant main effect of *early postnatal LPS* ($\lambda = 0.8379$, $F_{14,276} = 1.82$, $p = 0.03531$) and *interaction between early postnatal LPS, pedigree and sex* ($\lambda = 0.8010$, $F_{14,276} = 2.32$, $p = 0.00501$). Univariate ANOVAs showed a significant effect of: *pedigree* on time spent in the center ($F_{1,144} = 5.84$, $p = 0.01692$) and % distance in center ($F_{1,144} = 5.55$, $p = 0.01987$); *early postnatal LPS* on total distance ($F_{2,144} = 6.37$, $p = 0.00224$) and time rearing ($F_{2,144} = 4.35$, $p = 0.01467$); *sex* on total distance ($F_{1,144} = 4.27$, $p = 0.04057$); *interaction between early postnatal LPS and pedigree* on time rearing ($F_{2,144} = 4.94$, $p = 0.00841$); *interaction between pedigree and sex* on total distance ($F_{1,144} = 4.08$, $p = 0.045156$); and *interaction between early postnatal LPS, pedigree and sex* on total distance ($F_{2,144} = 4.71$, $p = 0.01040$) and entries into the center ($F_{2,144} = 3.13$, $p = 0.04682$). In this test all mice of the C57 pedigree spent less time in the center and walked less distance in this area compared to mice of the BALB/c pedigree (Fig. 4A and B). Male mice walked more than females, and the postnatal injection of the highest dose of LPS significantly reduced the locomotion in adult mice (Fig. 4C). Females of the C57 pedigree receiving the highest dose of LPS (50 μg/kg) greatly contributed to this last effect on locomotion (*interaction between early postnatal LPS, pedigree and sex*; Fig. 4C). In addition, *early postnatal LPS* had the effect of inhibit-

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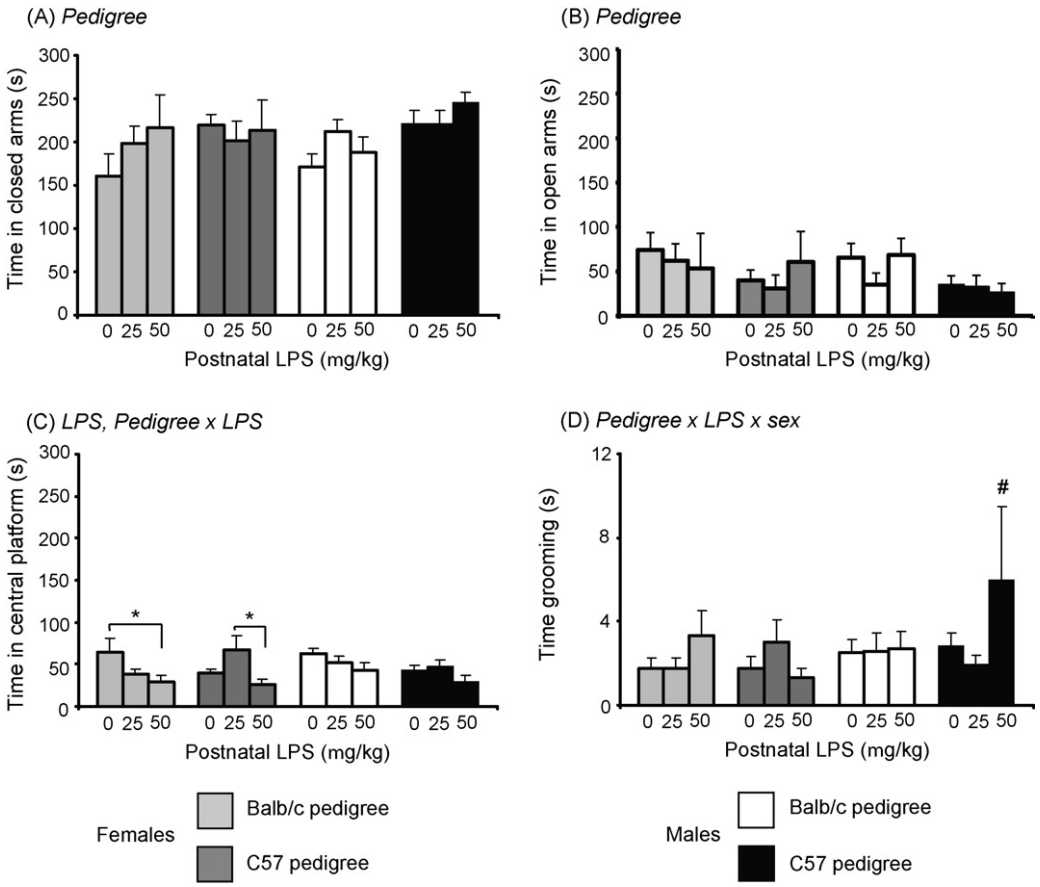


Fig. 3. Early postnatal LPS and pedigree effects on behavior in the EPM. Only variables that showed significant differences in ANOVA analysis are shown. (A) Time in closed arms, (B) Time in open arms, (C) Time in central platform, and (D) Time grooming. Mean ± SEM. Relevant Duncan's post hoc test, $^{*}p < 0.05$. # On this variable, this group showed significant differences ($p < 0.05$) when compared to each of the experimental groups. The effects revealed by ANOVA are indicated.

ing rearing behavior, but only in mice of the C57 pedigree (Fig. 4D). Finally, a dose-dependent effect of *early postnatal LPS* was observed in the number of entries into the center performed by females of the C57 pedigree (Fig. 4E).

MANOVA of anxiety-related behaviors in the LD revealed a significant main effect of *sex* ($\lambda = 0.9423$, $F_{2,\ 143} = 4.37$, $p = 0.01431$), with female mice spending less time in the light compartment and visiting this area less frequently than male mice.

MANOVA of anxiety-related behaviors in the NSF revealed a significant main effect of *sex* ($\lambda = 0.8947$, $F_{3,\ 142} = 5.57$, $p = 0.00121$). Univariate ANOVAs showed a significant *interaction between early postnatal LPS, pedigree and sex* on latency to eat the food pellet ($F_{2,144} = 3.22$, $p = 0.04271$). Males of the BALB pedigree treated with the lowest dose of *early postnatal LPS* (25 μg/kg) showed a longer latency to eat the food than males of the C57 pedigree injected with the same dose of LPS (Fig. 4F).

3.4. Analysis of adult depression-related behaviors

MANOVA of depression-related behaviors in the TS revealed a significant main effect of *sex* ($\lambda = 0.87508$, $F_{1,129} = 9.21$, $p = 0.00018$) and *interaction between early postnatal LPS, pedigree and sex* ($\lambda = 0.91928$, $F_{4,258} = 2.77$, $p = 0.02772$). Univariate ANOVAs showed a significant effect of *sex* ($F_{1,130} = 16.40$, $p = 0.00001$) and *interaction between early postnatal LPS, pedigree and sex* ($F_{2,130} = 5.40$, $p = 0.00557$) in the percentage of immobility time. Female mice spent more time immobile than males, and males of the C57 pedigree showed a dose-dependent effect of *early postnatal LPS* on this variable (Fig. 5).

No significant effect of *early postnatal LPS, pedigree, sex,* and/or interaction between these independent variables was observed in the FS.

3.5. Principal component analysis (PCA)

PCA of selected measures from all tests (Table 1) extracted three factors using the scree-plot solution, which accounted for 41.62% of total variance. It was confirmed that factors did not correlate (data not shown). Factor loadings obtained after Varimax rotation with Kaiser Normalization are shown in Table 1. The three emerging factors were:

1) Factor 1: The variables that loaded positively on this factor were total distance and time rearing in the EPM, and time rearing in the OF, whereas time in open arms and % entries into open arms in the EPM loaded negatively. This factor accounts for 19.7% of the variance, and was named "*Exploration and EPM-specific anxiety*", as higher scores show increased exploration but less visits to the anxiogenic arms of the EPM.
2) Factor 2: The variables that loaded positively on this factor were number of head dippings and time in central platform in the EPM, and total distance and entries into the center in the OF. This factor accounts for 12.6% of the variance, and was named "*Anxiety and risk assessment*", because higher scores show both reduced anxiety in the OF and a risk assessment coping strategy in the EPM.
3) Factor 3: The variables that loaded positively on this factor were time spent in lit compartment in the LD and % immobility time

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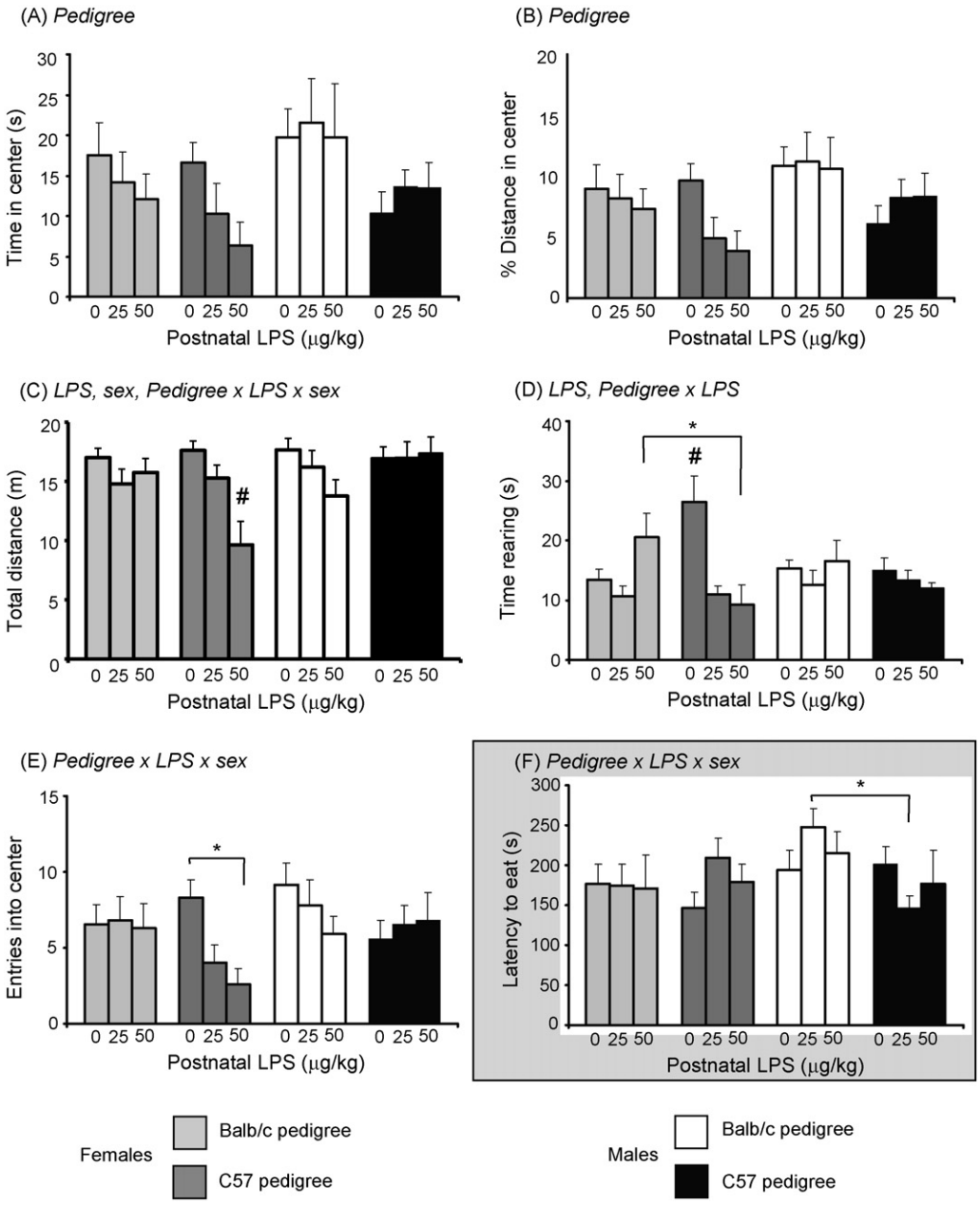


Fig. 4. Early postnatal LPS and pedigree effects on behavior in the OF and NSF. Only variables that showed significant differences in ANOVA analysis are shown. In the OF they were: (A) time in center, (B) percentage of distance in center, (C) total distance, (D) time rearing, and (E) visits in center. In the NSF: (F) latency to eat. Mean ± SEM. Relevant Duncan's post hoc test, $^{*}p < 0.05$. # On this variable, this group showed significant differences ($p < 0.05$) when compared to each of the experimental groups. The effects revealed by ANOVA are indicated.

in the FS, whereas entries into the lit compartment in the LD loaded negatively. It explains 9.3% of the variance. This factor was named "*Depression*".

MANOVA analysis was performed to evaluate the effect of *early postnatal LPS, pedigree, sex,* and/or interaction between these independent variables on factorial scores obtained from the different experimental groups. A significant main effect of *early postnatal LPS* was observed ($\lambda = 0.89128$, $F_{6,256} = 2.53$, $p = 0.02148$). Univariate ANOVAs showed a significant effect of *sex* ($F_{1,130} = 4.77$, $p = 0.03076$) and *early postnatal LPS* ($F_{2,130} = 6.35$, $p = 0.00234$) on Factor 2. This analysis showed that female mice had lower scores in this factor compared to males, showing an increased level of anxiety and reduced risk assessment behaviors in this gender. In addition the *early postnatal LPS* had the general effect of increasing the anxiety and reducing the risk assessment behavior described by Factor 2. This is remarkably true for female mice of the C57 pedigree (Fig. 6).

4. Discussion

We explored here whether maternal care and an early postnatal inflammatory stimulus could interact to program adult anxiety and depression-related behaviors. We first observed that specific variables of different behavioral tests were affected by each treatment, while seven variables showed an interaction between *pedigree* and *early postnatal LPS* treatment. The fact that independent variables affect differentially these behaviors suggests that they represent different strategies involved in a complex behavioral response to

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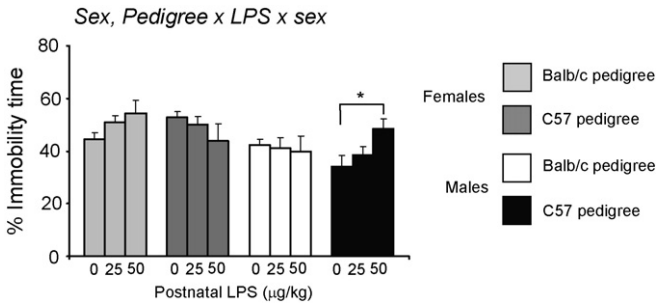


Fig. 5. Early postnatal LPS and pedigree effects on behavior in the TS. The only variables that showed significant differences in ANOVA were percentage of immobility time. Mean ± SEM. Relevant Duncan's post hoc test, $^{*}p < 0.05$. The effects revealed by ANOVA are indicated.

anxiogenic situations. In an attempt to extract a more significant variable across tests, we then performed a factorial analysis. In this case, we observed a general effect of *early postnatal LPS*, which was mainly due to an effect of this independent variable on Factor 2, "Anxiety and risk assessment." Our results show that the use of multiple behavioral tests combined with the study of the multidimensional structure of mouse anxiety-related behavior is relevant to the characterization of the long-term consequences of maternal care and early postnatal inflammation.

Many laboratories have previously opted to use animals of only one sex to perform behavioral analyses. We show here that five dependent variables showed an *interaction between pedigree, early postnatal LPS and sex*. This adds to previous observations that a variety of interventions and perturbations during the neonatal period, including maternal separation, neonatal handling, and immune challenge, result in gender specific alterations in the adult [33–40]. Because epidemiological studies in humans show a higher prevalence of anxiety and depression in women when compared to men [41,42], studying the gender contribution to developmental mechanisms regulating anxiety and depression is highly relevant.

Worth to mention here, because we tested the same animals across different behavioral paradigms, there is the possibility that an order effect may account for some of the effects observed.

4.1. Long-term effects of maternal care

C57 dams showed more maternal care than BALB/c dams. Even though profound differences in maternal care were observed, no differences in the weight of the animals at weaning or in adulthood were evidenced between the pedigrees. This pinpoints the fact that these natural differences in maternal care are either not strong enough to affect the gain of weight of the pups or that animals receiving poor maternal care (BALB/c pedigree) compensate for it by eating more during the shorter nursing period. Moreover, differences in milk composition could account for this lack of effect [43].

Moreover, no differences between the pedigrees were observed in the baseline levels of corticosterone, or in the levels of this hormone measured after an LPS challenge. Although there is a body of evidence showing that maternal behavior can influence HPA axis function in the pups [16,44–49], the effects appear to depend on the intensity of postnatal manipulation. In line with our results, a previous report on C57BL/6J × BALB/c F1 showed that basal corticosterone levels and corticosterone levels after a stressor (sudden loud noise) are similar in C57 and BALB/c pedigrees [50]. Here we extended those results using an immunological stressor (LPS) that induces a rapid and transient activation of the HPA axis, which did not reveal differences in the hormonal response in relation to animal pedigree. However, because we measured corticosterone levels only 2 h after LPS injection, we cannot rule out that maternal care affects the shape of the curve and that we are not revealing these differences.

Because it was previously shown that offspring of mothers that show increased maternal care are less anxious in adulthood (i.e. spend less time in the center in the OF; [6]), we expected mice of the C57 pedigree to show reduced anxiety-related behavior. Conversely, in this study offspring of the C57 pedigree spent less time in the open arms in the EPM and in the center in the OF, two variables considered to express anxiety. This suggests that either maternal care is having a less straightforward effect on anxiety as previously thought, or other factors are involved in the pedigree effect.

Even the behavioral analysis of BALB/c and C57BL/6J strains using EPM and OF tests has often been contradictory. BALB/c mice have been regarded as the least anxious, non-reactive strain [51], as an intermediately anxious strain [51], or as a highly anxious, not very active strain [52]. The same is true for the C57BL/6J strain. When these strains are compared, they either show opposite behaviors [52] or appear to have similar, intermediate levels of anxiety-related behavior [53]. Furthermore, the same mouse strain has been defined as 'anxious' in one test (i.e. EPM) and 'non-anxious' in another (i.e. OF) [53,54]. Combining different tests and/or including ethological variables such as grooming, rearing and head dipping, other authors have shown that the C57BL/6J and BALB/c strains respond to the same anxiogenic conditions with different coping strategies [55,56]. Taking this into account, it is difficult to establish *a priori* the behavioral phenotype of a F1 and more work like the one presented here is necessary to characterize the phenotype of F1 offspring.

It is worth to mention that C57 and BALB/c pedigree offspring differ not only in the maternal care received, but also in other factors such as differences in sexual chromosomes, differences in maternal milk composition, and genetic imprinting. However, different studies have proven that effects on emotional reactivity are mainly

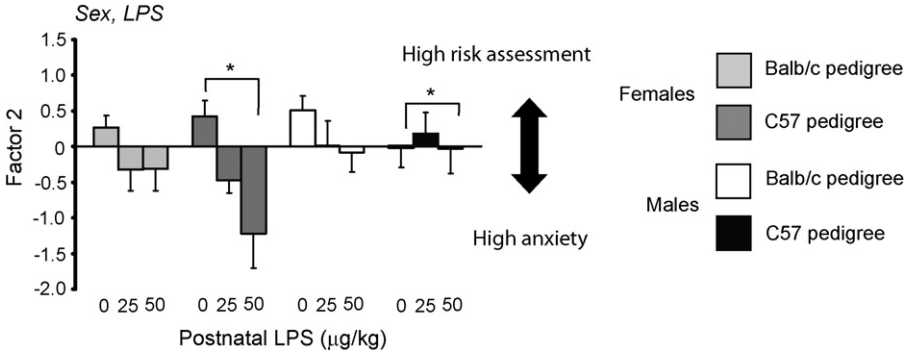


Fig. 6. Early postnatal LPS and pedigree effects on Factor 2. Mean ± SEM. Relevant Duncan's post hoc test, $^{*}p < 0.05$. The effects revealed by ANOVA are indicated.

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due to maternal behavior differences [16,18,33,49]. Therefore, F1 studies are a valuable experimental model for evaluating the contribution of maternal care on behavioral programming, and we propose that the effect observed here is in fact a consequence of differences in maternal care as previously shown [20,33].

4.2. Long-term effects of early postnatal LPS

We observed an effect of *early postnatal LPS* on specific variables related to anxiety and risk assessment. In particular, early postnatal LPS showed an effect on the time spent in the central platform in the EPM (a variable related to risk assessment) and on exploration in the OF (both total distance and time rearing). Moreover, we observed an effect of early postnatal LPS on the MANOVA of PCA factors, mainly due to a significant effect on Factor 2, "Anxiety and risk assessment". This effect was most evident on female mice of the C57 pedigree: in this group the highest LPS dose significantly reduced exploration in the OF.

The HPA axis is hyporeactive to different types of stress at P3 in the rat [57,58] and in the mouse [59]. We found, however, that *E. coli* LPS (serotype 0111:B4) is capable of inducing an acute, dose-dependent response on serum corticosterone in mice at P3. This elevation goes in line with previous work using endotoxin [15,27,60] or IL-1 [17,61] to stimulate HPA axis, and shows that this dose of LPS administered neonatally was biologically active and capable of activating the animal's HPA axis.

Moreover, the highest LPS dose altered the adult response to the same inflammatory stimulus, blunting the corticosterone response. This appears to add to contradictory previous evidence. On one hand, rats injected with *Salmonella enteritidis* endotoxin on P3 and P5 showed a more rapid increase in plasma corticosterone in comparison with control rats, but corticosterone levels 2 h after *E. coli* LPS were similar in both groups [25]. On the other hand, it was previously shown that early life exposure to LPS could alter the adult febrile response to LPS [62]. Although the actual differences in these studies need to be further clarified, our results suggest a dose-specific programming of HPA axis function and/or a dose-specific development of tolerance in the peripheral systems the detect LPS. A similar effect is observed in the adult rat only after repeated LPS injections (6 consecutive days; [63]).

The behavioral effect of early postnatal LPS also appears to depend on the dose used. Animals injected with the highest dose of LPS showed reduced exploration in the OF, although this effect was sex- and pedigree-dependent. This result goes in line with a previous report showing that a postnatal immunological challenge increased anxiety-related behavior in the EPM in the adult rat [15]. However, it is interesting to note that the behavioral and corticoid responses appear to be dissociated from one another: lower neuroendocrine responses do not necessarily translate as lower anxiety, and, at least in this case, both responses go in opposite directions.

Other reports have shown contradictory results. For example, in rats, injection of LPS at P7, P14, P21 or P28 did not result in alterations of behavior in the EPM [64]. Moreover, rats exposed to LPS at P14 showed no behavioral differences in the OF and FS paradigms, although they showed reduced exploration when exposed to novel objects [65]. Diverse reasons can account for these different effects of postnatal LPS treatment. Firstly, various inflammatory stimuli have been used. Although cytokines and endotoxins all elicit an inflammatory response, these responses could be dissimilar in quantity and quality [1,66]. Secondly, both acute and long lasting effects of immune challenges have been shown to depend on their occurrence during prenatal and postnatal susceptibility windows [13,59,67].

The developmental processes underlying these effects are not yet fully understood. One possibility is that cytokines released following endotoxin exposure in early life can alter the HPA axis and contribute to behavioral programming. Various cytokines (e.g. IL-1β) are released from peritoneal macrophages after LPS administration, and they can activate the HPA axis [68] or induce brain cytokines [69]. Brain cytokines can in turn alter behavior (e.g. [70,71]). A previous report has shown that IL-1β expression is higher in the hippocampus of adult rats injected at P4 with live *E. coli* [14]. Further studies are required to evaluate the role of any specific cytokine on programming of adult behavior.

4.3. Maternal care × early postnatal LPS interactions

We found an *interaction between pedigree and early postnatal LPS* on the Time Spent in the Central Platform in the EPM and on Time Rearing in the OF. Moreover, we observed an *interaction between pedigree, early postnatal LPS and sex* on five variables: time grooming in the EPM, total distance and entries into the center in the OF, latency to eat in the NSF, and % immobility time in the TS.

In the EPM, the main effect of this interaction was the dose-dependent decrease of time in the central platform spent by mice of the Balb/c pedigree, which was not observed in mice of the C57 pedigree. This behavior has been previously considered a risk assessment coping strategy, typically observed in Balb/c mice. In the OF, C57 pedigree females showed an effect of early postnatal LPS on variables that revealed increased anxiety and decreased exploration, which was not observed in the other experimental groups. In the NSF, the lower dose of LPS had opposite effects on males of the Balb/c and C57 pedigree, increasing the latency to eat in the former. Finally, only males of the C57 pedigree showed a dose-dependent effect of early postnatal LPS on the immobility time in the TS, increasing this depression-related behavior. In summary, we found that early postnatal LPS treatment resulted in increased anxiety and reduced exploration, but that this effect was dependent on the sex and pedigree of the animals.

It could be argued that LPS-treated pups were taken care of differently by their moms, and then early postnatal LPS would be an additional modulator of maternal care. As each litter in our experimental paradigm contained both saline- and LPS-treated pups, we were precluded from observing direct maternal interactions with their pups. However, it was previously shown that, although maternal behavior changes after immune challenge of neonates, mothers do not discriminate between LPS- and saline-treated pups [23]. This observation, along with our finding that LPS-treated pups appear to compete equally well with saline-treated pups for milk (as revealed by a similar weight gain 24 h after LPS treatment) suggests that there are no significant differences in maternal behavior towards LPS-treated pups when compared with their saline-treated littermates.

We hypothesize that the fact that both maternal care and early postnatal LPS treatment can interact in order to influence adult behavior reveals that they are acting on the same brain structure that regulates these coping strategies. However, the present study cannot rule out that the manipulations employed here affect different brain structures that independently modulate these behaviors. A future area of research will be to search and identify whether such a structure exists and what are the mechanisms by which maternal care and early postnatal LPS affect it. Several neurotransmitters, cytokines and hormones can alter behavior; therefore an unbiased search for molecular mechanisms (e.g. microarrays or proteomics) in different brain regions appears as an appropriate strategy.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbr.2010.04.032.

References

[1] Karrow NA. Activation of the hypothalamic–pituitary–adrenal axis and autonomic nervous system during inflammation and altered programming of the neuroendocrine-immune axis during fetal and neonatal development: lessons learned from the model inflammagen, lipopolysaccharide. Brain Behav Immun 2006;20:144–58.
[2] Heim C, Nemeroff CB. The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. Biol Psychiatry 2001;49:1023–39.
[3] Agid O, Shapira B, Zislin J, Ritsner M, Hanin B, Murad H, et al. Environment and vulnerability to major psychiatric illness: a case control study of early parental loss in major depression, bipolar disorder and schizophrenia. Mol Psychiatry 1999;4:163–72.
[4] Chapman DP, Whitfield CL, Felitti VJ, Dube SR, Edwards VJ, Anda RF. Adverse childhood experiences and the risk of depressive disorders in adulthood. J Affect Disord 2004;82:217–25.
[5] Kendler KS, Prescott CA, Myers J, Neale MC. The structure of genetic and environmental risk factors for common psychiatric and substance use disorders in men and women. Arch Gen Psychiatry 2003;60:929–37.
[6] Weaver IC, Meaney MJ, Szyf M. Maternal care effects on the hippocampal transcriptome and anxiety-mediated behaviors in the offspring that are reversible in adulthood. Proc Natl Acad Sci USA 2006;103:3480–5.
[7] Romeo RD, Fossella JA, Bateup HS, Sisti HM, Brake WG, McEwen BS. Maternal separation suppresses TGF alpha mRNA expression in the prefrontal cortex of male and female neonatal C57BL/6 mice. Brain Res Dev Brain Res 2004;152:73–7.
[8] Caldji C, Diorio J, Anisman H, Meaney MJ. Maternal behavior regulates benzodiazepine/GABAA receptor subunit expression in brain regions associated with fear in BALB/c and C57BL/6 mice. Neuropsychopharmacology 2004;29:1344–52.
[9] Caldji C, Tannenbaum B, Sharma S, Francis D, Plotsky PM, Meaney MJ. Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. Proc Natl Acad Sci USA 1998;95:5335–40.
[10] Hava G, Vered L, Yael M, Mordechai H, Mahoud H. Alterations in behavior in adult offspring mice following maternal inflammation during pregnancy. Dev Psychobiol 2006;48:162–8.
[11] Shi L, Fatemi SH, Sidwell RW, Patterson PH. Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. J Neurosci 2003;23:297–302.
[12] Meyer U, Yee BK, Feldon J. The neurodevelopmental impact of prenatal infections at different times of pregnancy: the earlier the worse? Neuroscientist 2007;13:241–56.
[13] Meyer U, Nyffeler M, Engler A, Urwyler A, Schedlowski M, Knuesel I, et al. The time of prenatal immune challenge determines the specificity of inflammation-mediated brain and behavioral pathology. J Neurosci 2006;26:4752–62.
[14] Bilbo SD, Newsum NJ, Sprunger DB, Watkins LR, Rudy JW, Maier SF. Differential effects of neonatal handling on early life infection-induced alterations in cognition in adulthood. Brain Behav Immun 2007;21:332–42.
[15] Walker FR, March J, Hodgson DM. Endotoxin exposure in early life alters the development of anxiety-like behaviour in the Fischer 344 rat. Behav Brain Res 2004;154:63–9.
[16] Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, et al. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic–pituitary–adrenal responses to stress. Science 1997;277:1659–62.
[17] del Rey A, Furukawa H, Monge-Arditi G, Kabiersch A, Voigt KH, Besedovsky HO. Alterations in the pituitary-adrenal axis of adult mice following neonatal exposure to interleukin-1. Brain Behav Immun 1996;10:235–48.
[18] Anisman H, Zaharia MD, Meaney MJ, Merali Z. Do early-life events permanently alter behavioral and hormonal responses to stressors? Int J Dev Neurosci 1998;16:149–64.
[19] Barbazanges A, Vallee M, Mayo W, Day J, Simon H, Le Moal M, et al. Early and later adoptions have different long-term effects on male rat offspring. J Neurosci 1996;16:7783–90.
[20] Calatayud F, Belzung C. Emotional reactivity in mice, a case of nongenetic heredity? Physiol Behav 2001;74:355–62.
[21] Lehmann J, Feldon J. Long-term biobehavioral effects of maternal separation in the rat: consistent or confusing? Rev Neurosci 2000;11:383–408.
[22] Granger DA, Hood KE, Ikeda SC, Reed CL, Block ML. Neonatal endotoxin exposure alters the development of social behavior and the hypothalamic–pituitary–adrenal axis in selectively bred mice. Brain Behav Immun 1996;10:249–59.
[23] Hood KE, Dreschel NA, Granger DA. Maternal behavior changes after immune challenge of neonates with developmental effects on adult social behavior. Dev Psychobiol 2003;42:17–34.
[24] Shanks N, Larocque S, Meaney MJ. Neonatal endotoxin exposure alters the development of the hypothalamic–pituitary–adrenal axis: early illness and later responsivity to stress. J Neurosci 1995;15:376–84.
[25] Shanks N, Windle RJ, Perks PA, Harbuz MS, Jessop DS, Ingram CD, et al. Early-life exposure to endotoxin alters hypothalamic–pituitary–adrenal function and predisposition to inflammation. Proc Natl Acad Sci USA 2000;97:5645–50.
[26] Bumiller A, Gotz F, Rohde W, Dorner G. Effects of repeated injections of interleukin 1beta or lipopolysaccharide on the HPA axis in the newborn rat. Cytokine 1999;11:225–30.
[27] Witek-Janusek L. Pituitary–adrenal response to bacterial endotoxin in developing rats. Am J Physiol 1988;255:E525–30.
[28] Greenberg PE, Sisitsky T, Kessler RC, Finkelstein SN, Berndt ER, Davidson JR, et al. The economic burden of anxiety disorders in the 1990s. J Clin Psychiatry 1999;60:427–35.
[29] Scott J, Dickey B. Global burden of depression: the intersection of culture and medicine. Br J Psychiatry 2003;183:92–4.
[30] Kajantie E. Fetal origins of stress-related adult disease. Ann N Y Acad Sci 2006;1083:11–27.
[31] Depino AM, Tsetsenis T, Gross C. GABA homeostasis contributes to the developmental programming of anxiety-related behavior. Brain Res 2008;1210:189–99.
[32] Carola V, Gross C. BDNF moderates early environmental risk factors for anxiety in mouse. Genes Brain Behav 2010.
[33] Calatayud F, Coubard S, Belzung C. Emotional reactivity in mice may not be inherited but influenced by parents. Physiol Behav 2004;80:465–74.
[34] Barr CS, Newman TK, Schwandt M, Shannon C, Dvoskin RL, Lindell SG, et al. Sexual dichotomy of an interaction between early adversity and the serotonin transporter gene promoter variant in rhesus macaques. Proc Natl Acad Sci USA 2004;101:12358–63.
[35] McCormick CM, Smythe JW, Sharma S, Meaney MJ. Sex-specific effects of prenatal stress on hypothalamic–pituitary–adrenal responses to stress and brain glucocorticoid receptor density in adult rats. Brain Res Dev Brain Res 1995;84:55–61.
[36] Papaioannou A, Gerozissis K, Prokopiou A, Bolaris S, Stylianopoulou F. Sex differences in the effects of neonatal handling on the animal's response to stress and the vulnerability for depressive behaviour. Behav Brain Res 2002;129:131–9.
[37] Park MK, Hoang TA, Belluzzi JD, Leslie FM. Gender specific effect of neonatal handling on stress reactivity of adolescent rats. J Neuroendocrinol 2003;15:289–95.
[38] Smythe JW, McCormick CM, Rochford J, Meaney MJ. The interaction between prenatal stress and neonatal handling on nociceptive response latencies in male and female rats. Physiol Behav 1994;55:971–4.
[39] Spencer SJ, Boisse L, Mouihate A, Pittman QJ. Long term alterations in neuroimmune responses of female rats after neonatal exposure to lipopolysaccharide. Brain Behav Immun 2005.
[40] Weinberg J, Krahn EA, Levine S. Differential effects of handling on exploration in male and female rats. Dev Psychobiol 1978;11:251–9.
[41] Cameron OG, Hill EM. Women and anxiety. Psychiatr Clin North Am 1989;12:175–86.
[42] Maier W, Gansicke M, Gater R, Rezaki M, Tiemens B, Urzua RF. Gender differences in the prevalence of depression: a survey in primary care. J Affect Disord 1999;53:241–52.
[43] Ragueneau S. Early development in mice. IV: Quantity and gross composition of milk in five inbred strains. Physiol Behav 1987;40:431–5.
[44] Levine S. The ontogeny of the hypothalamic–pituitary–adrenal axis. The influence of maternal factors. Ann NY Acad Sci 1994;746:275–88 [discussion 89–93].
[45] Macri S, Mason GJ, Wurbel H. Dissociation in the effects of neonatal maternal separations on maternal care and the offspring's HPA and fear responses in rats. Eur J Neurosci 2004;20:1017–24.
[46] Zaharia MD, Kulczycki J, Shanks N, Meaney MJ, Anisman H. The effects of early postnatal stimulation on Morris water-maze acquisition in adult mice: genetic and maternal factors. Psychopharmacology (Berl) 1996;128:227–39.
[47] Ladd CO, Thrivikraman KV, Huot RL, Plotsky PM. Differential neuroendocrine responses to chronic variable stress in adult Long Evans rats exposed to handling-maternal separation as neonates. PNEC 2005;30:520–33.
[48] Priebe K, Romeo RD, Francis DD, Sisti HM, Mueller A, McEwen BS, et al. Maternal influences on adult stress and anxiety-like behavior in C57BL/6J and BALB/cJ mice: a cross-fostering study. Dev Psychobiol 2005;47:398–407.
[49] Vallee M, Mayo W, Dellu F, Le Moal M, Simon H, Maccari S. Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: correlation with stress-induced corticosterone secretion. J Neurosci 1997;17:2626–36.
[50] Roy V, Merali Z, Poulter MO, Anisman H. Anxiety responses, plasma corticosterone and central monoamine variations elicited by stressors in reactive and nonreactive mice and their reciprocal F1 hybrids. Behav Brain Res 2007;185:49–58.
[51] Griebel G, Belzung C, Perrault G, Sanger DJ. Differences in anxiety-related behaviours and in sensitivity to diazepam in inbred and outbred strains of mice. Psychopharmacology (Berl) 2000;148:164–70.

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[52] Crawley JN, Belknap JK, Collins A, Crabbe JC, Frankel W, Henderson N, et al. Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. Psychopharmacology (Berl) 1997;132:107–24.
[53] Rogers DC, Jones DN, Nelson PR, Jones CM, Quilter CA, Robinson TL, et al. Use of SHIRPA and discriminant analysis to characterise marked differences in the behavioural phenotype of six inbred mouse strains. Behav Brain Res 1999;105:207–17.
[54] Trullas R, Skolnick P. Differences in fear motivated behaviors among inbred mouse strains. Psychopharmacology (Berl) 1993;111:323–31.
[55] Espejo EF. Effects of weekly or daily exposure to the elevated plus-maze in male mice. Behav Brain Res 1997;87:233–8.
[56] Carola V, D'Olimpio F, Brunamonti E, Mangia F, Renzi P. Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. Behav Brain Res 2002;134:49–57.
[57] Sapolsky RM, Meaney MJ. Maturation of the adrenocortical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. Brain Res 1986;396:64–76.
[58] Walker CD, Perrin M, Vale W, Rivier C. Ontogeny of the stress response in the rat: role of the pituitary and the hypothalamus. Endocrinology 1986;118:1445–51.
[59] Schmidt MV, Enthoven L, van der Mark M, Levine S, de Kloet ER, Oitzl MS. The postnatal development of the hypothalamic–pituitary–adrenal axis in the mouse. Int J Dev Neurosci 2003;21:125–32.
[60] Shanks N, Meaney MJ. Hypothalamic–pituitary–adrenal activation following endotoxin administration in the developing rat: a CRH-mediated effect. J Neuroendocrinol 1994;6:375–83.
[61] Besedovsky HO, del Rey A, Klusman I, Furukawa H, Monge Arditi G, Kabiersch A. Cytokines as modulators of the hypothalamus–pituitary–adrenal axis. J Steroid Biochem Mol Biol 1991;40:613–8.
[62] Boisse L, Mouihate A, Ellis S, Pittman QJ. Long-term alterations in neuroimmune responses after neonatal exposure to lipopolysaccharide. J Neurosci 2004;24:4928–34.
[63] Takemura T, Makino S, Takao T, Asaba K, Suemaru S, Hashimoto K. Hypothalamic–pituitary–adrenocortical responses to single vs. repeated endotoxin lipopolysaccharide administration in the rat. Brain Res 1997;767:181–91.
[64] Spencer SJ, Martin S, Mouihate A, Pittman QJ. Early-life immune challenge: defining a critical window for effects on adult responses to immune challenge. Neuropsychopharmacology 2006;31:1910–8.
[65] Spencer SJ, Heida JG, Pittman QJ. Early life immune challenge—effects on behavioural indices of adult rat fear and anxiety. Behav Brain Res 2005;164:231–8.
[66] Elin RJ, Sandberg AL, Rosentreich DL. Comparison of the pyrogenicity, Limulus activity mitogenicity and complement reactivity of several bacterial endotoxins and related compounds. J Immunol 1976;117:1238–42.
[67] Meyer U, Nyffeler M, Yee BK, Knuesel I, Feldon J. Adult brain and behavioral pathological markers of prenatal immune challenge during early/middle and late fetal development in mice. Brain Behav Immun 2008;22:469–86.
[68] Tilders FJ, DeRijk RH, Van Dam AM, Vincent VA, Schotanus K, Persoons JH. Activation of the hypothalamus–pituitary–adrenal axis by bacterial endotoxins: routes and intermediate signals. PNEC 1994;19:209–32.
[69] Pitossi F, del Rey A, Kabiersch A, Besedovsky H. Induction of cytokine transcripts in the central nervous system and pituitary following peripheral administration of endotoxin to mice. J Neurosci Res 1997;48:287–98.
[70] Cunningham C, Campion S, Teeling J, Felton L, Perry VH. The sickness behaviour and CNS inflammatory mediator profile induced by systemic challenge of mice with synthetic double-stranded RNA (poly I:C). Brain Behav Immun 2007;21:490–502.
[71] Depino AM, Alonso M, Ferrari C, del Rey A, Anthony D, Besedovsky H, et al. Learning modulation by endogenous hippocampal IL-1: blockade of endogenous IL-1 facilitates memory formation. Hippocampus 2004;14:526–35.