Molecular Psychiatry (2008) 1–12

www.nature.com/mp

ORIGINAL ARTICLE

Ontogeny of sensorimotor gating and immune impairment induced by prenatal immune challenge in rats: implications for the etiopathology of schizophrenia

E Romero[1,3], C Guaza[1], B Castellano[2] and J Borrell[1]

[1]*Group of Neuroimmunology, Functional and Systems Neurobiology Department, Instituto Cajal, Consejo Superior de Investigaciones Científicas, Madrid, Spain and* [2]*Unit of Histology, Faculty of Medicine, Autonomous University of Barcelona, Bellaterra, Spain*

It has been hypothesized that the maternal immune response to infection may influence fetal brain development and lead to schizophrenia. Animal experimentation has supported this notion by demonstrating altered sensorimotor gating (prepulse inhibition, PPI) in adult rats prenatally exposed to an immune challenge. In the present study, pregnant rats were exposed to the bacterial endotoxin lipopolysaccharide (LPS) throughout gestation and the offspring were examined by evaluating the PPI, dopaminergic function, brain protein expression and cytokine serum levels from weaning to late adulthood. Prenatal LPS exposure induced a deficit in PPI that emerged at 'puberty' and that persisted throughout adult life. This prenatal insult caused age-specific changes in accumbal dopamine levels and in synaptophysin expression in the frontal cortex. Moreover, serum cytokine levels were altered in an age- and cytokine-dependent manner. Here we show that prenatal LPS administration throughout pregnancy causes maturation-dependent PPI deficits and age-dependent alterations in dopamine activity, as well as in synaptophysin expression and cytokine levels.
Molecular Psychiatry advance online publication, 15 April 2008; doi:10.1038/mp.2008.44

Keywords: LPS; schizophrenia; prepulse inhibition; cytokine; dopamine; neurodevelopment

Introduction

Schizophrenia is a frequent and disabling psychiatric disorder that emerges in early adulthood, more frequently and with more severe effects in men than women.[1] Sensorimotor gating deficits are among the most robust features found in these patients and their relatives.[2] The etiology of schizophrenia is thought to be associated with disturbances during the development of the nervous system that usually remain silent until puberty, suggesting that postnatal brain maturation precipitates the emergence of the illness. Thus, rather than the consequences of an early acquired 'static' brain lesion, it is currently thought that adolescent brain maturation could interact with pre-existing developmental anomalies, thereby contributing to the pathophysiology of schizophrenia.[3]

Correspondence: Professor J Borrell, Group of Neuroimmunology, Functional and Systems Neurobiology Department, Instituto Cajal, Consejo Superior de Investigaciones Científicas Avda Doctor Arce 37, Madrid 28002, Spain.
E-mail: borrell@cajal.csic.es

[3]Current address: Institut de Génomique Fonctionnelle de Lyon, UMR CNRS 5242 INRA 1288, IFR128, Ecole Normale Supérieure de Lyon, 46 allée d'Italie, 69364 Lyon cedex 07, France.
Received 2 August 2007; revised 20 March 2008; accepted 24 March 2008

Maternal exposure to infection during pregnancy has been associated with an increased risk of offspring developing schizophrenia.[4] Although the mechanisms underlying this epidemiological relationship remain unclear, the maternal cytokine-associated inflammatory response to infection may be a crucial link, as the identity of the pathogen seems irrelevant.[5–8] Interestingly, maternal serum levels of the cytokine tumor necrosis factor-α (TNF-α)[7] or interleukin (IL)-8[5] are elevated in mothers of patients with schizophrenia. In addition to their immunological roles,[9] cytokines have been shown to affect the survival,[10] differentiation[11] and morphology[12,13] of developing neural cells. Thus, intense variations in the levels of inflammatory cytokines in the fetal environment may therefore adversely affect the development of the nervous system, and contribute to the development of future psychobehavioral and/or cognitive impairments.[14–21] The pattern of immune development can also be modulated by maternal immune events during gestation[22] and indeed, it has been suggested that postnatal immune responses are programmed *in utero*.[23]

Systemic administration of lipopolysaccharide (LPS) is a widely accepted model for emulating immune activation and it is known to release peripheral immunoregulatory cytokines[24] and also to stimulate cytokine expression in the central nervous

system.[25] We recently reported that maternal LPS-induced immune activation in rats produces prepulse inhibition (PPI) and immune disturbances in their adult progeny, which can be ameliorated by antipsychotics.[14,20] The present study was designed to assess the developmental time course of such prenatal LPS-induced pathologies.

Materials and methods

Animals and prenatal treatment

All procedures involved animals and their care complied with national and international laws and guidelines. In this study, Wistar rats from our in-house colony were used and they were kept under standard conditions ($22 \pm 2\,^{\circ}C$, 12:12 h light/dark cycle; lights on at 0700 hours), receiving food and water *ad libitum*.

Vaginal smears were taken daily from nulliparous female rats and when in estrus, they were coupled with male rats and coitus was determined the next morning by the presence of sperm in the vaginal smear (= day 0 of pregnancy). LPS dissolved in saline (LPS from *Escherichia coli*, Sigma L-3755 Serotype 026:B6) was administered subcutaneously to pregnant rats ($n = 16$) at a dose of $2\,mg\,kg^{-1}$, in a volume of $1.5\,ml\,kg^{-1}$, between 0900 and 1100 hours. Injections were given daily from days 1 to 21 of pregnancy and if a dam had not delivered before 1100 hours on day 22, an additional injection was administered. The control group consisted of pregnant rats ($n = 14$) that were administered saline alone following the same schedule. Animals were submitted to LPS or saline treatment at different seasons of the year during 18 months. The offspring of the LPS or saline-treated dams were used, they were weaned at 21 days of age and were caged in groups of three animals of the same sex that had been administered the same prenatal treatment. Rats from at least three different litters were used in each experiment. At least 5 animals per age and prenatal treatment conditions were used in the different analysis (see Table and Figure legends for detailed sample size). All rats were weighed immediately after the PPI test and then returned to their cage. Animals of 35, 70, 170 and 400 days of age were killed 96 h after the PPI testing and their brains were removed and their blood was collected to contribute to the biochemical and cytokine assays. It should be noted that PPI was not tested in 21-day-old but in 28-day-old rats for apparatus sensitivity reasons. The data with regard to the biochemical and cytokine assays appearing throughout the text as corresponding to the 70-, 170- and 400-day-old rats actually correspond to that of the 74-, 174- and 404-day-old rats, respectively.

PPI testing

The acoustic startle response was tested in a startle chamber on rats of the ages indicated using previously described methods.[14] After an initial 5-min period of accommodation to a 46 dB background noise (continuous throughout the session), 30 startle eliciting stimuli were presented (100 dB(A)). These initial startle pulses lead to some adaptation, which reduces the variability and stabilizes the baseline startle amplitude,[26] and thus, they were not used in the estimation of PPI. For PPI testing, 30 s after the last initial startle stimulus, 23 additional 100 dB(A) startle stimuli were given, consisting of 8 startle stimuli alone (pulse alone trials); 5 startle stimuli after an auditory prepulse of 6 dB above the background noise; 5 startle stimuli after an auditory prepulse of 12 dB above the background noise and 5 startle stimuli after a visual prepulse. The startle stimuli were given at a fixed interstimulus interval of 20 s and the type of trial to which the animals were subjected was semirandom, with the restriction that each type of trial had to occur in every block of three trials. The extent of PPI is expressed as the percentage decrease in the amplitude of the startle response caused by presentation of the prepulse (%PPI), according to the formula: (100−(startle amplitude on prepulse trials/startle amplitude on pulse alone trials) × 100).

Biochemical analysis of dopamine and its metabolites

Rats were killed by decapitation and their nucleus accumbens (NAC), striatum and frontal cortex were quickly dissected from the brain and stored at $-80\,^{\circ}C$ until they were assayed. The concentrations of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured in the NAC and striatum by HPLC using previously described methods.[14]

Immunoblotting

Frontal cortex tissue from LPS and control rats was homogenized in 500 µl of ice-cold 10 mM HEPES, pH 7.6, containing 1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 1 mM sodium orthovanadate, 2 mM NaF, 5 mM DTT and a mixture of protease inhibitors (CompleteTM, Roche Applied Science, Barcelona, Spain). Aliquots of the extracts (10 µg) were resolved on 10% SDS-polyacrylamide gels and transferred to nitrocellulose membranes (Amersham Biosciences, Barcelona, Spain). The membranes were blocked in 25 mM Tris, 137 mM NaCl, pH 7.4, 0.1% Tween-20, 5% dry skimmed milk and then incubated with the primary monoclonal antibodies against: synaptophysin (1:7000; Sigma, St Louis, MO, USA); GAP-43 (1:1500; Sigma); glycogen-synthase-kinase-3β (GSK-3β) (1:5000; Sigma) and α-tubulin (1:30 000; Sigma). After extensive washing, the membranes were incubated with a peroxidase-conjugated anti-mouse (Bio-Rad, Hercules, CA, USA) antibody and the protein bands were visualized by enhanced chemiluminescence (Amersham Biosciences). Finally, the films were scanned and quantified by densitometry (using a GS-800 Calibrated Densitometer, Bio-Rad), and the protein expression was normalized to that of α-tubulin. The values are expressed as the mean ± s.e.m. of three independent experiments performed.

Cytokine and corticosterone measurements
After decapitation, trunk blood was collected from each animal, and the serum was prepared by centrifugation at 15 000 *g* for 5 min and stored at −80 °C until it was assayed. Cytokine concentrations were determined using commercial ELISA kits in accordance with the manufacturer's instructions (BioSource International, Camarillo, CA, USA). Corticosterone was measured using a solid phase ^{125}I radioimmunoassay (Coat-A-Count Rat Corticosterone kit, Diagnostic Products Corp., Los Angeles, CA, USA); the detection limit was 5.7 ng ml^{-1}. All samples and standards were assayed in duplicate.

Statistical analysis
All values are reported as the mean ± s.e.m. Statistical analysis was performed using the SPSS package, applying the Student's *t*-test for pairs and analysis of variance (ANOVA), followed by the Student–Newman–Keuls *t*-test where appropriate for multigroup comparisons. The level of significance was set at $P<0.05$.

Results

Litter size and postnatal weights
No significant effect of prenatal LPS exposure on litter size was observed, neither in the number of male offspring (control: 4.8 ± 0.5; LPS: 3.7 ± 0.4, $F(1,44)=2996$; $P=0.091$) nor in the number of female offspring (control: 5 ± 0.6; LPS: 4.2 ± 0.4, $F(1,44)=1.451$; $P=0.234$). Analysis of offspring weight data at the time of testing revealed significant effect of sex ($F(1,233)=540.001$; $P<0{,}001$), with male offspring weighing significantly more than female offspring, and of age ($F(4,233)=1302.248$; $P<0{,}001$), with older animals weighing more than the younger ones. A significant sex/age interaction was observed ($F(4,233)=116.935$; $P<0{,}001$), weight increase with age was more pronounced in male than in female rats. Prenatal treatment failed to show a significant effect on the weight of the animals at any of the ages analyzed ($F(1,233)=2.405$; $P=0{,}122$, NS) (data not shown).

Developmental time course of PPI
To determinate whether the emergence of a sensorimotor gating deficit was induced by prenatal LPS administration, male and female offspring of rats exposed to LPS or saline throughout pregnancy were tested for PPI at 28, 35, 70, 170 and 400 days of age (Figure 1). ANOVA of the data revealed a significant effect of age ($F(4,230)=12.440$, $P<0.001$) since 28- and 35-day-old rats showed weaker PPI than older animals ($P<0.001$ for each comparison). As reported previously,[14,20] prenatal LPS treatment significantly disrupted PPI ($F(1,230)=48.820$, $P<0.001$). However, although the sex of the animal failed to produce any significant difference in PPI ($F(1,230)=0.054$, $P=$ NS), a significant sex/prenatal treatment interaction was observed ($F(1,230)=4.224$, $P=0.041$), as PPI deficit induced by LPS was more prominent and frequently observed in male than in female rats.

Post-hoc analysis of the data with regard to the PPI induced by the 6 dB prepulse type, revealed that LPS exposed male rats displayed significantly lower PPI than the corresponding controls at the ages of 70, 170 and 400 days ($P=0.001$, $P=0.005$ and $P<0.001$, respectively). Similarly, PPI was weaker in female rats exposed to LPS than in controls at the ages of 35 ($P<0.001$), 170 ($P=0.005$) and 400 ($P<0.001$) days. With regard to the 12 dB prepulse, the PPI values from LPS-exposed male rats were significantly weaker than in controls at the ages of 35 ($P=0.032$), 70 ($P=0.007$), 170 ($P=0.002$) and 400 ($P<0.001$) days, whereas in LPS-exposed female rats this prepulse only significantly impaired the PPI in animals aged 35 ($P=0.017$) and 400 days ($P=0.049$). With respect to the visual prepulse, both LPS-exposed male and female rats displayed a weaker PPI than the corresponding controls at the ages of 35 ($P=0.015$ in male rats and $P=0.012$ in female rats), 170 ($P=0.005$ in male rats, and $P=0.045$ in female rats) and 400 days ($P<0.001$ male rats, and $P=0.002$ female rats), whereas only male rats displayed a significant decrease at 70 days ($P=0.044$).

Monoamines in the NAC and striatum of rats prenatally exposed to LPS
The levels of DA and its metabolites were measured in male offspring (21, 39, 70, 170 and 400 days) of dams exposed to LPS or saline during pregnancy (Table 1).

In the NAC, a significant effect of age on DA ($F(3,83)=22.404$, $P<0.001$), DOPAC ($F(3,83)=10.612$, $P<0.001$) and HVA ($F(3,83)=7.892$, $P<0.001$) content was observed. Post-hoc analysis revealed that the tissue levels of DA were higher in 170-day-old rats than in other age groups ($P<0.001$ for each comparison). Similarly, DOPAC accumbal levels were higher in 170-day-old rats than in other age groups ($P=0.003$ vs 21 days, $P<0.001$ vs 39, 70 and 400 days), although they were lower in 70-day-old than in 21-day-old animals ($P<0.001$). Finally, the tissue levels of HVA were highest in 170-day-old rats ($P=0.004$ vs 21 days, $P<0.001$ vs 39, 70, 400 days) followed by the 21-day-old animals ($P=0.001$ vs 39- and 70-day-old, $P=0.022$ vs 400-day-old rats). Interestingly, there was a significant effect of the treatment on DA content ($F(3,83)=11.467$, $P<0.001$), whereas the treatment failed to show any significant effect on the accumulation of DA metabolites. Post-hoc analysis revealed that the DA concentration was lower in 39-day-old LPS-exposed animals than in the corresponding controls ($P=0.041$). However, the DA content in 170- and 400-day-old animals exposed to LPS was higher than in age-matched control rats ($P=0.007$ and $P=0.014$, respectively). Moreover, a significant interaction between age and prenatal treatment was observed for DA ($F(4,83)=6.192$, $P<0.001$) and DOPAC levels ($F(4,83)=2.952$, $P=0.025$), particularly due to a more prominent effect of age in the LPS than in the control animals (Table 1).

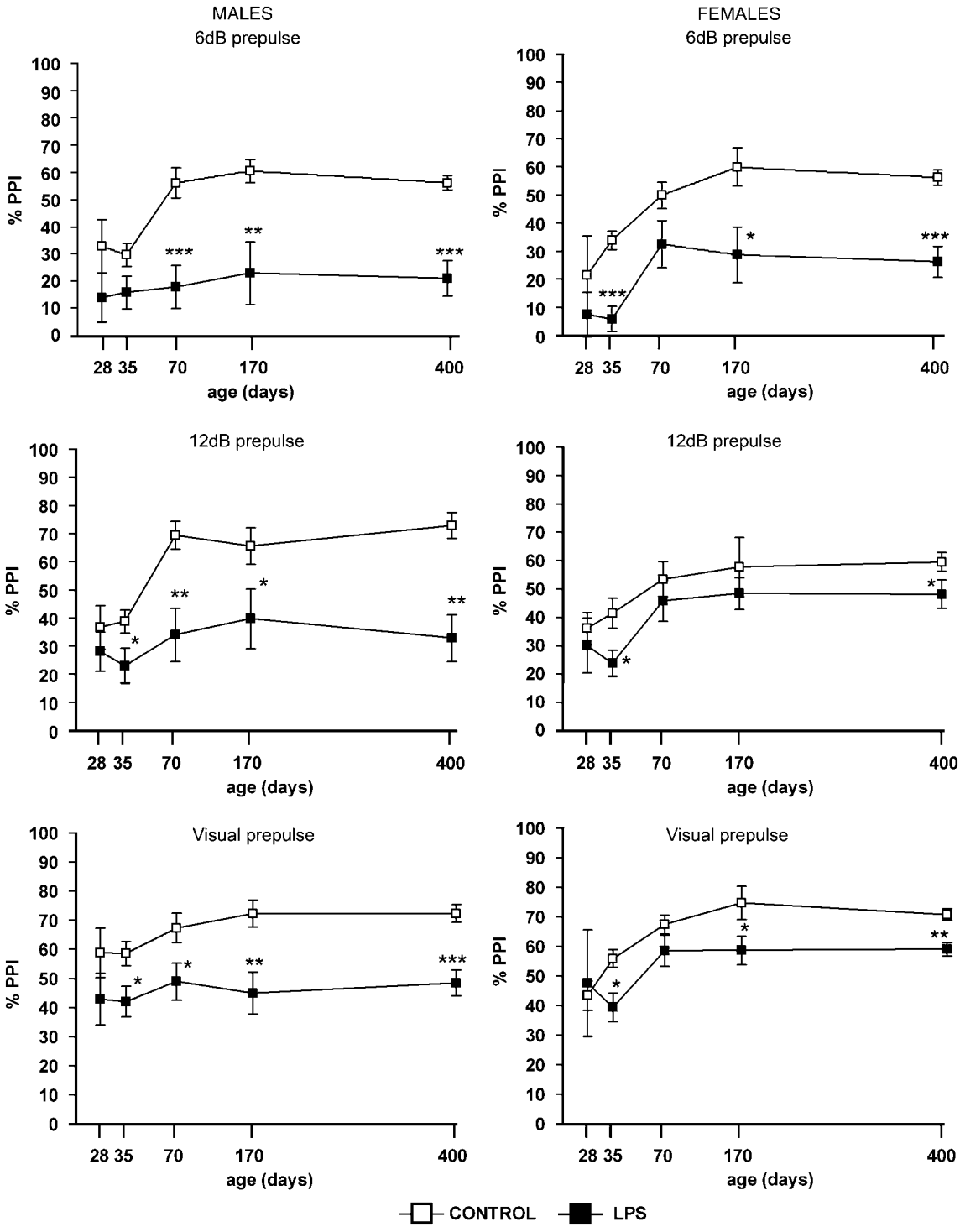

Figure 1 Effect of prenatal lipopolysaccharide (LPS) exposure on auditory (6 or 12 dB above background noise) and visual prepulse inhibition (PPI) in male and female rats (at 28, 39, 70, 170 or 400 days of age). Control group consisted of offspring of dams treated with saline throughout pregnancy. The results are expressed as the mean ± s.e.m. ($n = 7 \pm 2$ rats per group aged 28 days; $n = 25 \pm 4$ rats per group aged 35 days; $n = 15 \pm 4$ rats per group aged 70 days; $n = 9 \pm 1$ rats per group aged 170 days and $n = 7 \pm 1$ rats per group aged 400 days). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs corresponding control group (analysis of variance (ANOVA) followed by Student–Newman–Keuls *t*-test).

In the striatum, a significant effect of age was observed on DA, DOPAC and HVA content (DA: ($F(3,95) = 40.011$, $P < 0.001$); DOPAC: ($F(3,95) = 23.686$, $P < 0.001$); HVA: ($F(3,95) = 17.730$, $P < 0.001$)). Post-hoc analysis revealed that the accumulation of DA was higher in 170-day-old rats than in other age groups ($P < 0.001$ for each comparison). The lowest tissue level of DA was found in 21-day-old animals followed

Table 1 Tissue concentration of DA, DOPAC and HVA (pg μg^{-1} protein), in the NAC and striatum of 21-, 39-, 70-, 170- or 400-day-old male rats exposed to LPS or saline (controls) throughout gestation

			21 days	*39 days*	*70 days*	*170 days*	*400 days*
NAC	DA	Control	92.2 ± 10.9	118.3 ± 15.7	79.6 ± 11.6	176.2 ± 29.4	117.4 ± 22.1
		LPS	113.2 ± 12.3	72.1 ± 11.4*	94.4 ± 13.1	287.5 ± 15.4**	191.5 ± 16.4*
	DOPAC	Control	40.2 ± 9.8	35.9 ± 9.0	16.8 ± 2.9	57.6 ± 9.4	21.9 ± 3.9
		LPS	40.9 ± 7.5	16.9 ± 3.0	17.9 ± 2.1	71.2 ± 6.6	47.4 ± 8.0
	HVA	Control	14.1 ± 2.3	11.5 ± 2.4	6.8 ± 1.1	23.2 ± 2.9	9.6 ± 2.6
		LPS	20.9 ± 3.1	6.9 ± 1.3	7.9 ± 1.5	27.3 ± 4.2	14.7 ± 2.0
Striatum	DA	Control	89.7 ± 15.2	210.1 ± 30.0	340.5 ± 33.0	480.8 ± 33.1	383.0 ± 54.5
		LPS	114.8 ± 9.4	210.1 ± 19.8	288.2 ± 30.5	554.7 ± 46.1	378.7 ± 20.8
	DOPAC	Control	29.2 ± 5.7	50.3 ± 6.6	40.7 ± 4.3	81.6 ± 6.1	43.6 ± 5.8
		LPS	30.6 ± 2.4	39.2 ± 7.4	60.7 ± 8.5	113.7 ± 7.1	40.0 ± 3.7
	HVA	Control	15.9 ± 3.4	17.4 ± 3.5	21.4 ± 2.9	58 ± 6.5	32.2 ± 9.9
		LPS	22.0 ± 3.1	15.1 ± 2.6	18.2 ± 2.9	80 ± 11.2	30.5 ± 7.1

Abbreviations: DA, dopamine; DOPAC, dihydroxyphenylacetic acid; HVA, homovanillic acid; LPS, lipopolysaccharide; NAC, nucleus accumbens.
Results are expressed as the mean ± s.e.m. ($n = 10 \pm 2$ rats per group aged 21, 39, 70 and 400 days; $n = 5$–6 rats per group aged 170 days). *$P < 0.05$, ** $P < 0.01$ vs control group (ANOVA followed by Student–Newman–Keuls *t*-test).

by 39-day-old rats ($P < 0.001$ for each comparison). DOPAC levels were higher in 170-day-old rats than in the other age groups ($P < 0.001$ for each comparison) and lower in 21-day-old than in older rats ($P = 0.009$ vs 39-day-old, $P < 0.001$ vs 70- and 170-day-old, and $P = 0.035$ vs 400-day-old rats). HVA tissue levels were highest in 170-day-old rats ($P < 0.001$ for each comparison) followed by 400-day-old animals ($P = 0.026$ vs 21-day-old, $P = 0.007$ vs 39-day-old and $P = 0.034$ vs 70-day-old animals). There was no significant effect of the treatment; however, a significant interaction between age/prenatal treatment was observed for DOPAC levels ($F(4,95) = 3.435$, $P = 0.011$) due to a more prominent effect of age in the LPS than in the control groups.

Protein expression changes in the frontal cortex of male rats exposed prenatally to LPS

We assessed the effects of prenatal exposure to LPS on the expression of a series of proteins that may influence the activity of the frontal cortex. Accordingly, we analyzed the expression of synaptophysin, an integral membrane protein of the synaptic vesicle involved in neurotransmitter release,[27] of the growth-associated protein GAP-43, a presynaptic membrane protein concentrated in growth cones and axons[28] and of GSK-3β, a central component of the developmental Wnt signaling pathway.[29] We detected age-dependent changes in synaptophysin expression of LPS rats, and the expression of this presynaptic protein was lower in rats exposed prenatally to LPS than in control animals at 21 (~28%; $P < 0.05$) and 400 (~21%; $P < 0.05$) days of age (Figure 2). In contrast, synaptophysin expression was higher in 170-day-old animals prenatally exposed to LPS than in controls (~75%; $P < 0.001$).

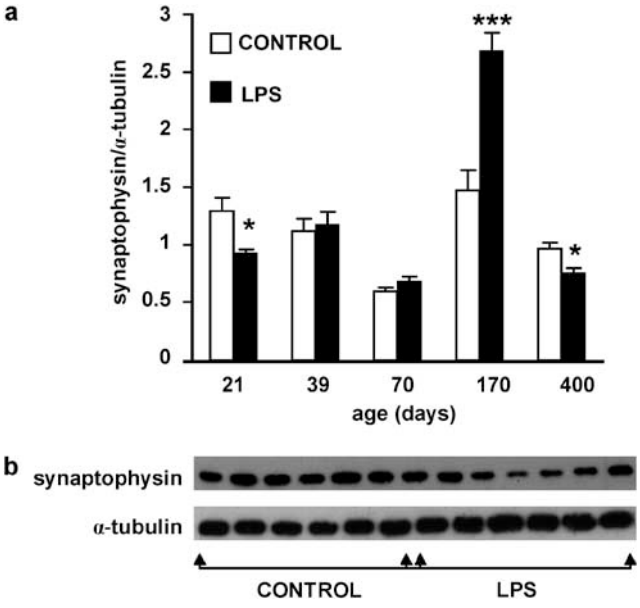

Figure 2 Expression of synaptophysin in the frontal cortex of male rats aged 21, 35, 70, 170 or 400 days exposed prenatally to lipopolysaccharide (LPS) or saline. (**a**) Densitometric analysis for synaptophysin (normalized to α-tubulin) represented as the mean ± s.e.m relative optical density corresponding to six LPS offspring and 6 control offspring. Each sample was assayed three times. (**b**) Representative western blots for synaptophysin and α-tubulin in the frontal cortex of 400-day-old LPS exposed ($n = 6$) and control offspring ($n = 6$). *$P < 0.05$, ***$P < 0.001$ vs control rats (Student's *t*-test).

No changes were observed in GAP-43 expression in the frontal cortex of LPS-exposed animals at any age evaluated (data not shown). However, the expression of GSK-3β diminished significantly in 400-day-old

rats prenatally exposed to LPS when compared to controls (~21%; $P<0.05$). In contrast, no difference in GSK-3β expression was found in LPS rats younger than 400 days (Figure 3).

Serum corticosterone level

Serum corticosterone level was measured in the male offspring of saline- and LPS-treated dams at 21, 39, 70, 170 and 400 days of age (Table 2). No significant effect of age was observed ($F(4,68)=0.73$, $P=$NS). Prenatally LPS-treated male rats displayed slightly higher corticosterone levels than controls at the age of 70 and 170 days without reaching the criteria for significance ($F(1,68)=2.773$, $P=$NS).

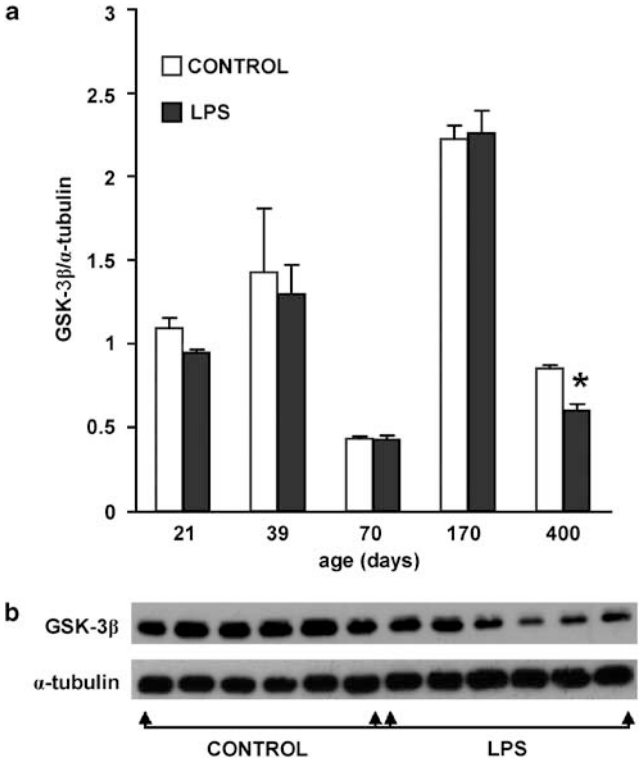

Figure 3 Expression of glycogen-synthase-kinase-3β (GSK-3β) in the frontal cortex of male rats aged 21, 39, 70, 170 or 400 days exposed prenatally to lipopolysaccharide (LPS) or saline. (**a**) Densitometric analysis for GSK-3β (normalized to α-tubulin) represented as the mean ± s.e.m relative optical density corresponding to six LPS offspring and six control offspring. Each sample was assayed three times. (**b**) Representative western blots for GSK-3β and α-tubulin in the frontal cortex of 400-day-old LPS ($n=6$) and control offspring ($n=6$). *$P<0.05$ vs control rats (Student's *t*-test).

Time course of the effect of LPS on serum cytokine levels

In order to profile the effect of maternal immune activation on serum cytokine levels from prepuberty until senescence, we measured the serum concentrations of IL-1β, IL-2, IL-6 and TNF-α from male offspring of dams exposed to LPS or saline throughout pregnancy, at the ages of 21, 39, 70, 170 and 400 days. We were unable to detect a significant effect of age ($F(4,90)=1.637$, $P=$NS) or prenatal LPS treatment ($F(1,125)=0.407$, $P=$NS) on the serum IL-1β levels (Figure 4a). However, a significant effect of age ($F(4,90)=4.660$, $P=0.002$), prenatal treatment ($F(1,145)=28.337$, $P<0.001$) and an age/treatment interaction ($F(1,145)=13.031$, $P<0.001$) was observed for the serum IL-2 concentrations. Indeed, 170-day-old control animals showed higher IL-2 levels than 21- ($P<0.001$), 39- ($P<0.001$), 70- ($P=0.060$) and 400-day-old ($P=0.020$) animals (Figure 4b). Moreover, LPS-exposed rats aged 70 and 170 days showed higher IL-2 levels than the corresponding control animals ($P<0.001$ vs both ages). Similarly, there was a significant effect of age ($F(4,90)=5.510$, $P=0.001$), treatment ($F(1,133)=21.985$, $P<0.001$) and an age/treatment interaction ($F(4,133)=27.618$, $P<0.001$) on IL-6 serum concentrations. Control animals aged 170 days showed higher IL-6 levels than at 21 ($P<0.001$), 39 ($P=0.020$), 70 ($P<0.001$) and 400 days of age ($P=0.008$). Furthermore, LPS-exposed rats aged 170 days displayed higher serum levels of this cytokine than the corresponding controls ($P<0.001$; Figure 4c). Finally, there was no significant effect of age on the serum TNF-α levels ($F(4,90)=1.790$, $P=$NS), although a significant effect of treatment ($F(1,143)=205.673$, $P<0.001$) and an age/treatment interaction ($F(4,143)=10.729$, $P<0.001$) was observed for this cytokine. LPS-exposed male animals showed higher levels of TNF-α than the corresponding controls at all ages evaluated ($P<0.001$ for each comparison; Figure 4d).

In order to identify a potential sexual dimorphism in the effect of prenatal LPS exposure on serum cytokines, we also measured the levels of IL-1β, IL-6 and TNF-α in the serum from female offspring at the ages of 39, 70, 170 and 400 days

Table 2 Serum concentration of corticosterone (pg ml^{-1}), in 21-, 39-, 70-, 170- or 400-day-old male rats exposed to LPS or saline (controls) throughout gestation

	21 days	*39 days*	*70 days*	*170 days*	*400 days*
Control	195.5 ± 69.6	123.2 ± 34	135.3 ± 33.2	114.7 ± 22.1	298.2 ± 84
LPS	223.8 ± 68.9	201.3 ± 51.7	265.4 ± 40.1	240.1 ± 43.9	186.3 ± 53.5

Abbreviation: LPS, lipopolysaccharide.
Results are expressed as the mean ± s.e.m. ($n=10\pm2$ rats per group aged 21 and 39 days; $n=17$–18 rats per group aged 70 days; $n=26$–12 rats per group aged 170 days and n = 6–9 rats per group aged 170 days).

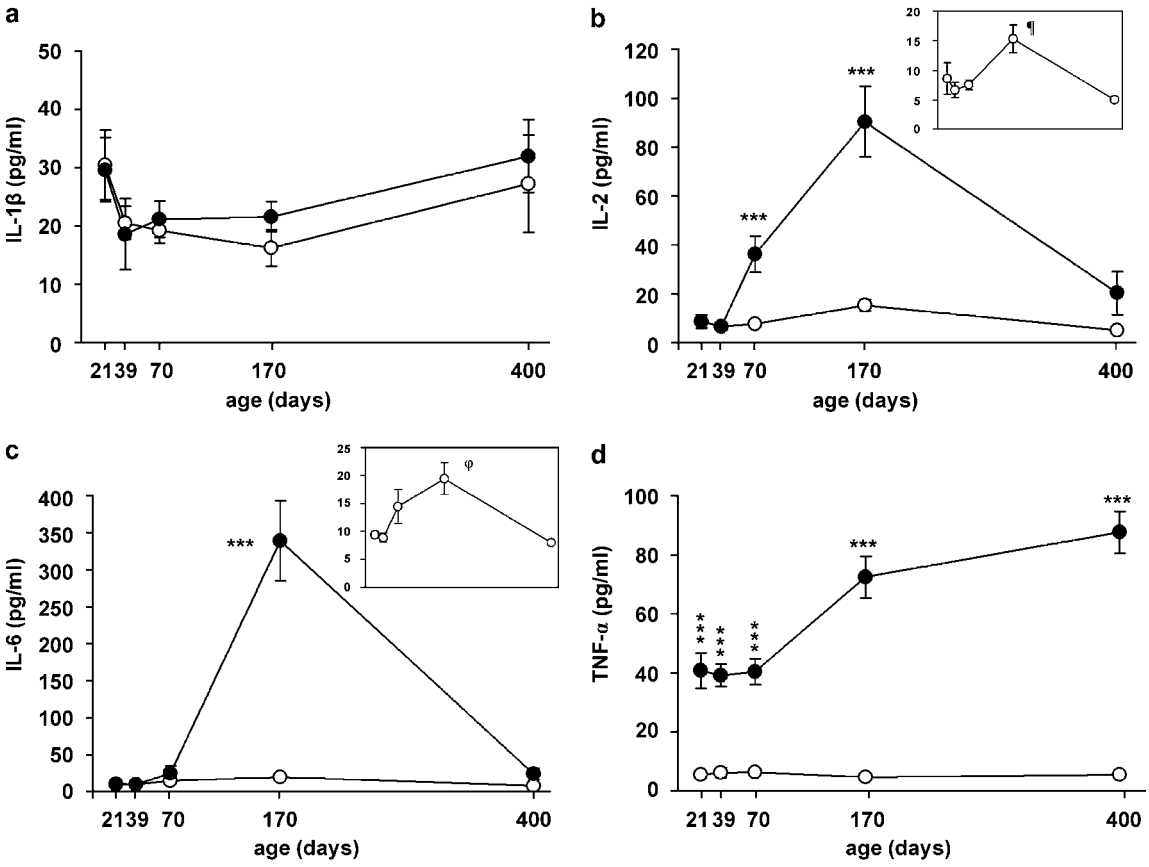

Figure 4 Serum cytokine levels of male rats exposed to lipopolysaccharide (LPS) (black circles) or saline (white circles) throughout the prenatal period at the age of 21, 39, 70, 170 or 400 days. Results are expressed as the mean ± s.e.m. (**a**) Serum interleukin (IL)-1β levels ($n = 9 \pm 2$ rats per group aged 21 and 39 days; $n = 32 \pm 4$ rats per group aged 70 days; $n = 13 \pm 1$ rats per group aged 170 days; $n = 6$ rats per group aged 400 days). (**b**) Serum IL-2 levels ($n = 13 \pm 2$ rats per group aged 21 and 39 days; $n = 25 \pm 5$ rats per group aged 70 days; $n = 20 \pm 2$ rats per group aged 170 days; $n = 6$ rats per group aged of 400 days). The upper part of the figure represents the IL-2 serum levels in the control group at the five ages cited above. (**c**) Serum IL-6 levels ($n = 15 \pm 1$ rats per group aged 21 days; $n = 11 \pm 1$ rats per group aged 39 days; $n = 20 \pm 3$ rats per group aged 70 and 170 days; $n = 6$ rats per group aged 400 days). The upper part of the figure represents the serum IL-6 levels in the control group at the five ages cited above. (**d**) Serum tumor necrosis factor-α (TNF-α) levels ($n = 9 \pm 2$ rats per group aged 21 days; $n = 6$ rats per group aged 39 and 400 days; $n = 26 \pm 2$ rats per group aged 170 days).***$P < 0.001$ vs corresponding control group; ¶$P < 0.05$ vs 70- or 400-day-old control animals and $P < 0.001$ vs 21- or 39-day-old control animals; ö $P < 0.05$ vs 70-day-old control animals and $P < 0.001$ vs 21-, 39- or 400-day-old control animals (analysis of variance (ANOVA) followed by Student–Newman–Keuls *t*-test).

(Table 3). No significant differences in serum IL-1β level due to age ($F(1,43) = 1.079$, P = NS) or treatment ($F(1,43) = 0.730$, P = NS) were observed. However, both IL-6 and TNF-α levels were significantly affected by age (IL-6: ($F(1,43) = 9.728$, $P = 0.005$), TNF-α: ($F(1,43) = 11.260$, $P < 0.001$) and treatment (IL-6: ($F(1,43) = 8.866$, $P = 0.005$), TNF-α: ($F(1,43) = 22.235$, $P < 0.001$)), and age/treatment interactions were observed (IL-6: ($F(4,43) = 4.058$, $P = 0.014$), TNF-α: ($F(4,43) = 8.948$, $P < 0.001$). The effect of prenatal LPS exposure on serum IL-6 level was observed only in 170-day-old female rats, which displayed higher serum level of this cytokine than the corresponding controls ($P < 0.001$). However, this prenatal insult increased serum level of TNF-α in female rats at all ages evaluated, except for the age of 170 days, where no significant differences were observed between female rats exposed to LPS or saline during their prenatal period ($P = 0.023$ vs 39-day-old, $P < 0.001$ vs 70-day-old and $P = 0.049$ vs 400-day-old control rats).

Discussion

This study reveals three important consequences in the offspring of maternal immune activation during pregnancy. First, it shows that such prenatal insults impair sensorimotor gating in a manner related to maturation, and more severely in male than in female rats. Second, it shows that a maternal immune challenge can compromise the integrity of neuronal circuits in the offspring throughout life, as reflected by the age-specific abnormalities in the abundance of DA in the NAC, and in synaptophysin in the frontal cortex. Finally, this study indicates that cytokine

Table 3 IL-1β, IL-6 and TNF-α serum levels (pg ml^{-1}) of female rats exposed to LPS or saline (control) throughout the prenatal period, at the age of 39, 70, 170 or 400 days

		39 days	*70 days*	*170 days*	*400 days*
IL-1β	Control	22.0 ± 3.1	25.2 ± 8.2	21.6 ± 2.5	31.3 ± 5.6
	LPS	19.5 ± 2.5	21.4 ± 4.0	18.6 ± 1.2	26.3 ± 9.3
IL-6	Control	16.8 ± 1.5	18.2 ± 1.9	22.5 ± 1.6	21.4 ± 1.9
	LPS	22.6 ± 1.0	19.9 ± 1.4	202.9 ± 53.5***	22.2 ± 3.7
TNF-α	Control	37.6 ± 10.8	11.2 ± 7.2	4.0 ± 0.0	19.7 ± 6.2
	LPS	100.3 ± 22.7*	81.2 ± 11.3***	6.2 ± 2.2	41.9 ± 7.5*

Abbreviations: IL, interleukin; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-α.
Results are expressed as the mean ± s.e.m. ($n = 6 \pm 2$ rats per group). *$P < 0.05$, ***$P < 0.001$ vs control rats (ANOVA followed by Student–Newman–Keuls *t*-test).

anomalies induced by prenatal immune activation are age dependent. In particular, the increase in TNF-α occurs earlier than the sensorimotor gating disruption. Although these data are discussed below, it is important to note that they indicate that maternal infection during pregnancy might direct the phenotypic expression of schizophrenic symptoms throughout life.

Sensorimotor gating is a fundamental brain function, by which excess or trivial stimuli are screened or 'gated out' by central inhibitory mechanisms in the early stage of information processing.[30] It is a process that emerges during infancy and matures during childhood.[31,32] In the present study, control animals showed a strong increase in PPI values from 35 to 70 days of age, suggesting that the formation of mature neural circuits for sensorimotor gating occurs in rats during this period. This maturation-dependent increase in sensorimotor gating was attenuated in the offspring of dams exposed to LPS during pregnancy, leading to an adult deficit in this brain function. This phenomenon was particularly robust in male rats, whose PPI score was not enhanced from 35 days of age, often considered as adolescence in the rat.[33] Thus, consistent with the clinical symptomatology of schizophrenia, the data presented show that a PPI deficit induced by prenatal exposure to LPS in rats emerges during adolescence and persists throughout life. These results are also in good agreement with the postpubertal changes in sensorimotor gating observed after prenatal administration of the synthetic cytokine inducing polyriboinosinic–polyribocytidylic acid (polyI:C) in mice[34] or rats.[19] As both LPS and polyI:C produce changes in the maternal and fetal cytokine levels,[35,36] cytokine induction during gestation could represent a link between maternal infection and adult sensorimotor gating pathologies. Moreover, it has been recently shown that a single maternal injection of IL-6 in mouse can induce sensorimotor gating deficits in the adult offspring.[37] Indeed, this behavioral feature may reflect the interaction of early developmental disturbances with adolescent brain maturation.

Prenatal LPS exposure strongly disrupted PPI in male rats at each prepulse intensity tested. In female rats, prenatal LPS exposure also produced a strong PPI deficit in response to the weaker auditory prepulse (6 dB above background); but to the stronger auditory (12 dB above background) or the visual prepulses the PPI deficit was significant only at some age points. Using an intermediate prepulse intensity (10 dB above background), we had reported that female rats prenatally exposed to LPS manifested a disruption in the PPI later than LPS-exposed male rats.[20] Hence, male rats appear to be more sensitive to the effects of prenatal immune activation on sensorimotor gating than female rats, supporting the idea of gender differences in PPI in pathological states. Interestingly, with a prepulse stimulus of 15 dB above background, men with schizophrenia display a weaker PPI than healthy men, whereas PPI in women with schizophrenia does not differ from that in healthy women.[38]

One of the most important elements of the complex circuits that modulate PPI is the dopaminergic input to the NAC.[39,40] Given that overactivity in this system reduces PPI, it is tempting to speculate that the prenatal disruption of PPI in LPS-exposed rats could be mediated by changes in the establishment of accumbal DA input during adolescence, which leads to DA overactivity in adulthood. This possibility is highlighted by the fact that LPS-exposed rats display lower accumbal levels of DA than controls in adolescence, normal levels during the juvenile period and higher DA levels than controls in adulthood. In the interpretation of these results, consideration must be given to the fact that multiple comparisons may render associations with *P*-values between 0.01 and 0.05 potentially relatively less meaningful. The changes observed in DA content in the NAC may reflect an abnormal basal DA synthesis and/or DA receptor reorganization during maturation. Indeed, DA receptors are regulated in response to the availability of DA[41] and dopaminergic systems undergo substantial reorganization during adolescence in both humans[42] and rats.[43] For example, basal DA synthesis peaks in the rat prefrontal cortex early in adolescence and subsequently wanes, whereas synthesis is low at this time in the NAC and it increases subsequently.[44] DA receptor overproduction and

elimination in adolescence displays regional differences[43,45] and it is much more pronounced in male than in female rats.[46] Thus, developmental events during adolescence may alter the relative balance of DA activity between the prefrontal cortex and the subcortical regions.

Synaptic abnormalities have been proposed as a cause of various sensory processing impairments associated with schizophrenia.[47] The onset of psychotic symptoms in adolescence or early adulthood has been related to an increase in the normal synaptic pruning that occurs in the prefrontal cortex.[48] In the present study, levels of synaptophysin, a marker of synaptic density,[49] were different throughout the lifetime of LPS rats when compared to the controls suggesting that the prenatal immune challenge provokes aberrant synaptic remodeling and plasticity in the frontal cortex in an age-dependent manner. The absence of changes in the expression of GAP-43, thought to influence the growth state of presynaptic terminals, in LPS-exposed rats may indicate that the altered synaptophysin levels in these LPS-animals are not the result of generalized changes of cortical neuropile but at individual terminals. The increased level of synaptophysin in the frontal cortex of adult (170 days) prenatally LPS-exposed rats is not consistent with the reduced levels found in the brains of patients with schizophrenia[50–52] and the implication of gray matter loss in the illness[53–58] as frequently reported. However, the emergence and timing of these deficits are difficult to be approached in humans. Interestingly, we observed a reduction of synaptophysin in the frontal cortex of 400-day-old rats prenatally exposed to LPS. This indicates that increased synaptophysin expression at the adult age could precede the reduced expression of this protein observed in the frontal cortex at senescence. Our findings agree with a previous report indicating that cortical levels of synaptic vesicle-associated proteins significantly decline with age in schizophrenic patients, but not in control subjects.[59] Moreover, although reduced synaptophysin levels in the brains of patients with schizophrenia are widely observed,[50–52,60] higher mRNA levels of cortical synaptophysin have been reported in 52–73-year-old schizophrenic patients than in the age-matched controls. However, no differences were found in schizophrenic patients older than 75 years.[59]

We found a slight reduction in the expression of GSK-3β in the frontal cortex of 400-day-old rats prenatally exposed to LPS, whereas no alterations were detected in this brain area of younger rats. GSK-3 abnormalities have previously been linked to schizophrenia.[61–64] Approximately 40% lower GSK-3β mRNA and protein levels, as well as GSK-3 kinase activity was found in postmortem tissue from the frontal cortex of subjects with schizophrenia when compared with controls. However, except for less GSK-3 mRNA, these differences were not confirmed in samples from a different brain source.[65] This difference between brain collections was also encountered by another group who found differences in GSK-3β protein levels from schizophrenic and control samples in one brain collection but not in another.[66,67] Our observations suggest that age could be an important confounding factor that may produce heterogeneity in the measurement of GSK-3β expression in schizophrenic brains.

The pregnant rats used in this study were submitted to the well-documented LPS-induced immune challenge.[20] Even though LPS could induce effects on maternal rearing, the data on serum corticosterone level and animal weight in the offspring at different ages studied made this explanation unlikely as a major source of PPI disruption. Although further studies will be needed to elucidate how prenatal LPS exposure disrupts PPI, our data show that this early environmental insult produces a postpubertal lifelong PPI deficit associated with altered accumbal DA levels and abnormal activity in the frontal cortex. Moreover, these results provide further support for the hypothesis that synaptic abnormalities in schizophrenia may be derived from differences in developmental synaptic density in the normal and diseased states. Accordingly, prenatal administration of polyI:C leads to postpubertal emergence of a sensorimotor gating deficit together with dopaminergic hyperfunction in mice[34] and rats.[19]

We found a dissociation between the developmental time course of the PPI deficit and the cytokine alterations induced by prenatal exposure to LPS. Specifically, although rats exhibited an 'adolescent level' in their sensitivity to prenatal LPS exposure in terms of PPI disruption, the consequences of this prenatal insult in the offspring's serum cytokine levels were readily observed in 21-day-old pups as an increase in the serum levels of TNF-α. Our study includes a wide range of postnatal ages in male and female rats and reveals that prenatal immune overactivation results in age-dependent cytokine-specific changes. The trends of IL-1β and IL-6 profiles were similar in both female and male rats prenatally exposed to LPS. However, it remains to be seen if the postpubertal rise in serum IL-2 observed in male rats appears similar in female rats as well. Interestingly, prenatal LPS exposure affects TNF-α level differently in male and female rats. In particular, an elevation of serum TNF-α was detected in male rats throughout the age range examined, whereas in female rats this increase was not observed at the age of 170 days. The postpubertal nature of this difference suggests that the effects of prenatal LPS exposure in cytokine levels are influenced by sex hormones.

There are several lines of evidence suggesting that immune system overactivation influences schizophrenia. Elevated serum levels of IL-2, IL-6 and TNF-α have been observed in several studies.[68–70] These levels have been correlated with the response to treatment,[71] younger age onset,[72] duration of the illness[73] and symptomatology.[74]

Among all the cytokine abnormalities, elevated serum TNF-α level seems to be the more persisting

feature in prenatally LPS-exposed rats and could be considered as an early phenotypic marker of the prenatal-LPS-induced pathology. Cytokines are able to cross the brain-blood-brain barrier (BBB)[75–77] and influence central monoamine activity.[78] Indeed, increased IL-6 facilitates the BBB disturbances.[79] It should be noted that IL-2 and IL-6 in the rodent brain are expressed in selected regions directly implicated in the gating circuitry.[80] The increase in serum TNF-α level prior to the expression of the PPI deficit suggests that this cytokine could participate directly, and/or by stimulation of proinflammatory cytokine release, in the anatomical damage to structures or to neurodevelopmental processes related to PPI. Further studies will elucidate if the LPS-induced PPI deficit reported in this work is associated with cytokine age-dependent changes in regions of the brain directly implicated in sensorimotor gating. However, it has been reported that maternal immune activation alters cytokine expression in the fetal brain[35,36,81] but not in the adult brain.[81] This raises the possibility that modifications in inflammatory-related responses in the brain, rather than cytokines themselves, could be the underlying relation between the peripheral cytokine changes and the PPI deficit associated with this prenatal insult. Supporting this hypothesis, it has been recently shown that TNF-α is able to induce in endothelial cell line, the transcription of inflammation-related genes which are upregulated in schizophrenia brains.[82] In adult rats, treatment with antipsychotics reversed the serum cytokine and PPI abnormalities induced by prenatal LPS exposure.[14] Interestingly, PPI is enhanced in both IL-2 knockout mice, and mouse strains that spontaneously develop systemic lupus-like autoimmune disease which present a marked deficit in IL-2 production.[83] It remains to be determined whether or not antibodies against IL-2, IL-6 or TNF-α could prevent or rescue PPI deficits induced by prenatal LPS exposure. Importantly, the benefits of antibodies against TNF-α as a promising therapy for schizophrenia have already been described.[84]

The precise mechanisms by which prenatal immune activation induces age-dependent changes in serum cytokines, and the relationship with the associated neuropathologies, remain largely unresolved. In addition to the relevance of this work in studying the link between maternal infection and schizophrenia, our results also point out the importance of maternal immunological insults during gestation for the future adult immunological responses. We might speculate that paying attention to these changes might open up new therapeutic approaches to neurodevelopmental diseases such as schizophrenia, where there is evidence of immune system overactivation.

Acknowledgments

We thank Rufino Fernández for his excellent technical assistance during the course of this research and María González for generating the HPLC data. This work was supported by the Fondo de Investigación Sanitaria (FIS, grant number 01/1138), Fundació La Marató de TV3 (grant number 014931) and the Comunidad de Madrid (grant number 08.5/0020/2001).

References

1 Lewis DA, Levitt P. Schizophrenia as a disorder of neurodevelopment. *Annu Rev Neurosci* 2002; **25**: 409–432.
2 Swerdlow NR, Talledo J, Sutherland AN, Nagy D, Shoemaker JM. Antipsychotic effects on prepulse inhibition in normal 'low gating' humans and rats. *Neuropsychopharmacology* 2006; **31**: 2011–2021.
3 Rapoport JL, Addington AM, Frangou S, Psych MR. The neurodevelopmental model of schizophrenia: update 2005. *Mol Psychiatry* 2005; **10**: 434–449.
4 Brown AS, Susser ES. In utero infection and adult schizophrenia. *Ment Retard Dev Disabil Res Rev* 2002; **8**: 51–57.
5 Brown AS, Hooton J, Schaefer CA, Zhang H, Petkova E, Babulas V *et al*. Elevated maternal interleukin-8 levels and risk of schizophrenia in adult offspring. *Am J Psychiatry* 2004; **161**: 889–895.
6 Pearce BD. Schizophrenia and viral infection during neurodevelopment: a focus on mechanisms. *Mol Psychiatry* 2001; **6**: 634–646.
7 Buka SL, Tsuang MT, Torrey EF, Klebanoff MA, Bernstein D, Yolken RH. Maternal infections and subsequent psychosis among offspring. *Arch Gen Psychiatry* 2001; **58**: 1032–1037.
8 Gilmore JH, Jarskog LF. Exposure to infection and brain development: cytokines in the pathogenesis of schizophrenia. *Schizophr Res* 1997; **24**: 365–367.
9 Curfs JH, Meis JF, Hoogkamp-Korstanje JA. A primer on cytokines: sources, receptors, effects, and inducers. *Clin Microbiol Rev* 1997; **10**: 742–780.
10 Yang L, Lindholm K, Konishi Y, Li R, Shen Y. Target depletion of distinct tumor necrosis factor receptor subtypes reveals hippocampal neuron death and survival through different signal transduction pathways. *J Neurosci* 2002; **22**: 3025–3032.
11 Jarskog LF, Xiao H, Wilkie MB, Lauder JM, Gilmore JH. Cytokine regulation of embryonic rat dopamine and serotonin neuronal survival *in vitro*. *Int J Dev Neurosci* 1997; **15**: 711–716.
12 Gilmore JH, Fredrik JL, Vadlamudi S, Lauder JM. Prenatal infection and risk for schizophrenia: IL-1beta, IL-6, and TNFalpha inhibit cortical neuron dendrite development. *Neuropsychopharmacology* 2004; **29**: 1221–1229.
13 Golan H, Levav T, Huleihel M. Distinct expression and distribution of vesicular proteins in the hippocampus of TNFa-deficient mice during development. *Synapse* 2004; **53**: 6–10.
14 Romero E, Ali C, Molina-Holgado E, Castellano B, Guaza C, Borrell J. Neurobehavioral and immunological consequences of prenatal immune activation in rats. Influence of antipsychotics. *Neuropsychopharmacology* 2007; **32**: 1791–1804.
15 Watanabe Y, Hashimoto S, Kakita A, Takahashi H, Ko J, Mizuno M *et al*. Neonatal impact of leukemia inhibitory factor on neurobehavioral development in rats. *Neurosci Res* 2004; **48**: 345–353.
16 Tohmi M, Tsuda N, Watanabe Y, Kakita A, Nawa H. Perinatal inflammatory cytokine challenge results in distinct neurobehavioral alterations in rats: implication in psychiatric disorders of developmental origin. *Neurosci Res* 2004; **50**: 67–75.
17 Meyer U, Feldon J, Schedlowski M, Yee BK. Towards an immunoprecipitated neurodevelopmental animal model of schizophrenia. *Neurosci Biobehav Rev* 2005; **29**: 913–947.
18 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.
19 Zuckerman L, Rehavi M, Nachman R, Weiner I. Immune activation during pregnancy in rats leads to a postpubertal emergence of disrupted latent inhibition, dopaminergic hyperfunction, and altered limbic morphology in the offspring: a novel neurodevelopmental model of schizophrenia. *Neuropsychopharmacology* 2003; **28**: 1778–1789.

20 Borrell J, Vela JM, Arevalo-Martin A, Molina-Holgado E, Guaza C. Prenatal immune challenge disrupts sensorimotor gating in adult rats. Implications for the etiopathogenesis of schizophrenia. *Neuropsychopharmacology* 2002; **26**: 204–215.
21 Fortier ME, Joober R, Luheshi GN, Boksa P. Maternal exposure to bacterial endotoxin during pregnancy enhances amphetamine-induced locomotion and startle responses in adult rat offspring. *J Psychiatr Res* 2004; **38**: 335–345.
22 Fagoaga OR, Nehlsen-Cannarella SL. Maternal modulation of neonatal immune system development. *Dev Immunol* 2002; **9**: 9–17.
23 Yamashita T, Freigang S, Eberle C, Pattison J, Gupta S, Napoli C *et al.* Maternal immunization programs postnatal immune responses and reduces atherosclerosis in offspring. *Circ Res* 2006; **99**: e51–e64.
24 Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science* 1990; **249**: 1431–1433.
25 Quan N, Stern EL, Whiteside MB, Herkenham M. Induction of pro-inflammatory cytokine mRNAs in the brain after peripheral injection of subseptic doses of lipopolysaccharide in the rat. *J Neuroimmunol* 1999; **93**: 72–80.
26 Campeau S, Davis M. Prepulse inhibition of the acoustic startle reflex using visual and auditory prepulses: disruption by apomorphine. *Psychopharmacology (Berl)* 1995; **117**: 267–274.
27 Alder J, Kanki H, Valtorta F, Greengard P, Poo MM. Overexpression of synaptophysin enhances neurotransmitter secretion at Xenopus neuromuscular synapses. *J Neurosci* 1995; **15**(1 Part 2): 511–519.
28 Benowitz LI, Routtenberg A. GAP-43: an intrinsic determinant of neuronal development and plasticity. *Trends Neurosci* 1997; **20**: 84–91.
29 Grimes CA, Jope RS. The multifaceted roles of glycogen synthase kinase 3beta in cellular signaling. *Prog Neurobiol* 2001; **65**: 391–426.
30 Braff D, Stone C, Callaway E, Geyer M, Glick I, Bali L. Prestimulus effects on human startle reflex in normals and schizophrenics. *Psychophysiology* 1978; **15**: 339–343.
31 Ornitz EM, Guthrie D, Sadeghpour M, Sugiyama T. Maturation of prestimulation-induced startle modulation in girls. *Psychophysiology* 1991; **28**: 11–20.
32 Ornitz EM, Guthrie D, Kaplan AR, Lane SJ, Norman RJ. Maturation of startle modulation. *Psychophysiology* 1986; **23**: 624–634.
33 Spear LP. The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav Rev* 2000; **24**: 417–463.
34 Ozawa K, Hashimoto K, Kishimoto T, Shimizu E, Ishikura H, Iyo M. Immune activation during pregnancy in mice leads to dopaminergic hyperfunction and cognitive impairment in the offspring: a neurodevelopmental animal model of schizophrenia. *Biol Psychiatry* 2006; **59**: 546–554.
35 Ashdown H, Dumont Y, Ng M, Poole S, Boksa P, Luheshi GN. The role of cytokines in mediating effects of prenatal infection on the fetus: implications for schizophrenia. *Mol Psychiatry* 2006; **11**: 47–55.
36 Gilmore JH, Jarskog LF, Vadlamudi S. Maternal poly I:C exposure during pregnancy regulates TNF alpha, BDNF, and NGF expression in neonatal brain and the maternal-fetal unit of the rat. *J Neuroimmunol* 2005; **159**: 106–112.
37 Smith SE, Li J, Garbett K, Mirnics K, Patterson PH. Maternal immune activation alters fetal brain development through interleukin-6. *J Neurosci* 2007; **27**: 10695–10702.
38 Kumari V, Aasen I, Sharma T. Sex differences in prepulse inhibition deficits in chronic schizophrenia. *Schizophr Res* 2004; **69**: 219–235.
39 Humby T, Wilkinson LS, Robbins TW, Geyer MA. Prepulses inhibit startle-induced reductions of extracellular dopamine in the nucleus accumbens of rat. *J Neurosci* 1996; **16**: 2149–2156.
40 Swerdlow NR, Braff DL, Geyer MA, Koob GF. Central dopamine hyperactivity in rats mimics abnormal acoustic startle response in schizophrenics. *Biol Psychiatry* 1986; **21**: 23–33.
41 Gelbard HA, Teicher MH, Baldessarini RJ, Gallitano A, Marsh ER, Zorc J *et al.* Dopamine D1 receptor development depends on endogenous dopamine. *Brain Res Dev Brain Res* 1990; **56**: 137–140.
42 Seeman P. The absolute density of neurotransmitter receptors in the brain. Example for dopamine receptors. *J Pharmacol Methods* 1987; **17**: 347–360.
43 Teicher MH, Andersen SL, Hostetter Jr JC. Evidence for dopamine receptor pruning between adolescence and adulthood in striatum but not nucleus accumbens. *Brain Res Dev Brain Res* 1995; **89**: 167–172.
44 Teicher MH, Dumont NL, Andersen SL. The developing prefrontal cortex: is there a transient interneuron that stimulates catecholamine terminals? *Synapse* 1998; **29**: 89–91.
45 Andersen SL, Thompson AT, Rutstein M, Hostetter JC, Teicher MH. Dopamine receptor pruning in prefrontal cortex during the periadolescent period in rats. *Synapse* 2000; **37**: 167–169.
46 Andersen SL, Rutstein M, Benzo JM, Hostetter JC, Teicher MH. Sex differences in dopamine receptor overproduction and elimination. *Neuroreport* 1997; **8**: 1495–1498.
47 Feinberg I. Schizophrenia: caused by a fault in programmed synaptic elimination during adolescence? *J Psychiatr Res* 1982; **17**: 319–334.
48 Mirnics K, Middleton FA, Lewis DA, Levitt P. Analysis of complex brain disorders with gene expression microarrays: schizophrenia as a disease of the synapse. *Trends Neurosci* 2001; **24**: 479–486.
49 Wiedenmann B, Franke WW. Identification and localization of synaptophysin, an integral membrane glycoprotein of Mr 38 000 characteristic of presynaptic vesicles. *Cell* 1985; **41**: 1017–1028.
50 Karson CN, Mrak RE, Schluterman KO, Sturner WQ, Sheng JG, Griffin WS. Alterations in synaptic proteins and their encoding mRNAs in prefrontal cortex in schizophrenia: a possible neurochemical basis for 'hypofrontality'. *Mol Psychiatry* 1999; **4**: 39–45.
51 Eastwood SL. The synaptic pathology of schizophrenia: is aberrant neurodevelopment and plasticity to blame? *Int Rev Neurobiol* 2004; **59**: 47–72.
52 Mirnics K, Levitt P, Lewis DA. Critical appraisal of DNA microarrays in psychiatric genomics. *Biol Psychiatry* 2006; **60**: 163–176.
53 Rapoport JL, Giedd J, Kumra S, Jacobsen L, Smith A, Lee P *et al.* Childhood-onset schizophrenia. Progressive ventricular change during adolescence. *Arch Gen Psychiatry* 1997; **54**: 897–903.
54 DeLisi LE, Sakuma M, Ge S, Kushner M. Association of brain structural change with the heterogeneous course of schizophrenia from early childhood through five years subsequent to a first hospitalization. *Psychiatry Res* 1998; **84**: 75–88.
55 Giedd JN, Jeffries NO, Blumenthal J, Castellanos FX, Vaituzis AC, Fernandez T *et al.* Childhood-onset schizophrenia: progressive brain changes during adolescence. *Biol Psychiatry* 1999; **46**: 892–898.
56 Mathalon DH, Sullivan EV, Lim KO, Pfefferbaum A. Progressive brain volume changes and the clinical course of schizophrenia in men: a longitudinal magnetic resonance imaging study. *Arch Gen Psychiatry* 2001; **58**: 148–157.
57 Sowell ER, Thompson PM, Holmes CJ, Jernigan TL, Toga AW. *In vivo* evidence for post-adolescent brain maturation in frontal and striatal regions. *Nat Neurosci* 1999; **2**: 859–861.
58 Thompson PM, Vidal C, Giedd JN, Gochman P, Blumenthal J, Nicolson R *et al.* Mapping adolescent brain change reveals dynamic wave of accelerated gray matter loss in very early-onset schizophrenia. *Proc Natl Acad Sci USA* 2001; **98**: 11650–11655.
59 Tcherepanov AA, Sokolov BP. Age-related abnormalities in expression of mRNAs encoding synapsin 1A, synapsin 1B, and synaptophysin in the temporal cortex of schizophrenics. *J Neurosci Res* 1997; **49**: 639–644.
60 Eastwood SL, Cairns NJ, Harrison PJ. Synaptophysin gene expression in schizophrenia. Investigation of synaptic pathology in the cerebral cortex. *Br J Psychiatry* 2000; **176**: 236–242.
61 Kozlovsky N, Belmaker RH, Agam G. Low GSK-3beta immunoreactivity in postmortem frontal cortex of schizophrenic patients. *Am J Psychiatry* 2000; **157**: 831–833.
62 Kozlovsky N, Belmaker RH, Agam G. Low GSK-3 activity in frontal cortex of schizophrenic patients. *Schizophr Res* 2001; **52**: 101–105.
63 Kozlovsky N, Nadri C, Agam G. Low GSK-3beta in schizophrenia as a consequence of neurodevelopmental insult. *Eur Neuropsychopharmacol* 2005; **15**: 1–11.

64 Kozlovsky N, Shanon-Weickert C, Tomaskovic-Crook E, Kleinman JE, Belmaker RH, Agam G. Reduced GSK-3beta mRNA levels in postmortem dorsolateral prefrontal cortex of schizophrenic patients. *J Neural Transm* 2004; **111**: 1583–1592.
65 Nadri C, Dean B, Scarr E, Agam G. GSK-3 parameters in postmortem frontal cortex and hippocampus of schizophrenic patients. *Schizophr Res* 2004; **71**: 377–382.
66 Beasley C, Cotter D, Khan N, Pollard C, Sheppard P, Varndell I *et al.* Glycogen synthase kinase-3beta immunoreactivity is reduced in the prefrontal cortex in schizophrenia. *Neurosci Lett* 2001; **302**: 117–120.
67 Beasley C, Cotter D, Everall I. An investigation of the Wnt-signalling pathway in the prefrontal cortex in schizophrenia, bipolar disorder and major depressive disorder. *Schizophr Res* 2002; **58**: 63–67.
68 Monteleone P, Fabrazzo M, Tortorella A, Maj M. Plasma levels of interleukin-6 and tumor necrosis factor alpha in chronic schizophrenia: effects of clozapine treatment. *Psychiatry Res* 1997; **71**: 11–17.
69 Gaughran F. Immunity and schizophrenia: autoimmunity, cytokines, and immune responses. *Int Rev Neurobiol* 2002; **52**: 275–302.
70 Pae CU, Yoon CH, Kim TS, Kim JJ, Park SH, Lee CU *et al.* Antipsychotic treatment may alter T-helper (TH) 2 arm cytokines. *Int Immunopharmacol* 2006; **6**: 666–671.
71 Lin A, Kenis G, Bignotti S, Tura GJ, De Jong R, Bosmans E *et al.* The inflammatory response system in treatment-resistant schizophrenia: increased serum interleukin-6. *Schizophr Res* 1998; **32**: 9–15.
72 Maes M, Bosmans E, Calabrese J, Smith R, Meltzer HY. Interleukin-2 and interleukin-6 in schizophrenia and mania: effects of neuroleptics and mood stabilizers. *J Psychiatr Res* 1995; **29**: 141–152.
73 Ganguli R, Yang Z, Shurin G, Chengappa KN, Brar JS, Gubbi AV *et al.* Serum interleukin-6 concentration in schizophrenia: elevation associated with duration of illness. *Psychiatry Res* 1994; **51**: 1–10.
74 Muller N, Riedel M, Ackenheil M, Schwarz MJ. The role of immune function in schizophrenia: an overview. *Eur Arch Psychiatry Clin Neurosci* 1999; **249**(Suppl 4): 62–68.
75 Banks WA, Kastin AJ, Gutierrez EG. Penetration of interleukin-6 across the murine blood-brain barrier. *Neurosci Lett* 1994; **179**: 53–56.
76 Gutierrez EG, Banks WA, Kastin AJ. Murine tumor necrosis factor alpha is transported from blood to brain in the mouse. *J Neuroimmunol* 1993; **47**: 169–176.
77 Waguespack PJ, Banks WA, Kastin AJ. Interleukin-2 does not cross the blood-brain barrier by a saturable transport system. *Brain Res Bull* 1994; **34**: 103–109.
78 Brebner K, Hayley S, Zacharko R, Merali Z, Anisman H. Synergistic effects of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha: central monoamine, corticosterone, and behavioral variations. *Neuropsychopharmacology* 2000; **22**: 566–580.
79 Brett FM, Mizisin AP, Powell HC, Campbell IL. Evolution of neuropathologic abnormalities associated with blood-brain barrier breakdown in transgenic mice expressing interleukin-6 in astrocytes. *J Neuropathol Exp Neurol* 1995; **54**: 766–775.
80 Swerdlow NR, Geyer MA. Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. *Schizophr Bull* 1998; **24**: 285–301.
81 Meyer U, Murray PJ, Urwyler A, Yee BK, Schedlowski M, Feldon J. Adult behavioral and pharmacological dysfunctions following disruption of the fetal brain balance between pro-inflammatory and IL-10-mediated anti-inflammatory signaling. *Mol Psychiatry* 2007; **13**: 208–221.
82 Saetre P, Emilsson L, Axelsson E, Kreuger J, Lindholm E, Jazin E. Inflammation-related genes up-regulated in schizophrenia brains. *BMC Psychiatry* 2007; **7**: 46.
83 Petitto JM, Huang Z, Hartemink DA, Beck Jr R. IL-2/15 receptor-beta gene deletion alters neurobehavioral performance. *Brain Res* 2002; **929**: 218–225.
84 Skurkovich SV, Aleksandrovsky YA, Chekhonin VP, Ryabukhin IA, Chakhava KO, Skurkovich B. Improvement in negative symptoms of schizophrenia with antibodies to tumor necrosis factor-alpha and to interferon-gamma: a case report. *J Clin Psychiatry* 2003; **64**: 734–735.