Prenatal immune activation leads to multiple changes in basal neurotransmitter levels in the adult brain: implications for brain disorders of neurodevelopmental origin such as schizophrenia

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Abstract

Maternal infection during pregnancy enhances the offspring’s risk for severe neuropsychiatric disorders in later life, including schizophrenia. Recent attempts to model this association in animals provided further experimental evidence for a causal relationship between in-utero immune challenge and the postnatal emergence of a wide spectrum of behavioural, pharmacological and neuroanatomical dysfunctions implicated in schizophrenia. However, it still remains unknown whether the prenatal infection-induced changes in brain and behavioural functions may be associated with multiple changes at the neurochemical level. Here, we tested this hypothesis in a recently established mouse model of viral-like infection. Pregnant dams on gestation day 9 were exposed to viral mimetic polyriboinosinic-polyriboctydilic acid (PolyI:C, 5 mg/kg i.v.) or vehicle treatment, and basal neurotransmitter levels were then compared in the adult brains of animals born to PolyI:C- or vehicle-treated mothers by high-performance liquid chromatography on post-mortem tissue. We found that prenatal immune activation significantly increased the levels of dopamine and its major metabolites in the lateral globus pallidus and prefrontal cortex, whilst at the same time it decreased serotonin and its metabolite in the hippocampus, nucleus accumbens and lateral globus pallidus. In addition, a specific reduction of the inhibitory amino acid taurine in the hippocampus was noted in prenatally PolyI:C-exposed offspring relative to controls, whereas central glutamate and \( \gamma \)-aminobutyric acid (GABA) content was largely unaffected by prenatal immune activation. Our results thus confirm that maternal immunological stimulation during early/middle pregnancy is sufficient to induce long-term changes in multiple neurotransmitter levels in the brains of adult offspring. This further supports the possibility that infection-mediated interference with early fetal brain development may predispose the developing organism to the emergence of neurochemical imbalances in adulthood, which may be critically involved in the precipitation of adult behavioural and pharmacological abnormalities after prenatal immune challenge.

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Key words: Animal model, dopamine, infection, schizophrenia, serotonin.

Introduction

Maldevelopment of the central nervous system (CNS) is implicated in several neuropsychiatric disorders, including schizophrenia (Rapoport et al., 2005; Weinberger, 1987). Epidemiological evidence indicates that maternal viral or bacterial infections during critical periods of pregnancy increase the risk for this disabling brain disorder in the offspring (Brown and Susser, 2002; Brown et al., 2004; Babulas et al., 2006; Patterson, 2007). According to one hypothesis, maternal infection during pregnancy may interfere with normal fetal brain development and thereby
predispose the developing organism to the emergence of postnatal neuropathology and psychopathology (Gilmore and Jaruskog, 1997; Meyer et al., 2008a,b; Patterson, 2002).

Recent attempts to model this association in animals has yielded substantial support for a causal relationship between in-utero immune challenge and the postnatal emergence of brain and behavioural abnormalities (reviewed in Meyer et al., 2007; Nawa and Takei, 2006). A variety of structural and functional abnormalities have been detected in rats and mice following prenatal exposure to bacterial endotoxin (Borrell et al., 2002; Fortier et al., 2004, 2007; Romero et al., 2007), human influenza virus (Fatemi et al., 1999, 2002; Shi et al., 2003), a synthetic viral mimic (Meyer et al., 2005, 2006a,b, 2008a,b; Nyffeler et al., 2006; Ozawa et al., 2006; Shi et al., 2003; Smith et al., 2007; Zuckerman et al., 2003), or the pro-inflammatory cytokine interleukin (IL)-6 (Samuelsson et al., 2006; Smith et al., 2007). Many of the prenatal infection-induced functional deficits in rodents are implicated in schizophrenia and psychosis-related behaviour, including deficits in sensorimotor gating (Braff et al., 2001), selective associative learning (Lubow, 2005; Weiner, 2003), and working memory (Goldmann-Rakic, 1994), as well as potentiated sensitivity to acute dopaminergic and glutamatergic drug treatment (Lahti et al., 2001; Laruelle et al., 1996, 1999). The myriad of behavioural and pharmacological deficits emerging after prenatal immune activation may be indicative of dysfunctions in multiple brain areas and neurotransmitter systems. Indeed, the behavioural and pharmacological aberrations detected in prenatally immune challenged animals are paralleled by various abnormalities at the neuroanatomical level. For example, recent experimentation in rodents has demonstrated that prenatal immune activation can lead to long-term changes in the central dopaminergic, glutamatergic and GABAergic systems, including increased striatal immunoreactivity (IR) for the dopamine (DA)-related marker tyrosine hydroxylase (Borrell et al., 2002; Meyer et al., 2008b), reduced prefrontal cortical DA D1 and D2 receptor IRs (Meyer et al., 2008b,c), decreased expression of the GABAergic markers reelin and parvalbumin in the medial prefrontal cortex and hippocampus (Fatemi et al., 1999; Meyer et al., 2006b, 2008c), enhanced hippocampal and amygdalar expression of γ-aminobutyric acid (GABA)A subunits (Nyffeler et al., 2006), as well as reduced hippocampal expression of NMDA receptor subunits (Meyer et al., 2008c). The prenatal infection-induced disturbances in pre- and post-synaptic neuronal markers may also be associated with multiple neurochemical imbalances at the neurotransmitter levels. However, evidence for this possibility is still lacking. A direct examination of the efficacy of prenatal immune activation to affect basal neurotransmitter levels in the adult CNS is therefore clearly warranted.

In the present study, we addressed this issue by evaluating in mice whether maternal viral-like immune activation in early/middle gestation may induce long-lasting neurochemical changes in the adult offspring. To this end, pregnant dams on gestation day (GD) 9 were exposed to the synthetic analogue of double-stranded RNA, polyriboinosinic-polyribocytidlic acid (PolyI:C), and the central neurotransmitter contents were then compared in the brains of adult offspring born to PolyI:C- and vehicle-treated mothers. PolyI:C is known to mimic the acute phase response to viral infection, which is accompanied by the presence of high levels of pro-inflammatory cytokines and numerous other inflammatory molecules (Cunningham et al., 2007; Fortier et al., 2004; Traynor et al., 2004). This immunological treatment also increases pro-inflammatory cytokine levels in the fetal brain (Meyer et al., 2006b, 2008a), lending credence to the use of the prenatal PolyI:C model in mice as an experimental tool to study the long-term consequences of fetal brain inflammation on subsequent brain and behavioural development. The efficacy of prenatal PolyI:C exposure in early/middle pregnancy in mice (i.e. on GD 9) to induce long-term behavioural, pharmacological, and neuroanatomical abnormalities in adult life has been verified previously by numerous studies (Meyer et al., 2005, 2006a,b, 2008a–c; Nyffeler et al., 2006; Shi et al., 2003).

Here, we measured the effects of prenatal PolyI:C-induced immune challenge on the basal levels of DA and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), as well as serotonin (5-HT) and its major metabolite 5-hydroxyindoleacetic acid (5-HIAA) by high-performance liquid chromatography (HPLC) on post-mortem tissue. In addition, the excitatory amino acid glutamate, the inhibitory amino acids GABA, and the inhibitory amino sulfonic acid taurine were also quantified by HPLC analyses. All neurochemical investigations were conducted in numerous cortical and subcortical brain areas, including the prefrontal cortex (PFC), caudate putamen (CPU), nucleus accumbens (NAc), lateral globus pallidus (LGP), hippocampus (HPC), amygdala (AM), ventral tegmental area (VTA), and substantia nigra pars compacta (SNc).
Method

Animals

Female and male C57BL/6J breeders were obtained from the in-house (Laboratory of Behavioural Neurobiology, ETH Zurich, Switzerland) specific pathogen-free (SPF) colony at the age of 10–14 wk. Littermates of the same sex were kept in groups of 3–5 mice. Breeding began after 2 wk of acclimatization to the new animal holding room. The breeding procedure and the verification of pregnancy have been fully described elsewhere (Meyer et al., 2005). All procedures described in the present study had been previously approved by the Cantonal Veterinarian’s Office of Zurich, and are in agreement with the Principles of Laboratory Animal Care (NIH publication No. 86-23, revised 1985).

Prenatal treatment

Pregnant dams on gestation day (GD) 9 received a single injection of 5 mg/kg PolyI:C (potassium salt; Sigma-Aldrich, Buchs, St Gallen, Switzerland). PolyI:C was dissolved in isotonic 0.9% NaCl solution to yield a final concentration of 1 mg/ml and administered via the intravenous route (i.v.) at the tail vein under mild physical constraint as previously described (Meyer et al., 2005). Control dams received an equivalent volume of vehicle (0.9% NaCl solution) only. The dose of PolyI:C and gestational period were selected based on previous studies (see e.g. Meyer et al., 2005, 2006a,b).

A total of 10 pregnant mice were included, half of which were subjected to PolyI:C treatment, and the other half to vehicle treatment. All animals were returned to their home cages immediately after the injection procedures and left undisturbed until weaning of the offspring.

Collection of adult brain samples

The offspring born to PolyI:C- and vehicle-treated mothers were weaned and sexed at postnatal day 21 as described previously (Meyer et al., 2005). They were maintained under an ad-libitum food (Kliba, Klibamühlen, Kaiseraugst, Switzerland) and water diet, and kept in a temperature- and humidity-controlled (21 ± 1 °C, 55 ± 5%) animal vivarium under a 12 h reversed light–dark cycle (lights off 07:00 hours).

The offspring assigned to the neurochemical tests derived from multiple independent litters (five PolyI:C litters, five control litters). Two male offspring per litter were included in all the neurochemical assays, yielding to a total number of n = 10 per treatment group. They were killed by decapitation at age 12 wk. The brains were extracted from the skull within <20 s, snap-frozen in liquid nitrogen, and stored at −80 °C until required. Frozen coronal sections (0.5-mm-thick) were prepared using a cryostat at the following coordinates with respect to bregma: AP (+2.3 to +1.3), (+1.3 to +0.3), (−0.1 to −0.6), (−1.2 to −2.2), and (−2.8 to −3.8). Tissue samples from both hemispheres (seven samples per brain) were processed from the PFC (including cingulate and prelimbic cortices), CPu, NAc (including shell and core subregions), LGP, HPC (including parts of the fields CA1-3 and dentate gyrus), AM (including the lateral and medial division and capsular part), VTA, and SNc via micropunches of 1 mm diameter.

Post-mortem neurochemical analyses

Brain samples were homogenized by ultrasonication in 20 vol of 0.1 N perchloric acid at 4 °C immediately after collection via micropunches. A total of 100 μl of the homogenate was added to equal volumes of 1 N sodium hydroxide for measurement of protein content. The remaining homogenate was centrifuged at 17 000 g and 4 °C for 10 min. Aliquots of the supernatants were added to equal volumes (20 μl) of 0.5 M borate buffer and stored at −80 °C for subsequent analyses of amino acids. The remaining supernatants were used for immediate measurement of monoamines and their metabolites.

The levels of monoamines (DA and 5-HT) and their metabolites (DOPAC, HVA, and 5-HIAA) were measured by HPLC with electrochemical detection as previously described (Felice et al., 1978; Sperk et al., 1981; Sperk, 1982). Briefly, the perchloric acid extracts were separated on a column (Prontosil 120-3-C18-SH; length 150 mm, inner diameter 3 mm; Bischoff Analysetechnik und -geräte GmbH, Leonberg, Germany) at a flow rate of 0.55 ml/min. The mobile phase consisted of 80 mM sodium dihydrogen phosphate, 0.85 mM octane-1-sulfonic acid sodium salt, 0.5 mM ethylenediaminetetraacetic acid disodium salt, 0.92 mM phosphoric acid and 4% 2-propanol (all chemicals Merck KGaA, Darmstadt, Germany). Monoamines were detected using an electrochemical detector (41 000, Chromsystems Instruments & Chemicals GmbH, Munich, Germany) at an electrode potential of 0.8 V. For calibration, 0.1 M perchloric acid containing 0.1 μM DOPAC, HIAA, HVA, 3-MT and 5-HT and 1 μM DA was injected into the HPLC system before and after sample analysis. Sample analysis was performed based on peak areas using a computer-based chromatography.
data system (CSW 1.7, DataApex Ltd, Praha, Czech Republic) in relation to the mean of the applied calibration solutions.

Glutamate, GABA, and taurine were determined using methods described previously (Piepponen and Skujins, 2001). Briefly, amino acids were precolumn-derivatized with o-phthalaldehyde-2-mercaptoethanol using a refrigerated autoinjector and then separated on a HPLC column (ProntoSil C18 ace-EPS) at a flow rate of 0.6 ml/min and a column temperature of 40 °C. The mobile phase was 50 mM sodium acetate (pH 5.7) in a linear gradient from 5% to 21% acetonitrile. Derivatized amino acids were detected by their fluorescence at 450 nm after excitation at 330 nm.

**Statistical analysis**

All data were analysed using independent Student’s t tests. Statistical significance was set at $p \leq 0.05$ (two-tailed). Analyses were conducted using the statistical software StatView (version 5.0; SAS Institute, Cary, NC, USA) implemented on a personal computer running the Windows XP operating system.

**Results**

**DA and its metabolites DOPAC and HVA**

DA was detectable in all brain areas examined: high levels of DA were found in the CPu and NAc, intermediate levels of DA were detected in the LGP, and relatively low levels of DA were measured in the PFC, HPC, AM and VTA/SNc (Figure 1a). Most importantly, prenatal exposure to the inflammatory agent PolyI:C significantly increased basal DA content in the PFC ($p = 0.05$) and LGP ($p = 0.002$) relative to prenatal control treatment (Figure 1a). In comparison to adult control offspring, mice born to PolyI:C-exposed mothers displayed a 43% and 102% increase in DA levels in the PFC and LGP, respectively (Figure 1a). In contrast, prenatal immune activation did not significantly affect basal DA content in the other brain regions examined (i.e. CPu, NAc, HPC, AM, VTA/SNc; $p > 0.05$; Figure 1a).

In addition to the observed changes in central DA content, prenatal immune activation led to a significant increase in the levels of the DA metabolite DOPAC in the PFC (+46%, $p = 0.04$), and the LGP (+50%, $p = 0.008$) relative to prenatal control treatment (Figure 1b). Prenatal immune activation did not significantly affect basal DOPAC content in the other brain regions examined (i.e. CPu, NAc, HPC, AM and VTA/SNc; $p > 0.05$; Figure 1b).

**Figure 1.** Mean values ± S.E.M. (in nM/mg protein) of (a) dopamine (DA), (b) dihydroxyphenylacetic acid (DOPAC), (c) homovanillic acid (HVA) in the prefrontal cortex (PFC), caudate putamen (CPu), nucleus accumbens (NAc), lateral globus pallidus (LGP), hippocampus (HPC), amygdala (AM), ventral tegmental area (VTA), and substantia nigra pars compacta (SNc) of adult control offspring (□) and mice born to PolyI:C-exposed mothers (■). For individual regions, significance levels are denoted by * $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$, based on independent Student’s t tests. The inset in panel (a) depicts DA levels in the PFC in a magnified manner in order to facilitate the comparison of DA content in this specific brain area between control and PolyI:C offspring. n.d., Not detectable.
Prenatal immune challenge also significantly increased the content of the DA metabolite HVA in the NAc (+28%, \( p = 0.001 \)) and LGP (+80%, \( p = 0.0007 \)) relative to prenatal control treatment (Figure 1c). A 46% increase in HVA levels were also noted in the VTA/SNc of prenatally PolyI:C-exposed animals compared with control offspring, but this effect only attained statistical trend level (\( p = 0.06 \)). In contrast, prenatally immune challenged and control offspring did not significantly differ with respect to HVA levels in the CPu, and VTA/SNc (Figure 1c). HVA levels were below detection limit in the PFC, HPC, and AM.

In order to further directly assess the impact of prenatal PolyI:C exposure on DA turnover in the adult brain, ratios of DOPAC/DA and HVA/DA were analysed. These analyses revealed no significant group differences in any of the brain regions examined (Table 1). This indicated that the turnover of central DA was largely unaffected by the prenatal immunological manipulation.

**5-HT and its metabolite 5-HIAA**

5-HT was detectable in all brain regions studied at relatively high levels (Figure 2a). Prenatal immune challenge significantly decreased basal 5-HT content in the NAc, LGP, and HPC relative to prenatal control treatment (Figure 2a). In comparison to adult control offspring, mice born to PolyI:C-exposed mothers displayed a 38% (\( p = 0.043 \)), 30% (\( p = 0.014 \)) and 42% (\( p = 0.035 \)) decrease in 5-HT levels in the NAc, LGP, and HPC, respectively (Figure 2a). On the other hand, prenatal immune activation did not significantly affect basal 5-HT content in the PFC, CPu, AM or VTA/SNc.

Prenatal immune activation also led to decreases in 5-HIAA in the LGP (−29%, \( p = 0.021 \)) and HPC.

### Table 1. Mean values ± S.E.M. (in nM/mg protein) of the DOPAC/DA and HVA/DA ratios in the brain regions of adult control offspring, and mice born to PolyI:C-exposed mothers

<table>
<thead>
<tr>
<th></th>
<th>PFC</th>
<th>CPu</th>
<th>NAc</th>
<th>LGP</th>
<th>HPC</th>
<th>AM</th>
<th>VTA/SNc</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DOPAC/DA ratio</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.68 ± 0.15</td>
<td>0.08 ± 0.01</td>
<td>0.11 ± 0.01</td>
<td>0.12 ± 0.03</td>
<td>1.82 ± 0.40</td>
<td>0.43 ± 0.03</td>
<td>0.67 ± 0.07</td>
</tr>
<tr>
<td>PolyI:C</td>
<td>1.69 ± 0.10</td>
<td>0.09 ± 0.01</td>
<td>0.11 ± 0.02</td>
<td>0.10 ± 0.01</td>
<td>2.00 ± 0.35</td>
<td>0.45 ± 0.11</td>
<td>0.67 ± 0.03</td>
</tr>
<tr>
<td><strong>HVA/DA ratio</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>n.d.</td>
<td>0.05 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.16 ± 0.03</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.36 ± 0.17</td>
</tr>
<tr>
<td>PolyI:C</td>
<td>n.d.</td>
<td>0.06 ± 0.02</td>
<td>0.09 ± 0.03</td>
<td>0.14 ± 0.01</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.45 ± 0.13</td>
</tr>
</tbody>
</table>

DOPAC, dihydroxyphenylacetic acid; DA, dopamine; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid; PFC, prefrontal cortex; CPu, caudate putamen; NAc, nucleus accumbens; LGP, lateral globus pallidus; HPC, hippocampus; AM, amygdala; VTA, ventral tegmental area; SNc, substantia nigra pars compacta; n.d., not determined.

All ratios were calculated based on DA, DOPAC and DA levels.
A 30% decrease in 5-HIAA levels were also noted in the NAc of prenatally PolyI:C-exposed animals compared with control offspring, but this effect only attained statistical trend level ($p = 0.06$).

5-HIAA levels in the PFC, CPu, AM and VTA/SNc were largely comparable between adult offspring born to vehicle- and PolyI:C-treated mothers (Figure 2b). In order to assess the effect of prenatal PolyI:C exposure on 5-HT turnover in the adult brain, ratios of 5-HIAA/5-HT were analysed. These analyses revealed no significant group differences in 5-HIAA/5-HT ratios in any of the brain regions examined (Table 2), suggesting that central 5-HT turnover was largely unaffected by the prenatal immune activation.

Glutamate, GABA and taurine

Glutamate was detectable in all brain regions examined, with highest levels found in the PFC (see Table 3). Prenatal exposure to the inflammatory agent PolyI:C did not significantly affect glutamate levels in any of the brain regions examined (Table 3). Similarly, prenatal immune challenge did not lead to significant alterations in the adult offspring’s central GABA content in comparison with prenatal control treatment (Table 3). GABA was detectable in all brain regions studied, and the two experimental groups were largely comparable with respect to GABA levels measured in each of the brain areas of interest.

However, a 23% ($p < 0.05$) reduction of the inhibitory amino sulfonic acid taurine was noticed in the HPC of prenatally PolyI:C-exposed offspring relative to controls (Figure 3). This effect of immune activation appeared to be restricted to HPC, because prenatal PolyI:C exposure did not significantly affect taurine levels in the other brain areas examined (i.e. PFC, CPu, NAc, LGP, AM, VTA/SNc) relative to prenatal control treatment.

Discussion

The present study shows that a single maternal exposure to the inflammatory agent PolyI:C in early/middle pregnancy in mice is sufficient to induce long-lasting changes in multiple neurotransmitter levels in the brains of adult offspring. Here, we found that prenatal immune activation significantly affected the basal levels of central DA and its metabolites DOPAC.

Table 2. Mean values ± S.E.M. (in nm/mg protein) of the 5-HIAA/5-HT ratio in brain regions of adult control offspring, and mice born to PolyI:C-exposed mothers

<table>
<thead>
<tr>
<th></th>
<th>PFC</th>
<th>CPu</th>
<th>NAc</th>
<th>LGP</th>
<th>HPC</th>
<th>AM</th>
<th>VTA/SNc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.43 ± 0.02</td>
<td>0.69 ± 0.05</td>
<td>0.52 ± 0.08</td>
<td>0.57 ± 0.05</td>
<td>0.54 ± 0.08</td>
<td>0.45 ± 0.03</td>
<td>0.41 ± 0.03</td>
</tr>
<tr>
<td>PolyI:C</td>
<td>0.38 ± 0.09</td>
<td>0.71 ± 0.1</td>
<td>0.51 ± 0.05</td>
<td>0.58 ± 0.05</td>
<td>0.66 ± 0.06</td>
<td>0.44 ± 0.03</td>
<td>0.38 ± 0.03</td>
</tr>
</tbody>
</table>

For abbreviations see Table 1. All ratios were calculated based on 5-HIAA and 5-HT levels.

Table 3. Mean values ± S.E.M. (in nm/mg protein) of ω-aminobutyric acid (GABA) and glutamate in brain regions of adult control offspring, and mice born to PolyI:C-exposed mothers

<table>
<thead>
<tr>
<th></th>
<th>GABA</th>
<th>Glutamate</th>
<th>GABA</th>
<th>Glutamate</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFC</td>
<td>11.82 ± 1.24</td>
<td>89.33 ± 8.28</td>
<td>12.83 ± 1.12</td>
<td>88.53 ± 8.72</td>
</tr>
<tr>
<td>CPu</td>
<td>11.84 ± 1.13</td>
<td>70.05 ± 6.85</td>
<td>10.83 ± 0.86</td>
<td>64.65 ± 5.64</td>
</tr>
<tr>
<td>NAc</td>
<td>29.20 ± 3.14</td>
<td>60.53 ± 5.14</td>
<td>24.52 ± 2.64</td>
<td>61.82 ± 6.15</td>
</tr>
<tr>
<td>LGP</td>
<td>27.93 ± 3.36</td>
<td>52.43 ± 6.82</td>
<td>32.04 ± 4.12</td>
<td>52.85 ± 4.97</td>
</tr>
<tr>
<td>HPC</td>
<td>14.54 ± 2.47</td>
<td>64.82 ± 11.31</td>
<td>14.82 ± 1.45</td>
<td>64.54 ± 6.35</td>
</tr>
<tr>
<td>AM</td>
<td>26.51 ± 2.99</td>
<td>78.04 ± 10.14</td>
<td>25.22 ± 2.75</td>
<td>69.52 ± 10.21</td>
</tr>
<tr>
<td>VTA/SNc</td>
<td>39.70 ± 6.03</td>
<td>46.43 ± 6.72</td>
<td>35.97 ± 4.77</td>
<td>41.21 ± 7.15</td>
</tr>
</tbody>
</table>

For abbreviations see Table 1. (−26%; $p = 0.045$) relative to prenatal control treatment (Figure 2b). A 30% decrease in 5-HIAA levels were also noted in the NAc of prenatally PolyI:C-exposed animals compared with control offspring, but this effect only attained statistical trend level ($p = 0.06$). 5-HIAA levels in the PFC, CPu, AM and VTA/SNc were largely comparable between adult offspring born to vehicle- and PolyI:C-treated mothers (Figure 2b).

In order to assess the effect of prenatal PolyI:C exposure on 5-HT turnover in the adult brain, ratios of 5-HIAA/5-HT were analysed. These analyses revealed no significant group differences in 5-HIAA/5-HT ratios in any of the brain regions examined (Table 2), suggesting that central 5-HT turnover was largely unaffected by the prenatal immune activation.
and HVA (Figure 1), 5-HT and its metabolite 5-HIAA (Figure 2), as well as the amino sulfonic acid taurine (Figure 3) in adulthood. In contrast, maternal immunological stimulation during pregnancy failed to induce significant changes in GABA and glutamate content in the brains of adult offspring (Table 2). This indicates that the long-term effects of prenatal immune activation in early/middle gestation on central neurotransmitter levels in adulthood are restricted to distinct neurotransmitter systems. Furthermore, the changes in basal levels of DA, 5-HT and taurine in adult CNS of prenatally immune challenged animals were clearly dependent on the brain area examined, suggesting that distinct brain structures may have a differential vulnerability for prenatal infection-induced imbalances in basal neurotransmitter levels. This suggestion is consistent with the observations that the long-term neuroanatomical consequences of prenatal immune challenge in rodents are restricted to specific brain areas (Borrell et al., 2002; Meyer et al., 2006b, 2008b,c; Nyffeler et al., 2006). Interestingly, the existing within-group variances in basal neurotransmitter and metabolite levels in the two experimental groups (i.e. PolyI:C and control offspring) are largely comparable with those revealed in behavioural and pharmacological tests (see e.g. Meyer et al., 2005, 2006a,b, 2008a–c). Hence, even though the extent to which maternal PolyI:C treatment during pregnancy can induce brain and behavioural deficits in the progeny may vary from one offspring to another, prenatally PolyI:C-treated offspring as a group significantly differ from control offspring in many aspects of adult behaviour, pharmacology and neurochemistry.

The finding of increased basal DA content in the PFC of prenatally PolyI:C-exposed mice supports the possibility that in-utero immune activation in early/middle gestation may induce long-lasting dopaminergic abnormalities in the PFC. Meyer and colleagues (2008a,b) have recently shown that maternal PolyI:C-induced immune activation on GD 9 in mice significantly reduces DA D_1 and D_2 receptors in the PFC of adult offspring. Together with the findings of the present study, this may indicate that down-regulation of DA receptors in the PFC of prenatally immune challenged subjects may represent a compensatory post-synaptic mechanism for enhanced basal DA levels in this brain area.

The lack of a significant effect of prenatal PolyI:C exposure on DA turnover in striatal regions (i.e. CPu and NAc) is inconsistent with the recent study by Ozawa and colleagues (2006), who reported enhanced levels of both DOPAC and HVA in the striatum of prenatally PolyI:C-exposed offspring, leading to increased striatal DA turnover (as indexed by the DOPAC/HVA/DA ratio). One possible explanation for these discrepancies would be the timing and duration of the prenatal immunological manipulation. In the study by Ozawa et al. (2006), PolyI:C was administered to pregnant mice on six consecutive days from GD 12–17, thereby mimicking a subchronic inflammatory reaction from middle to late gestation. Here, pregnant mice were exposed to a single PolyI:C injection on GD 9, which induces an acute maternal/fetal inflammatory reaction in early/middle gestation. However, our findings are compatible with the findings by Zuckerman and colleagues (2003), who demonstrated that a single exposure to PolyI:C in middle/lateral gestation (GD 15) in rats does not affect basal striatal DA release in vitro but leads to enhanced striatal DA release only following KCl-induced stimulation.

The null effect of prenatal PolyI:C exposure on basal GABA content in the adult brain (Table 2) does not support the possibility that the up-regulation of hippocampal GABA_A receptors previously described in prenatally PolyI:C-exposed mice (Meyer et al., 2008c; Nyffeler et al., 2006) may constitute a compensatory reaction to reduced basal GABA levels in the HPC. However, in the present study we revealed that prenatal PolyI:C exposure significantly reduced the hippocampal content of taurine (Figure 3), which is considered an endogenous GABA analogue that selectively activates GABA_A (but not GABA_B) receptors...
The impact of prenatal infection on the integrity of serotonergic systems in the adult CNS has received only little attention so far. Here, we found that prenatal exposure to inflammatory treatment significantly reduced 5-HT levels in the NAc, HPC and LGP at adult age (Figure 2a). These effects were paralleled by decreases in accumbal, hippocampal and pallidal 5-HIAA levels (Figure 2b), which is the major metabolite of 5-HT. The concomitant reduction of both 5-HT and 5-HIAA in prenatally PolyI:C-exposed animals readily suggests that prenatal immune activation affects basal 5-HT levels without changing 5-HT turnover. Indeed, the 5-HT/5-HIAA ratios were highly comparable between adult offspring of vehicle- and PolyI:C-exposed mothers (Figure 2c). The reduction in basal 5-HT levels in the brains of prenatally PolyI:C-exposed subjects may provide an interesting link between the causal relationship of prenatal immune challenge and reduction of postnatal hippocampal neurogenesis (Meyer et al., 2006b). Indeed, there is considerable evidence indicating that 5-HT has beneficial effects on adult hippocampal neurogenesis (reviewed in Gould, 1999). It would therefore be of great interest to explore whether administration with pharmacological compounds known to enhance central levels of 5-HT may restore the decrease in postnatal neurogenesis after prenatal immune challenge (Banasr et al., 2004; Meyer et al., 2006b).

Moreover, there is recent evidence from human postmortem studies showing that impaired adult neurogenesis is linked to schizophrenia (Reif et al., 2006), although the precise roles of serotonergic dysfunctions in this association remain to be explored.

Notably, the observed neurochemical changes in the adult CNS of prenatally PolyI:C-exposed offspring (Figures 1–3), together with the previously reported long-term neuroanatomical consequences of this prenatal immunological manipulation (Meyer et al., 2006b, 2008b,c; Nyffeler et al., 2006) may readily contribute to some of the known behavioural, cognitive and pharmacological dysfunctions emerging after prenatal immune challenge in early/middle gestation in mice (Meyer et al., 2005; 2006a,b; 2008a–c; Ozawa et al., 2006; Shi et al., 2003; Smith et al., 2007). For example, it has long been recognized that normal working-memory functions are crucially dependent on the integrity of the prefrontal cortical DA system: both insufficient and excessive prefrontal DA signalling can lead to working-memory deficits, especially in the spatial domain (reviewed in Williams and Castner, 2006). It is thus conceivable that the emergence of impaired working memory after prenatal immune activation in early/mid gestation (see Meyer et al., 2005) may be linked to alterations in the PFC DA system as observed in the present study (Figure 1a) and previously (Meyer et al., 2008b,c). Furthermore, altered DA-associated signalling in the PFC may also be involved in the precipitation of sensorimotor gating deficits [in the form of reduced prepulse inhibition (PPI)] seen in adult mice after prenatal PolyI:C-induced immune activation (Meyer et al., 2005; 2008a,c; Ozawa et al., 2006; Shi et al., 2003; Smith et al., 2007). Although the precise role of the prefrontal DA system in the regulation and modulation of PPI still remains to be explored (Bast et al., 2002; Koch and Bubser, 1994; Swerdlow et al., 2005, 2006), selective enhancement of dopaminergic activity in the PFC by micro-infusion of the direct DA receptor agonist apomorphine has been shown to disrupt PPI in rats (Broersen et al., 1999; Lacroix et al., 2000). Interestingly, this effect is enhanced by simultaneous blockade of DA D2 receptors in the PFC (de Jong and van den Buuse, 2006). The concomitant reduction in prefrontal DA D2 receptors (Meyer et al., 2008b,c) and increase in DA levels (Figure 1a) may thus readily contribute to the PPI deficiency in mice exposed to in-utero immune activation in early/middle gestation (Meyer et al., 2005; 2008a,c; Ozawa et al., 2006; Shi et al., 2003; Smith et al., 2007). An alternative (but not mutually exclusive) possibility would be that disturbances in accumbal DA and 5-HT systems may be involved in the precipitation of PPI deficits following prenatal immune activation. Indeed, the NAc is known to play an essential role in the regulation and modification of PPI (Koch, 1999; Koch and Schnitzler, 1997; Swerdlow et al., 2001). Of particular interest in this context may be the PolyI:C-induced reductions in central 5-HT levels (see Figure 2a), since depletion of central 5-HT is known to disrupt PPI in rats (Fletcher et al., 2001; Prinssen et al., 2002). Additional studies are thus clearly warranted in order to dissect the relative contributions of distinct neurochemical abnormalities to sensorimotor gating dysfunctions following prenatal immune challenge.

It is known that DA signalling in the LGP is responsive to a variety of physiological stimuli known to modulate DA activity in the PFC, NAc or CPu, including acute stress and reward (Fuchs et al., 2005). The LGP is an essential component and major relay
nucleus of the indirect pathway of the basal ganglia connecting the CPu with the output structures of the basal ganglia, that is the substantia nigra pars reticulata (SNr) and nucleus entopeduncularis (Chesselet and Delfs, 1996; Parent and Hazrati, 1995). The LGP is innervated by GABAergic neurons arising from the CPu, and it sends prominent GABAergic projections to the output structures of the basal ganglia, thereby controlling basal ganglia information flow (Lindvall and Bjorklund, 1979). The LGP also receives DA afferents from the SNc (Arluison et al., 1984; Fuchs and Hauber, 2004; Lindvall and Bjorklund, 1979). Interestingly, it has recently been shown that the LGP is responsive to DA-stimulating drugs such as amphetamine (Amph) and cocaine (Fuchs et al., 2005). This effect may be of particular interest in the present context, because previous experiments in mice have demonstrated that prenatal PolyI:C-induced immune challenge leads to a potentiated sensitivity to the indirect DA receptor agonist Amph (Meyer et al., 2005, 2008a–c; Ozawa et al., 2006; Zuckerman et al., 2003). It is thus intriguing to speculate that increased DA transmission in the LGP (Figure 1a) may contribute to the enhanced sensitivity to acute Amph treatment seen after prenatal immunological stimulation (Meyer et al., 2005; 2008a–c; Ozawa et al., 2006; Zuckerman et al., 2003). On the other hand, it is well known that reductions in central serotonergic neurotransmission can increase the sensitivity to systemic Amph treatment (for a review see Marek, 2007). The concomitant decrease and increase in 5-HT (Figure 2) and DA (Figure 1) levels, respectively, may thus also suggest that intricate interactions between abnormal central 5-HT and DA activities may underlie the potentiation of Amph sensitivity in prenatally immune-challenged animals (Meyer et al., 2005; 2008a–c; Ozawa et al., 2006; Zuckerman et al., 2003).

There is recent evidence that the amino sulfonic acid taurine is involved in the regulation and modulation of anxiety-like behaviour (Chen et al., 2004; Kong et al., 2006). Exogenously applied taurine has been shown to exert anxiolytic effects in a number of animal models of anxiety, including the elevated plus maze and open-field exploration tests (Chen et al., 2004; Kong et al., 2006). Increased anxiety-like behaviour is one of the well established long-term behavioural effects of prenatal immune challenge in early/middle gestation in mice (Meyer et al., 2005, 2006b; Shi et al., 2003; Smith et al., 2007). Here, we provide the first piece of evidence that prenatal PolyI:C-induced immune activation reduces the basal levels of endogenous taurine in the adult CNS (Figure 3). Our finding may thus highlight an interesting link between prenatal infection-induced changes in central taurine levels and the emergence of enhanced anxiety-like behaviour (Meyer et al., 2005, 2006b; Shi et al., 2003). One possibility to further explore this possible association is to investigate whether exogenously applied taurine would normalize anxiety-like behaviour in prenatally immune-challenged offspring animals.

The present findings of altered basal neurotransmitter levels in the adult CNS of prenatally immune-challenged animals capture some of the critical neurochemical imbalances found in patients with schizophrenia. For example, there is biochemical evidence for enhanced HVA in the basal ganglia of schizophrenia patients (Toru et al., 1982, 1988). Interestingly, this effect is associated with increased tyrosine hydroxylase activity in these brain regions (Toru et al., 1982, 1988), similar to what is found in adult mice and rats following exposure to in-utero immune challenge (Borrell et al., 2002; Meyer et al., 2008b). In addition, the findings of reduced 5-HT and 5-HIAA levels in various brain areas of prenatally PolyI:C-exposed mice (Figure 2) is in agreement with numerous neuroanatomical, pharmacological, and CSF studies indicating a deficit in 5-HT function in schizophrenia patients especially with pronounced negative symptoms (Abi-Dargham et al., 1997). Finally, the effects of prenatal immune challenge on hippocampal taurine levels (Figure 3) parallels the findings of reduced basal levels of this amino sulfonic acid in the CSF of drug-free schizophrenia patients (Do et al., 1995).

In conclusion, the present data confirmed our expectation that the emergence of a multitude of behavioural, pharmacological and neuroanatomical abnormalities after PolyI:C-induced prenatal immunological stimulation (Meyer et al., 2005, 2006a,b, 2008a–c; Ozawa et al., 2006; Shi et al., 2003; Smith et al., 2007; Zuckerman et al., 2003) may be associated with significant alterations in several neurotransmitter systems at adult age. Our findings thus further support the hypothesis that infection-mediated interference with early normal brain development may have long-lasting consequences for the integrity of neuronal systems at adult age (Meyer et al., 2007). The constellation of the neurochemical abnormalities reported here, together with the neuroanatomical findings described in previous experimental investigations of the long-term effects of prenatal immune challenge in rodents, strongly supports the biological and neuro-immunological plausibility for a causal relationship between maternal infection and/or inflammation during pregnancy and a higher risk for schizophrenia-related neuropathology in the adult offspring.
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Statement of Interest
None.

References


