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Modulation of schizophrenia-related genes in the forebrain of adolescent and adult rats exposed to maternal immune activation

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Abstract

Maternal immune activation (MIA) is an environmental risk factor for schizophrenia, and may contribute to other developmental disorders including autism and epilepsy. Activation of pro-inflammatory cytokine systems by injection of the synthetic double-stranded RNA polyriboinosinic-polyribocytidilic acid (Poly I:C) mediates important neurochemical and behavioral corollaries of MIA, which have relevance to deficits observed in schizophrenia. We examined the consequences of MIA on forebrain expression of neuregulin-1 (NRG-1), brain-derived neurotrophic factor (BDNF) and their receptors, ErbB4 and trkB, respectively, genes associated with schizophrenia. On gestational day 14, pregnant rats were injected with Poly I:C or

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KBS and NMR designed the study, wrote the protocols, and supervised the project. AMH, RA, SLB and KHL performed the research. AMH, NMR and KBS wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest:

The authors disclose the following relationships:

Dr. N. Richtand is a member of the speaker's bureaus of Otsuka America Pharmaceutical and Sunovion Pharmaceuticals and serves on the advisory board of Otsuka America Pharmaceutical, outside the submitted work. All other authors declare that they have no conflicts of interest in relation to this study.

vehicle. Utilizing *in situ* hybridization, expression of NRG-1, ErbB4, BDNF, and trkB was examined in male rat offspring at postnatal day (P) 14, P30 and P60. ErbB4 mRNA expression was significantly increased at P30 in the anterior cingulate (AC Ctx), frontal, and parietal cortices, with increases in AC Ctx expression continuing through P60. ErbB4 expression was also elevated in the prefrontal cortex (PFC) at P14. In contrast, NRG-1 mRNA was decreased in the PFC at P60. Expression of BDNF mRNA was significantly upregulated in the PFC at P60 and decreased in the AC Ctx at P14. Expression of trkB was increased in two regions, the piriform cortex at P14 and the striatum at P60. These findings demonstrate developmentally and regionally selective alterations in the expression of schizophrenia-related genes as a consequence of MIA. Further study is needed to determine contributions of these effects to development of alterations of relevance to neuropsychiatric diseases.

Keywords

Poly I:C; BDNF; Neuregulin-1; trkB; ErbB4

1. Introduction

Maternal infections during pregnancy are implicated in elevated risk for several mental disorders, including schizophrenia, autism, and epilepsy (Patterson, 2002; Brown, 2006; Brown and Patterson, 2011). The strongest evidence for this linkage is in schizophrenia, a neuropsychiatric disorder affecting 1% of the population resulting from the combined influence of genetic and environmental factors. Compelling evidence demonstrates that maternal immune activation (MIA), i.e. maternal exposure to infection during pregnancy, accounts for up to one third of attributable environmental risk for schizophrenia (Brown & Derkits 2010). Evidence demonstrates that the immune response itself, rather than a specific infectious agent, is responsible for elevated risk for schizophrenia (Smith et al., 2007; Brown and Derkits, 2010).

In animal models attempting to replicate this environmental risk, prenatal exposure to infectious agents, including influenza, lipopolysaccharide and the viral mimetic polyriboinosinic-polyribocytidilic acid (Poly I:C), results in cellular, neurochemical, and behavioral alterations relevant to schizophrenia (Meyer et al., 2009; Brown and Derkits, 2010). Key alterations stem from the maternal inflammatory response rather than the virus itself, as behavioral abnormalities were induced in the absence of viral infection by injection of Poly I:C, which stimulates maternal cytokine expression (Shi et al 2003). The synthetic double-stranded Poly I:C RNA is commonly used to create a strong, acute, non-specific immune reaction via the Toll-like receptor 3, resulting in cytokine release (Fortier et al., 2004). Cytokines are polypeptides involved in the inflammatory response and whose prenatal elevation is linked to increased risk of brain damage in offspring (Yoon et al., 1997). Cytokine levels are elevated in mothers of offspring later diagnosed with schizophrenia (Brown et al., 2004). In the MIA model, both behavioral and transcriptional alterations observed in MIA offspring were also inducible through maternal exposure to the cytokine interleukin–6 (IL-6) (Smith et al., 2007). In contrast to the alterations of cytokine levels, viral RNA is not detected in the tissue of animals exposed to infections prenatally

despite later adult abnormalities (Shi et al., 2005). These observations suggest stimulation of maternal pro-inflammatory cytokine systems by injection of Poly I:C mediates important neurochemical and behavioral consequences of MIA (Ozawa et al., 2006; Smith et al., 2007; Pratt et al., 2013).

Maternal immune activation offspring display behavioral abnormalities into adulthood of relevance to schizophrenia. These behavioral abnormalities include altered locomotor responsiveness to stress, amphetamine, and novel environment, increased anxiety and depressive symptomology, deficits in prepulse inhibition of startle (PPI) and latent inhibition and increased responsiveness to stimulant drugs (Shi et al., 2005; Meyer et al., 2005; Brown and Patterson, 2011; Missault et al., 2014). Animals also display impaired working memory, place preference, and novel object recognition (Meyer et al., 2009; Richtand et al., 2012; Lukasz et al., 2013). Neuroanatomically, MIA elicits many brain alterations, including increases in γ-aminobutyric (GABA) receptor expression in the hippocampus and cerebellum, and widely reported developmental and functional abnormalities of the dopaminergic mesotelencephalic system (Bakos et al 2004, Ozawa et al 2006, Romero et al 2010, Vuillermot et al 2010). Prefrontal cortical abnormalities observed in MIA offspring include elevated basal extracellular glutamate (Roenker et al 2011) and altered synaptophysin expression (Romero et al 2010). Within the hippocampus, MIA offspring exhibit glutamate system defects including decreased N-Methyl-D-aspartate (NMDA) receptor-dependent synaptic current and plasticity (Escobar et al 2011, Lante et al 2007) and elevated basal extracellular glutamate (Ibi et al 2009). Imaging studies of this animal model consistently observe increased ventricular size in MIA offspring, with volume reductions in the hippocampus, prefrontal cortex (PFC), and striatum (Meyer et al., 2009; Piontkewitz et al., 2011b). Striatal and hippocampal volume reductions preceded the onset of behavioral abnormalities in both sexes, with structural abnormalities appearing developmentally earlier in males (Piontkewitz et al 2011a, Piontkewitz et al 2011b, Piontkewitz et al 2009). Of interest, antipsychotic medications impact some of these behavioral alterations, including the increased responsiveness to amphetamine, PPI, latent inhibition and alterations in the volume of brain regions (Meyer and Feldon, 2010; Piontkewitz et al., 2011b; Richtand et al., 2011).

Exposure to maternal infection during development may interact with expression of genes mediating schizophrenia risk. Neurotrophic factors are signaling molecules important during many stages of neurodevelopment including proliferation, differentiation, and migration and continue to support neuronal health and synaptic maintenance into adulthood (Knusel et al., 1991; Poo, 2001; Yarden and Sliwkowski, 2001; Buonanno and Fischbach, 2001; Seroogy et al., 2013). Neuregulin-1 (NRG-1) and its receptor ErbB4, as well as brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, are associated with the development of schizophrenia (Stefansson et al., 2002; Chong et al., 2008; Rybakowski, 2008; Ray et al., 2014). ErbB4 and NRG-1 are associated with familial inheritance of schizophrenia and the interaction between the two genes increases the risk of schizophrenia in some studies (Stefansson et al., 2003; Nicodemus et al., 2006; Norton et al., 2006; Silberberg et al., 2006). Dysregulation of NRG-1 signaling may also contribute to the development of positive schizophrenic symptoms (Shamir et al., 2012). Whereas studies directly linking BDNF gene mutations to schizophrenia are conflicting, multiple reports find

that BDNF (both mRNA and protein), as well as its high-affinity receptor trkB, are reduced in cortical regions of schizophrenia patients (Durany et al., 2001; Weickert et al., 2003, 2005; Pandya et al., 2013). We therefore sought to examine the effects of MIA on mRNA expression of these genes throughout the postnatal period in the rat forebrain, testing the hypothesis that MIA will result in aberrant regulation of NRG-1, BDNF and their respective receptors. The regions and time points examined were chosen for their relevance to the manifestation of behavioral symptoms in the MIA model.

2. Methods

2.1 Animals

Nulliparous female Sprague Dawley rats, aged 3–5 months, were obtained from Harlan Laboratories (Indianapolis, IN). Male breeders were produced within the animal facility. All animals were housed under standard conditions with food and water available *ad libitum* and a 12:12 light/dark cycle. All procedures were in accordance with the Guide for Care and Use of Laboratory Animals, with approval by our Institutional Animal Care and Use Committee.

2.2 Prenatal Poly I:C treatment

The female rats were acclimated for at least two weeks, and then placed in the same cage overnight with a male Sprague Dawley rat. Males were removed the next morning, considered gestational day (G) 0 (Taylor, 1986). The female rats were singly housed throughout the pregnancy. On G14, pregnant dams were weighed (weight gain > 40 grams) and injected with Poly I:C (Sigma, St. Louis, MO) (8 mg/kg, i.p.) (Bronson et al., 2011) dissolved in saline or with saline vehicle control (1 ml/kg, i.p.). Dams were weighed again 24 hours following the Poly I:C or saline injection to identify anorexia and weight loss resulting from an inflammatory response (Fortier et al., 2004). The MIA dams without weight loss and saline dams with weight loss were removed from study as the offspring of these animals have a different phenotypic response (Bronson et al., 2011). On postnatal day (P) 1, litters were culled to eight. Pups were weaned on P21 and housed 2–3 per cage by sex and litter. No more than 2 rats per litter were used in each experimental group to avoid litter effect confounds. On P14, P30 and P60 male MIA offspring were sacrificed and brains were collected and frozen in dry ice to be subsequently processed for in situ hybridization.

2.3 In situ hybridization

Fresh-frozen brains (n = 5–6 per condition) were serially sectioned (at 10-μm thickness) throughout the PFC and striatal levels using a cryostat, thaw-mounted onto Superfrost plus microslides (VWR, Batavia, IL), and stored at −20°C until hybridization. Semi-adjacent sections were processed for the in situ hybridization localization of BDNF, trkB, NRG-1 and ErbB4 mRNAs using lineralized cDNA plasmids and were labeled using the proper polymerase (T3 for BDNF and T7 for NRG-1, ErbB4 and trkB) and ^{35}S-UTP (PerkinElmer, Boston, MA), as previously described (Seroogy and Herman, 1997; Numan et al., 2005; Dickerson et al., 2009; Hemmerle et al., 2012). The BDNF cRNA (a gift from Drs. Christine Gall and Julie Lauterborn, University of California-Irvine) included 384 bases complementary to the rat BDNF mRNA coding region (nucleotides 388–771; Isackson et al., 1991; Gall et al., 1992). The trkB riboprobe detects the kinase-specific, full-length form

of the rat trkB receptor (nucleotides 1358–1558; Middlemas et al., 1991; Goodness et al., 1997). The pan-NRG-1 plasmid was contained in a pCR-TOPO vector and consisted of 500 bp. The ErbB4 cRNA (kindly provided by Dr. Harley Kornblum, UCLA) was transcribed from a PCR 2.1 vector containing a 1.8 kb fragment complementary to a region of the ErbB4 receptor extracellular domain (Kornblum et al., 2000).

For hybridization pretreatment, slides were brought to room temperature and placed in 4% paraformaldehyde (pH 7.4) for 10 minutes to fix tissue. The slides were then placed in a series of five-minute washes involving phosphate-buffered saline (PBS) (twice), followed by 0.1 M PBS/0.2% glycine (twice), and again 0.1 M PBS (twice). All washes were made with diethyl pyrocarbonate (DEPC)-treated water. Triethanolamine (pH 8.0) containing 0.25% acetic anhydride was then used for 10 minutes for acetylation of the slides. Finally, slides were dehydrated in a series of ethanol washes, delipidated in chloroform and air-dried prior to hybridization.

Following pretreatment, the sections were hybridized with 50 μl of hybridization solution and subsequently coverslipped. The hybridization solution for each probe included the following: 20mM Tris-HCl, 10mM EDTA, 1X Denhardt's, 335mM NaCl, 50% deionized formamide, 10% dextran sulfate, 0.3 mg/ml denatured salmon sperm DNA, 0.15 mg/ml tRNA, 40mM dithiothreitol, DEPC H_2O, and the appropriate ^{35}S-labeled cRNA probe. The resulting concentration of the hybridization solution was 1 x 10^6 cpm/slide. The slides were incubated inside a sealed, humidified chamber at 60°C for 16 hours. After hybridization, the coverslips were removed and the slides were washed in a series of standard sodium citrate washes of decreasing concentration and RNase buffer and air-dried. After the post-hybridization washes, the hybridized slides were exposed to BioMax MR Film (Kodak, Rochester, NY) for appropriate periods for each probe (NRG-1: 12 days, ErbB4: 9 days, BDNF: 7 days, trkB: 4 days). Kodak GPX developer and fixer was then used to develop the films.

2.4 Analysis

Film autoradiograms were analyzed by densitometry using Scion Image software (NIH) to compare optic densities (OD) of hybridization (mean corrected gray level) in select brain regions of the MIA versus control offspring. The following brain regions were examined: PFC, anterior, frontal, and parietal cortices, striatum, piriform cortex, and hippocampus. Control measurements for background were taken from unlabeled regions on each section and subtracted from each OD value to give a corrected OD value. Densitometric measurements were taken of each area from at least six sections per animal for each probe and averaged to obtain the mean corrected OD. The MIA offspring data are presented as a percentage of the control offspring values. Group differences were determined by t-test (GraphPad Prism) and considered significant when $p < 0.05$.

3. Results

3.1 NRG-1

The PFC of MIA offspring exhibited a slight, but significant decrease in NRG-1 expression at P60 compared to saline-treated animals ($t(10) = 3.069$, $p < 0.05$) (Fig. 1). No alterations

were observed in NRG-1 expression in the other cortical regions examined, in the striatum or in the hippocampus (see Table 1).

3.2 ErbB4

Several regions exhibited changes in ErbB4 expression in the MIA offspring group compared to controls. Hybridization for ErbB4 mRNA in the PFC was increased at P14 in MIA offspring ($t(10) = 2.923$, $p < 0.05$) (Fig. 2). In the anterior cingulate cortex (AC Ctx), ErbB4 mRNA expression was significantly elevated at both P30 ($t(10) = 3.285$, $p < 0.05$) and P60 ($t(10) = 2.777$, $p < 0.05$) when compared to the control offspring (Fig. 3A–E). Additionally at P30, levels of ErbB4 mRNA expression in the frontal ($t(10) = 3.184$, $p < 0.05$) and parietal ($t(10) = 3.015$, $p < 0.05$) cortices were increased in the MIA offspring (Fig. 3A–B, F–G), whereas there was a trend towards an increase in mRNA expression at P60 in these two regions ($t(10) = 2.170$, $P = 0.08$) (frontal); $t(10) = 2.024$, $p = 0.09$ (parietal)) (Fig. 3C–D, F–G). Finally, in the piriform cortex, a decrease in mRNA expression was noted at P30 in the MIA offspring group compared to the control offspring ($t(10) = 2.379$, $p < 0.05$) (Fig. 4). No alterations in ErbB4 hybridization were observed in the striatum or hippocampus (Table 1).

3.3 BDNF

Two regions examined exhibited alterations in BDNF expression in MIA compared to the control offspring. In the PFC, BDNF expression in the MIA offspring was elevated at the P60 time point ($t(9) = 3.094$, $p < 0.05$) (Fig. 5A–B, E). In contrast, the BDNF mRNA levels were decreased in the AC Ctx of MIA offspring at P14 ($t(9) = 2.618$, $p < 0.05$) (Fig. 5C–D, F). There were no changes in expression found in the frontal, parietal, and piriform cortices, hippocampus or striatum (Table 1).

3.4 trkB

Hybridization levels of trkB were elevated in select regions. In the piriform cortex, compared to controls, trkB expression in MIA offspring was increased at P14 ($t(9) = 2.555$, $p < 0.05$) (Fig. 6A–B, E). Levels of trkB mRNA were also upregulated in the striatum of MIA in comparison to control offspring at P60 ($t(9) = 2.459$, $p < 0.05$) (Fig. 6C–D, F). No significant changes were observed in the prefrontal, frontal, or parietal cortices or in the hippocampus (Table 1).

4. Discussion

Expression of schizophrenia-related genes after MIA was altered in multiple forebrain regions throughout brain development (Table 1). These regions include the prefrontal, anterior cingulate, frontal, parietal and piriform cortices. The subcortical striatum also exhibited modifications in gene expression, but only in adulthood. In contrast, no changes in gene expression were noted in the hippocampus. Our study encompassed multiple periods of rat neurodevelopment with postnatal (P14), adolescent (P30) and adult (P60) (McCormick et al., 2010) time points examined. This allowed us to observe how gene expression was altered throughout the major developmental stages including equivalent time spans of behavioral symptom manifestation in adolescence and adulthood. This is especially

important in this model, as many of the behavioral and cognitive adaptations do not emerge until the animals reach adulthood (Meyer and Feldon, 2010). The present altered levels of schizophrenia-related genes in juveniles and adults may have implications for the later behavioral deficits observed in animals prenatally exposed to immune activation.

Several candidate genes for increased schizophrenia risk include genes associated with neuronal plasticity and synaptic formation or impairment thereof (Le Strat et al., 2009). The genes examined in this study are important in neuronal maturation, including migration, axon formation, and myelination and the continued neuronal plasticity observed in adulthood. Clinical studies have found the expression of BDNF, trkB, NRG-1, and ErbB4 altered in the post-mortem tissue of schizophrenic patients (Weickert et al., 2003; Law et al., 2006; Chong et al., 2008; Joshi et al., 2014; Ray et al., 2014). In addition to the ligands and the receptors themselves, the signal cascades stimulated by ErbB4 and trkB receptor activation, including the PI3K-Akt, PLCγ and Raf-MAP kinase pathways, are specifically implicated in the pathology of schizophrenia (Stornetta and Zhu, 2011; Zheng et al., 2012). Upregulation of neurotrophic factors is often viewed as a beneficial compensatory signaling mechanism. However, alteration in trophic factor expression can paint a complicated picture, as increased levels are not always an advantageous outcome. For example, in the forced swim test, elevation of BDNF in the mesolimbic pathway of rats contributes to behavioral alterations of relevance to depression (Eisch et al., 2003).

This study is believed to be the first to examine NRG-1/ErbB4 gene expression in offspring exposed to MIA. ErbB4 is a receptor tyrosine kinase that can form both homo- and heterodimers with other receptors in its family after ligand binding. Of the multiple members of the neuregulin family that bind with ErbB4, NRG-1 is the most strongly linked to schizophrenia at this time (Buonanno, 2010), though some studies indicate mutations of NRG-3 may increase risk as well (Kao et al., 2010). Neuregulin-1 has numerous different isoforms via various promoters and alternative splicing (Buonanno and Fischbach, 2001; Deng et al., 2013; Seroogy et al., 2013), and in our study we used a pan-NRG-1 probe to detect expression of multiple forms of NRG-1. It is possible that a riboprobe targeting more specific isoforms would uncover more subtle variations in NRG-1 gene expression. Whereas there was only modulation of NRG-1 mRNA in the PFC at P60, this change occurred long after exposure to the maternal immune response had taken place, suggesting that prenatal exposure to the immune activation may result in long-term plasticity not evident until adulthood. ErbB4 appears to be more malleable in this model, with alterations observed in various regions, including the PFC and AC Ctx, at all three developmental time points.

Activation of the ErbB4 receptor via NRG-1 is essential in the functional development of major neurotransmitter systems linked to schizophrenia, including dopamine, glutamate and GABA (Laruelle et al., 2003; Lewis et al., 2005) and interference in ErbB4 signaling in these systems can have detrimental effects (Stefansson et al., 2002). Additionally, ErbB4 activation is increased in the PFC of schizophrenic brains and may be specifically related to deficits in NMDA receptor (NMDAR) signaling in schizophrenia patients (Hahn et al., 2006). Interestingly, in our model, an increase in ErbB4 expression was found in cortical regions during later developmental time points, comparable to the time period of symptom manifestation in humans. Consonant with our findings, elevated expression of ErbB4 mRNA

has been shown in the PFC of schizophrenic brains (Joshi et al., 2014). Research has suggested that NRG-1 signaling via ErbB4 can result in a decrease in NMDAR activation (Huang et al., 2000; Gu et al., 2005), relating to the glutamatergic hypoactivity hypothesis of schizophrenia. However, knockout of ErbB4 in neurons/glia produced lower motor activity and reduced grip strength, which may correlate with schizophrenia symptoms (Golub et al., 2004; Esper et al., 2006). These studies present an imprecise picture of the relationship between ErbB4 levels and behavioral changes, but it is possible that there is a distinct pattern of changes in ErbB4 expression found depending on the brain region.

The other major neurotrophic factor examined, BDNF, is a member of the neurotrophin family and BDNF/trkB signaling is believed to play a large role in the normal function of the nervous system, including having a major role in synaptic efficacy and plasticity (Thoenen, 1995; Poo, 2001; Waterhouse and Xu, 2009). Like ErbB4, the expression of BDNF is vital to the function of forebrain GABAergic, dopaminergic, and glutamatergic circuits (Itami et al., 2000; Glorioso et al., 2006; Pillai, 2008). Schizophrenia patients display reduction of BDNF serum and plasma levels in several studies (Palomino et al., 2006; Grillo et al., 2007), though how this precisely correlates with brain BDNF levels is unknown at this time. Postmortem brains of schizophrenia patients show increases of BDNF protein in the frontal and parietal cortices and decreases in BDNF protein and mRNA in the PFC (Durany et al., 2001; Weickert et al., 2003; Pillai, 2008). Loss of trkB signaling in the PFC of schizophrenia patients appears to be a factor in the dysfunction of GABAergic neurons (Hashimoto et al., 2005). Similar to findings in ErbB4 studies, there is inconsistent data on BDNF alterations in the hippocampus and cortex of schizophrenia patients (Angelucci et al., 2005; Autry and Monteggia, 2012). Interestingly, other studies have described an interaction between trkB and ErbB4 signaling pathways in cortical neurons, with trkB being necessary for NRG-1 phosphorylation of NRB2 prior to synaptogenesis (Pandya and Pillai, 2014). Moreover, NRG-1 is able to increase the release of BDNF (Pandya and Pillai, 2014). Reduction of trkB/ErbB4 interaction is also observed in the prefrontal cortex of schizophrenia patients (Pandya and Pillai, 2014). In our study, most areas with alterations in BDNF/trkB expression did not also exhibit changes in NRG-1/ErbB4 and vice versa, a major exception being the PFC where BDNF was upregulated and ErbB4 was decreased at P60.

In previous studies, animals receiving Poly I:C acutely in adulthood displayed alterations in BDNF mRNA levels in the cortex and hippocampus, potentially linked to behavioral changes seen in the animals (Kranjac et al., 2012; Gibney et al., 2013). Decreases in BDNF within the frontal cortex are found in additional developmental models of relevance to schizophrenia including maternal stress and genetic models (Pillai and Mahadik, 2008; Matrisciano et al., 2012). Other studies utilizing the MIA model have identified decreased BDNF in the placentas of treated dams, whereas whole brain modification of BDNF protein content was undetectable on P1 (Gilmore et al., 2005). Locating regions of BDNF perturbation is important, as studies of behaviors of relevance to schizophrenia in BDNF knockout models suggest that BDNF contributes to the development of positive symptoms like hyperactivity and elevated responsiveness to stimulant drugs (Autry and Monteggia, 2012). In the current study, we found changes in BDNF and trkB expression in cortical regions and the striatum at P14 or P60, but no changes were observed in the hippocampus.

This variance from other studies could be explained by the use of alternative models, as several involved injections of Poly I:C into the adult animals themselves (Kranjac et al., 2012; Gibney et al., 2013).

Mechanisms underlying this developmental modulation of schizophrenia-related genes is not known, but may be related to the cytokine release resulting from the MIA. After administration of Poly I:C to pregnant dams, cytokines, including the pro-inflammatory cytokines IL-1α, IL-1β, IL-6, tumor neurosis factor-α (TNF-α), and interferon-γ, are altered in the offspring in a region-specific manner throughout development (Meyer et al., 2008; Garay et al., 2013; Pratt et al., 2013). Some anti-inflammatory cytokines and chemokines appear to be altered as well (Garay et al., 2013). The enduring modulation of cytokine levels in the MIA model may have clinical relevance, as increased cytokine levels have been reported in schizophrenia patients, including IL-6 and TNF-α (Strous and Shoenfeld, 2006; Miller et al., 2011). The release of cytokines, particularly IL-6, appears to be essential for the behavioral and physiological effects observed in the MIA model (Smith et al., 2007). There are, however, conflicting data as to whether the number and density of microglia themselves are altered (Garay et al., 2013; Van den Eynde et al., 2014).

While we did not measure regional alterations of cytokines in the present experiments, there is strong evidence linking cytokine levels to regulation of the neurotrophic factors examined in this study (Imai et al., 2007; Noga et al., 2007; Marballi et al., 2010; Skaper et al., 2012; Calabrese et al., 2014). Other prenatal models of relevance to schizophrenia, including prenatal restraint stress, also detect changes in forebrain expression of BDNF and ErbB4 (Matrisciano et al., 2012; Stevens et al., 2013). Neurotrophic factor alterations could be a fundamental indication of processes underlying the deficits observed in different models. Cytokine-induced alterations of trophic factors such as BDNF may therefore identify a mechanism linking immune activation to the brain plasticity underlying behavioral deficits in these developmental models (Calabrese et al., 2014).

Why particular brain regions are more susceptible to neurotrophic factor modifications is unknown at this time. It is believed that the neuroanatomical changes observed in developmental models are a result of alterations in neurodevelopment rather than neurodegeneration (Meyer and Feldon, 2010). Given prior studies identifying brain parenchymal alterations in the MIA model, it is possible that differential loss of certain cell types may contribute to the altered mRNA expression observed in our study. Reductions in volume of the hippocampus, prefrontal cortex, and striatum have been consistently observed in MIA offspring. Striatal and hippocampal volume reductions preceded the onset of behavioral abnormalities in both sexes, with structural abnormalities appearing developmentally earlier in males (Piontkewitz et al., 2009,2011a,b). Future studies utilizing co-localization of cell-specific markers could help to further elucidate this potential mechanism.

One caveat in the relevance of the MIA neurodevelopmental model to schizophrenia is that gene expression in our study was examined in prenatally challenged Sprague Dawley rats presumably devoid of schizophrenia risk-allele genes. Previous studies demonstrate significant increase in schizophrenia risk in human MIA offspring with a family history of

psychotic disorder, whereas enhancement of schizophrenia risk was not detectable in offspring lacking a family genetic risk for the illness (Clarke et al., 2009). Thus, the interaction between the environmental influence of MIA and schizophrenia susceptibility alleles is likely a requirement for elevation of schizophrenia risk. Evidence supporting this gene/environment interaction has also been demonstrated in other rodent animal models, where activation of the prenatal and neonatal immune system by Poly I:C in DISC1 mutant mice results in offspring with behavior and cellular effects worsened compared to mutant animals alone (Abazyan et al., 2010; Ibi et al., 2010; Nagai et al., 2011). In heterozygous knockouts of NRG-1, it appears that NRG-1 is necessary for the reduced sociability and spatial working memory deficits observed in MIA offspring, whereas PPI deficits (reflecting sensorimotor gating impairment) were observed in both knockout and wild-type mice (O'Leary et al., 2014). This suggests that NRG-1 plays a key role in the manifestation of many, but not all, of the behavioral deficits observed in the MIA model. To draw a more complete picture, it would be important to examine alterations of neurotrophic factors in genetic models, like DISC1 mutant mice, after exposure to MIA to determine if the gene expression changes are exacerbated compared to wild-type animals.

An additional consideration for data interpretation in our study is that alterations in mRNA expression may not directly correlate with protein expression, the ultimate effectors of biological action. While mRNA and protein expression vary in a synchronous fashion in some systems (e.g. Wang et al., 1995; Ohnuma et al., 2003; Dickerson et al., 2009), there are other examples of genes for which mRNA and protein expression do not demonstrate complete correlation (e.g. Conner et al., 1997; Gygi et al., 1999). Protein expression may also vary at the subregional level as well (see Weickert et al., 2003). The differences in mRNA expression observed in our data set should also be considered preliminary because we did not correct for multiple comparisons. While the data presented therefore provide a framework for trophic factor plasticity in the MIA model, future confirmatory studies would ideally analyze both mRNA and protein levels to provide a more complete understanding of cellular mechanisms. It will likewise be intriguing to examine the potential linkage of gene expression alterations in regions like the PFC and piriform cortex to behavioral dysfunctions observed in MIA models such as PPI and novel object recognition (Ozawa et al., 2006; Lukasz et al., 2013).

In conclusion, animals exposed to MIA via Poly I:C administration display region-specific, aberrant expression of key schizophrenia-related genes at different stages of postnatal development. These modifications appear to predominately affect neocortical regions of the brain compared to the hippocampus, with ErbB4 displaying the most plastic properties. Further study is needed to determine contributions of these effects to development of behavioral and neurochemical alterations of relevance to neuropsychiatric disease states. Deciphering these alterations will aid in uncovering the biological basis of schizophrenia-related behavioral symptoms and allow development of more targeted therapies.

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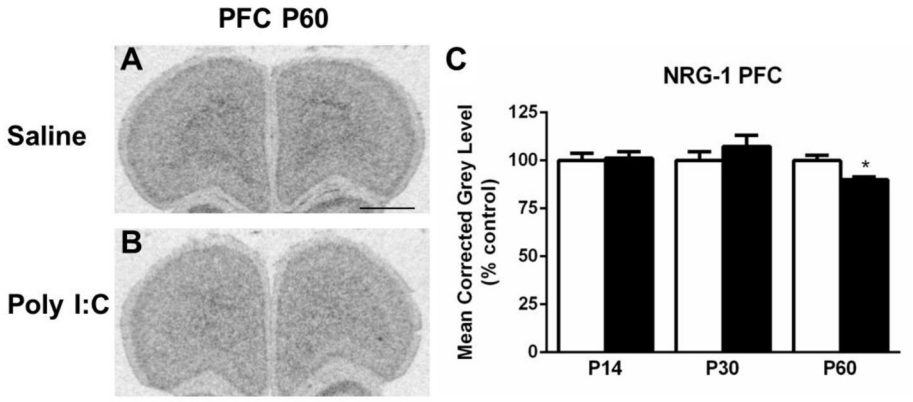

Figure 1.

A–B. Representative autoradiograms of the prefrontal cortex (PFC) at P60 showing cRNA-labeling of neuregulin-1 (NRG-1) under saline (control) and MIA offspring conditions. **C.** Quantification of hybridization signal revealed that levels of NRG-1 mRNA are decreased in the PFC at P60. Open bar, control offspring; black bar, MIA offspring. Data are expressed as mean ± SEM. *$p < 0.05$ compared to respective control values. Scale bar in A = 1000 μm for A,B.

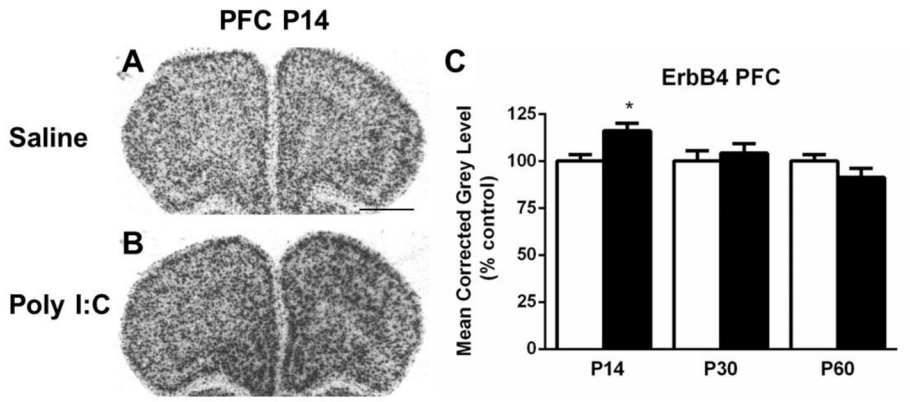

Figure 2.

A–B. Representative autoradiograms of the prefrontal cortex (PFC) at P14 showing labeling of ErbB4 mRNA under saline (control) and MIA offspring conditions. **C.** Quantification of ErbB4 mRNA expression in the PFC demonstrates increased hybridization signal at P14. Open bar, control offspring; black bar, MIA offspring. Data are expressed as mean ± SEM. *$p < 0.05$ compared to respective control values. Scale bar in A = 1000 μm for A,B.

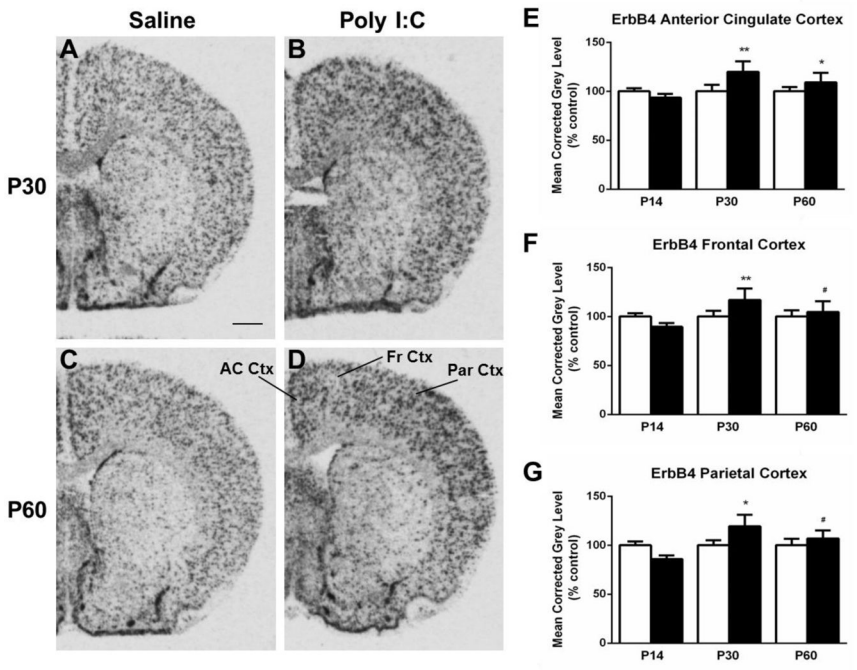

Figure 3.

A–D. Representative autoradiograms of ErbB4 mRNA expression in the anterior cingulate cortex (AC Ctx), frontal cortex (Fr Ctx) and parietal cortex (Par Ctx) at P30 (**A–B**) and P60 (**C–D**) under saline (control) and MIA offspring conditions. **E.** Quantification of the hybridization signal revealed increased expression of ErbB4 at P30 and P60 in the AC Ctx in MIA offspring **F.** Levels of ErbB4 mRNA expression in the Fr Ctx increased at P30, and exhibited a trend towards an increase at P60. **G.** In the Par Ctx there is an increase of mRNA expression at P30, also with a trend towards elevation at P60. Open bar, control offspring; black bar, MIA offspring. Data are expressed as mean ± SEM. #p=0.08, *p < 0.05, **p < 0.01 compared to respective control values. Scale bar in A = 1000 μm for A–D.

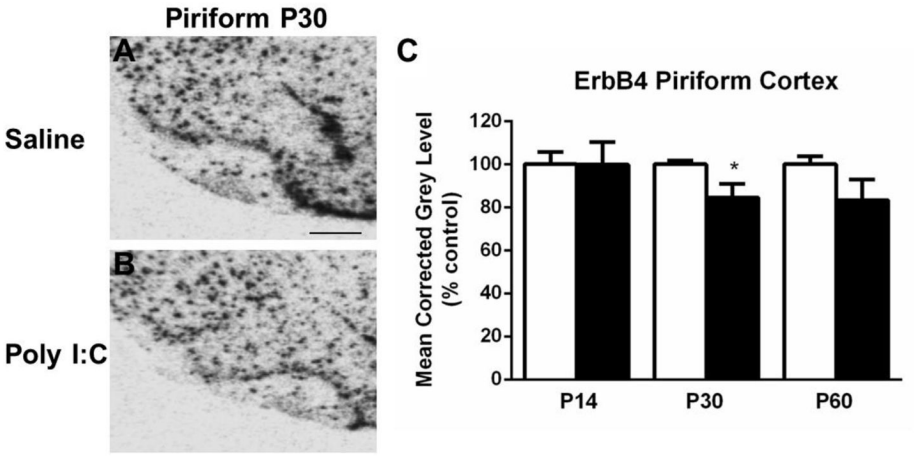

Figure 4.

A–B. Representative autoradiograms of the piriform cortex (Pir Ctx) at P30 showing cRNA-labeling of ErbB4 mRNA under saline (control) and MIA offspring conditions. **C.** In the Pir Ctx a decrease in mRNA expression was detected at P30. Open bar, control offspring; black bar, MIA offspring. Data are expressed as mean ± SEM. *$p < 0.05$ compared to respective control values. Scale bar in A = 500 μm for A,B.

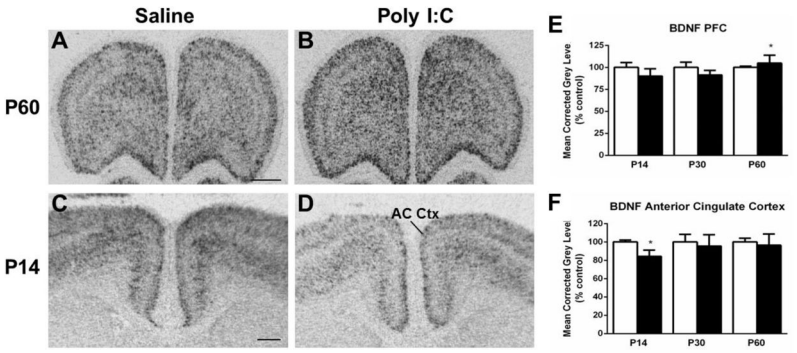

Figure 5.

Representative autoradiograms of BDNF mRNA expression in the prefrontal cortex (PFC) at P60 (**A–B**) and in the anterior cingulate cortex (AC Ctx) at P14 (**C–D**) under saline (control) and MIA offspring conditions. **E.** Quantification of BDNF mRNA expression in the prefrontal cortex (PFC) indicated an increased level of expression at P60. **F.** There was a decrease of BDNF mRNA in the anterior cingulate cortex (AC Ctx) of Poly I:C-treated rats at P14. Open bar, control offspring; black bar, MIA offspring. Data are expressed as mean ± SEM. *$p < 0.05$ compared to respective control values. Scale bar in A = 1000 μm for A,B; C = 500 μm for C,D.

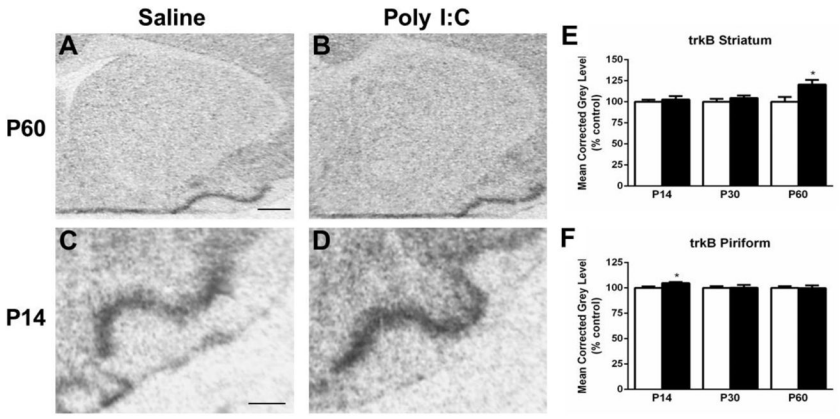

Figure 6.

Representative autoradiograms of trkB mRNA expression in the striatum at P60 (**A–B**) and in the piriform cortex at P14 (**C–D**) under saline (control) and MIA offspring conditions. **E.** Measurement of hybridization signal in the striatum revealed an increase in trkB mRNA levels in Poly I:C-treated animals at P60. **F.** Levels of trkB mRNA in the piriform cortex are elevated at P14 in Poly I:C animals compared with saline-treated animals. Open bar, control offspring; black bar, MIA offspring. Data are expressed as mean ± SEM. *$p < 0.05$ compared to respective control values. Scale bar in A = 1000 μm for A,B; C = 500 μm for C,D.

Table 1

Summary of Poly I:C-Induced Alterations in Neurotrophic Factor/Receptor Expression by Forebrain Region

	Neurotrophic Factor/Receptor											
	BDNF			trkB			NRG-1			ErbB4		
Brain Region	**P14**	**P30**	**P60**	**P14**	**P30**	**P60**	**P14**	**P30**	**P60**	**P14**	**P30**	**P60**
Prefrontal Cortex	↔	↔	↑	↔	↔	↔	↔	↔	↓	↑	↔	↔
Anterior Cingulate Cortex	↓	↔	↔	↔	↔	↔	↔	↔	↔	↔	↑	↑
Frontal Cortex	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↑	↑
Parietal Cortex	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↑	↑
Piriform Cortex	↔	↔	↔	↑	↔	↔	↔	↔	↔	↔	↓	↔
Striatum	NA	NA	NA	↔	↔	↑	↔	↔	↔	↔	↔	↔
Hippocampal Region CA1	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔
Hippocampal Region CA3	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔
Dentate Gyrus	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔

Abbreviations: NA, not applicable, i.e., no measurable expression found in this region; BDNF, brain-derived neurotrophic factor; trkB, tropomyosin receptor kinase B; NRG-1, neuregulin-1; ↑, increased expression; ↑, strong trend towards increased expression; ↓, decreased expression; ↔ no change in expression.