The Neurodevelopmental Impact of Prenatal Infections at Different Times of Pregnancy: The Earlier the Worse?

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Environmental insults taking place in early brain development may have long-lasting consequences for adult brain functioning. There is a large body of epidemiological data linking maternal infections during pregnancy to a higher incidence of psychiatric disorders with a presumed neurodevelopmental origin in the offspring, including schizophrenia and autism. Although specific gestational windows may be associated with a differing vulnerability to infection-mediated disturbances in normal brain development, it still remains debatable whether and/or why certain gestation periods may confer maximal risk for neurodevelopmental disturbances following the prenatal exposure to infectious events. In this review, the authors integrate both epidemiological and experimental findings supporting the hypothesis that infection-associated immunological events in early fetal life may have a stronger neurodevelopmental impact compared to late pregnancy infections. This is because infections in early gestation may not only interfere with fundamental neurodevelopmental events such as cell proliferation and differentiation, but it may also predispose the developing nervous system to additional failures in subsequent cell migration, target selection, and synapse maturation, eventually leading to multiple brain and behavioral abnormalities in the adult offspring. The temporal dependency of the epidemiological link between maternal infections during pregnancy and a higher risk for brain disorders in the offspring may thus be explained by specific spatiotemporal events in the course of fetal brain development. NEUROSCIENTIST 13(3):241–256, 2007. DOI: 10.1177/1073858406296401

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Disturbance of normal brain development is implicated in a number of neuropsychiatric disorders, including autism and schizophrenia. Both are characterized by a heterogeneous group of symptoms with relatively ill-defined etiologies. Besides a clear genetic contribution (Harrison and Weinberger 2005), various environmental factors appear to increase the risk for schizophrenia and/or related disorders (reviewed in McDonald and Murray 2000; Dean and Murray 2005; Opler and Susser 2005). Many of these factors operate at prenatal stages of life, that is, during the critical periods of central nervous system (CNS) development.

Epidemiological research over the past two decades has indicated that the risk for schizophrenia and autism is enhanced in offspring exposed to viral or bacterial infections in utero (for recent reviews, see Brown and Susser 2002; Arndt and others 2005; Cannon and Clarke 2005). There is also considerable epidemiological evidence for the possibility that specific gestational periods may correspond to time windows with differing vulnerability to infection-mediated disturbances in fetal brain development and associated adult psychopathology. However, the neuroimmunological mechanisms underlying this temporal dependency are poorly understood. Here, we integrate both epidemiological and experimental findings of prenatal infectious events in the etiology of schizophrenia and related disorders. A special emphasis is put on the impact of the precise times of prenatal immune challenge on the specification of postnatal psychopathology and neuropathology, thereby highlighting critical neuroimmunological mechanisms involved in shaping the vulnerability to prenatal infection-mediated disturbances in normal brain development.

The Identification of Critical Time Windows by Epidemiological Studies

Many early epidemiological studies found a significant association between maternal infection during pregnancy and a higher incidence of schizophrenia in the progeny only when the maternal host was infected in the second trimester of human pregnancy (reviewed in Machón and others 1995). This readily suggested that second trimester infections might confer maximal risk for neurodevelopmental disturbances and associated psychopathology in the offspring. However, because most of the initial epidemiological reports were based on ecologic research

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designs, several methodological limitations may have undermined the validity of the conclusions drawn from these reports (Brown and Susser 2002). Such limitations may include imprecise measurements of the infectious exposure in the population studied, that is, exposure is defined on the basis of the dates of epidemics in the population or on the maternal recall of infection after pregnancy. This may readily explain why several attempts have failed to find significant associations between maternal infections during mid-pregnancy (i.e., around the second trimester) and the higher incidence of schizophrenia and related disorders in the offspring (see, e.g., Crow and Done 1992; Selten and Slaets 1994; Morgan and others 1997; Mino and others 2000).

The subsequent establishment of prospective approaches has advanced the research on prenatal environmental risk factors of neurodevelopmental disorders in general, and schizophrenia in particular. One of them, the Prenatal Determinants of Schizophrenia (PDS), features a large birth cohort of well-characterized pregnancies with archived aliquots of maternal sera collected throughout pregnancy, and thorough diagnostic classifications of schizophrenia outcomes (for a detailed description, see Susser and others 2000). Hence, the PDS not only allows for verifying possible epidemiological associations between prenatal exposure to infections and higher risk for schizophrenia-like postnatal pathology on the basis of prospectively collected maternal sera, but it also ascertains the immunological characterization and quantification of the specific pathogen invading the maternal host during pregnancy.

Research in the context of the PDS has generally provided further support for a causal relationship between prenatal infections and higher risk for psychopathology in later life. Most importantly, however, findings from the PDS have suggested that the time window with maximal risk for infection-mediated disturbances in brain development is earlier than the second trimester of human pregnancy. For example, there is serologic evidence for prenatal exposure to influenza and a sevenfold higher risk for schizophrenia when the maternal host was infected in the first trimester. In contrast, no increased risk was found after prenatal influenza exposure at a later gestational stage (Brown, Begg, and others 2004). Moreover, genital and/or reproductive infections increase the incidence of schizophrenia in the offspring when the exposure takes place around the time of conception or in the first few weeks of pregnancy (Babulas and others 2006). In this latter report, the time window of susceptibility was relatively narrow too, as no elevated risk was associated with genital/reproductive infections at later gestational times.

Hence, epidemiological reports using prospectively collected and quantifiable measurements have raised the possibility that the first rather than the second trimester of human pregnancy may be considered as a gestational time window with maximal vulnerability for infection-mediated disturbances in fetal brain development. In fact, serologic confirmation of prenatal infections in the etiology of schizophrenia has highlighted that the critical gestational period may be earlier than previously assumed, that is, in the first weeks of gestation rather than around mid-pregnancy.

There is also considerable epidemiological evidence that links failures in early fetal brain development (i.e., around the first few weeks of gestation) to a higher risk for autism (reviewed in Arndt and others 2005; Libbey and others 2005; Miller and others 2005). For example, the incidence of autistic spectrum disorder is significantly higher in children prenatally exposed to the anticonvulsant agent valproic acid (Moore and others 2000) or the immunomodulatory agent thalidomide (Stromland and others 1994) early in the first trimester of gestation. Similarly, the association between maternal rubella infection during pregnancy and the higher risk for autism in the resulting offspring is also believed to be a consequence of disturbances in early fetal brain development (Chess 1971; Ueda and others 1979; Chess and Fernandez 1980). Hence, the risk for autism and schizophrenia appears to be particularly increased after the exposure to environmental insults in early fetal life. This further supports the hypothesis that similar neurodevelopmental mechanisms may be involved in the etiopathogenesis of these two disabling brain disorders (Levitt and others 2004; Fatemi 2005A; DiCicco-Bloom and others 2006; see also Box 1).

Examination of the Epidemiological Link between Prenatal Infection and Postnatal Neuropathology in Animal Models

Recent experimentation in animals has yielded additional support for a causal relationship between prenatal immunological disturbances and the emergence of postnatal brain and behavioral dysfunctions. A multitude of behavioral, cognitive, and psychopharmacological abnormalities has been detected in adult mice and rats following the prenatal exposure to the bacterial endotoxin lipopolysaccharide (LPS) (Borrell and others 2002; Fortier, Joober, and others 2004; Golan and others 2005), human influenza virus (Shi and others 2003), the viral mimic polyriboinosinic-polyribocytidilic acid (PolyI:C) (Shi and others 2003; Zuckerman and others 2003; Zuckerman and Weiner 2003, 2005; Meyer and others 2005; Meyer, Feldon, and others 2006; Meyer, Nyffeler, and others 2006; Meyer, Schwendener, and others 2006; Ozawa and others 2006), and the pro-inflammatory cytokine interleukin (IL)-6 (Samuelsson and others 2006). The spectrum of behavioral abnormalities induced by the various prenatal immunological manipulations is summarized in Table 1. Many of the functional deficits could be related to the endophenotypes of schizophrenia and related disorders (Lipska and Weinberger 2000; Meyer and others 2005), and the responsiveness of some of the behavioral deficits to known antipsychotic drug treatment has further added to the predictive validity of these models for schizophrenia-related psychopathology. A summary of the behavioral paradigms commonly used to model schizophrenia- and autism-related phenotypes in animals is provided in Box 1. In addition to the alterations at the functional levels, long-term alterations at the cellular and

Box 1. Animal Behavioral Paradigms to Model Schizophrenia- and Autism-Related Phenotypes

Behavioral Paradigm	Neuropsychological/-Chemical Processes Involved	Symptom in Schizophrenia	Symptom in Autism Spectrum Disorder	References
Prepulse inhibition	Sensorimotor gating	Impaired	Impaired	Braff and others (2001); Weiss and Feldon (2001); Perry and others (2006)
Latent inhibition	Selective associative learning and attentional control	Reduced in patients with positive symptoms; increased in patients with negative symptoms	Not determined	Feldon and Weiner, (1992); Moser and others (2000); Weiner (2003)
US-pre-exposure effect	Associative/emotive control of selective learning; blocking	Deficient	Not determined	Jones and others (1992); Moran and others (2003); Meyer and others (2004)
Working memory	Working memory	Impaired	Impaired	Goldman-Rakic, (1994); Castner and others (2004); Hill (2004)
Discrimination reversal learning	Behavioral and/or cognitive flexibility	Enhanced in patients with positive symptoms; decreased in patients with negative symptoms (perseverative behavior)	Decreased (perseverative behavior)	Crider (1997); Rapin and Katzman (1998); Yogev and others (2003); Hill (2004); Zuckerman and Weiner (2005); Meyer, Nyffeler, and others (2006)
Social interaction test	Social interaction	Reduced especially in patients with negative symptoms	Reduced	McGlashan and Fenton (1992); Lord and others (2000); Shi and others (2003); Moy and others (2006)
Open field exploration	Exploratory behavior; stereotypies; anxiety-related behavior	Stereotypies	Restricted interests; increased anxiety	Lord and others (2000); Garner and others (2003); Shi and others (2003); Evans and others (2005); Meyer, Nyffeler, and others (2006); Stoppelbein and others (2006)
Locomotor reaction to systemic amphe-tamine	DA-associated neurotransmission	Increased sensitivity to dopaminergic drug treatment; enhanced striatal dopamine release	No major changes in DA-associated neurotransmission	Laruelle and others (1999, 2003); Lam and others (2006); Zuckerman and others (2003); Meyer and others (2005)

(continued)

Box 1. (continued)

Behavioral Paradigm	Neuropsychological/-Chemical Processes Involved	Symptom in Schizophrenia	Symptom in Autism Spectrum Disorder	References
Locomotor reaction to systemic dizocilpine (MK-801) and phencyclidine (PCP)	NMDA-receptor-associated neurotransmission	NMDA-receptor hypofunctions	Enhanced glutamate-associated signaling; increased AMPA- (but not NMDA-) receptor expression	Carlsson and others (2001); Purcell and others (2001); Zuckerman and Weiner (2005); McDougle and others (2005); Eyjolfsson and others (2006)

gene expression levels have been noted in the brains of neonatal and adult offspring subjected to in utero immune challenge (Fatemi, Sidwell, Akhter, and others 1998; Fatemi, Sidwell, Kist, and others 1998; Fatemi and others 1999, 2002, 2004, 2005; Zuckerman and others 2003; Golan and others 2005; Meyer, Nyffeler, and others 2006; Nyffeler and others 2006; Samuelsson and others 2006), some of which are closely associated with the pathophysiology of schizophrenia and autism (Table 2).

Among the various animal models thus far established, only a few have directly examined the influence of the precise times of prenatal immune activation on the emergence of brain and behavioral pathology in later life. However, as discussed in detail below, there is already some experimental support for the possibility that the specificity of the structural and functional consequences of fetal brain inflammation critically depends on the precise times of prenatal infection.

Prenatal Exposure to PolyI:C

We have recently performed a series of experiments testing the critical window hypothesis by investigating in mice whether maternal immune stimulation at very early to late gestation stages is associated with distinctive psychopathological profiles in the adult offspring. PolyI:C, a synthetic analogue of double-stranded RNA, was used in these studies to challenge pregnant mice immunologically at different times of gestation (Fig. 1). Systemic exposure to PolyI:C results in an intense but time-limited inflammatory cytokine response in the host without the production of specific antibodies (Kimura and others 1994; Alexopoulou and others 2001; Fortier, Kent, and others 2004). Hence, its application allows the experimenter to set precisely the times of the maternal inflammatory response corresponding to specific periods of fetal development. The use of PolyI:C also offers the advantage over the alternative use of virulent pathogens to specifically mimic a general cytokine-associated inflammatory response in the maternal host, considering furthermore that the exact identity of a pathogen seems irrelevant in the epidemiological link between prenatal immune challenge and the emergence of brain and behavioral pathology in postnatal life (see below).

In the first study, we compared the phenotypic profiles of offspring born to PolyI:C-treated mothers on gestation day (GD) 6, 9, 13, or 17 (Meyer, Feldon, and others 2006). It was demonstrated that adult mice subjected to prenatal immune challenge on GD 6 and 9 displayed marked selective learning deficits in the form of latent inhibition (LI) deficiency; this effect was marginal in mice treated with PolyI:C on GD 13 and was absent in offspring subjected to prenatal PolyI:C exposure on GD 17. Hence, the efficacy of prenatal immune challenge to impair LI in adulthood appeared to decrease as prenatal development progressed. On the other hand, the expression of another selective learning paradigm, the US-pre-exposure effect (USPEE), was abolished by the prenatal immunological manipulation regardless of the precise times of maternal immune challenge. Together these findings provided experimental evidence that early prenatal immune challenge in mice can exert more extensive neurodevelopmental impact in comparison to late treatment, at least in the precipitation of long-term deficits in selective associative learning. In a subsequent study, we demonstrated a functional double dissociation of the long-term behavioral consequences between prenatal PolyI:C exposure on GD 9 and GD 17 in mice. Whereas the prenatal manipulation conducted on GD 9 suppressed spatial exploration in adulthood, the same immunological challenge conducted on GD 17 led to response perserveration in a discrimination reversal learning task (Meyer, Nyffeler, and others 2006). This yielded additional support for the suggestion that the specificity of postnatal behavioral and cognitive dysfunctions is critically determined by the precise times of prenatal immune challenge.

The dissociable functional consequences of early/mid and late prenatal PolyI:C exposure further indicated that the precise times of maternal immune activation can differentially affect the development of the relevant neural substrates involved. This expectation has also been confirmed by our recent demonstration that the long-term

Table 1. Summary of current animal models demonstrating behavioral, cognitive, and pharmacological long-term consequences of prenatal exposure to various immunostimulatory agents, including human influenza virus, polyriboinosinic-polyribocytidilic acid (PolyI:C), lipopolysaccharide (LPS), and interleukin (IL)-6. The precise timing of prenatal immune activation is specified in gestation days (GD). Upward and downward arrows indicate an enhancement and impairment of the particular functional phenotype, respectively. The asterisks signify normalization by antipsychotic drug treatment. The hyphens indicate that no significant changes were detected. The behavioral paradigms used to assess schizophrenia- and autism-related phenotypes in rodents are also summarized in Box 1. ND = not determined; DA = dopamine; NMDA = *N*-methyl-D-aspartate.

			Functional Brain Abnormalities in Adult Offspring								
Immunostimulatory Agent	**Species**	**Gestation Period**	**Spatial Exploration**	**Prepulse Inhibition**	**Latent Inhibition**	**Working Memory**	**Reference Memory**	**Reversal Learning Effect**	**Sensitivity to DA Agonists**	**Sensitivity to NMDA Antagonists**	**References**
Influenza Virus	Mouse	GD 9	↓	↓*	ND	ND	ND	ND	ND	↑	Shi and others (2003)
PolyI:C	Rat	GD 15	ND	ND	↓*	ND	—	↓*	↑	↑	Zuckerman and others (2003); Zuckerman and Weiner (2005)
	Mouse	GD 6	ND	ND	↓	ND	ND	ND	ND	ND	Meyer, Feldon, and others (2006)
	Mouse	GD 9	↓	↓	↓	↓	ND	↓	↑	ND	Shi and others (2003); Meyer and others (2005); Meyer, Nyffeler, and others (2006); Meyer, Schwendener, and others (2006)
	Mouse	GD 13	ND	ND	↓	ND	ND	ND	ND	ND	Meyer, Feldon, and others (2006)
	Mouse	GD 17	—	—	—	↓	ND	↑	↑	↑	Meyer, Nyffeler, and others (2006); Meyer, Yee, Feldon (unpublished observation)
	Mouse	GD 12-17	↑	↓	ND	↓*	ND	ND	↑	ND	Ozawa and others (2006)
LPS	Rat	Throughout gestation	ND	↓*	ND	ND	ND	ND	ND	ND	Borrell and others (2002)
	Rat	GD 18-19	ND	—	ND	ND	ND	ND	↑	ND	Fortier, Joober, and others (2004)
	Mouse	GD 17	—	ND	ND	ND	↑	ND	ND	ND	Golan and others (2005)
IL-6	Rat	GD 8, 10, and 12	ND	ND	ND	ND	↓	ND	ND	ND	Samuelsson and others (2006)
	Rat	GD 16, 18, and 20	ND	ND	ND	ND	↓	ND	ND	ND	Samuelsson and others (2006)

Table 2. Summary of current animal models demonstrating morphological and cellular abnormalities in brains of offspring subjected to prenatal exposure to various immunostimulatory agents, including human influenza virus, polyriboinosinic-polyribocytidilic acid (PolyI:C), lipopolysaccharide (LPS), and interleukin (IL)-6. The precise timing of prenatal immune activation is specified in gestation days (GD). Upward and downward arrows indicate an increase and decrease of the particular structural phenotype, respectively. The hyphens denote that no changes were detected. ND = not determined; $GABA_A$-R = gamma-aminobutyric acid(A) receptors; NMDA-R = *N*-methyl-D-aspartate receptors; TH = tyrosine hydroxylase.

			Structural Brain Abnormalities in Offspring								
Immunostimulatory Agent	**Species**	**Gestation Period**	**Corticogenesis/ Neurogenesis**	**Pyramidal Cells**	**Gliosis**	**Apoptosis**	**Reelin**	**$GABA_A$-R**	**NMDA-R**	**TH**	**References**
Influenza Virus	Mouse	GD 9	↓	Increased density; atrophy	↑	ND	↓	ND	ND	ND	Fatemi and others (1999, 2002, 2004)
PolyI:C	Rat	GD 15	ND	Pyknotic	ND	ND	ND	ND	ND	ND	Zuckerman and others (2003)
	Mouse	GD 9	↓	—	—	—	↓	↑	ND	ND	Meyer, Nyffeler, and others (2006); Nyffeler and others (2006)
	Mouse	GD 17	↓	—	—	↑	—	ND	ND	ND	Meyer, Nyffeler, and others (2006)
LPS	Rat	Throughout gestation	ND	ND	↑	ND	ND	ND	ND	↑	Borrell and others (2002)
	Mouse	GD 17	ND	Increased density; shrinkage	ND	ND	ND	ND	ND	ND	Golan and others (2005)
IL-6	Rat	GD 8, 10, and 12	ND	Pyknotic; loss	↑	—	ND	—	—	ND	Samuelsson and others (2006)
	Rat	GD 16, 18, and 20	ND	Pyknotic; loss	↑	↑	ND	↑	↑	ND	Samuelsson and others (2006)

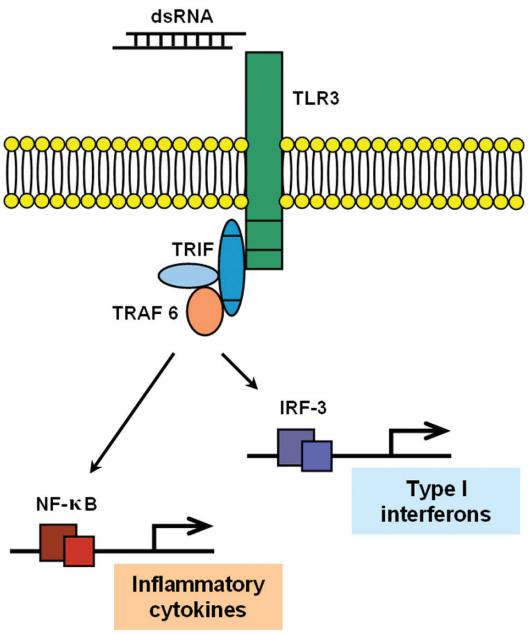

Fig. 1. Simplified diagram illustrating the induction of cytokine responses during viral-like infection. Double-stranded RNA (dsRNA) and its synthetic analogue polyriboinosinic-polyribocytidilic acid (PolyI:C) are recognized by the transmembrane protein toll-like receptor 3 (TLR3). dsRNA is generated during viral infection as a replication intermediate for single-stranded RNA (ssRNA) or as a by-product of symmetrical transcription in DNA viruses. Recognition of dsRNA or PolyI:C by TLR3 recruits adaptor proteins TRIF (toll/IL-1 receptor domain-containing adaptor-inducing IFN-beta) and TRAF6 (TNF receptor-associated factor 6), thereby leading to the activation of the transcription factors NF-κB (nuclear factor kappa-B) and IRF-3 (IFN regulatory factor-3). The activation of the NF-κB pathway results in the induction of various inflammatory cytokines, including IL-6, IL-12, and TNF-α, whereas IRF-3 activation leads to the induction of type I interferons (IFN-α/β). For a detailed description of TLR3-mediated signaling, please refer to Matsumoto and others (2004), Schröder and Bowie (2005), and Akira and others (2006).

neuropathological consequences are readily distinguishable between middle (i.e., GD 9) and late (i.e., GD 17) gestational immune stimulation (Meyer, Nyffeler, and others 2006). Animals subjected to prenatal PolyI:C exposure on GD 9 displayed a severe reduction in Reelin-positive cells and adult neurogenesis in the hippocampal formation. On the other hand, PolyI:C treatment on GD 17 only resulted in a marginal decrease in hippocampal Reelin-immunoreactivity but impaired adult neurogenesis to an extent that is similar to that following prenatal immune activation in mid-gestation. The protein Reelin is involved in fetal and postnatal development by acting as a positioning and detachment signal for newly generated neurons (Hack and others 2002), and its expression is reduced in a number of neuropsychiatric disorders of presumed neurodevelopmental origin, including schizophrenia and autism (Fatemi 2005B). In addition, our findings also coincide with the discovery that impaired adult neurogenesis is linked to schizophrenia (Reif and others 2006), although the specific etiopathological contributions of abnormal neurogenesis to this disease remain to be determined.

Prenatal immune challenge in late gestation (GD 17) also selectively enhanced postnatal apoptosis in the dentate gyrus, whereas this effect was marginally reversed in offspring subjected to prenatal inflammation in mid-pregnancy (Meyer, Nyffeler, and others 2006). Inasmuch as abnormal apoptotic mechanisms have also been implicated particularly in schizophrenia (Jarskog and others 2005), the correspondence between prenatal immune challenge in late gestation and the emergence of increased apoptosis in the postnatal brain may highlight an important etiopathological mechanism that leads to dysfunctional brain apoptosis in schizophrenia.

In a recent study, Zuckerman and Weiner (2005) failed to find differences between the long-term functional consequences of prenatal PolyI:C exposure on GD 15 and GD 17. In both cases, the prenatal immunological manipulation led to equivalent behavioral and cognitive deficits in the adult offspring compared to prenatal control animals. This indicated that the temporal distinction between GD 15 and GD 17 in rats may not be sensitive enough to influence the specificity of infection-mediated postnatal pathology. These findings are thus in support of the general impression that comparison between PolyI:C-induced immune activations at early and at late pregnancy yields qualitatively more distinct patterns of pathology than the comparison between mid- and late-pregnancy treatment (Meyer, Feldon, and others 2006).

Prenatal Exposure to Bacterial Endotoxin and Influenza Virus

The administration of the bacterial endotoxin LPS or human influenza virus to pregnant rodents was among the first animal models examining the long-term consequences of prenatal immune challenge on adult brain functioning (e.g., Fatemi, Sidwell, Akhter, and others 1998; Fatemi, Sidwell, Kist, and others 1998; Fatemi and others 1999; Borrell and others 2002; see also Tables 1 and 2). Although a direct evaluation of the impact of the precise times of maternal immune challenge is still missing in these two models, differences in the exact timing of maternal LPS exposure among diverse research groups may also account for the distinct behavioral and neuroanatomical effects emerging in adult offspring.

In the study of Borrell and others (2002), pregnant rats were repeatedly exposed to LPS on alternate days throughout gestation, thereby mimicking a chronic inflammatory response from early to late pregnancy. In addition to their neuroanatomical findings (Table 2), a severe sensorimotor gating deficit in the form of reduced prepulse inhibition was revealed in the adult offspring (Table 1). In a subsequent study, Fortier, Joober, and others (2004) evaluated the long-term behavioral and pharmacological effects of prenatal LPS administered on days GD 17 and 18 in rats.

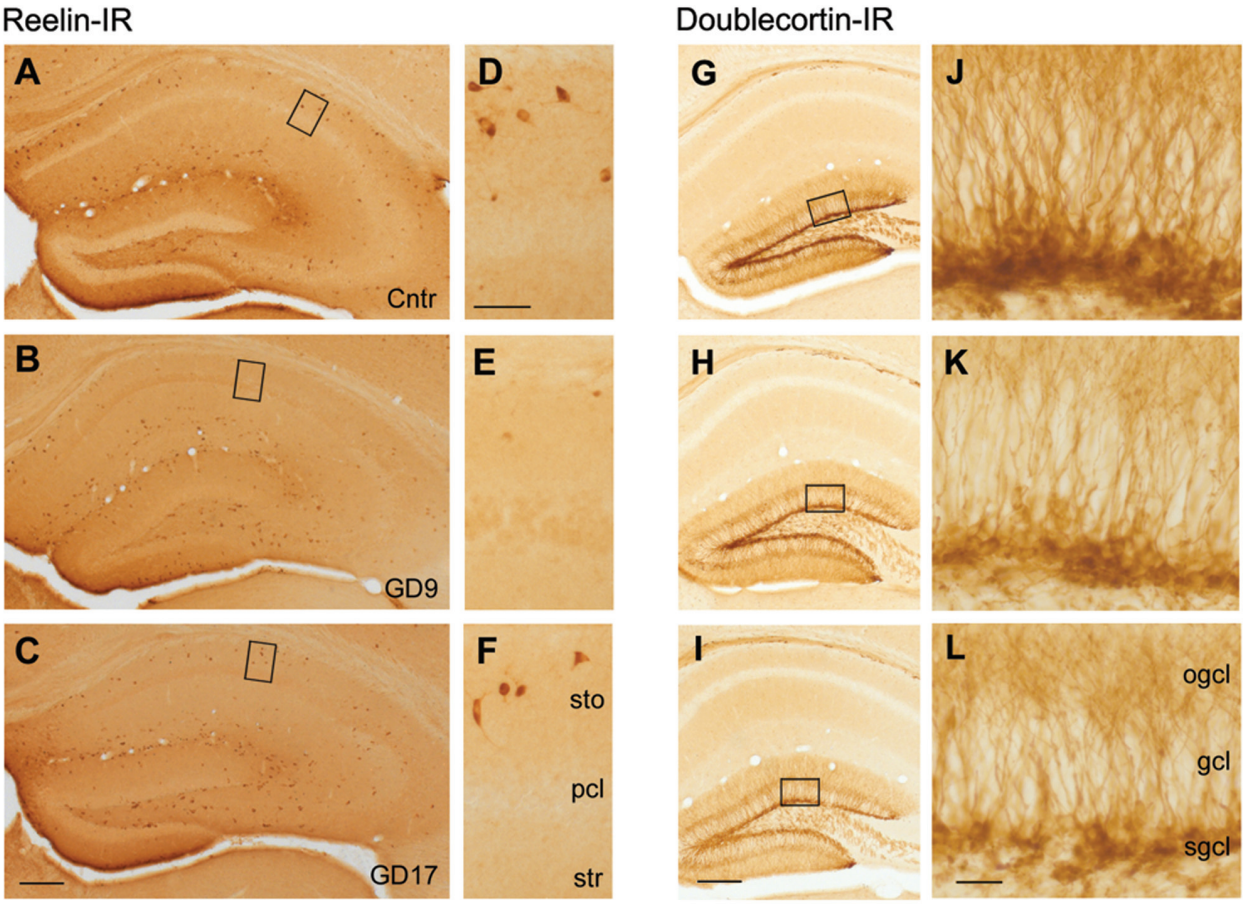

Fig. 2. Differential postnatal neuropathology following prenatal immune activation in early/mid and late gestation. Images *A-F* show the distribution of Reelin-immunoreactive (IR) in the hippocampal formation and dentate gyrus of representative mice born to control mothers (Cntr; *A*, *D*), and to mothers having been treated with PolyI:C (5 mg/kg, i.v.) on gestation day (GD) 9 (*B*, *E*) or GD 17 (*C*, *E*). As evident in the images at a higher magnification (indicated by the square in *A-C*), animals subjected to prenatal PolyI:C exposure on GD 9 display a severe reduction in Reelin-positive cells particularly in the stratum oriens of the CA1 subfield (*E*) in comparison to control animals (*D*). On the other hand, prenatal immune activation on GD 17 only leads to a marginal decrease in hippocampal Reelin-IR (*F*). However, prenatal PolyI:C-induced immunological stimulation in mice impairs postnatal neurogenesis independent of the precise times of immune challenge. The photomicrographs *G-L* represent immunoperoxidase stainings of the hippocampal formation and dentate gyrus from representative control mice (*G*, *J*) and animals subjected to prenatal PolyI:C (5 mg/kg, i.v.) exposure on GD 9 (*H*, *K*) or GD 17 (*I*, *L*) using anti-Doublecortin antibody. As evident in the images at a higher magnification (indicated by the squares in *G-I*), reduced Doublecortin-IR can be observed in both the outer granule cell layer and the subgranular cell layer of the dentate gyrus after prenatal PolyI:C exposure on GD 9 (*K*) or GD 17 (*L*) relative to control treatment (*J*). Adapted from Nyffeler and others (2006), with permission of the Society of Neuroscience. gcl = granule cell layer; ogcl = outer granule cell layer; pcl = pyramidal cell layer; sgcl = subgranular cell layer; sto = stratum oriens; str = stratum radiatum. Scale bars: *A-C* and *G-I*, 500 µm; *D-F* and *J-L*, 50 µm.

This immunological manipulation led to an increased response to the locomotor-stimulating effects of amphetamine in the adult offspring, but it largely spared sensorimotor gating. Hence, clear functional differences are also noted between chronic prenatal LPS exposure and its application restricted to late gestational stages. It will therefore be of great interest to further evaluate whether LPS treatment in early/mid gestation alone, that is, instead of chronic exposure from early to late pregnancy (Borrell and others 2002), would be sufficient to interfere with sensorimotor processes in later life. This would not only help to further characterize the impact of the precise times of prenatal immune challenge on postnatal brain pathology in prenatal LPS models, but it would also help to consolidate the generality of the critical window hypothesis across different prenatal immunological manipulations in experimental models.

Prenatal Exposure to Specific Cytokines

By treating pregnant rats repeatedly with the inflammatory cytokine IL-6 on either days 8, 10, and 12 (early treatment) or days 16, 18, and 20 (late treatment), Samuelsson and others (2006) also reported a structural and functional

dissociation of the long-term effects of early/mid and late prenatal immune stimulation. The authors showed that adult rats subjected to late prenatal IL-6 treatment displayed a marked increase in hippocampal astrogliosis and apoptosis, together with an enhanced expression of the $GABA_A$ α_5- and *N*-methyl-D-aspartate (NMDA)-NR1-receptor subunits. In contrast, postnatal astrogliosis was only modest in rats exposed to IL-6 in early/mid gestation, and hippocampal apoptosis and NMDA/$GABA_A$-receptor dysregulations were virtually absent following the prenatal immunological manipulation in early/mid gestation. The distinct anatomical and neurochemical effects between early/mid and late gestational IL-6 exposure were also paralleled by a functional dissociation: Late prenatal IL-6 treatment led to a robust spatial learning deficit in the Morris watermaze, whereas this effect was only modest in adult animals subjected to prenatal IL-6 exposure in early/mid gestation.

Hence, both prenatal PolyI:C and IL-6 administration can have distinct structural and functional long-term effects in the resulting offspring depending on the precise times of prenatal exposure (Meyer, Feldon, and others 2006; Meyer, Nyffeler, and others 2006; Samuelsson and others 2006). This is not surprising, because systemic application of PolyI:C to pregnant dams results in a strong elevation of IL-6 in the maternal serum and of elevated IL-6 protein and/or gene expression levels in the fetal brain (Meyer, Nyffeler, and others 2006). Interestingly, the maternal IL-6 response to systemic PolyI:C treatment does not seem to differ between early/mid (GD 9) and late (GD 17) pregnancy in mice; however, significant differences in IL-6 levels are found in the fetal brain, which is most clearly observed in terms of fetal IL-6 gene expression levels (Meyer, Nyffeler, and others 2006). It thus follows that the specificity of cytokine events in the fetal brain may have a fundamental role in determining the precise patterns of structural and functional brain abnormalities in later life. This possibility will be discussed in the following sections. Here we would like to emphasize that the comparisons between the long-term consequences of prenatal immune challenge conducted in early/mid and late gestation using either the viral mimic PolyI:C (Meyer, Feldon, and others 2006; Meyer, Nyffeler, and others 2006) or the inflammatory cytokine IL-6 (Samuelsson and others 2006) in experimental animal models readily support the hypothesis that early/mid and late gestation periods correspond to two developmental windows with differing vulnerability to adult behavioral dysfunctions and associated neuropathology.

The Roles of Cytokines in Mediating the Link between Maternal Infection during Pregnancy and Neurodevelopmental Defects in the Offspring

The Prenatal Cytokine Hypothesis of Schizophrenia and Related Disorders

A higher risk for schizophrenia has been demonstrated following prenatal exposure to a variety of infectious agents, including influenza, rubella, measles, polio, varicella zoster, herpes simplex type 2, and diphtheria and pneumonia (reviewed in Machón and others 1995; McDonald and Murray 2000; Brown and Susser 2002; Ashdown and others 2006). Hence, the association between in utero exposure to infection and the emergence of postnatal psychopathology is not limited to a single pathogen, suggesting therefore that immunological factors common to all infections must be involved in this causal link.

Cytokines are critical mediators of the early defense against a variety of infectious agents. These molecules belong to a class of low-molecular-weight proteins with wide-ranging roles in the innate and adaptive immune systems (Curfs and others 1997). In the CNS, cytokines can also influence various neurodevelopmental processes, including cell differentiation, maturation, and survival (see below). Infection-mediated elevation of cytokines in the maternal host, and thereafter in the fetal environment, may thus adversely affect normal brain development and contribute to the disease process implicated in schizophrenia and related disorders (Fig. 3) (Gilmore and Jarskog 1997; Patterson 2002). This hypothesis has been substantiated by recent epidemiological reports (Buka and others 2001; Brown, Hooton, and others 2004) and various experimental findings (e.g., Urakubo and others 2001; Gilmore and others 2005; Ashdown and others 2006; Meyer, Nyffeler, and others 2006).

At the cellular and molecular levels, two major processes may account for cytokine effects on brain development. These involve direct effects on neuronal populations, in addition to indirect effects via glial cell functions (see graphical illustration in Fig. 4).

Cytokine Specificity and Concentrations

Different members of the cytokine family can be grouped according to their main production sites and functions in the peripheral immune system (Curfs and others 1997). For example, IL-1β, IL-6, and tumor necrosis factor (TNF)-α are often classified as pro-inflammatory cytokines because of their critical roles in the early defense against infection and the initiation and/or progression of inflammation. On the other hand, IL-10 and transforming growth factor (TGF)-β are cytokines with strong anti-inflammatory properties. They can limit the production and action of pro-inflammatory molecules and are therefore fundamental to cytokine and immune cell homeostasis.

Different classes of cytokines also appear to exert differing effects on the development of neuronal cells. Among the variety of pro- and anti-inflammatory cytokines, IL-1β is the most capable of inducing the conversion of rat mesencephalic progenitor cells into a dopaminergic phenotype in vitro (Ling and others 1998; Potter and others 1999), and IL-6 is highly efficacious in decreasing the survival of fetal brain serotonin neurons (Jarskog and others 1997). In contrast, IL-1β and IL-6 (and to a lesser extent TNF-α) appear to have an equivalent capacity to negatively regulate the survival of fetal midbrain dopaminergic neurons at low to

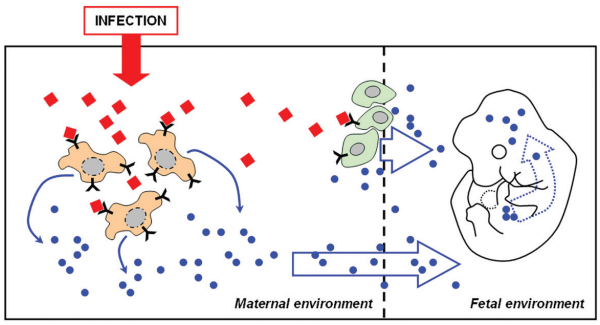

Fig. 3. Cytokine responses in the maternal and fetal compartments following maternal infection during pregnancy. Bacterial and viral pathogens (represented by red diamonds) invading the maternal host are recognized by peripheral immune cells (orange) via binding to various pattern recognition receptors (PRRs) such as toll-like receptors. This leads to the rapid production and release of pro- and anti-inflammatory cytokines (represented by blue dots) in the maternal environment (see also Fig. 2). Many PRRs are also expressed by cells of the maternal-fetal interface (dashed line), including trophoblasts and uterine epithelial cells (green). These cells may thus mount additional cytokine responses upon maternal infection. Transplacental transfer of maternally produced cytokines and production of cytokines at the maternal-fetal interface lead to an elevation of these molecules in the fetal environment, including the fetal brain. The fetal system at late but not early gestation (Meyer, Nyffeler, and others 2006) contributes to this process by endogenously synthesizing and secreting cytokines following maternal immune activation (indicated by the dashed blue arrow). Once in the fetal brain, cytokines can affect ongoing neurodevelopmental processes depending on the fetal developmental stage and cytokine specificity. The latter is critically influenced by various factors, including the genetic background and gestational stage (see main text). Immunological stimulation is also strongly associated with the activation of maternal stress response axes such as the hypothalamic-pituitary-adrenal (HPA) axis (not shown), which may additionally be involved in the precipitation of aberrant fetal brain development following maternal infection during pregnancy (see Koenig and others 2002; Koenig 2006).

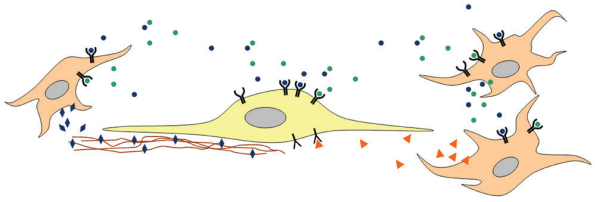

Fig. 4. Direct versus indirect effects of cytokines on neurodevelopmental processes. Cytokine receptors are expressed on many neuronal cell types during prenatal and postnatal CNS development (Benveniste 1998; Dame and Juul 2000; Gilmore and others 2004). Different species of cytokines (green and blue dots) may therefore directly influence the development of neuronal cells (yellow) by activating intracellular signaling cascades after binding to the appropriate cell surface receptors. In contrast, cytokines may affect the development of neuronal cells indirectly through their actions on glial cells (light orange). Glial cells have prominent functions in the normal development of the CNS by providing local signaling cues and neurotrophic factors (orange triangles), as well as extracellular matrix proteins (blue diamonds embedded in brown matrix). Hence, cytokine stimulation may induce glial cells to secrete specific signaling molecules, which in turn may bind to appropriate receptors located on neuronal cells and eventually may affect neurodevelopmental programs.

medium concentrations (Jarskog and others 1997), whereas the same cytokines can enhance the survival of these cells at higher concentrations (Kushima and others 1992; Akaneya and others 1995). A similar dependency on cytokine specificity and/or concentration has also been found in a recent study by Gilmore and others (2004), who have demonstrated that only TNF-α can affect the cortical neuron's dendrite development at low doses, whereas the same effects can be achieved by the exposure of fetal cortical neurons to higher doses of either IL-1β, IL-6, or TNF-α.

Although the underlying molecular mechanisms have yet to be identified, the results from various in vitro studies clearly illustrate that the effects of cytokines on neurodevelopmental processes critically depend on their specificity and/or exposure dose. This may also offer a parsimonious explanation for the observation that some of the long-term functional deficits emerging after PolyI:C-induced prenatal immune challenge in mice are dependent on the precise dose of PolyI:C used (Shi and others 2003; Meyer and others 2005). Specific members of the cytokine family, including IL-1 and epidermal growth factor, have also been demonstrated to trigger and precipitate differing functional long-term consequences when administered to neonatal rodents (for a recent review, see Nawa and Takei 2006). This highlights that cytokine specificity may also be a critical determinant in the precipitation of postnatal neurodevelopmental disturbances.

Factors Contributing to Cytokine Specificity and Concentration

In a complex biological system such as the fetal brain, several factors modulate cytokine specificity and/or concentration upon maternal immunological stimulation. First, the maternal genetic background plays a fundamental role in shaping the immune response against infection (Segal and Hill 2003). This may therefore also determine cytokine specificity and/or concentration in the fetal environment. Second, the progression of pregnancy is associated with considerable hormonal fluctuations, which may similarly contribute to varying maternal and fetal cytokine responses following infection (Sargent 1993; Entrican 2002). Third, fetal brain cytokine specificity and concentration may be modulated by the fetal immune system as such. The establishment of a functional immune system requires a sequential series of well-coordinated developmental events that begin early in fetal life (Holt and Jones

2000). In most mammals, however, the fetal immune system is relatively poorly developed in early/mid pregnancy and functional maturation is achieved only at late gestational and postnatal stages of development (Holsapple and others 2003). As a consequence, the fetal immunological reaction to maternal infection is also dependent on the precise times of gestation. This is highlighted by the observations in rats and mice that maternal exposure to the immunostimulatory agents LPS and PolyI:C induces significant increases in fetal brain cytokine gene expression in late but not early/mid gestation (Cai and others 2000; Meyer, Nyffeler, and others 2006).

Hence, apart from the genetic background, different gestational stages are associated with fluctuations in maternal and fetal cytokine levels. This may play an important role in determining the eventual fetal brain cytokine response in pathological conditions such as maternal infection, thereby influencing the short- and long-term neurodevelopmental effects in the offspring. However, the responsiveness and/or sensitivity of developing cells to many signaling cues, including cytokines, can considerably differ as neurodevelopment progresses. Cytokine specificity and/or concentration alone may therefore not be sufficient for the specification of the precise neurodevelopmental impact on fetal brain development. As highlighted in the next section, the eventual neurodevelopmental consequences of cytokine challenge are thus expected to be dependent on the precise stage of brain development.

Affecting Neuronal Development at Different Stages of Fetal Life

Early (pre- and postnatal) development of the CNS follows a tightly regulated time course marked by discrete phases of proliferation, migration, differentiation, target selection, and maturation of different neuronal and glial populations. The presence of specific environmental factors, including cytokines and related molecules, may therefore have differential effects on ongoing neurodevelopmental processes depending on the precise stage of brain development. Here, we illustrate this possibility using the prenatal development of the dopaminergic system as an example. Imbalances in the central dopaminergic system are suggested to be one of the key features of psychotic behavior (Carlsson 1974; Snyder 1976; Seeman 1987), even though dysfunctions in many other neurotransmitter systems, including the glutamatergic, serotonergic, and GABAergic systems, may also similarly contribute to behavioral and cognitive dysfunctions observed in schizophrenia and related disorders (reviewed in Carlsson and others 1999; Laruelle and others 2003).

In the adult mammalian brain, the vast majority of dopaminergic cell bodies are located in discrete mesencephalic structures, namely, the substantia nigra (SN) and the ventral tegmental area (VTA). Dopaminergic cell bodies of the VTA primarily project to the medial and ventral parts of the caudate putamen, nucleus accumbens, hippocampus, amygdala, and prefrontal cortex, which together form the mesocorticolimbic dopamine system. This pathway has long been recognized to play crucial roles in the regulation and modulation of cognitive, attentional, emotional, and reward-related behaviors (for recent reviews, see Spanagel and Weiss 1999; Nieoullon 2002). On the other hand, dopaminergic cell bodies located in the SN project to dorsal and lateral parts of the caudate putamen, representing the mesostriatal dopaminergic pathway. Integrated in a complex network that includes other subthalamic and cortical areas, the mesostriatal system is fundamental to the control of voluntary movements and body posture (Groenewegen 2003).

Mesencephalic dopaminergic neurons arise from a common set of precursor cells early in fetal development (Fig. 5). In mice, proliferation of dopaminergic progenitor cells starts around GD 9.5~10.5 within the caudal region of the developing mesencephalon and peaks between GD 12 and 13 (Bayer and others 1995; Marti and others 2002). Subsequently, genesis of dopaminergic neurons declines. These cells then migrate to the rostral part of the mesencephalon during which they continue to develop as their axons and dendrites form and consolidate connections with their targets (GD 14 through early postnatal life) (Riddle and Pollock 2003). In rats, the same developmental phases occur approximately two days later (Altman and Bayer 1981; Voorn and others 1988). Dopamine synthesizing neurons in the human fetal brain are also established very early in development, namely, at around five weeks postconception (Pickel and others 1980; Brana and others 1996; Aubert and others 1997). Hence, the cascade of developmental events that leads to the establishment of the central dopaminergic system in both rodents and humans starts early in fetal life.

It follows that the normal development of the dopaminergic system may be particularly vulnerable to environmental insults in early gestation. Upon maternal infection in early pregnancy, cytokine-associated inflammatory events in the fetal brain primarily affect the processes of cell proliferation and differentiation. Hence, mesencephalic-derived progenitor cells can be driven to convert into dopamine neurons following exposure to the pro-inflammatory cytokine IL-1β (Ling and others 1998; Potter and others 1999). Most importantly, neuronal target selection and synapse maturation may additionally be affected as a consequence of early cytokine-associated interventions. It is thus expected that prenatal immune challenge in early/mid pregnancy can interfere with the normal development of the dopaminergic system at multiple levels, including cellular density, distribution, and connectivity. Although direct evidence for this possibility is still lacking, this may readily explain why prenatal immune challenge in early/mid gestation can lead to multiple brain and behavioral dysfunctions in later life, including sensorimotor gating deficits, impaired selective learning and working memory, enhanced behavioral switching, reduced spatial exploration, and increased sensitivity to dopamine-receptor agonists (Table 1). Many of these behavioral aberrations emerging in adulthood are associated with imbalances in the central dopaminergic system (Zuckerman and others 2003; Meyer and others

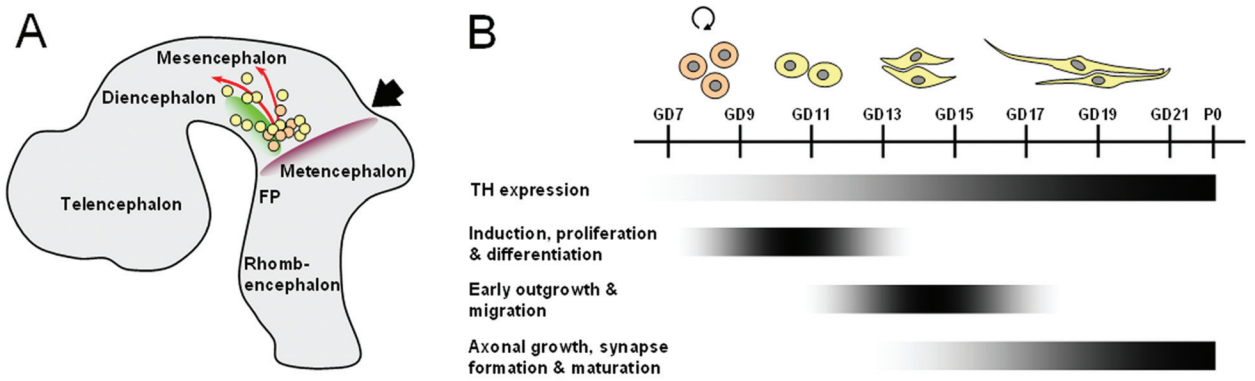

Fig. 5. The prenatal development of the mesolimbic dopaminergic system. *A*, The figure illustrates a sagittal view of a mouse fetal brain at around gestation day (GD) 10~11. Midbrain dopaminergic neurons are generated close to two important signaling centers located near the mid-hindbrain boundary (black arrow). Neural precursor cells (orange) proliferate within the caudal region of the developing mesencephalon and are exposed to early inductive signals, including sonic hedgehog (green underlay) and fibroblast growth factor ligands (purple underlay). Subsequent to neuronal induction, the cells begin to acquire a dopaminergic phenotype (yellow) and migrate to the rostral part of the mesencephalon, where they form and consolidate connections with their targets. FP = floor plate. *B*, A schematic representation of the temporal events taking place in the prenatal development of the dopaminergic system in the mouse. Induction and proliferation of dopaminergic progenitor cells start relatively early in fetal life, namely, around GD 9.5~10.5, and peaks around GD 12. As the genesis of dopaminergic cells declines, the cells extend thin processes and begin to migrate (at around GD 13). After reaching their targets, the cells extend more elaborate axonal and dendritic processes and refine the connections with their targets (GD 14 through early postnatal life). The expression of tyrosine hydroxylase (TH) increases as development of the dopaminergic systems progresses. Adapted from Riddle and Pollock (2003), with permission from Elsevier.

2005) and thus resemble some of the behavioral and pharmacological dysfunctions observed particularly in schizophrenic patients with positive symptoms (reviewed in Carlsson and others 1999; Laruelle and others 2003).

In contrast, prenatal brain inflammation in late gestation especially interferes with cell migration, organization, and synapse maturation (Gilmore and others 2004), the long-term consequences of which may be more restricted at both the functional and structural levels compared to early/mid gestational immune challenge. Indeed, prenatal immune challenge in late pregnancy may specifically lead to cognitive deficits (Meyer, Nyffeler, and others 2006; Samuelsson and others 2006) and increased sensitivity to NMDA-receptor antagonism (Meyer, Feldon, Yee, unpublished observation; see also Zuckerman and Weiner 2005), while sparing spatial exploration, sensorimotor gating, and selective associative learning. The long-term pharmacological and cognitive consequences of prenatal immune activation in late gestation may thus be more closely related to negative symptoms of schizophrenia (Sullivan and others 2006). However, the relative contributions of particular neurotransmitter systems to these dysfunctions are not yet delineated and need to be subjected to further investigations.

Summary and Conclusion

There is accumulating epidemiological as well as experimental support for the hypothesis that the vulnerability to infection-mediated disturbances in fetal brain development and associated adult psychopathology varies with gestational times. Most importantly, recent prospective epidemiological studies have highlighted that infections in early gestation (i.e., in the first trimester of human pregnancy) are associated with the highest risk for schizophrenia and related disorders in the offspring. This has thus challenged the prevailing view that infections at the second trimester of pregnancy might confer the maximal risk for the offspring to develop schizophrenia and related disorders in adulthood.

Modeling the epidemiological link in animals has yielded additional support for the suggestion that immunological challenge at different times of prenatal development may have distinct neurodevelopmental consequences. The biological plausibility for this hypothesis is given by the fact that fetal brain inflammation in early gestation may not only disrupt fundamental neurodevelopmental processes such as cell proliferation and differentiation, but also predisposes the developing nervous system to additional failures in subsequent cell migration, target selection, and synapse maturation. In this sense, inflammatory events taking place in early fetal brain development may have more severe neurodevelopmental sequelae compared to late pregnancy infection, and may therefore lead to multiple postnatal brain dysfunctions and associated symptom heterogeneity in schizophrenia and related disorders. In contrast, maternal infections in late pregnancy may especially affect late neurodevelopmental programs in the developing fetus, thereby inducing a more restricted pathological phenotype in the grown offspring, or even spare the emergence of postnatal brain and behavioral pathology (Fig. 6).

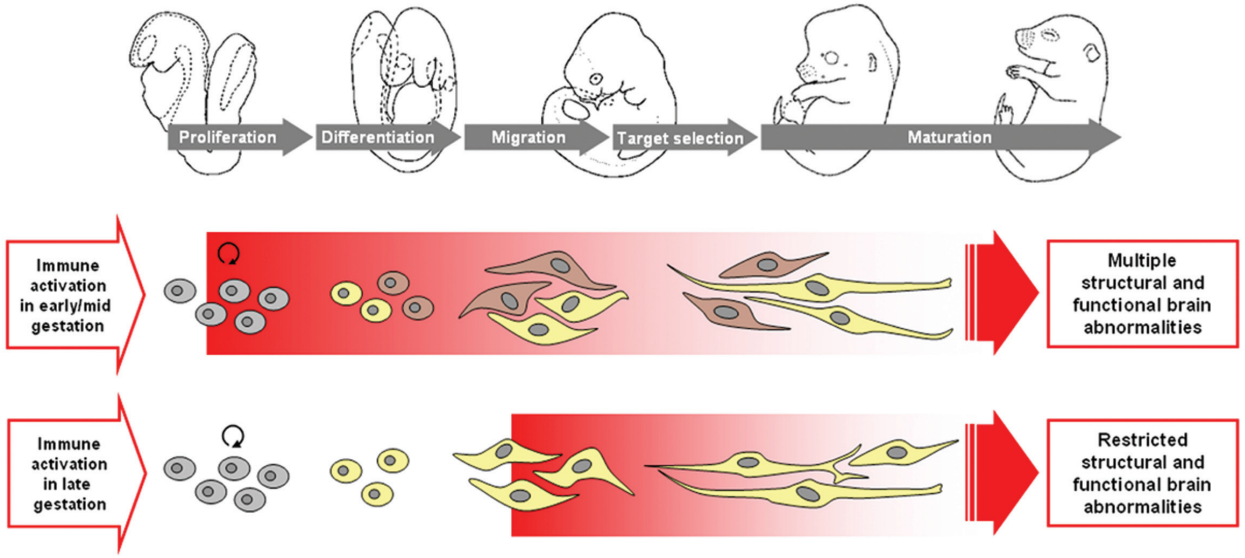

Fig. 6. Hypothesized model of the differential neurodevelopmental impact of prenatal immune challenge in early/mid and late gestation. Maternal infection in early/mid pregnancy may affect early neurodevelopmental events in the fetal brain, thereby influencing the differentiation of neural precursor cells (gray) into a particular neuronal phenotype (yellow or brown). This may predispose the developing fetal nervous system to additional failures in subsequent cell migration, target selection, and maturation (as indicated by the elongated shape of the cells), eventually leading to multiple structural and functional brain abnormalities in later life. In contrast, prenatal immune challenge in late gestation may particularly interfere with late neurodevelopmental programs in the developing fetus, including cell migration, target selection, and synaptogenesis. This may lead to a more restricted pathological phenotype in the grown offspring compared to immunological stress in early/mid gestation.

The exact correspondence of fetal developmental progression between human and rodents remains to be worked out. This highlights one of the difficulties in equating the windows identified in laboratory rodents to human epidemiological studies, and vice versa. A better understanding of the spatiotemporal events in prenatal brain development across different species is therefore indispensable for the delineation of the precise neurodevelopmental impact of in utero infections in humans as well as in rodents. This will also clearly help to better characterize the critical neuroimmunological mechanisms involved in aberrant brain development following the prenatal exposure to infections.

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