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Sex effects on neurodevelopmental outcomes of innate immune activation during prenatal and neonatal life

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Abstract

Humans are exposed to potentially harmful agents (bacteria, viruses, toxins) throughout our lifespan; the consequences of such exposure can alter central nervous system development. Exposure to immunogens during pregnancy increases the risk of developing neurological disorders such as schizophrenia and autism. Further, sex hormones, such as estrogen, have strong modulatory effects on immune function and have also been implicated in the development of neuropathologies (e.g., schizophrenia and depression). Similarly, animal studies have demonstrated that immunogen exposure *in utero* or during the neonatal period, at a time when the brain is undergoing maturation, can induce changes in learning and memory, as well as dopamine-mediated behaviors in a sex-specific manner. Literature that covers the effects of immunogens on innate immune activation and ultimately the development of the adult brain and behavior is riddled with contradictory findings, and the addition of sex as a factor only adds to the complexity. This review provides evidence that innate immune activation during critical periods of development may have effects on the adult brain in a sex-specific manner. Issues regarding sex bias in research as well as variability in animal models of immune function are discussed.

Keywords

Innate immune activation; Sex steroids; LPS; Poly(I:C); Development

Introduction

There is strong evidence to support the notion that disturbances in neural developmental processes *in utero* caused by prenatal exposure to immunogens are a risk factor for the development of various psychopathologies such as schizophrenia (Brown et al., 2004; Mednick et al., 1988; O'Callaghan et al., 1994), attention deficit/hyperactivity disorder (Vuillermot et al., 2012), depression (Ling et al., 2004; Winter et al., 2009) and autism (Miller et al., 2005; Rodier and Hyman, 1998). In fact, both human and animal studies of prenatal immune activation have revealed abnormalities in adult brain cytoarchitecture, such as reduced dendritic arborization and abnormal neuronal migration (Deutsch et al., 2010;

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Fatemi and Folsom, 2009), suggesting a neurodevelopmental origin for some psychiatric illnesses that takes place prior to the onset of symptoms. Epidemiological studies show that specific time points during the gestational period may be time windows of vulnerability to infection-mediated disturbances in fetal brain development (Boksa, 2010). For example, maternal infection during the first and second trimesters of pregnancy leads to a higher incidence of schizophrenia in offspring (reviewed in Machon et al., 1995; Susser et al., 2000). Similarly, animal studies have revealed that maternal immune activation during a period equivalent to the first trimester of human pregnancy produces alterations in dopamine-mediated processes such as deficits in prepulse inhibition and latent inhibition in the offspring (Meyer et al., 2006a).

Whereas the fetus exists in an environment where it is isolated from pathogens by the placental barrier, the neonate faces a world replete with bacteria; in fact, the maternal vagina and feces provide an early source of commensal bacteria for the neonate. This exposure is believed to be beneficial, particularly with respect to the colonization of the gastrointestinal tract and for subsequent development of normal gastrointestinal function (Wagner, 2008). There is now good evidence that normal commensal bacteria can alter neurological and behavioral development (Foster et al., 2008) however, the presence of pathogenic microorganisms that can prematurely activate the innate immune system can also permanently alter adult immune function, physiology and behavior. Neonatal immune activation has been associated with such psychiatric disorders as Alzheimer's disease, schizophrenia, cerebral palsy and autism (Garnier et al., 2003; Hornig et al., 1999; Huleihel et al., 2004; Nelson and Willoughby, 2000; Rantakallio et al., 1997; Shi et al., 2003). Cytoarchitectural changes can also be seen in the adult animals exposed to immunogen as neonates such as enlargement of bilateral ventricles and alterations in axonal and dendritic arborization in the parietal cortex and substantia nigra (Fan et al., 2011). Common functional deficits seen in rodent offspring exposed to prenatal or neonatal immune activation include cognitive and social dysfunctions (Bilbo et al., 2005; Bitanihirwe et al., 2010; Ibi et al., 2009; Wang et al., 2010).

Although there is a wealth of animal research published on the effects of early immune activation on brain development and behavior, the majority of studies cited include male subjects exclusively (Boksa, 2010). This is problematic since sex differences are observed in a broad range of neuropsychiatric disorders such as autism, depression and schizophrenia (see Aleman et al., 2003; Beery and Zucker, 2011; Flor-Henry, 1990; Wald and Wu, 2010). The aim of this review is to provide a brief overview of studies that have examined the long-term effects of early life immune activation on brain development and behavior in both sexes, with particular emphasis on factors contributing to the broad variability in results.

Animal models of pathogenic immune activation

The immune system can be activated in response to a variety of agents. In this review, we will limit our discussion to the effects of innate immune activation, as opposed to those that involve acquired immunity responsible for antibody generation.

Substances that activate the innate immune system are recognized by specific pattern activated receptors called Toll-like receptors (TLRs) on immune cells in the periphery and

brain (reviewed by Iwasaki and Medzhitov, 2004). Different pathogens activate different TLRs. For example, gram negative bacteria activate TLR4 and viruses activate TLR3. Upon activation, these TLRs stimulate the production and release of proinflammatory cytokines, such as interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α (Dobrovolskaia et al., 2003; Doyle and O'Neill, 2006; Iwasaki and Medzhitov, 2004). Also it has been shown that TLR3 activation can result in the release of interferons (IFN)-α and (IFN)-β (Katafuchi et al., 2003; Toth et al., 1990; Voss et al., 2006). While cytokines are best known for their actions in cell–cell communication between immune cells, they also mediate functions in the non-immune tissues, by binding to cytokine receptors on a wide variety of cells. Cytokine receptor activation in the periphery and on the vasculature of the brain (Bsibsi et al., 2002) results in activation of NF-κB and AP-1 followed by the production of other mediators including prostaglandins and nitric oxide (Voss et al., 2007). Further synthesis of cytokines in the brain is also observed (Doukas et al., 1994; Katafuchi et al., 2003; Voss et al., 2007; Voss et al., 2006). As a consequence, neurological function is altered, resulting in activation of the hypothalamic–pituitary–adrenal (HPA) axis, and alterations in autonomic outputs (e.g., metabolism) (Dunn, 1988), sensory function (hyperalgesia) (Safieh-Garabedian et al., 2002) and behavior (Nelson et al., 1997).

Two of the most commonly used pathogenic agents to induce pre-natal and neonatal immune activation are polyinositic:polycitidylic acid (poly(I:C)), a double stranded RNA which mimics a viral infection and activates TLR3; and lipopolysaccharide (LPS) a cell wall component of gram negative bacteria that activates TLR4. Acute exposure to either of these agents elicits fever and reduces feeding and social behaviors, while chronic exposure produces chronic inflammation and a subsequent increase in sickness behavior.

Upon maternal immune activation, there are elevated levels of pro-inflammatory cytokines in the maternal circulation, as well as in the placenta and fetal circulation (Ashdown et al., 2006; Bell et al., 2004; Urakubo et al., 2001). However, a number of studies report that neither immune molecules (e.g., LPS) nor maternal cytokines enter fetal circulation suggesting that fetal cytokine levels are elevated due to exposure to placental cytokines or self-production (e.g., Cai et al., 2000; Gayle et al., 2004; Gilmore et al., 2005; Urakubo et al., 2001). The mechanism by which cytokines, once induced in the fetal brain, cause their effects is not yet known, however they have been found to regulate cell growth and differentiation (Heinrich et al., 1998).

Cytokine–sex steroid–immune system interactions

The sex steroids (estrogens, androgens and progesterone) are well known to have important roles in regulating the immune system (see Chrousos, 2010; Munoz-Cruz et al., 2011; Pittman, 2011 for recent reviews). In general, estrogen increases the immune response (Grossman, 1984) and androgens inhibit it (Spinedi et al., 1992) such that females have more pronounced responses than males to immune activation. Sex steroids also influence HPA axis activity, since estrogen positively (Vamvakopoulos and Chrousos, 1993) and androgen negatively (Bingaman et al., 1994) modulate corticotrophin releasing hormone (CRH) production such that females exhibit higher plasma corticosterone (CORT) levels compared to males. Studies examining adrenal function following endotoxin (LPS)

administration in mice, have found that female plasma CORT levels are significantly higher than male levels regardless of the post-administration time point examined (Spinedi et al., 2004). There are also important developmental alterations in the innate immune response. For example, the HPA axis is markedly suppressed until near the end of the second postnatal week in rodents (Levine, 2001); at later time points, Spinedi et al. (1992) found LPS-stimulated CORT secretion to be higher in postnatal day (P) 30 than P45 males, and highest in P45 females, indicating that pubertal changes in gonadal activity results in sexual dimorphism of immune–neuroendocrine interactions. Thus exposure to an immunogen during the first postnatal week will result in a very different response than at later periods. The changing influence of the endocrine system is also evident during pregnancy; as a consequence of changing hormone levels throughout pregnancy, the magnitude of the innate immune response changes, with it being suppressed near term (see Mouihate et al., 2008; Spencer et al., 2008 for review); thus the impact upon the fetus will also be different.

Methodological considerations

A number of different approaches and times have been employed in early immune activation studies, which produce varied results depending upon how inflammation is induced and the age of the animal. In some cases (Bilbo et al., 2005; Shi et al., 2003) a true infection is induced, whereas in other cases, inflammation is induced using LPS (e.g., Boisse et al., 2004; Lante et al., 2007), poly(I:C) (Ellis et al., 2006; Meyer et al., 2006a) or a cytokine (e.g., Samuelsson et al., 2006; Smith et al., 2007). These varied approaches may be important as Schwarz and Bilbo (2011) recently showed that LPS and neonatal (n) *E. coli* infection in 4 day old rat pups elicit markedly different inflammatory profiles in the brain (similar findings were seen with maternal IL-6 and poly(I:C), in Hsiao and Patterson, 2011). Likewise, it has been suggested that the inflammatory response arising from a single cytokine exposure may be much more limited than that resulting from TLR activation by LPS or poly(I:C) (Ellis et al., 2006). An additional confounding factor is that inflammation is often induced at different pre- and post-natal ages. This is important for several reasons. First, innate immune function matures over the first few days and weeks of life (Bortolussi et al., 1992; Pittman, 2011) and the early postnatal period represents a critical window. In terms of developmental biology, the gestational period in rats and mice only covers the first and second trimester of human pregnancy, the first 2 weeks of postnatal life corresponds to the third trimester of human pregnancy (Clancy et al., 2007). Secondly, rats and mice are born altricial; their brains continue to undergo maturational processes following parturition. Clancy et al. (2007) suggested that, at birth, the non-cortical and limbic structures of the rat brain are equivalent to second trimester human development, while the cortical structures are comparable to third trimester human development; it is not until postnatal day (P) 18 that the rat brain can be compared to human brain development at birth (Clancy et al., 2007). Thus environmental factors have the potential to alter rat and mouse brain development during both gestational and neonatal periods. It should be noted that even though it is expected that fetal development progresses at different rates in rodents and humans, there are also significant differences between rats and mice. In fact, many key developmental events occur approximately 2 days later in rats compared to mice during prenatal and perinatal periods (Bayer et al., 1995; Clancy et al., 2007). The gestational length is 19–21 days in mice and 21–23 days in rats. However, since rats and mice show similar alterations in brain structure

and function as well as behavior following early immune activation (see review Meyer et al., 2009), studies on both of these closely related species are discussed throughout the present review.

Because cytokines have important developmental effects on modulation of synapse formation and regulation of neurogenesis (Boulanger, 2009; Carpentier et al., 2009; Fourgeaud and Boulanger, 2010) those that appear in the brain subsequent to an inflammation may alter normal developmental processes. Similarly, cytokines have important effects on neuronal excitability and neurotransmitter function (reviewed in Galic et al., 2012) and altering the balance of inhibitory and excitatory activity at a critical period may have important long term effects. Furthermore, as different areas of the brain develop at different time points in the neonatal period, this may result in different long term effects, depending upon the time of inflammation. Even the immune competent microglia in the brain appear at different times in different structures; for example the hypothalamus and the hippocampus vary significantly in microglia number in early development (Schwarz et al., 2012).

Research on the impact of prenatal and postnatal (neonatal) immune activation on brain development and behaviors such as emotionality and learning, has produced mixed results and evaluation of sex-specific effects has been limited. The majority of studies examining sex differences do not reveal sex-dependent changes in brain and behavior (e.g., Shi et al., 2003; Zuckerman et al., 2003); however there are a few studies that show clear sex-dependent effects (e.g., Meyer et al., 2008a; Schwendener et al., 2009). The variability in findings is highlighted in the following section. It is clear that a number of factors can contribute to the inconsistent results presented, including age of pathogen exposure, dose used, time of testing and sex.

Effect of immune activation on brain, behavior, and immunity

Learning and memory

Prenatal effects—Several studies have reported that molecules such as LPS and cytokines produce learning deficits and disruption in memory formation in adulthood (e.g., Kohman et al., 2008a). Prenatal immune activation induces both structural and functional abnormalities in the hippocampus (e.g., Meyer et al., 2008b), an area implicated in learning and memory (e.g., Broersen, 2000; Moser et al., 1993). Further, viral infections have been reported to reduce synaptic density and bring about neuronal loss (Sharma et al., 2002) and pyramidal cell atrophy in the hippocampus (Fatemi et al., 2002).

Although there are a number of articles on prenatal immune activation and spatial learning deficits in particular, deficits produced in this form of learning are inconsistent (Boksa, 2010) and are often limited to one sex (Fatemi et al., 2002; Sharma et al., 2002).

Although limited, the literature that exists on the effect of prenatal immune activation on synaptic plasticity, learning and memory with sex as a factor reveal that prenatal immune activation can impair learning and memory (e.g., Howland et al., 2012; Lante et al., 2007;

Zhang et al., 2011), but not all show sex-specific deficits (e.g., Samuelsson et al., 2006; Wang et al., 2010).

Studies that have reported no sex differences in spatial learning and memory as a function of prenatal immune activation, have shown that prenatal treatment with LPS (early-to-middle gestation) or IL-6 (middle and late gestation) induce spatial learning and memory impairments in the radial arm water maze (RAWM, Wang et al., 2010) and the Morris water maze (MWM, Samuelsson et al., 2006) in rodent offspring aged 20 weeks and older, regardless of sex. These findings align with those of others who have found maternal immune activation at late gestation induces similar learning and memory impairments in the MWM (Golan et al., 2005, 2006). However, Samuelsson et al. (2006) found that even though prenatal IL-6 exposed male and female rats showed learning and memory deficits, females showed more impairment in spatial memory (increased escape latency). Furthermore, they also found sex- and region-specific hippocampal neuronal loss in prenatal IL-6 exposed offspring, predominantly in males. Specifically, prenatal IL-6 treatment produced neuronal loss mainly in the hilus, CA2 and CA3 of male offspring and in the hilus and CA1 of female offspring (Samuelsson et al., 2006).

Others have also shown that learning and memory deficits are sex-dependent, and can be influenced by prenatal immune activation. For example, Lante et al. (2007) found LPS-induced inhibition of LTP, and spatial learning and memory deficits in prenatally immune challenged male offspring. Specifically, male Sprague Dawley (P28) offspring exposed to maternal LPS during late gestation (GD19) showed spatial learning and memory deficits in the MWM compared to male controls. There were no spatial learning and memory deficits observed in female offspring exposed to maternal LPS compared to female controls (Lante et al., 2007). In the same study, Lante et al. (2007) found that prenatal LPS induced inhibition of NMDA receptor-dependent processes in male rat offspring. They found a significant increase in AMPA/NMDA ratio suggesting that NMDA had less of a contribution to basal synaptic transmission, demonstrating that impairment in LTP seen in prenatal LPS-exposed male offspring was the result of direct alteration of synaptic NMDA receptors, consistent with findings described by others (Escobar et al., 2011; Oh-Nishi et al., 2010). Howland et al. (2012) also demonstrated that hippocampal dependent learning and memory is detrimentally affected in male rat offspring exposed to the prenatal viral-like immune activation (Howland et al., 2012). They found that poly(I:C) treatment on GD15 produces recognition memory deficits in male, not female offspring. Specifically, associative learning memory was assessed using the object-in-place memory test, in which memory is inferred when an animal spends more time exploring objects that switched locations compared to objects that remain in the same location across baseline sampling and test trials. Howland et al. (2012) found that during the test phase, saline-treated male rats showed intact object-in-place memory, however prenatal poly(I:C) male offspring failed to do so. Female offspring of both prenatal treatment groups failed to show intact object-in-place memory (Howland et al., 2012). These sex-specific deficits contrast with those seen by others (Gue et al., 2004; Zhu et al., 2004) who demonstrated deficits that are more pronounced in female than male offspring using different prenatally-induced stressors.

Examination of other forms of learning and memory, such as those that involve executive functioning (prefrontal cortex mediated), using the prenatal immune activation model have also revealed sex-specific deficits in memory task performance in adult offspring (Zhang et al., 2011). Sex-specific effects on cognitive flexibility (assessed with the attentional set-shifting and reversal learning tasks) were found in young adult offspring (P60 to P90) that received prenatal exposure to poly(I:C) on GD15 (Zhang et al., 2011). Zhang et al. (2011) found that prenatal poly(I:C) exposure significantly impaired strategy set-shifting from a visual- to response-based strategy in males. However, the prenatal poly(I:C) male offspring performed better than male control offspring in the reversal learning phase. Prenatal poly(I:C) treatment did not affect female offspring performance on either task (Zhang et al., 2011).

Neonatal effects—Neonatal immune activation may induce cognitive deficits later in life (e.g., Bilbo et al., 2006; Kohman et al., 2008a). It has been shown that immune system activation by proinflammatory cytokines such as IL-1β, IL-6 and TNF-α produce learning and memory deficits in adult rodents (Kohman et al., 2007; Pugh et al., 1998). Adult LPS exposure has been found to impair hippocampal dependent learning and memory in tasks such as the MWM and contextual fear conditioning (Kohman et al., 2008b; Pugh et al., 1998; Shaw et al., 2001; Sparkman et al., 2005). There is evidence to suggest that neonatal immune activation alters such responses to LPS administration in adulthood. Bilbo et al. (2005) reported that exposure to neonatal (n) *E. coli* infection on P4 impaired memory for a recently explored context as assessed using a fear conditioning paradigm and produced a rapid increase in hippocampal IL-1β levels in male rats that received an adult LPS challenge on P60 (Bilbo et al., 2005). The authors also showed that the memory impairment seen in the n *E. coli* rats could be prevented by blocking brain IL-1β synthesis just prior to the context preexposure task (Bilbo et al., 2005). Similarly the group demonstrated that n *E. coli* treated male rats showed selective impairments in memory for contextual fear conditioning (hippocampal dependent task) but not memory for auditory-cue fear conditioning (hippocampal independent task) following adult LPS challenge on P90 (Bilbo et al., 2006).

Our group has shown that nLPS at P5, P14 or P30 causes deficits in adult males in MWM probe trials and in fear conditioning tests, but only in the P5 and P30 groups. This was associated with alterations in expression of some NMDA receptor subunits (Harre et al., 2008). There is mixed evidence that neonatal immune activation produces learning and memory deficits in a sex specific manner. Kohman et al. found that nLPS (P4 and P5) treated male mice performed significantly fewer avoidance responses than saline treated males in a two-way active avoidance paradigm, a finding not observed in nLPS treated females on P70 (Kohman et al., 2008a; Kohman et al., 2008b). In another study however, no sex effects were detected on performance in a passive avoidance test in P21 rats administered LPS intracerebrally (1 mg/kg) on P5 (Fan et al., 2011). The authors found learning and memory deficits on this task in both nLPS males and females relative to nSal controls.

Overall, LPS- or poly(I:C)-induced immune activation during the gestational and neonatal periods produce learning and memory deficits in hippocampal- and prefrontal cortex-mediated tasks. Sex specific effects are occasionally observed (e.g., Lante et al., 2007; Zhang et al., 2011).

Innate anxiety-related behaviors

Prenatal effects—Maternal immune challenge literature is extremely limited in its discussion of sex effects on emotionality. Innate anxiety-related behaviors, as assessed by elevated plus maze (EPM) and open field test (OFT), appear to be the only variables that warrant discussion since other behaviors have not been thoroughly assessed by independent investigators.

Generally, prenatal immune challenge does not produce sex-specific effects on anxiety-related behaviors. For example, both male and female mouse offspring born to dams treated with poly(I:C) on GD17 do not spend more time in the periphery of an open field nor spend less time in the open arms of an EPM compared to saline-treated control offspring regardless of age at testing (Schwendener et al., 2009) suggesting that maternal immune activation does not produce overt changes in innate anxiety-related behaviors in offspring. However, Wang et al. (2010) found that female mouse offspring of dams that received LPS (GD 8–15) spend more time in the periphery of an open field (suggesting anxiety) at P200 compared to prenatal LPS male offspring (Wang et al., 2010). No other sex or pretreatment differences were observed at other time points (e.g., P70, P400, P600), although previous studies have shown decreased anxiety in P600 males prenatally exposed to acute LPS (Golan et al., 2005, 2006). Compared to prenatal immune activation by pathogens, other forms of prenatal stressors (such as restraint stress) seem to consistently produce anxiety-like behaviors in both prenatally stressed offspring compared to non-stressed control (Kohman et al., 2008a; Laviola et al., 2004).

Neonatal effects—Existing literature on the effects of neonatal immune activation on innate anxiety-related behaviors shows mixed findings. Compared to vehicle controls, adult (P80) and senescent (P400), but not adolescent (P45) male rats that received nLPS (P3 and P5) spent more time in the closed arm of an elevated plus maze (EPM) reflecting heightened anxiety (Walker et al., 2004). Kohman et al. (2008a,b) however, found no effects of nLPS (P4 and P5) treatment on open arm activity in both adult male and female mice (Kohman et al., 2008b). These findings are consistent with Spencer et al. (2005) who found that neonatal LPS (P14) exposure did not alter anxiety-like behavior in the EPM (Spencer et al., 2005). Sex differences were observed in nLPS (P3 and P5) treated rats on the EPM in adulthood. Specifically, adult nLPS males who received exposure to a three-day stress protocol (restraint stress, isolation stress) spent less time in the open arms (exhibiting anxiogenic effect) compared to nLPS females and all other treatment groups (Walker et al., 2009). Lastly, Fan et al. (2011) reported that nLPS (P5) male and female rats on P21 showed anxiolytic effects (spent more time in the open arms) on the EPM compared to nSal controls (Fan et al., 2011). The anxiolytic effects observed have been previously seen by others (Imhof et al., 1993; Masur et al., 1980; Walker et al., 2004).

The findings suggest that early life immune system activation may interfere with innate anxiety-motivated behaviors, producing anxiolytic or anxiogenic effects, irrespective of sex.

Dopamine-mediated behaviors

Spontaneous locomotor activity

Prenatal effects: Though limited, there is evidence for prenatal immune activation having sex-specific effects on locomotion. Prenatal LPS exposure (GD 8–15) produces elevated locomotor activity in P200 female, but not male mouse offspring. No other prenatal treatment and sex effects were observed when mice were tested on P70, P400 and P600 (Wang et al., 2010). Previous research has shown however that adult (P600) male offspring that were prenatally exposed to acute LPS showed an increase in locomotor activity compared to pre-natal saline-treated controls (Golan et al., 2005, 2006). Similarly, young adult (P56–57) female rat offspring of dams treated with poly(I:C) on GD15 were more active in a 30-min open field test compared to male counterparts (Howland et al., 2012). Contrary to above-mentioned findings, in mice Meyer et al. (2008a) found that prenatal poly(I:C) (GD9) treatment does not produce sex-specific changes in locomotor activity in an open field test during pre-pubescence (P35) and young adulthood (P100–110) (Meyer et al., 2008a). This discrepancy could be attributable to a number of factors, one of which may be time of prenatal treatment (early versus late gestation).

Neonatal effects: The effects of neonatal immune activation on locomotor activity have also been mixed. No locomotor effects on open field test (OFT) were observed in n *E. coli* infected (P4) treated rats relative to controls (Bilbo et al., 2006). Kohman et al. (2008b) however, found a sex specific effect in locomotor activity in the EPM. Specifically, the group reported that nSal (P4 and P5) treated female mice that received LPS on P70 showed a decrease in total distance travel term should be traveled, not travel relative to saline treated controls. However, nLPS treated female mice that received adult LPS showed attenuated response (i.e., nLPS–aLPS were more active than nSal–aLPS female rats). Irrespective of neonatal treatment, male rats exposed to adult LPS challenge traveled a shorter distance compared to male rats exposed to saline in adulthood (Kohman et al., 2008b). These findings contrast with Spencer et al. (2006) who found that regardless of neonatal treatment condition, female rats treated at P14 did not differ in locomotor activity in the OFT following adult LPS challenge (Spencer et al., 2006). Walker et al. (2009) found that only nLPS (P3 and P5) male rats exposed to repeated stress over 3 days in adulthood were less active than male controls and female counterparts in an OFT (Walker et al., 2009). Hyperactivity in an OFT was observed in male and female nLPS (P5) treated rats on P13–P17 (Fan et al., 2011).

Thus, it is evident that studies reporting effects of pathogen immune activation on locomotion have found mixed results; in some cases locomotor effects are absent but in others, locomotor effects do exist in a sex dependent manner.

Prepulse inhibition (PPI)—Inhibition of the acoustic startle reflex (ASR) by low intensity acoustic stimuli is referred to as prepulse inhibition (PPI). PPI may reflect underlying sensorimotor processes involved in filtering of exteroceptive stimuli (Kline et al., 1998). The phenomenon is thought to initiate an inhibitory process that attenuates the startle response.

Maternal immune activation by administration of poly(I:C) on GD15 produces disruptions in PPI in pre- and post-pubescent male and female rat offspring (Howland et al., 2012). Similarly, Borrell et al. (2002) found that prenatal LPS treatment disrupted PPI later in life in a sex-independent manner. However, they found that even though all prenatally-treated offspring showed PPI deficits, the manifestation occurred later (P100) in female compared to male rat (P60) offspring of dams that received prenatal LPS treatment every other day during pregnancy. The authors also found that prenatal LPS-exposed male offspring displayed a significantly lower baseline startle response than control male offspring, an effect not seen in pre-natal LPS-exposed female offspring compared to control females (Borrell et al., 2002). Romero et al. (2010) also found that prenatal LPS treatment produced PPI deficits in male and female rat offspring, however the effects were more pronounced in males at each prepulse intensity tested, whereas female offspring showed a strong PPI deficit in response to a weak auditory prepulse (Romero et al., 2010). These findings suggest that male rats are more sensitive to the prenatal effects of immune challenge on sensorimotor gating than female rats. Furthermore, haloperidol and clozapine, both dopamine antagonists, administered prior to PPI testing, significantly reversed the PPI deficits seen in both male and female rat offspring that received prenatal LPS exposure (Borrell et al., 2002). The findings suggest that PPI-induced deficits resulting from prenatal LPS exposure are likely mediated by alterations in brain dopaminergic function (Swerdlow and Geyer, 1998).

Latent inhibition (LI)—Pre-exposure to a conditioned stimulus (CS) proactively interferes with associative learning, a cognitive phenomenon called latent inhibition (LI). LI reflects a selective attention mechanism that enables organisms to modify their behavior to better take advantage of changing contingencies of reinforcement (Meyer et al., 2006b).

Deficits in LI are typically seen in adult rodent offspring exposed to poly(I:C) between GD 9 and 15 (Meyer et al., 2005, 2006a; Piontkewitz et al., 2011) regardless of sex. However, Bitanihirwe et al. (2010) found that male offspring of poly(I:C) (GD17) mouse dams exhibited abnormally enhanced LI in associative learning compared to prenatal saline-treated males (Bitanihirwe et al., 2010). Poly(I:C) female offspring did not differ from control female offspring.

Innate immune response

Prenatal effects—As cytokines are the major signaling molecules to the brain during immune activation, it is instructional to examine if there are differences in cytokine expression after early immune activation and if these differ between males and females. Although the existing literature-base is limited, several pro-inflammatory cytokines have been examined to reveal sex-specific effects of prenatal immune activation, including IL-6, IL-1β and TNF-α (Hodyl et al., 2007, 2008; Samuelsson et al., 2006). The bulk of these studies suggest that there are no sex differences in serum and mRNA cytokine expression as a function of prenatal (LPS, IL-6 or saline) treatment (Hodyl et al., 2007, 2008; Samuelsson et al., 2006). However, in some studies, cytokine levels differ as a function of prenatal treatment. For example, Samuelsson et al. (2006) reported that offspring of rat dams exposed to IL-6 on GD 16, 18 and 20 (late gestation) exhibit higher basal levels of serum IL-6 compared to controls at 4, 8 and 24 weeks of age (Samuelsson et al., 2006). On the other

hand, Hodyl et al. (2007) have shown that offspring of rat dams exposed to LPS on GD 16, 18 and 20 show no increase in IL-1β serum levels between 0 (baseline) and 4 h post LPS challenge (on P19, pre-weaning day), whereas control offspring showed an increase in both IL-1β and TNF-α serum levels, a finding that does not hold at other ages (in puberty and adulthood). The only sex difference found was at senescence (13 months), when female rat offspring showed significantly higher TNF-α serum levels than male offspring at 4 h post-LPS challenge, despite equivalent baseline levels and independent of prenatal treatment (Hodyl et al., 2007). Borrell et al. (2002) found prenatal LPS treatment had no effect on serum levels of IL-1β or CORT levels in male and female adult rat offspring. However, serum levels of IL-6 were significantly higher in 100 day old prenatally-treated offspring, while IL-2 levels were significantly elevated in both 100 and 300 day old prenatal LPS-exposed males, and only in 300 day old prenatal LPS-exposed females (Borrell et al., 2002).

Neonatal effects—In 1995, Shanks et al. reported that LPS given during the first week of life was associated with altered levels of glucocorticoid receptors in the rat brain (Shanks et al., 1995) and subsequent studies suggested that LPS pretreated animals were more responsive to adult LPS and exhibited reduced levels of inflammation to adjuvant-induced arthritis (Shanks et al., 2000). With the use of a similar neonatal LPS administration, Walker et al. (2006) observed a marked attenuation in febrile response in nLPS (P3–5) treated males exposed to an adult LPS challenge, but no changes in CORT level was observed. In contrast, Bilbo et al. (2010) used n *E. coli* infection on P4 rats and found that adult febrile responses to LPS were largely unchanged. In addition, neonatally treated animals also exhibited an altered sensitization of the effects of nLPS to adult LPS challenge (Bilbo et al., 2010). Our group has carried out extensive investigations of the effects of nLPS administered at P14 on adult male neuroimmune responses; to summarize, we found that neonatally LPS treated animals exhibit significantly attenuated febrile response to adult LPS challenge due to amplified corticosteroid responses that suppress cytokine production (Ellis et al., 2005; Galic et al., 2009). This was also seen with neonatal poly(I:C) treatment (Ellis et al., 2006). The mechanism underlying the amplified CORT response appears to be associated with higher levels of TLR4 and COX-2 in the liver that provide an early bolus of circulating prostaglandin E1 (PGE1) to activate the HPA axis (Mouihate et al., 2010). An equally dramatic reduction in febrile responses was also seen in adult nLPS treated female rats (Spencer et al., 2006) as well as similar reductions in plasma IL-6 and in brain COX-2 following adult LPS challenge (Kentner et al., 2010). In the latter study, females were assessed at all stages of the estrous cycle. We also compared nLPS exposed male and female rats with much higher, septic doses (1 or 3 mg/kg) of LPS in adulthood (Spencer et al., 2010). A 1 mg/kg dose of LPS caused hypothermia and this effect was attenuated in both males and females that had been previously exposed to nLPS. However, when rats were given an even higher dose of LPS (3 mg/kg) a sex difference emerged in that nLPS males displayed reduced hypothermia, followed by a greater hyperthermia to LPS in adulthood, whereas females did not differ as a function of neonatal treatment. Differences in febrile response were paralleled by changes in CORT responses after LPS, i.e., there were sex differences in CORT levels. Similarly Saia et al. (2011) reported that a much higher dose of LPS (10 mg/kg, a dose chosen by the authors to induce endotoxemic shock, which did not result in rat mortality) administered in adulthood produced an attenuated febrile and pro-

inflammatory response in P14 nLPS females, but these differences disappeared after ovariectomy (Saia et al., 2011).

Discussion

Though brief, the literature covered in the preceding sections demonstrate several key issues that limit our understanding of the influence infectious agents such as influenza and viruses can have on early brain development and subsequent learning, memory, and behavior.

Male bias in the neurosciences

Of grave concern is the under representation of female subjects in both human and basic research. A survey of 2000 basic research papers in 2009 (Beery and Zucker, 2011) revealed a male bias in 8 out of 10 disciplines, most pronounced in neuroscience and pharmacology. What is more alarming is that many articles failed to report the sex of the subjects used and when both sexes were used in a study, about two-thirds of those studies did not analyze the data by sex (Beery and Zucker, 2011; Wald and Wu, 2010). Factors that continue to encourage male bias in basic research include the cyclic nature of reproductive hormones and the subsequent increased cost of using females in studies; more female subjects are necessary to account for cyclic hormone changes in the rodent's 4-day estrous cycle (Beery and Zucker, 2011; Wald and Wu, 2010). Furthermore, the majority of studies that evaluate sex differences covered in this review did not consider the rodents' estrous cycle (i.e., did not monitor cycle phase) in the analyses, nor did they attempt to test the females in a particular phase of the 4-day cycle. The importance of this observation is highlighted in studies examining the effects of sex steroids on sensorimotor gating (PPI). Koch reported that examination of PPI across the estrous cycle of adult female rats revealed that PPI is altered, such that when levels of estrogen are highest (proestrus), PPI scores are lowest (Koch, 1998). In proestrus, females also exhibit lower PPI activity scores than males (scores equivalent to those produced by females in diestrous and estrous). These findings directly contradict those of Van den Buuse and Eikelis (2001), who found estrogen treatment administered to ovariectomized females produced a dose-dependent increase in PPI of the acoustic response, in the absence of startle habituation (Van den Buuse and Eikelis, 2001). The latter observation is in line with human studies that documented symptoms of schizophrenia are most severe during the late luteal and premenstrual phases, when estrogen levels are low (Angermeyer et al., 1998; Hafner et al., 1993). More importantly, since sex steroids play an important modulatory role in the regulation of immune function, and a variety of autoimmune (e.g., rheumatoid arthritis, Grave's disease) and neurological (e.g., anxiety, depression) disorders are more prevalent in females than males (reported in (Beery and Zucker, 2011; Gaillard and Spinedi, 1998), it is necessary to remove sex bias from neuroscience and biomedical sciences in general.

Controlling for variability

As thoroughly discussed previously (Boksa, 2010; Meyer et al., 2009), it is very difficult to draw conclusions from CNS alterations seen in rodent models of maternal immune activation. This concern extends to research examining brain and behavioral outcomes following neonatal immune activation because laboratories differ in their selection of

immune activation models used. As illustrated here, studies vary drastically in type of infectious agent administered, dose used, pre- or post-natal age of immunogen administration, post-injection time of testing, species used and dependent variable measured. In addition to sex, any one or a combination of the aforementioned variables can account for the variability seen across studies examining effects of early life immune activation on adult neurological and behavioral development. A clear example of this comes from research conducted by Meyer et al. (2006a,b) who found that timing of prenatal poly(I:C) exposure during pregnancy was critical to performance deficits in different cognitive tasks; that is, early pregnancy poly(I:C) exposure (GD 6 and 9) produced offspring that displayed selective learning deficits (e.g., LI), but not following GD17 exposure (Meyer et al., 2006a). Similarly, Meyer et al. (2006b) found that GD9 poly(I:C) treated offspring showed suppressed spatial exploration, but GD17 poly(I:C) treated offspring exhibited impairments in a discrimination reversal learning task (Meyer et al., 2006b). These findings illustrate that precise timing of an early life immune activation produces distinctive behavioral and cognitive dysfunctions in adulthood (see Meyer et al., 2007 for an extensive discussion of this topic).

Conclusion

Many studies designed to examine the effects of prenatal exposure to stressors (e.g., restraint stress) have demonstrated clear sex differences in brain development and behavior (Mueller and Bale, 2007, 2008; Weinstock, 2007). However, relatively little research has addressed the effects of early life immune activation on development in both sexes. What we can glean from the few studies conducted is that some, but not all, early immune activation effects are strongly sex dependent. Factors such as type of immunogen used (e.g., LPS, poly(I:C), cytokines), dose used, and time of administration (early vs. late gestation, neonatal period) may interact with sex to play a significant role in the variability observed. Therefore, we must encourage uniformity in methodology employed across laboratories to allow for direct comparison of results in general. Further, it is imperative that more studies examine possible sex-related differences in brain development and subsequent functional, morphological and behavioral alterations as a consequence of early immune activation, not only because females are grossly underrepresented in research, but also because there are a number of autoimmune and neurological disorders that are gender-biased. The more we understand the mechanisms underlying sex differences in brain development and behavior, the better we are able to treat some of the most debilitating mental and physical disorders.

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