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Review

Connections between Early-life Neuroinflammation, Neural Stem Cells and Progenitors and Origins of Neuropsychiatric Disorders

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Abstract

A number of studies have highlighted the connection between infections during pregnancy in mothers and increased risk for neuropsychiatric disorders later in life leading to the view that maternal immune activation is a significant contributor to psychiatric illnesses. Meta-analyses have revealed associations between the incidence of premature birth and perinatal inflammation with smaller total brain volumes, cognitive, motor and behavioral deficits in childhood and adolescents. In animal studies where inflammation has been induced during the perinatal period, parallel changes in cognition and behavior have been seen reminiscent of those observed in human clinical studies. Several cytokines and in particular IL-1ß and IL-6 that are produced maternally can cross the placenta as well as the blood-brain-barrier to affect the developing brain, and they may positively or negatively regulate the stem cells and progenitors that reside in the brain's germinal matrices. Therefore, here we will review the literature towards the goal of highlighting how IL-1 and IL-6 affect the proliferation and differentiation of the stem cells and progenitors of the major germinal zones of the developing brain, and discuss how changes in the progenitor cell population can contribute to psychiatric disorders such as autism and schizophrenia.

Keywords
Subventricular zone; subgranular zone; neurogenesis; gliogenesis; maternal infection

1. Maternal Immune Activation is a Risk Factor for Psychiatric Disorders

Studies over the past two decades have established that abnormal fetal brain development puts children at risk for neuropsychiatric disorders later in life [1, 2]. These studies also indicate that there is an interplay between genetic variations and environmental factors that contribute to the etiology of these complex disorders [3]. Maternal infections comprise the most widely implicated environmental factors for abnormal fetal brain development. Epidemiological studies suggest an association between maternal viral infections and psychiatric disorders such as attention deficit and hyperactivity disorder, autism and schizophrenia [4, 5]. Over time, other infectious agents have been added to the list suggesting that a diverse group of pathogens that infect mothers can produce similar neurodevelopmental problems. Furthermore, clinical studies have concluded that the maternal immune response to viral and bacterial infections, and not direct fetal infections, are responsible for the higher incidence of psychiatric disorders in their infants [6]. Experimental studies in rodents have validated the consequences of maternal influenza infections which produce behavioral deficits reminiscent of autism where no virus can be detected in the fetus [7]. Although the underlying mechanisms of this epidemiological association remain unclear, maternal cytokine production in response to infection remains a crucial link [8-11].

2. Animal Models of Maternal Infection

Polyinosinic-polycytidylic acid (Poly(I:C)), is a double stranded RNA molecule that mimics a viral infection and stimulates an immune response by activating toll-like receptor-3 [12]. In the past decade, many laboratories have injected Poly(I:C) into pregnant rodents as means of activating an maternal immune response (MIA) in a controlled and temporally restricted manner [13]. Poly(I:C) induced MIA models vary in dose, route of administration, number of injections and gestational timing, that produce dramatically different maternal cytokine profiles [14] and subsequently different brain abnormalities and behavioral outcomes in the offspring [7, 15, 16]. For example, when poly(I:C) was injected into gestational day (GD)-9 pregnant mice their offspring exhibited a fear of being in open spaces, whereas this behavioral phenotype was not observed when poly(I:C) was injected into the dams at GD-17. By contrast, GD-17 immune activation produced perseverative behaviors in the offspring supporting the above conclusion that the gestational age at the time of infection will produce different consequences [14, 17]. This makes sense when one considers the theorem that elevated levels of cytokines might affect the output of the neural stem cells and progenitors (NSPs). These NSPs produce a variety of cell types in the brain, and it has been well established that each cell type is born during specific epochs over the course of neural development [18]. For example, during the first trimester in primates there are waves of neurogenesis and cell migration during which the brain's long projection neurons are produced. During the second trimester, the stem cells and progenitors in the brain are producing local circuit interneurons and both the projection neurons and the local circuit neurons are making synaptic

connections with each other. Then during the third trimester, the non-neuronal cells and especially the myelinating oligodendrocytes are being produced. Accordingly, establishing which neurodevelopmental processes are affected by MIA and how these affect the trajectory of neurogenesis, neural circuit formation, myelination and ultimately behaviors are key areas of investigation concerning the fetal origins of adult neurological and psychiatric diseases.

An alternative MIA animal model is the maternal administration of the bacterial endotoxin lipopolysaccharide (LPS) which mimics the innate acute phase response to bacterial infections and does so in the absence of live bacteria. LPS, which is a cell wall component of gram-negative bacteria, is recognized by the pathogen recognition receptor toll-like receptor 4 [19]. Upon binding to this receptor, LPS stimulates the production of a wide array of innate immune response proteins that include the synthesis and release of proinflammatory cytokines. There are some notable similarities between the cytokine-associated inflammatory responses triggered by LPS and poly(I:C) [19, 20]. Therefore, it should not be surprising that prenatal LPS treatment, similarly to poly(I:C), precipitates a number of behavioral and neurochemical changes relevant to psychiatric illnesses. Despite the apparent similarities between the LPS- and poly (I:C)-induced effects, there also are some noticeable differences between the two models with respect to the nature of brain and behavioral changes. For example, early prenatal poly(I:C) treatment in mice has been shown to increase the number of midbrain dopamine neurons produced [21], whereas prenatal LPS exposure decreases the numbers of midbrain dopamine neurons produced [22]. Prenatal LPS exposure in rhesus monkey also causes a significant increase in global white matter volume [23], whereas an opposite pattern (i.e., decreased white matter volume) has been noted in rhesus monkeys born to influenza-infected mothers [24]. Such differences in the long-term outcomes between prenatal exposures to bacterial-like and viral/viral-like immunogens support the thesis that different pathogens can induce distinct sets of neuroimmune abnormalities across brain development. Accordingly, many questions remain regarding the timing and the type of inflammatory stimuli associated with disease-specific outcomes such as ASD, Schizophrenia and other CNS disorders [25].

3. Roles of Cytokines in Normal Brain Development

Cytokines, which may be defined as small intercellular signaling proteins, shape brain development beginning very early in fetal life. Several families of these signals, such as the TGFβ family (which includes the Bone Morphogenic Proteins) and the IL-6 family (which includes IL-6, Leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF) and others) play important roles throughout brain development. These cytokines can be detected in the human fetus as early as 5 weeks of gestation [26], which is when early patterning events are occurring during human brain development. Other studies have detected their presence in the fetal brains of non-human species beginning in early gestation [14, 27]. Cytokines can modulate stem cell self-renewal, progenitor cell proliferation, cell specification, differentiation, cell migration [28, 29], as well as dendrite growth, dendritic complexity [8] synaptogenesis and neuronal survival [30], thereby affecting multiple aspects of CNS development. As they play important roles during normal development, it follows that an imbalance in the levels of cytokines in the fetal circulation or in the developing brain could adversely affect neurodevelopmental processes resulting in disorders as disparate as attention deficit and hyperactivity disorder, Schizophrenia, Autism and intellectual disability.

Factors that will contribute to the ultimate outcome of a maternal infection include whether the cytokines are derived from the maternal or the fetal system, whether there is a change in the balance of neuroprotective vs. cytotoxic cytokines, and the stage of fetal brain development and/or immune system development.

4. Cytokine Levels Increase in Placenta and Amniotic Fluid in Prenatal Infection

Several models of MIA, including influenza infection, LPS and Poly I:C administration, increase levels of cytokines in the maternal serum, placenta and fetal brain [14, 31-35]. In the Poly (I:C) model both maternal serum interleukin-1 (IL-1β) and interleukin-6 (IL-6) protein levels were elevated and comparable when pregnant dams were injected at early (GD-9) and late (GD-17) stages of fetal development. Notably, poly (I:C) induced a stronger IL-6 response at GD-17 than at GD-9 [14]. Moreover, studies have provided evidence that the increased levels of cytokines in the maternal circulation can cross the placenta to enter the fetal circulation. For example, high levels of cytokines, such as IL-2 and IL-6, were detected in the amniotic fluid and fetal tissues after administering cytokines into the maternal jugular vein [36, 37]. Interestingly, IL-6 is transferred bi-directionally across healthy term human placentas whereas the cytokines IL-1α and TNF-α are transferred only minimally through the placenta [38].

To determine whether elevated levels of cytokines in the fetal circulation cross the blood-barrier to enter the brain, Urakubo et al., 2001 injected GD-16 pregnant rats with a low-dose (0.5 mg/kg) of LPS [33] and collected amniotic fluid, placenta and fetal brains 2 - 8 hours later. Protein levels of IL-1 β, IL-6 and TNFα were quantified by ELISA. They showed that LPS significantly increased placental levels of IL-1β, IL-6 and TNFα. Of these three cytokines, only IL-6 levels were significantly increased in the amniotic fluid. In a similar study, pregnant rats were injected systemically with 100 μg/kg of LPS at gestation day 18 [35]. Subsequently, maternal blood, amniotic fluid, placenta and fetal brains were collected. Analyses of maternal serum showed a significant increase in IL-6 and IL-1β and increased levels of these cytokines achieved a maximum in the amniotic fluid 6 hours after LPS treatment.

Key to implicating elevated levels of cytokines in altered fetal brain development is establishing whether these pro-inflammatory cytokines can pass from the maternal circulation into the fetus and from the fetal circulation into the brain. In one study, pregnant rats were infused with radioactive IL-6 at mid-pregnancy (GD 11-13) and late- pregnancy (GD 17-19) [36]. When fetal tissue was sampled, a 40-fold passage of IL-6 across the placental barrier to fetal tissue was detected at mid-pregnancy compared to late pregnancy (which showed little passage of IL-6). In another study where radioactively labeled IL-6 and IL-1β were injected into the jugular veins of adult mice, there was a linear relationship between brain/serum ratios for both IL-6 and IL-1β, suggesting that these cytokines can cross the murine blood-brain barrier. Furthermore, administering unlabeled IL-6 or IL-1β along with the radioactively labeled IL-6 or IL-1β competitively reduced the levels of radioactive cytokines into the brain suggesting the presence of saturable transporters for these cytokines [39]. Banks et al., 1994 showed that IL-6 was transported into the murine brain and could be inhibited by unlabeled IL-6 but not by unlabeled IL-1α or TNFα, providing evidence that the transporter for IL-6 is distinct from that used by IL-1α and TNFα. They also found that most of the IL-6 could be recovered from the brain parenchyma, demonstrating that blood-borne IL-6 can penetrate the blood-brain barrier. Despite intense

research, the identity of the transporter has not yet been established. In addition, a recent study showed that subsequent to a systemic maternal inflammation stimulating a fetal inflammatory response, that levels of IL-6 mRNA increase within the fetal CNS indicating that local production of IL-6 within the CNS also occurs [40]. Given the importance of the cell proliferation to produce the variety of cells within the developing brain, and the evidence that IL-6 can cross the blood-brain barrier and can be produced locally, a key question is whether elevated levels of cytokines in the developing brain will alter the proliferation of the neural stem cells and/or the neural progenitors and if so, whether this disruption of normal brain histogenesis will contribute to the etiology of psychiatric disorders.

5. Adverse Effects of IL-6 on Fetal Brain Development

Studies have established that IL-6 is elevated in maternal serum, amniotic fluid and serum of children subsequently diagnosed with Autism Spectrum Disorder (ASD). Moreover, independent studies have established that children and adults with ASD have higher circulating IL-6 levels compared to controls [41-43]. These increases are associated with impaired communication skills, irritability, socialization and intelligence [44]. Accordingly, IL-6 is increasingly being used as a biomarker for ASD [45]. Experimental studies in mice also highlight the role of IL-6 in disrupting the brain development. Indeed, a single injection of IL-6 into a pregnant mouse at GD12.5 produced cognitive and behavioral deficits similar to those seen in neurodevelopmental disorders like ASD and Schizophrenia [46]. Moreover, administering a blocking antibody to IL-6, but not to IL-1α, TNFα or IFNγ reversed all of the behavioral deficits seen in this mouse model of MIA. This study further showed that Poly I:C injections into pregnant IL-6 knockout mice didn't produce the behavioral problems seen in wild-type mice, lending strong support to the conclusion that IL-6 is the key mediator of the effects of MIA on fetal brain development. Those data are further supported in a LPS model of MIA, where treatment with the anti-inflammatory drug Pioglitazone abolished ASD-like behaviors and reduced elevated plasma IL-6 levels [47].

Recent studies have aimed at uncovering the underlying mechanisms leading to the cognitive and behavioral deficits seen in ASD. For instance, exposure to diesel exhaust gases during intrauterine development, an environmental factor associated with ASD, leads to behavioral alterations in adult mice reminiscent of ASD [48]. These mice displayed elevated brain IL-6 at birth, as well as abnormally high levels of phosphorylated STAT3 and decreased levels of reelin. At P60, reelin co-localizes with an accumulation of calretinin+ cells in the inner layers of the cortex, suggesting that increased levels of IL-6 during development inhibits interneuron migration and consequently disrupts proper cortical lamination and, therefore, upsets the balance of inhibitory and excitatory synapses [48].

6. Adverse Effects of IL-6 on SVZ Neurogenesis

Normal brain development is highly dependent on timely, regulated proliferation and differentiation of NSPs. Hence, neurogenesis is observed at high levels throughout the brain during the early stages of development, being later restricted to a few specific niches in the adult brain. In the developing brain, neurogenesis and gliogenesis are vulnerable to changes in the fetal environment, and especially to neuroinflammation. For example, Gallagher et al., 2013 evaluated the long-lasting effects of maternal IL-6 on embryonic NSPs [49]. They administered a single pulse

of IL-6 into pregnant mice at gestational day 13.5, the equivalent of the human 1st trimester [18], and evaluated the effects on NSP proliferation in adult subventricular (SVZ) of 2-month old offspring. Using the marker Sox2 along with BrdU, they found increased numbers of double labeled cells in the adults that had been exposed to IL-6 at E13.5. They concluded that IL-6 increased the proliferation of NSCs in the SVZ, as they didn't find any differences in other NSP subpopulations when labeled using antibodies to GFAP, Olig2 and Mash1. However, as Sox-2 is expressed by both progenitors and stem cells, these data do not conclusively establish which of these populations was increased.

Gallagher et. al., 2013, also used the neurosphere assay and reported an increase in SVZ neurosphere initiating cells cultured from adults exposed to IL-6 as fetuses. In contrast, when we treated cultured SVZ cells from P5 mice for 6 days with IL-6 (5 ng/ml), it decreased both the number and sizes of the neurospheres. Typically, a decrease in neurosphere number is interpreted as a reduction in number of NSCs. However, when we differentiated the neurospheres that were treated with IL-6 there was an increase in the percentage of multipotential neurospheres compared to control. One limitation of the neurosphere assay is that it can't distinguish between spheres formed by stem cells vs. those formed by progenitors [50]. In order to resolve this issue, we used a multi-color flow cytometry panel to more completely establish how IL-6 affects the NSPs of the SVZ. As we will explain in more detail below, IL-6 stimulated the proliferation of a multipotential progenitor at the expense of the NSCs. This result is reminiscent of a study in adult mice, where a transient surge in IL-6 activated the putative adult NSCs in the short term but depleted the NSC pool in the long term [51]. This same study also showed that accompanying the depletion of the NSCs, there was reduced olfactory bulb neurogenesis. These results demonstrate that increasing IL-6 levels at different stages of brain development has different consequences.

6.1 Multipotential Progenitors within the Neonatal SVZ

Many studies have demonstrated that cells isolated from the SVZ are epidermal growth factor and/or fibroblast growth factor (FGF) responsive, but more recent studies have determined that another subset of cells in the SVZ express receptors that respond to platelet–derived growth factor (PDGF) [52, 53]. PDGF stimulates the growth of PDGFRα and PDGFRβ positive cells from both the embryonic and the adult mouse SVZ [53, 54]. While PDGF responsive radial glia are rare in the mouse brain, they are important for human neocortical development [55]. Using a flow cytometry panel, that can distinguish between eight different cell types found in the SVZ, we have identified NSCs, 4 multipotential progenitors (MPs) and 3 bipotential progenitors [56]. We also have established the growth factor dependency of these NSPs. Five of the 8 subpopulations are multipotential progenitors when differentiated *in vitro*, yielding neurons, oligodendrocytes and astrocytes. These were the CD133+LeX+NG2–CD140a– (NSC), CD133–LeX+NG2–CD140a– (MP1), CD133+LeX+NG2+ CD140a– (MP2), CD133+LeX+NG2+CD140a+ (MP4) and CD133–LeX+NG2+CD140a+ (referred to as PDGFRα -FGF2-responsive MPs: PFMP). This PFMP is likely the NG2+ multipotential progenitor that has been isolated from the embryonic forebrain [54] and is similar to the PDGF responsive progenitors present in the neonatal rat SVZ as described by Moore et al., 2014 [57].

6.2 Modeling Maternal Infections by Systemically Injecting IL-6

Mice are born precociously, thus the brain of a newborn mouse is equivalent to that of an early 3rd trimester human infant [17]. Previous studies have measured 2-3 fold increases in the blood plasma IL-6 levels in children with ASD [41, 43]. Therefore, to determine the dose of IL-6 provided to mice needed to mimic the elevated levels of IL-6 seen in children who will develop ASD, we injected neonatal mice with increasing doses of IL-6 vs PBS twice on postnatal day 4 (P4) and once on P5 and then measured plasma levels of IL-6 two hours after the 3rd injection. We used this paradigm to produce prolonged and elevated levels of IL-6 for at least 36 hours. Administering 75 ng of IL-6 increased blood plasma levels by 2- fold; therefore, decided to administer 75 ng of IL-6 to mimic the 2-fold increase in circulating IL-6 level observed in ASD patients.

6.3 Elevated Levels of Il-6 Increase the Proliferation of Pdgf-Responsive Multipotential Progenitors

Administering 75 ng of IL-6 twice on P4 and once on P5 altered the neonatal SVZ NSP cell pool by specifically increasing the proportion of PFMPs 2-fold while reducing the proportions of NSCs, MP1s, BNAP/GRP1s and GRP2s, as determined using flow cytometry [58]. This result begins to explain how IL-6 treatment can reduce neurosphere number and size, while increasing multipotentiality. We also found that IL-6 increased the percentage of EdU$^+$ PFMPs by 2-fold compared to a decrease in EdU$^+$ NSCs *in vitro* and confirmed our *in vitro* results *in vivo* using intraperitoneal injections of 75 ng of IL-6, which increased the percentage of EdU$^+$ PFMPs [58]. We did not see any significant differences in NSC proliferation in situ, but that can be attributed to the long cell cycle length of the NSCs, which was not captured, as we evaluated a single time point. Altogether, these data lead to the conclusion that IL-6 expands the PFMPs at the expense of the NSCs.

As discussed above, different progenitor cell types are differentially responsive to IL-6 and that IL-6 can either promote or suppress cell proliferation. For example, studies have analyzed the expression of the IL-6 receptor and gp130 on hematopoietic progenitors and demonstrated that most CD34$^+$ progenitors are IL6Rα negative and are unresponsive to IL-6 alone [59]. By contrast, Monje et al., 2003 showed that the hippocampal NSPs express IL-6 receptors and IL-6 inhibited hippocampal neurogenesis [60]. Gallagher et. al., 2013 also showed that fetal Sox2$^+$ VZ/SVZ progenitors express the IL-6R. To determine whether IL-6 acts directly on the PFMPs we used flow cytometry to show that the PFMPs as well as another multipotential and one bipotential NSP in neonatal SVZ express IL-6Rα, whereas the IL-6Rα was not expressed by the NSCs or several other SVZ NSPs. Therefore, the cell types that express membrane-bound receptor can be stimulated directly in the presence of increased level of IL-6, whereas the rest can be stimulated by IL-6, but only through trans-signaling which will require the presence of sIL-6Rα.

6.4 Signaling Pathways Activated by IL-6 Family Cytokines

A number of signaling pathways have been shown to be activated by IL-6 in different CNS cell types such as the Janus kinases (JAK) and, to a lesser extent, TYK, which, in turn, activate a number of proteins including STATs. IL-6 can also activate the RAS-RAF-MAPK, the phosphatidyl inositol-3 kinase pathway and the NFkB pathway [61]. Studies have investigated the activation of pSTAT1 vs.

pSTAT3 by IL-6 in highly enriched astrocyte compared to microglial cultures [62]. The astrocytes responded with significant increases in both pSTAT1 and pSTAT3, in contrast IL-6 treated microglia activated pSTAT1 significantly, but not pSTAT3. Therefore, we evaluated signal transduction pathways activated in SVZ NSPs in response to IL-6 and LIF, a related cytokine that shares GP130R with IL-6 and has been shown to affect SVZ NPs [56]. We found that IL-6 significantly increased pSTAT3 signal, with no effect on pSTAT1 and no effect on either pAKT or p44/p42 MAPK. In contrast, LIF significantly increased both pSTAT1 and pSTAT3 in these SVZ NSPs and it increased phosphorylation of both P42 and P44 MAPK [58].

STAT3 has been shown to be present and active in the developing mouse CNS as early as GD-7.5 [63]. The loss of STAT3 resulted in fewer NSPs suggesting that the STAT3 functions first to promote NSP proliferation. STAT3 activity increases again later in development during cell fate commitment, showing its dual role during CNS development. Interestingly, Jak/Stat3 activation by IL-6 has been shown to promote the proliferation of mesenchymal stem cells in bone cancer and inhibiting STAT3 decreased cell proliferation, migration and invasion with down-regulation of mRNA expression of CyclinD, Bcl-xL and Survivin, which enhanced apoptosis [64]. Also, treatment with a STAT3 inhibitor decreased tumor growth with reduced protein levels of IL-6, pSTAT3 and PCNA indicating a role for IL-6 in mesenchymal stem cell proliferation, supporting our data that IL-6 mediated activation of pSTAT3 in SVZ NSPs increases PFMP cell proliferation. However, this hypothesis needs to be further tested.

6.5 Transcription Factors Induced in SVZ Neural Progenitors by IL-6

We postulated that the IL-6 mediated increase in PFMP proliferation could be due to increased expression of transcription factors involved in self-renewal of the NSPs. Therefore, we analyzed the self-renewal genes Ascl1, Dlx2, Klf4, Id2, Olig2, Fbx15, Gsx1, Gsx2 and Bmi-1, which have been shown to be important for neural progenitor proliferation. Neurospheres maintained in medium supplemented with IL-6 had 2.5 fold higher levels of Ascl1 than controls [58]. Using chromatin immunoprecipitation, it has been shown that Ascl1 induces E2f1 (a transcriptional activator of genes promoting G1/S transition) and its co-activators Ep400, Cdca7, Tead1, Tead2, and FoxM1, to promote G2/M transition [65]. Additionally, acute loss of Ascl1 impairs progenitor cell divisions in the SVZ. These data support the conclusion that Ascl1 directly regulates the genes involved in cell-cycle progression.

Gsx1 and Gsx2 represent a family of homeobox genes that are expressed in discrete progenitors of the embryonic brain, including the ventral telencephalon where they have been shown to govern the early specification of lateral ganglionic eminence progenitors [66-69]. Gsx genes are not only required for the patterning, but also for the control of cell proliferation. Gain-of-function studies suggest that Gsx1 promotes progenitor maturation and acquisition of neuronal phenotypes [70]. We found that IL-6 significantly increased Gsx1 in SVZ NSPs consistent with the view that the Gsx1 transcription factor is involved in the proliferative response of the PFMPs to IL-6 [58].

7. Elevated Perinatal Systemic IL-6 Increases Anxiety

Perinatal inflammatory challenges with LPS have been shown to promote behavioral alterations in adolescent (P35) and adult (P70) male mice, reminiscent of human psychiatric disorders such as

ASD. One the hallmarks of these behavioral alterations is an increase in anxiety [71]. The elevated plus maze is a well characterized behavioral paradigm and is one of the most widely used tests for anxiety research. This test relies upon the animal's natural tendency to stay in enclosed spaces and their unconditioned fear for open spaces and heights. In our own experiments, mice injected with IL-6 from P3-P5 were then analyzed as adolescents (P32). They spent significantly more time in the closed arm compared to the PBS group, suggesting that elevating IL-6 perinatally increases anxiety [72]. Furthermore, LPS-induces anxiety-like behaviors in several animal models that are associated with hippocampal neuroinflammation and increased production of inflammatory mediators such as IL-6 and IL-1β [73]. Earlier studies in our lab have shown that increased levels of IL-1β affect hippocampal based functions and that mice treated with IL-1β between P1-P5 postnatally displayed anxiety-like behavior both in the open-field test and elevated plus maze when tested as adults [74].

8. Role of Ventral Hippocampus and Basolateral Amygdala in Anxiety Behavior

The amygdala is the brain structure that is typically associated with anxiety and social behaviors [75, 76] and it has robust and reciprocal connections to the ventral hippocampus (vHPC)[77, 78]. Human and rodent studies have demonstrated that the vHPC is important for the expression of fear- and anxiety-related behaviors as well as for social interactions [79-81]. In non-human primates, vHPC lesions lead to abnormal responses to social signals and to degradation of social bonds [82]. Experiments using social interaction paradigms in rodents have also provided evidence that the vHPC is involved in social behaviors [83-85]. In a recent study, investigators showed that newly added adult dentate granule neurons decrease the excitability of pre-existing vHPC granule neurons. When they experimentally inhibited adult neurogenesis the animals were more anxiogenic when placed into a stressful situation [86].

As anxiety is correlated with deficits in social interaction, and both the amygdala and vHPC are involved in both anxiety and social behaviors, investigators have studied the role the basolateral amygdala-vHPC circuit in social behavior. Using an optogenetic approach to target basolateral amygdala terminals in the vHPC, it has been found that inhibiting basolateral amygdala-vHPC projections increased sociability in both the juvenile-intruder test and the three-chamber sociability test, while exciting this pathway decreased sociability [87]. As these brain structures play important roles in these behaviors, it will be important in future studies to determine whether elevated levels of IL-6 affect neurogenesis and the function of this circuit.

9. Effects of Inflammation on the Stem Cells and Progenitors of the Developing Hippocampus

It has long been reported that preterm births are usually a consequence of MIA [88]. Neuroimaging and cognitive studies have suggested that abnormal hippocampal development and function in premature infants may be a result of MIA [89]. Similarly, decreased neurogenesis in the hippocampus has been reported in murine models of gestational inflammation using LPS [90] and poly (I:C) [91]. However, the long-term effects of maternal infections on hippocampal development are still poorly understood. In a rat model for prenatal systemic inflammation, consecutive LPS administrations during late gestation resulted in a decrease in DCX labeled cells at adolescence, suggesting a persisting effect on the NSPs of the SGZ. These deficits were rescued with postnatal administration of IL-1ra [92]. Another study administered LPS for two days at mid-

and late-gestation and showed a reduction in the number of immature neurons produced in the dentate gyrus that persisted into adulthood [93]. Additional reports using other inflammation inducers showed similar results. For instance, exposure of mice from P1 to P17 to silica nanoparticles inhibited progenitor cell proliferation in the SGZ in a dose-dependent manner, associated with social interaction deficits and increased anxiety. Importantly, perinatal silica exposure did not produce deficits in locomotion, short-term memory or depression-like behavior. Similarly, injecting Poly(I:C) into late-gestation mice leads to a strong inflammatory response in the hippocampus of the offspring, associated with impaired hippocampal neurogenesis in prepubertal males, cognitive deficits and anxiety-like behavior. These animals displayed high microglial activation, which was rescued by treatment with a peroxisome proliferator-activated receptor gamma agonist [91].

The observed cellular and behavioral neurodevelopmental outcomes of prenatal exposure to LPS and poly I:C are correlated with an elevation of proinflammatory cytokines in maternal and fetal systems [94-96]. Additionally, systemic inflammation decreases hippocampal neurogenesis in the adult brain [97], suggesting that proinflammatory cytokines can negatively regulate both the proliferation and differentiation of NSPs. As should be clear from the discussion above, progenitors are differentially responsive to cytokines, and IL-6 can either promote or suppress cell proliferation, depending on the context and developmental stage of the inflammatory challenges. For example, Monje et al., 2003 showed that LPS challenge increases the production of the IL-6 receptor in neural progenitors and that IL-6 inhibited hippocampal neurogenesis [60]. In fact, treatment with IL-6 neutralizing antibodies can restore the negative effects of inflammation on adult hippocampal neurogenesis [60, 98]. It also has been shown that the effects of the LPS challenge persist into the late pro-inflammatory response, with increased levels of IL-6 and microglial activation, correlating with decreased hippocampal neurogenesis [99]. It is worth noting, however, that the same effects are not reproduced by repeated intermittent injection of LPS [99]. Accordingly, administering an immunopotentiator like thymosin alpha-1 (Ta1), which can decrease IL-6 levels, negated the LPS-induced impairment of hippocampal neurogenesis [100].

Other studies have more dramatically manipulated the levels of IL-6 and reported that the hippocampal NSP pool is altered, but with mixed results. In a study of IL-6 germ-line null mice, where IL-6 is deficient, Bowen et al., 2011 showed compromised neurogenesis with reduced NSP cell proliferation, survival and maturation in the subgranular zone (SGZ) and SVZ [101]. By contrast, Vallieres et al., (2002) used transgenic mice to constitutively over-express IL-6 in astrocytes and reported that the proliferation, survival, and differentiation of NSPs were all reduced in the hippocampal SGZ [102]. Also, the injection of IL-6 into late pregnant Wistar rats, leads to structural hippocampal abnormalities and neuronal loss and decreased spatial learning in adulthood [103].

Thus, hippocampal NSPs appear to be sensitive to both excessively high and excessively low levels of IL-6 *in vivo*. *In vitro*, the soluble factors released by human olfactory epithelial neural stem/progenitor cells, of which IL-6 is an important component, reduced neurogenesis of murine hippocampal NSPs, promoting astrocytic differentiation [104]. However, in another *in vitro* model in which hippocampal neurogenesis was reduced by treatment with either interferon-α or its downstream effectors interferon-stimulated gene 15 and ubiquitin-specific peptidase 18, IL-6 had no direct impact on neurogenesis. Despite the high levels of IL-6 elicited by interferon-α,

treatment with IL-6 alone was not sufficient to recapitulate the neurogenic effect, although, it reproduced the interferon-α-induced increase in apoptosis [105].

10. Interleukin-1 and Hippocampal Neurogenesis

The regulated expression of both IL-1R1 [106] and IL-1β during early postnatal development of the hippocampus [107] suggests that this cytokine may have an important role during hippocampal development. IL-1β belongs to the IL-1 family of cytokines, which includes IL-1α, IL-1ra, and IL-18, which play important roles in both innate and adaptive immunity. IL-1α and IL-1β bind respectively to two receptors, Il-1R1 and Il-1R2. However, only IL-1R1 binding results in proinflammatory cell signaling, as Il-1R2 lacks the intracellular domain necessary for signal transmission. Both receptors can be alternatively spliced and secreted, acting as soluble decoys that sequester IL-1 without activating inflammatory signaling [108].

IL-1β is considered a prototypical proinflammatory cytokine, as it is released by activated macrophages along with other major proinflammatory molecules, such as IL-6, IL-12, TNFα, and the chemokine CXCL8. IL-1β contributes to a host of local and systemic activities. Local effects include vascular endothelium activation, lymphocyte activation, local tissue destruction, and enhancing effector immune cell infiltration. Systemic effects include inducing a fever, lymphocyte activation, and increasing IL-6 production through MAPK signaling cascades [109].

IL-1β is quickly upregulated in the brain following different forms of experimental brain injury [110, 111]. In these experimental models, blocking either IL-1β or its receptor reduces the neuroinflammation seen after the brain injury. [112, 113]. IL-1R1 is widely expressed by resident brain cells, and signaling through this receptor in neurons, astrocytes, microglia, and endothelial cells results in a range of both toxic and protective effects in the brain [114-120]. In neurons, IL-1β appears to exert a dose-dependent effect that results in depolarization at lower concentrations and hyperpolarization and synaptic transmission inhibition at higher concentrations [115]. Additionally, IL-1β has been shown to mediate excitotoxicity, regulate NMDA receptor phosphorylation [114, 116, 118] and induce the expression of neuronal chemokines that act on local microglia to influence acute neuroinflammation [120]. In astrocytes, IL-1β signaling via IL-1R1 induce astrogliosis and the production of neurotoxic, neuroprotective and inflammatory mediators via activation of the MAPK and NFκB signaling pathways [117]. The brain endothelium is also activated by IL-β via the MAPK and NFκB signaling pathways, resulting in the expression of cell adhesion molecules and various chemokines that aid in leukocyte infiltration [119]. Therefore, the widespread expression and/or responsiveness to IL-1β in the brain infiltrating immune cells indicate that signaling through this pathway is a major regulator of neurodevelopment throughout the brain. However, the hippocampus appears to be particularly sensitive to IL-1, since the IL-1R1 is expressed at extraordinarily high levels within the dentate gyrus [121].

In vitro studies have shown that IL-1β signaling negatively affects rat embryonic hippocampal neurogenesis by decreasing the proliferation of NSPs [122] and suppressing neuronal differentiation [123], via activation of NF-κB signaling [122, 124]. Interestingly, IL-1β-induced activation of NF-κB also suppresses the expression of TLX (the orphan nuclear receptor tailless homolog), that promotes NSP proliferation and inhibits neuronal differentiation. Lentiviral overexpression of TLX was shown to counteract the negative effects of IL-1β on the proliferation of embryonic rat NSPs *in vitro* [122].

Studies on adult hippocampal neurogenesis can shed light on the effects of IL-1β on fetal neurogenesis. For example, Koo and Duman performed *in vitro* studies of adult rat hippocampal NSPs and showed that NSPs express IL-1R1 and that activation of IL-1R1 by IL-1β decreased NP proliferation via NF-κB signaling [124]. Similarly, *in vitro* studies on embryonic rat hippocampal NPs by Green and colleagues revealed decreased NSP proliferation and decreased neuronal differentiation as a result of IL-1β signaling through IL-1R1 [123], supporting the conclusion that IL-1R1 signaling negatively affects hippocampal NPs. A recent study showed that the cannabinoid system can modulate NSP proliferation and have a neuroprotective role, rescuing hippocampal neurogenesis during HIV-1 neurotoxic insults [125, 126]. Activating a candidate cannabinoid receptor, GPR55, suppressed the pro-inflammatory responses of IL-1β treatment on adult rat hippocampal NSCs in vitro, rescuing the negative effects of IL-1β on neurogenesis [127].

By contrast, a separate study showed that treating hippocampal NSPs in vitro with IL-1α increased proliferation of adolescent (P21) hippocampal NSPs, while treatment with IL-6 produced an inverse effect. In an animal model of chemically induced hippocampal injury using trimethyltin (TMT) IL-1R1 signaling was shown to activate IκB/NFκB in the SGZ of adolescent (P21) mice, whereas in adults, the same injury activated IL-6/gp130 signaling via Ras/MAPK more prominently [128]. These data suggest that IL-1 can affect hippocampal neurogenesis both directly and indirectly by inducing IL-6.

In our studies to elucidate the effects of increased levels of IL-1 on hippocampal neurogenesis we have established that inducing systemic inflammation by daily administration of IL-1β during the first 5 days of life increased hippocampal levels of IL-1α and acutely reduced the proliferation of $Tbr2^{+}$ progenitors in the dentate gyrus of mice. *In vitro*, both IL-1α and IL-1β produced G1/S cell cycle arrest that resulted in reduced progenitor cell proliferation within the transient amplifying progenitor cell cohort. By contrast, IL-1β treatment did not reduce neural stem cell self-renewal. By contrast treating hippocampal NSPs with IL-1 increased neural stem cell frequency[74].

An earlier study that used this same model of *in vivo* perinatal inflammation showed that mice that received IL-1β as neonates displayed memory deficits which suggested abnormal hippocampal function [129]. To evaluate whether other cognitive and behavioral traits associated with hippocampal function would also be altered in these mice, we administered tasks designed to assess exploratory and anxiety behavior and working and spatial memory. Interestingly, mice that received IL-1β as neonates showed signs of anxiety in both the open field test and the elevated plus maze during adolescence that were also evident in adulthood. These mice did not display working memory deficits in adulthood, but they did display deficits in long-term spatial memory [74]. Altogether, these da support the view that perinatal inflammation negatively affects the developing hippocampus and results in behavioral problems that persist into adulthood. Future studies will contribute new insights into the origins of the cognitive and behavioral impairments seen in prematurely born sick infants.

11. Modulating Neuroinflammation as a Therapeutic Strategy

Given the potent effects that elevated levels of IL-1 and IL-6 can exert on the developing brain, a natural conclusion is that reducing the levels of these cytokines to baseline levels or decreasing their ability to activate their cognate receptors will be beneficial. While we are not aware of any clinical trials to date that have attempted to manipulate these specific cytokines to prevent

psychiatric disorders, the data reviewed above from pre-clinical studies show that there are existing approaches that are technically feasible. For example, as shown by Wang et al., 2017, administering the immunopotentiator thymosin alpha-1 (Ta1), decreased IL-6 levels and negated the LPS-induced impairment of hippocampal neurogenesis [100]. An alternative approach would be to administer a neutralizing antibody to IL-6 maternally to antagonize circulating levels of IL-6 [130] or to administer polyethylene glycol linked IL-6 binding peptides that have been generated and which antagonize IL-6 with sub-nanomolar potency and persists in circulation for several days. Neutralizing antibodies to IL-6 have been shown as a feasible approach for reducing IL-6 efficacy in a model of gestational inflammation induced by LPS [131]. Girard et al., 2012, showed that administering the IL-1 receptor antagonist postnatally after prenatal LPS administration restored neurogenesis in the hippocampus demonstrating that treatments directed to the fetus and neonate may well restore brain development [92]. Accordingly, it is certainly feasible to administer reagents to modify the actions of these cytokines; however, systematic studies will be necessary to determine the timing and doses of these modulators to obtain optimal therapeutic benefits.

12. Summary

Increased levels of maternal cytokines, especially IL-1β and IL-6, which are positively associated with an increased risk of psychiatric disease in the offspring as discussed in two excellent reviews [132, 133] can cross the fetal placenta and cross the BBB through saturable transporters, where they can affect the developing brain. While many studies have evaluated their effects on neuronal survival, astrogliosis and microgliosis and failure of oligodendrocyte maturation. However, it is clear that they can produce other effects. In particular, as we've reviewed here, they can influence (either negatively or positively) the proliferation of the neural progenitors that reside with within the subventricular and subgranular zones, which in turn may affect the final cellular composition of the brain, deviating the potentially the balance of excitatory and inhibitory neurons as well as the neuroglial composition (summarized in Figure 1). Collectively, the studies reviewed here warrant further investigations into the role of these two cytokines on neurogenesis and how they may contribute to cognitive, motor and behavioral problems seen in childhood and adolescents born prematurely [134-136].

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Author Contributions

EK, FV and SWL performed literature reviews and contributed to writing the manuscript. EK produced Figure 1. All authors discussed the contents of the article, provided comments on drafts and approved the final manuscript.

Competing Interests

The authors confirm that they have no conflicts of interest to declare for this publication.

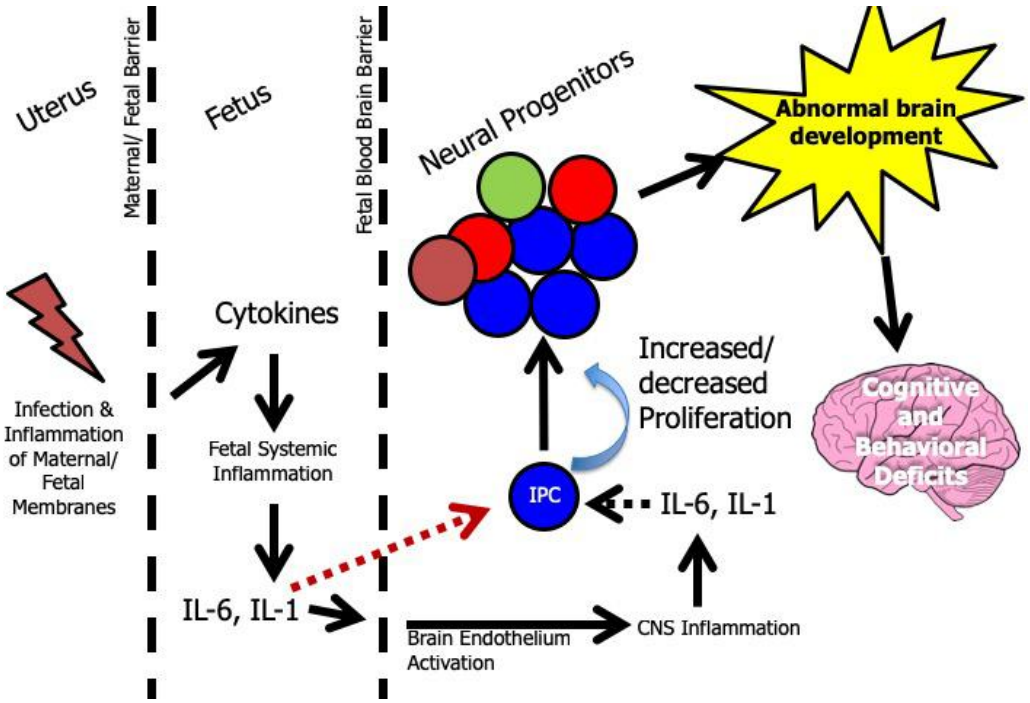

Figure 1 Model for effects of perinatal inflammation on neural development**.** Infection induced inflammation during pregnancy increases the levels of circulating cytokines, reaching the uterine membranes and the placenta. Cytokines that cross the placental barrier will trigger fetal systemic inflammation, with production of interleukins such as IL-6 and IL-1. These in turn can affect the endothelium resulting on local cytokine production or they can be transported across the endothelium to directly affect intermediate progenitor cells (IPC) in the subventricular zone and the hippocampal subgranular zone, to increase or decrease the proliferation of specific subsets of neurons and glial cells. The alterations in the types of cells produced will, in turn, affect the formation of neural circuits which in turn will lead to abnormal cognition and behavior.

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