Published in final edited form as:

Horm Behav. 2013 May ; 63(5): 684–691. doi:10.1016/j.yhbeh.2013.02.017.

FRANK A. BEACH AWARD: Programming of Neuroendocrine Function by Early-Life Experience: A Critical Role for the Immune System

Staci D. Bilbo

Department of Psychology and Neuroscience, Duke Institute for Brain Sciences (DIBS), Duke University, 572 Research Drive, Box 91050, Durham, NC 27708

Abstract

Many neuropsychiatric disorders are associated with a strong dysregulation of the immune system, and several have a striking etiology in development as well. Our recent evidence using a rodent model of neonatal *E. coli* infection has revealed novel insight into the mechanisms underlying cognitive deficits in adulthood, and suggests that the early-life immune history of an individual may be critical to understanding the relative risk of developing later-life mental health disorders in humans. A single neonatal infection programs the function of immune cells within the brain, called microglia, for the life of the rodent such that an adult immune challenge results in exaggerated cytokine production within the brain and associated cognitive deficits. I describe the important role of the immune system, notably microglia, during brain development, and discuss some of the many ways in which immune activation during early brain development can affect the later-life outcomes of neural function, immune function, and cognition.

Keywords

neonate; infection; microglia; development; learning; memory; Interleukin (IL)-1 β ; obesity; TLR4

Introduction

Well-known for its critical role in host defense, the immune system also plays a critical role in brain development. The resident immune cells of the brain, microglia, and their releasable factors, cytokines and chemokines, have a central role in many processes of neural development including cell proliferation, neurogenesis, synaptic formation and pruning, and programmed cell death. In addition, the immune system represents a particularly critical interface between the environment, physiology, and brain of an individual such that external stimuli can affect not only the immediate development but also the later-life function of the brain and behavior. Thus, neuroendocrine-immune activation during prenatal or early postnatal development can have profound and long-lasting effects on the brain. The purpose of this review is to provide a review of data from my lab on the long-term effects of early-life immune activation on adult brain function, a current perspective on the long-term mechanisms by which these effects are propagated from development to adulthood, and the

© 2013 Elsevier Inc. All rights reserved.

staci.bilbo@duke.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

implications of this work for additional early-life events that may enduringly alter adult neuroendocrine function and behavior via their impact on the developing immune system.

Microglia and Neurodevelopment

Colonization of Microglia in the Developing Rodent Brain

Microglia originate early in the life of the rodent fetus and can be very long-lived, meaning that microglia are believed to reside in the brain for the life of the organism, similar to neurons. Microglial progenitor cells begin colonizing the rodent brain around embryonic day (E) 9-10 via the infiltration of primitive macrophage precursors from the yolk sac (Chan et al., 2007; Ginhoux et al., 2010). Primitive microglia have been localized to subcortical regions of the developing brain including the hippocampus and the corpus callosum (Wang et al., 2002; Xu et al., 1993), entering the brain parenchyma via the blood stream and ventricles (Cuadros and Navascues, 1998). After their initial colonization of the brain, microglia migrate to their final destination within the brain where they continue to proliferate throughout pre- and postnatal brain development.

To date, it is not well-known what factors drive the infiltration and migration of immature microglia into the brain parenchyma. Some researchers have noted that the invasion of microglia within the developing brain coincides with the naturally-occurring programmed cell death during early brain development (Ashwell, 1991; Perry et al., 1985); however, very little is known about the exact relationship between developmental cell death in the brain and microglial colonization of neural tissues. While it is possible that the factors released from dying cells within the developing brain may in turn control the migration and infiltration of microglia into the parenchyma, it is also possible that other factors may attract the infiltration and migration of immature microglia throughout the developing nervous system. Many chemokines (**chemotactic cytokines**) have a demonstrated role in microglial migration and neural development within the healthy brain (Cowell and Silverstein, 2003; Cowell et al., 2006; Rezaie et al., 2002), and a significant number of chemokines are highly up-regulated within the rat hippocampus/cortex at birth, when compared to the adult brain. These include chemokine (C-C motif) ligand (CCL) 2, CCL3, CCL6, CCL7, and CCL12 (Schwarz et al., 2012). A few of these cytokines (CCL2, CCL3, and CCL7) are expressed by astrocytes and neural progenitor cells and have a demonstrated role in regulating the development of microglial cells within the human brain (Hahn et al., 2010; McKimmie and Graham, 2010; Rezaie and Male, 1999; Rezaie et al., 2002). Monocyte-chemoattractant protein (MCP)-1 and intercellular cell adhesion molecule (ICAM)-2 may also play a role (Rezaie and Male, 1999), and a recent report shows that mice lacking the receptor for colony-stimulating factor (CSF)-1 do not develop microglia (Ginhoux et al., 2010). Thus, neural progenitor cells and immature astrocytes may release these chemokines, even in the absence of cell death, as a mechanism for attracting primitive macrophages and immature microglia into the developing brain.

Microglia Have a Distinct Morphology throughout Neural Development

Developing microglia have a distinct morphology and function within the neonatal brain. Microglia shift morphology and function throughout brain development from an immature/amoeboid morphology to a mature/ramified morphology. This shift in microglial morphology occurs in an age- and brain region-dependent manner, such that microglial number, morphology, and cytokine/chemokine production coincide with the neural development of each brain region. For example, IL-1 β is produced at detectable levels within the cortex from approximately E14 to postnatal day (P) 7 (Giulian et al., 1988). In contrast, the cerebellum, which develops significantly later, just prior to birth in rodents, has a peak in IL-1 β levels that occurs from P2 to P14 (Giulian et al., 1988). The developmental

shift in microglial morphology and function is best-characterized within the hippocampus, cortex, and amygdala, brain regions that are important for cognitive processes such as learning, memory, attention, and a number of social behaviors in adulthood.

Within the embryonic hippocampus, cortex, and amygdala of the rat, microglia have a predominantly round amoeboid morphology, even days prior to birth. At this time, very few microglia have short, stout processes that extend from the enlarged soma. At birth, however, the microglial population begins to exhibit a significantly different morphology, as more microglia display short or long, thick processes at this time. Just four days later, on postnatal day (P) 4, there is a significant increase in the *number* of microglia within these brain regions and a dramatic shift in *morphology* such that microglia begin displaying smaller cell bodies and thinner processes. These findings indicate that even at this early time point in neurodevelopment, microglia are rapidly maturing and shifting into a more ramified morphology (Schwarz et al., 2012).

Coincident with the striking difference in morphology, developing microglia also exhibit a significantly different biochemistry than microglia in the adult brain. For example, between birth and P4 the expression of Interleukin (IL)-1 β , the enzyme which cleaves IL-1 β into its active form (Caspase 1), and the IL-1 “decoy” receptor (IL1r2) are significantly increased approximately 6-, 6-, and 10-fold respectively when compared to the adult hippocampus and cortex, suggesting that immune molecules and signaling pathways such as these may have a more ubiquitous role within the brain than originally thought (Schwarz et al., 2012).

Further research must be done to expand the current knowledge of microglial ontogeny and function throughout the developing brain. Understanding the mechanisms of microglial colonization will lend greater insight into the mechanisms by which the brain develops under normal circumstances, and the mechanisms by which the developing brain might respond and subsequently be affected by an early-life immune challenge that occurs at the peak of microglial colonization, when cytokine and chemokine production are quite distinct from that in the adult brain.

The Functional Role of Microglia and Cytokines in Brain Development

Taking into consideration the morphology of immature microglia and the increased production of cytokines within the developing brain described above, one might assume that the primary role of microglia within the developing brain is related to their role as brain macrophages, specifically that they are actively engaged in the phagocytosis of cellular debris of apoptotic cells as well as the induction of apoptosis in other cells (Bessis et al., 2007; Marin-Teva et al., 2004). However, recent work suggests that microglia, cytokines, and chemokines have a more complex role in the developing immune system.

In addition to phagocytosing dying cells and cellular debris, microglia have a critical role in the phagocytosis of spurious synapses throughout development (Schafer et al., 2012; Stevens et al., 2007). Synapse elimination is an important process of neural development and is critical for the formation of functional neural circuits. C1q, the initiating protein within the classical complement cascade of the immune system, localizes to synapses within the postnatal brain intended for elimination. Microglia expressing the complement receptor for this protein are subsequently activated for phagocytosis of these individual synapses (Schafer et al., 2012; Stevens et al., 2007). A large number of cytokines and other immune molecules, many of which are microglial-derived, have been characterized for their importance in many neurodevelopmental processes such as neurogenesis, neuronal and glial cell migration, proliferation, differentiation, and synaptic maturation and pruning. These include members of the gp130, bone morphogenetic protein (BMP), and transforming growth factor beta (TGF β) super-families, as well as many traditionally defined “pro-

inflammatory” cytokines (e.g., IL-1 β , TNF α) (Boulanger, 2009; Deverman and Patterson, 2009; Garay and McAllister, 2010; Merrill, 1992). Chemokines also have a more ubiquitous function within the developing brain. For example, the chemokine (C-X-C motif) CXCL12 (SDF-1) and its exclusive receptor CXCR4 have a critical role in the migration of different neuronal populations to their final destination within brain regions such as the developing cerebellum, dentate gyrus, cortex, and hypothalamus (see (Deverman and Patterson, 2009) for review). These data suggest that similar to their chemoattractant role within the immune system, chemokines may be produced and/or secreted by other cell types within the brain and as such guide the migration of neurons to their final destination within their respective functional circuits. We anticipate that future research will identify additional mechanisms by which immune molecules and microglia guide the normal development of the nervous system.

The Long-term Consequences of Early-Life Infection: Neuroimmune and Cognitive Dysfunction

Given the evidence presented above describing the important role of the immune system in normal brain development, it is likely no surprise that elevated levels of pro-inflammatory cytokines, caused by perinatal infection (bacterial or viral), have been linked with abnormal brain development and an increased risk of neurodevelopmental disorders in humans (Cai et al., 2000; Meyer et al., 2006; Pang et al., 2003; Urakubo et al., 2001). Bacterial infection represents the number one cause of perinatal infection in newborns worldwide and is a significant cause and consequence of premature births (Osrin et al., 2004; Skogstrand et al., 2008). Human newborns are particularly impaired in their ability to mount an immune response against bacteria, and this results in increased infant mortality especially within developing countries (Marshall-Clarke et al., 2000; Osrin et al., 2004). Viral infections, such as maternal influenza infection, result in increased cytokine production via the activation of the maternal immune system, the fetal immune system, and immune cells within the placenta. The increased synthesis of cytokines following maternal influenza infection has been linked to an increased risk of schizophrenia in offspring, as well as a host of other cognitive disorders (Brown, 2006; Brown et al., 2004; Mortensen et al., 1999; Patterson, 2009).

Recent advances in maternal and perinatal medicine have greatly increased survival rates following perinatal infection, particularly among populations in developed countries. Despite this, it remains to be determined what the total impact of perinatal infection may be on the physiology and behavior of surviving individuals. One of the most common long-term consequences of perinatal infection or inflammation is *cognitive dysfunction* (Bauman et al., 1997), which includes general deficits in learning, memory, and attention; however, the exact mechanisms underlying these deficits are unknown. In addition, neuropsychiatric conditions as diverse as Alzheimer’s disease, autism, and depression are similarly associated with neuroimmune abnormalities, including exaggerated microglial activation and altered cytokine expression (Dantzer and Kelley, 2007); yet the proposed etiology of these disorders, such as a single precipitous event or infection, has been either insufficient or may simply not be well-understood. Thus, the early-life immune history of an individual may be the key to understanding the later-life risk of developing not only general cognitive deficits but also the risk of other neuropsychiatric disorders.

Developmental or “fetal programming” is an area of research that is based on the idea that experiences during the pre- or postnatal period may modulate or “program” the trajectory of a particular developmental processes, with the result that adult outcomes such as behavior are significantly and often permanently affected. This concept has been well-described with regards to the long-term consequences of early-life stress and resulting glucocorticoid

exposure, maternal interactions, fetal growth, and metabolism (Champagne, 2008; de Boo and Harding, 2006; Owen et al., 2005). In contrast, the concept of perinatal programming of the immune system and the potential effects on brain and behavior in adulthood remains relatively unexplored despite strong evidence that perinatal exposure to infectious agents has a number of influences on later life outcomes including disease susceptibility, and as mentioned above, increased vulnerability to cognitive and/or neuropsychiatric disorders (Hornig et al., 1999; Nelson and Willoughby, 2000; Shi et al., 2009).

I have hypothesized the developing brain is particularly sensitive to early-life immune activation and the associated risk of later-life cognitive disorders because 1) microglia are long-lived such that previously activated/functionally altered microglia (i.e. microglia exposed to an early-life immune challenge) may remain within the brain into adulthood, 2) immature microglia within the developing brain are functionally and/or immunologically different than microglia within the adult brain such that early-life immune activation can have greater consequences for neuroimmune function when compared to the adult brain, and 3) microglia and their inflammatory products are critical for normal cognitive function and behavior such that neuroimmune dysfunction results in cognitive dysfunction. I present evidence within the following sections that directly support this hypothesis and describe techniques that can be used to explicitly test these general ideas for researchers interested in understanding the long-term consequences of early-life immune challenge on later-life behavior.

A Rodent Model of Early-Life Infection and Later-Life Cognitive Dysfunction

Infection of rat pups with a non-lethal dose of *Escherichia coli* (*E. coli*) on P4, a developmental time point in rodents that is similar to the early third trimester of prenatal development in humans, produces a robust immune response within the periphery and the brain. Within the hippocampus, neonatal infection causes a prolonged (~24 hour) increase in cytokine expression, with a distinct increase in the expression of genes from the IL-1 family of cytokines. *E.coli* increases the expression of IL-1 β , Caspase 1 (which cleaves IL-1 β from its pro- to its active form), IL-18, and the IL-1 receptors 1 and 2 within the hippocampus (Bilbo et al., 2005a; Schwarz and Bilbo, 2011). Cytokine receptors are distributed throughout the developing and adult brain and the hippocampus, in particular, has one of the highest densities of microglia and cytokine receptors within the brain, including receptors for IL-1 β (Cunningham and De Souza, 1993).

The hippocampus is a brain region critical for learning and memory, spatial navigation, and attention in adulthood. The hippocampus is particularly vulnerable to damage from events such as chronic/severe stress, epilepsy, stroke, hypoxia/ischemia, or cardiac arrest that occur during development or in adulthood [see (Williamson and Bilbo, 2013) for review]. The developing hippocampus is similarly vulnerable to an early-life infection, as a single neonatal infection can affect cognitive processes such as learning and memory in adulthood. However, there are two potential mechanisms by which a neonatal infection might influence adult neural function and associated learning and memory. Early-life immune activation could permanently damage or disrupt the development of neural pathways important for learning and memory within the hippocampus, or alternatively early-life immune activation could re-program immune function thereby negatively affecting how the adult immune system responds to a subsequent immune challenge via either prolonged or exaggerated pro-inflammatory cytokine production or decreased anti-inflammatory regulation. In this case, abnormal levels of cytokines and chemokines would *indirectly* impair the neural processes important for learning and memory.

To distinguish between these two potential hypotheses, adult rats infected neonatally with *E. coli* were tested in a modified version of contextual fear conditioning known as the context

pre-exposure task, a simple yet robust method for assessing a rat's memory for a recently explored context (Rudy et al., 2004). In this task, when a rat is placed into a specific context and *immediately* shocked, he displays little or no conditioned fear (freezing) to the context the next day. This absence of fear to the context is thought to occur because the rat does not have the opportunity to adequately sample the environment and thus store a representation of its features (a hippocampal-dependent process) prior to an immediate shock. If, however, a rat is pre-exposed to the context for several minutes the day before, an immediate shock the following day will produce substantial freezing on a subsequent test day. The benefit of this technique is that a representation of the context can be learned and thus stored a day prior to immediate shock and the memory test. Thus, an immune challenge given immediately after the context pre-exposure can affect learning without directly affecting the behavioral output during the memory test given days later.

Neonatally infected rats or controls received no injection, saline, or a low dose of LPS (which by itself does not cause memory impairments) immediately following the context pre-exposure. If the adult LPS challenge causes an exaggerated or prolonged immune response in neonatally infected rats that would interfere with learning the context, then only rats that experience the combination of a neonatal infection and LPS after context pre-exposure would display impaired memory. However, if neonatal *E. coli* infection directly alters the development of neural pathways that support memory formation, one would find that neonatally infected rats should display impaired memory regardless of the adult immune challenge. The results of these experiments demonstrate that only rats that experience the **combination** of neonatal infection and subsequent LPS exposure display impaired memory for the explored context (Bilbo et al., 2005a; Bilbo et al., 2005b). In contrast, neonatally infected rats that do not receive an adult immune challenge at the time of context pre-exposure do not exhibit memory deficits, similar to controls. Interestingly, this treatment paradigm has no effect on conditioned fear to a tone that was paired with the shock (Bilbo et al., 2006), indicating the long-term consequences of early-life infection on cognitive function are specific to hippocampal-dependent learning and memory. Taken together, these data support the hypothesis that neonatal immune activation increases the risk of cognitive deficits *indirectly*, via long-term programming of neuroimmune responses which subsequently interferes with learning and memory. In addition, these data suggest that the hippocampus is particularly sensitive to the effects of early-life infection.

Is There a Sensitive Period for the Long-Term Cognitive Effects of Early-Life Infection?

While the model presented above indicates that neonatal infection can program neuroimmune function for the life of the rat thereby affecting adult cognition, these data could alternatively be explained by a general sensitization of the neuroimmune system caused by a single *E. coli* infection, *regardless of age*. If the neonatal period is a particularly sensitive time point for long-term programming of neuroimmune function and adult cognition, an infection given to developing rats at a later time point should yield no significant impairment on learning and memory in adulthood. In fact, treatment of juvenile rats (P30) with a non-lethal, though significantly larger (2.5×10^{-8} colony forming units versus the 1×10^{-6} colony forming units given to neonates) dose of *E. coli* produces a significant increase in cytokine expression within the periphery and brain at the time of the *E. coli* infection (Campisi et al., 2003), but produces no long-term deficits in learning and memory into adulthood (Bilbo et al., 2006). Thus, these data provide critical evidence that neonatal development is a particularly sensitive period for the long-term consequences of early-life infection on later-life neuroimmune function and associated cognitive dysfunction. These data also support the hypothesis that microglia are functionally and/or immunologically different during early brain development than microglia within the adult, or even juvenile/early adolescent brain.

What is the Mechanism by which Early-Life Infection can Impact Later-Life Cognitive Function?

The evidence presented thus far indicate that a neonatal infection can re-program the neuroimmune system such that a second immune challenge in adulthood results in an exaggerated immune response which interferes with the processes of learning and memory. An increasing body of literature indicates that immune molecules have a critical role in the molecular and cellular processes of learning and memory. For example, IL-1 β is induced within the hippocampus in response to normal learning and is critical for maintaining long-term potentiation (LTP), a cellular process of synaptic strengthening thought to underlie learning and memory (Ross et al., 2003; Schneider et al., 1998). Decreased levels of IL-1 β or IL-1 β activity in mice lacking the gene for IL-1 β , or mice over-expressing the endogenous IL-1 receptor antagonist (IL1ra) exhibit markedly impaired HP-dependent learning and memory (Goshen et al., 2007; Spulber et al., 2009). In contrast, exaggerated levels of IL-1 β can similarly impair memory (Barrientos et al., 2002). These data suggest that IL-1 β is important for normal learning and memory, and that either low or exaggerated levels of this particular cytokine can impair learning and memory. In support of the hypothesis that exaggerated IL-1 β synthesis interferes with memory consolidation, treatment of neonatally-infected rats with an inhibitor of Caspase-1, the enzyme which synthesizes the active form of IL-1 β , at the time of LPS administration completely prevents the memory impairment in neonatally-infected rats (Bilbo et al., 2005a), providing causal evidence that exaggerated IL-1 β within the hippocampus of neonatally-infected rats following context pre-exposure and LPS treatment can significantly interfere with learning and memory.

Analysis of brain-derived neurotrophic factor, BDNF, within the hippocampus at the time of learning has revealed significant insight into the potential mechanisms by which exaggerated IL-1 β interferes with contextual memory formation. BDNF is well-characterized for its role in learning and memory, is a molecule necessary for inducing/maintaining the long-term potentiation of hippocampal circuits, and is up-regulated specifically within the hippocampus following contextual learning (Hall et al., 2000). Following contextual fear conditioning, BDNF expression is similarly elevated within the hippocampus of control and neonatally-infected rats that were treated with saline at the time of context pre-exposure, consistent with its role in learning and memory and the data presented above indicating that the mechanisms of learning are not globally disrupted in neonatally-infected rats. In contrast, LPS treatment significantly decreases BDNF production in all hippocampal regions. Neonatally-infected rats treated with LPS immediately after context pre-exposure exhibit the greatest decrease in BDNF within the CA1 of the hippocampus when compared to LPS treated controls, indicating that BDNF may never reach a threshold necessary for consolidating the representation of the context in neonatally-infected rats (Bilbo et al., 2008). In addition, this significant *decrease* in BDNF within the hippocampus of neonatally-infected rats occurs shortly after the exaggerated *increase* in IL-1 β production following LPS administration (Bilbo et al., 2008). Thus, over-production of IL-1 β within the hippocampus caused by long-term programming of neuroimmune function by early-life infection can produce significant cognitive deficits, as well as significant alterations in the cellular mechanisms underlying normal learning and memory.

Interestingly, a slight variation of the previous experimental paradigm provides an entirely new perspective on the role of IL-1 β in learning and memory and the long-term programming of microglial function and cognition. Treatment of neonatally-infected rats with LPS *24 hours prior* to contextual fear conditioning produces a deficit in learning and memory similar to when neonatally-infected rats are injected with LPS immediately *after* the context pre-exposure in the context pre-exposure task (Bilbo et al., 2006). This impairment in learning and memory cannot be accounted for by an exaggerated LPS-induced immune response in neonatally-infected rats because IL-1 β is undetectable in both control and

neonatally-infected rats 24 hours after the LPS treatment (Bilbo et al., 2005a). In contrast, microglia within the hippocampus of neonatally-infected rats continue to show an activated profile, characterized by increased levels of the activation markers cluster of differentiation (CD) 11b and glial fibrillary acidic protein (GFAP), 24 hours after LPS administration (Bilbo et al., 2005a). Given these findings and data indicating that IL-1 β is normally increased during processes of learning and memory (Schneider et al., 1998) and necessary for learning and memory, one might hypothesize that neonatally-infected rats have exaggerated IL-1 β levels within the hippocampus *directly as a consequence of learning* which is subsequently interfering with memory selectively in these rats. In fact, IL-1 β is detected at low but physiological levels within the hippocampus 2 hours after contextual fear conditioning even in control rats (Williamson et al., 2011). In contrast, IL-1 β is undetectable within the adjacent parietal cortex or prefrontal cortex following contextual fear conditioning, confirming that IL-1 β is significantly and selectively elevated within the hippocampus at the time of context learning. In addition, IL-1 β is undetectable within the hippocampus following either exposure to the context (with no shock) or footshock alone, suggesting that IL-1 β is only synthesized within the hippocampus during a **learning experience**. Twenty-six hours after LPS administration and 2 hours after fear conditioning, IL-1 β levels were significantly *exaggerated* within the hippocampus of neonatally-infected rats, while IL-1 β levels were not significantly increased above learning alone in control rats (Williamson et al., 2011). These data indicate that LPS given 24 hours prior to learning and memory causes a significant shift in microglial function in neonatally-infected rats such that learning itself results in exaggerated IL-1 β production which impairs learning and memory.

Consistent with this interpretation, rapid isolation and separation of microglia (CD11b+ cells) and other neuronal cell types (CD11b-) using fluorescence activated cell sorting (FACS) for analysis of IL-1 β expression indicates that microglia are the sole source of IL-1 β production at the time of learning, and similarly the sole source of exaggerated IL-1 β in neonatally-infected rats (Williamson et al., 2011). Inhibiting microglia with minocycline either at the time of LPS treatment or at the time of learning can reverse the memory impairment in neonatally-infected rats (Williamson et al., 2011). These data directly indicate that 1) Microglia have a critical role in learning and memory (via the production of IL-1 β), 2) IL-1 β is increased within the hippocampus during learning and necessary for memory formation; and 3) Neuroimmune dysfunction (exaggerated IL-1 β) directly results in cognitive dysfunction. As a result, early-life programming of microglial function can significantly impact cognitive function, potentially for the entire lifespan, via its effects on neuroimmune function.

The Long-Term Consequences of Early-Life Infection: Aging-related Glial Activation and Associated Cognitive Decline

One of the most novel and important conclusions from the previously described set of experiments is that early-life immune activation can significantly shift the developmental trajectory of microglia, thereby altering the function of these cells to subsequent challenges, potentially *for the life of the individual*. Normal aging is associated with increased glial activation and inflammation within the brain, characterized by increases in the expression of major histocompatibility (MHC) II on microglia and astrocytes and increased expression of pro-inflammatory cytokines, including IL-1 β [see (Godbout and Johnson, 2009; Lynch, 2010) for review]. Given that neonatally-infected rats are more vulnerable to exaggerated pro-inflammatory cytokine production following an LPS challenge in adulthood, one might similarly hypothesize that neonatally-infected rats may also be more vulnerable to aging-related changes in glial function and neuroinflammation and thus more vulnerable to associated cognitive decline. In fact, aged rats (16 months of age) infected neonatally with

E. coli show significant cognitive deficits in an *ambiguous cue* fear-conditioning task when compared to their age-matched controls. In contrast to the contextual fear-conditioning task, this version of fear conditioning determines whether animals can distinguish between a light cue which consistently predicts a shock and a tone cue which only occasionally predicts a shock (and thus is “ambiguous”) (Bilbo, 2010). This task is also a robust measure of learning and memory yet can detect much more subtle deficits in learning and memory than contextual fear conditioning. At two months of age, there were no significant differences between neonatally-infected rats and control rats in their ability to correctly associate the light with the shock; however, at 16 months of age, neonatally-infected rats froze equally to the light and the tone, an effect that was not seen in controls (Bilbo, 2010). These results indicate that only *aged* neonatally-infected rats were inaccurate in acquiring the associative memory between the light and the shock, and instead generalized the fear conditioning to both cues. Notably, neither treatment group received an LPS “second hit” at either 2 or 16 months of age.

In a second task to assess spatial hippocampal-dependent learning and memory, the Morris water maze, both young and old rats (neonatally infected and controls) learned the location of a hidden/submerged platform in a large swimming pool at the same rate over the course of 24 training trials. However, during a probe trial given 24 hours after the final training session, 16 month old neonatally infected rats spent significantly less time searching in the target quadrant of the maze for the hidden platform than did their age-matched controls, suggesting they forgot the location of the hidden platform. A similar effect was seen 96 hours after the final training session (Bilbo, 2010). The results of this cognitive task indicate that while the older neonatally-infected rats were able to learn the task correctly, they were unable to maintain this spatial representation of the task just 24 or 96 hours later (Bilbo, 2010). Thus, neonatal immune activation increases the risk for later-life cognitive dysfunction such that neonatally-infected rats fair far worse after the onset of aging; and aging, which is associated with a general increase in microglial activation and cytokine production, is acting as a “second hit” (similar to the LPS challenge described above) for these vulnerable rats.

The analysis of microglial activation markers (CD11b and MHC II) and astrocyte activation markers (GFAP) within the brains of neonatally-infected and control rats support this interpretation. Aged rats have significantly higher levels of all three proteins within the hippocampus than their younger counterparts; however, neonatally-infected rats have significantly elevated levels of CD11b within all subregions of the hippocampus and significantly elevated levels of GFAP within the dentate gyrus at 16 months of age when compared to their age matched controls (Bilbo, 2010). In conclusion, aging itself is similar to an immune challenge during which time microglia shift into a more reactive phenotype and function (Godbout et al., 2005; Godbout and Johnson, 2009; Griffin et al., 2006; Lynch, 2010; Nolan et al., 2005).

PAMPs, DAMPs and PRRs: Implications for Immune Activation During Brain Development

Much of this discussion has focused on the impact of early-life infection on brain development and its long-term consequences, but I believe the important role of immune molecules in brain development has implications for a wide number of insults or environmental stimuli that might activate the developing immune system either directly or indirectly, and in so doing exert enduring effects on neural function and behavior. The innate immune system represents a complete set of tissues, cells, and molecules critical for the detection, containment, and elimination of a large number of pathogens, insults, and damage to cells. **Pathogen-associated molecular patterns (PAMPs)** are a diverse set of

evolutionarily-conserved molecules present on micro-organisms such as bacteria and viruses, which are recognized by the innate immune system via the activation of the endogenous **pattern recognition receptors** (PRRs), most notably the Toll-like Receptors (TLRs). TLR4 is critical for the recognition of PAMPS such as bacterial molecules (i.e. LPS) and bacteria themselves (i.e. *E. coli*). However, pathogens are not the only agents that are considered “foreign” or dangerous to our body’s physiology. Thus, the immune system must identify and respond to other sources of potential danger, which could be defined broadly as anything that might cause tissue stress or damage. The function of some TLRs, including TLR2 and 4, extends beyond that of PAMP recognition to “**danger**” associated **molecular patterns** (DAMPs) recognition more broadly (Matzinger, 2002a, b). DAMPs include endogenous “alarmins” that are released in response to cellular or tissue stress and damage (Bianchi, 2007), which are recognized by TLRs and induce activation of the immune system. There are many putative factors that have been identified as alarmins, including IL-1 α , hyaluronan, high mobility group box (HMGB) 1, and heat shock proteins [see (Bianchi, 2007) for review]. A few of these alarmin molecules have an established role in brain development, neural function, and behavior [see (Bilbo and Schwarz, 2012) for recent review].

There is also growing evidence that diverse environmental stimuli can trigger TLR signaling, either directly or indirectly via an alarmin pathway, to induce proinflammatory cytokine signaling. These include toxins such as constituents of air pollution (e.g., diesel exhaust (Inoue et al., 2006a; Inoue et al., 2006b)), drugs of abuse including morphine, amphetamines, and alcohol (Hutchinson et al., 2010a; Hutchinson et al., 2010b; Hutchinson et al., 2011), and dietary fatty acids (Schaeffler et al., 2009). If such factors are present (or present in *abnormal* concentrations) during prenatal or postnatal development, they may have the capacity to program long-term neuroimmune function, in a similar manner to infection, via the common activation of TLR mechanisms, and subsequently have long-term effects on the brain and behavior into adulthood. For instance, we have explored the impact of high fat diet-induced maternal obesity on fetal brain development, with the hypothesis that excess saturated fatty acids may alter the trajectory of offspring brain and behavioral development via a specific impact on TLRs and/or innate immunity.

Long-term Effects of a Maternal High-Fat Diet on Brain and Behavior

Maternal obesity is a growing public health concern with serious health risks for both the mother and the baby, including gestational diabetes, stillbirth, and preeclampsia leading to preterm birth (Cedergren, 2004). Perhaps most troubling is that maternal obesity may program the offspring for obesity and lifelong metabolic disorders; the children of obese women exhibit increased body mass index, body fat percentage, and insulin resistance (Freeman, 2010; Grattan, 2008; McGuire et al., 2010; Simmons, 2008), and these same traits are exhibited by the offspring of rodent mothers fed a high-fat diet (Armitage et al., 2004; Chen et al., 2008; Dunn and Bale, 2009). Notably, TLR4 function on microglia within the hypothalamus is altered as a consequence of high fat diet, and is critical in the induction of metabolic changes such as insulin resistance. Specifically, adult rats fed a diet of saturated fatty acids have significant activation of TLR4 on microglia within the hypothalamus, the site of many nuclei critical for the regulation of feeding behavior (e.g. arcuate nucleus, lateral hypothalamus, and the dorsolateral portion of the ventromedial nucleus of the hypothalamus). The activation of TLR4 by fatty acids causes the downstream activation of signaling proteins and kinases that are normally induced following a “typical” immune challenge, such as bacteria or LPS. These include the recruitment of MyD88 (an intracellular TLR4 signaling molecule), phosphorylation of cJun Kinase, and phosphorylation of Extracellular signal-regulated kinase (ERK), along with significantly increased synthesis of many cytokines including TNF α , IL-1 β , IL-6, and IL-10 within the hypothalamus (Milanski

et al., 2009). Most importantly, these neuroimmune changes are necessary for inducing leptin resistance in the rats fed a high-fat diet, and have been strongly linked to other metabolic disorders mediated by TLR4 activation (Fessler et al., 2009).

Knowing this, one might hypothesize that pregnant dams fed a high-fat diet during gestation might cause similar glial activation as an infection within the brains of their offspring, and thus change the developmental trajectory of microglia in these rats to increase their risk of neuroinflammation, obesity, and potentially behavior into adulthood. In support of this hypothesis, TLR4 expression and other markers of microglial activation are up-regulated the day after birth within the hippocampus of offspring whose mothers were fed a high-fat diet (Bilbo and Tsang, 2010). Most strikingly, activation of the immune system by maternal high fat diet also causes long-term programming of the neuroimmune system and behavior into adulthood, similar to an early-life infection. Specifically, microglial activation is increased basally in the hippocampus of adult offspring from mothers fed a high fat diet. In addition, the male offspring of mothers fed a high fat diet have increased levels of anxiety as adults, as well as changes in cognition. Finally, these animals exhibit markedly exaggerated levels of IL-1 β within the hippocampus in response to an LPS challenge, compared to controls born to lean mothers (Bilbo and Tsang, 2010). Though the mechanisms remain to be fully explored, it is clear that factors other than pathogens are capable of activating the innate immune receptors on microglia within the developing brain, with striking consequences for brain and behavior into adulthood. Factors such as diet may also lay the groundwork for greater damage by co-occurring infection or injury if overlaid on top of an already “inflammatory” neural environment during development.

Conclusions

In closing, my research over the past several years has revealed that early-life programming of later-life neuroimmune function, caused by a single neonatal infection, is a vulnerability factor such that later-life immune challenges and microglial activation is exacerbated in these rats, with significant negative consequences for cognition. These effects of early-life infection are relatively subtle. Specifically, neonatally-infected rats are *vulnerable*, not universally impaired. They are capable of learning cognitive tasks, and impairments are only revealed by a second immune challenge or increasing age, indicating that the neural circuitry necessary for learning and memory is intact. At the same time, the effects of early-life infection are profound. Specifically, a minor yet significant shift in microglial function has significant negative consequences for cognition. Finally, the effects of early-life infection are very persistent. The immediate immune response to a neonatal infection occurs over the course of a few days, after which point the infection is completely cleared. Despite this, neonatal immune activation that occurs during this narrow time frame in development has long-term consequences on the developmental trajectory of microglia in the brain, significantly affecting microglial function for months and even years after the initial insult.

I propose that this model of early-life immune activation reveals novel insights into the mechanisms of cognitive dysfunction and the many neuropsychiatric disorders that display a strong immune dysregulation and a strong developmental component to their etiology. Moreover, these data provide a reference for the understanding of a potentially wide range of environmental factors that impact the developing immune system, such as diet, environmental toxins, drugs of abuse, and others (e.g., via TLR activation), which thereby enduringly alter neuroendocrine function. While the field of “neuroendocrinology” in general is rapidly expanding, our current knowledge regarding the role of the immune system in neural development is only in its infancy. A greater understanding of the specific mechanisms underlying the role of the immune system in neural development will allow researchers and medical professionals to expand their current view of immune molecules,

traditionally considered important only during sickness, to a broader role for these fascinating molecules in many basic physiological processes. Undoubtedly, this review and the data presented within merely skim the surface of what could potentially be a greater understanding of the developmental risk factors related to immune activation that may be associated with the later-life etiology of many neuropsychiatric and cognitive disorders.

Acknowledgments

I am honored and humbled to have received the Frank Beach Award in Behavioral Neuroendocrinology. I would like to thank my many wonderful mentors over the past years, including Walter Wilczynski, Lainy Day, Randy Nelson, Firdaus Dhabhar, Steven Maier, Linda Watkins, and Jerry Rudy. I'd also like to thank my first postdoctoral colleague Dr. Jaclyn Schwarz, and many talented post-docs, graduate, and undergraduate students, for their collaboration over my early years as a new primary investigator at Duke. Finally, I would like to acknowledge funding during this time by the NIMH (R01 MH083698), NIDA (R01 DA025978), NIEHS (P30 ES011961), and the Duke Institute for Brain Sciences for a Research Incubator Award.

References

- Armitage JA, Khan IY, Taylor PD, Nathanielsz PW, Poston L. Developmental programming of the metabolic syndrome by maternal nutritional imbalance: how strong is the evidence from experimental models in mammals? *The Journal of physiology*. 2004; 561:355–377. [PubMed: 15459241]
- Ashwell K. The distribution of microglia and cell death in the fetal rat forebrain. *Brain research. Developmental brain research*. 1991; 58:1–12. [PubMed: 2015649]
- Barrientos RM, Higgins EA, Sprunger DB, Watkins LR, Rudy JW, Maier SF. Memory for context is impaired by a post context exposure injection of interleukin-1 beta into dorsal hippocampus. *Behavioural brain research*. 2002; 134:291–298. [PubMed: 12191816]
- Bauman ML, Filipek PA, Kemper TL. Early infantile autism. *Int Rev Neurobiol*. 1997; 41:367–386. [PubMed: 9378598]
- Bessis A, Bechade C, Bernard D, Roumier A. Microglial control of neuronal death and synaptic properties. *Glia*. 2007; 55:233–238. [PubMed: 17106878]
- Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. *Journal of leukocyte biology*. 2007; 81:1–5. [PubMed: 17032697]
- Bilbo SD. Early-life infection is a vulnerability factor for aging-related glial alterations and cognitive decline. *Neurobiology of learning and memory*. 2010; 94:57–64. [PubMed: 20388544]
- Bilbo SD, Barrientos RM, Eads AS, Northcutt A, Watkins LR, Rudy JW, Maier SF. Early-life infection leads to altered BDNF and IL-1beta mRNA expression in rat hippocampus following learning in adulthood. *Brain, behavior, and immunity*. 2008; 22:451–455.
- Bilbo SD, Biedenkapp JC, Der-Avakian A, Watkins LR, Rudy JW, Maier SF. Neonatal infection-induced memory impairment after lipopolysaccharide in adulthood is prevented via caspase-1 inhibition. *The Journal of neuroscience: the official journal of the Society for Neuroscience*. 2005a; 25:8000–8009. [PubMed: 16135757]
- Bilbo SD, Levkoff LH, Mahoney JH, Watkins LR, Rudy JW, Maier SF. Neonatal infection induces memory impairments following an immune challenge in adulthood. *Behavioral neuroscience*. 2005b; 119:293–301. [PubMed: 15727533]
- Bilbo SD, Rudy JW, Watkins LR, Maier SF. A behavioural characterization of neonatal infection-facilitated memory impairment in adult rats. *Behavioural brain research*. 2006; 169:39–47. [PubMed: 16413067]
- Bilbo SD, Schwarz JM. The immune system and developmental programming of brain and behavior. *Frontiers in neuroendocrinology*. 2012; 33:267–286. [PubMed: 22982535]
- Bilbo SD, Tsang V. Enduring consequences of maternal obesity for brain inflammation and behavior of offspring. *The FASEB journal: official publication of the Federation of American Societies for Experimental Biology*. 2010; 24:2104–2115.
- Boulanger LM. Immune Proteins in Brain Development and Synaptic Plasticity. *Neuron*. 2009; 64:93–109. [PubMed: 19840552]

- Brown AS. Prenatal infection as a risk factor for schizophrenia. *Schizophrenia bulletin*. 2006; 32:200–202. [PubMed: 16469941]
- Brown AS, Begg MD, Gravenstein S, Schaefer CA, Wyatt RJ, Bresnahan M, Babulas VP, Susser ES. Serologic evidence of prenatal influenza in the etiology of schizophrenia. *Archives of General Psychiatry*. 2004; 61:774–780. [PubMed: 15289276]
- Cai Z, Pan ZL, Pang Y, Evans OB, Rhodes PG. Cytokine induction in fetal rat brains and brain injury in neonatal rats after maternal lipopolysaccharide administration. *Pediatric research*. 2000; 47:64–72. [PubMed: 10625084]
- Campisi J, Hansen MK, O'Connor KA, Biedenkapp JC, Watkins LR, Maier SF, Fleshner M. Circulating cytokines and endotoxin are not necessary for the activation of the sickness or corticosterone response produced by peripheral *E. coli* challenge. *Journal of applied physiology* (Bethesda, Md. 2003; 1985; 95:1873–1882.
- Cedergren MI. Maternal morbid obesity and the risk of adverse pregnancy outcome. *Obstetrics and gynecology*. 2004; 103:219–224. [PubMed: 14754687]
- Champagne FA. Epigenetic mechanisms and the transgenerational effects of maternal care. *Frontiers in neuroendocrinology*. 2008; 29:386–397. [PubMed: 18462782]
- Chan WY, Kohsaka S, Rezaie P. The origin and cell lineage of microglia: new concepts. *Brain Research Reviews*. 2007; 53:344–354. [PubMed: 17188751]
- Chen H, Simar D, Lambert K, Mercier J, Morris MJ. Maternal and postnatal overnutrition differentially impact appetite regulators and fuel metabolism. *Endocrinology*. 2008; 149:5348–5356. [PubMed: 18635655]
- Cowell RM, Silverstein FS. Developmental changes in the expression of chemokine receptor CCR1 in the rat cerebellum. *The Journal of comparative neurology*. 2003; 457:7–23. [PubMed: 12541321]
- Cowell RM, Xu H, Parent JM, Silverstein FS. Microglial expression of chemokine receptor CCR5 during rat forebrain development and after perinatal hypoxia-ischemia. *Journal of neuroimmunology*. 2006; 173:155–165. [PubMed: 16516309]
- Cuadros MA, Navascues J. The origin and differentiation of microglial cells during development. *Progress in neurobiology*. 1998; 56:173–189. [PubMed: 9760700]
- Cunningham ET Jr, De Souza EB. Interleukin 1 receptors in the brain and endocrine tissues. *Immunology today*. 1993; 14:171–176. [PubMed: 8499077]
- Dantzer R, Kelley KW. Twenty years of research on cytokine-induced sickness behavior. *Brain, behavior, and immunity*. 2007; 21:153–160.
- de Boo HA, Harding JE. The developmental origins of adult disease (Barker) hypothesis. *The Australian & New Zealand Journal of Obstetrics & Gynaecology*. 2006; 46:4–14.
- Deverman BE, Patterson PH. Cytokines and CNS development. *Neuron*. 2009; 64:61–78. [PubMed: 19840550]
- Dunn GA, Bale TL. Maternal high-fat diet promotes body length increases and insulin insensitivity in second-generation mice. *Endocrinology*. 2009; 150:4999–5009. [PubMed: 19819967]
- Fessler MB, Rudel LL, Brown JM. Toll-like receptor signaling links dietary fatty acids to the metabolic syndrome. *Current opinion in lipidology*. 2009; 20:379–385. [PubMed: 19625959]
- Freeman DJ. Effects of maternal obesity on fetal growth and body composition: implications for programming and future health. *Seminars in fetal & neonatal medicine*. 2010; 15:113–118. [PubMed: 19853544]
- Garay PA, McAllister AK. Novel roles for immune molecules in neural development: implications for neurodevelopmental disorders. *Frontiers in synaptic neuroscience*. 2010; 2:136. [PubMed: 21423522]
- Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF, Conway SJ, Ng LG, Stanley ER, Samokhvalov IM, Merad M. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science (New York, N.Y.)*. 2010; 330:841–845.
- Giulian D, Young DG, Woodward J, Brown DC, Lachman LB. Interleukin-1 is an astroglial growth factor in the developing brain. *The Journal of neuroscience: the official journal of the Society for Neuroscience*. 1988; 8:709–714. [PubMed: 3257519]
- Godbout JP, Chen J, Abraham J, Richwine AF, Berg BM, Kelley KW, Johnson RW. Exaggerated neuroinflammation and sickness behavior in aged mice following activation of the peripheral

- innate immune system. The FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 2005; 19:1329–1331.
- Godbout JP, Johnson RW. Age and neuroinflammation: a lifetime of psychoneuroimmune consequences. *Immunol Allergy Clin North Am*. 2009; 29:321–337. [PubMed: 19389585]
- Goshen I, Kreisel T, Ounallah-Saad H, Renbaum P, Zalstein Y, Ben-Hur T, Levy-Lahad E, Yirmiya R. A dual role for interleukin-1 in hippocampal-dependent memory processes. *Psychoneuroendocrinology*. 2007; 32:1106–1115. [PubMed: 17976923]
- Grattan DR. Fetal programming from maternal obesity: eating too much for two? *Endocrinology*. 2008; 149:5345–5347. [PubMed: 18936494]
- Griffin R, Nally R, Nolan Y, McCartney Y, Linden J, Lynch MA. The age-related attenuation in long-term potentiation is associated with microglial activation. *Journal of neurochemistry*. 2006; 99:1263–1272. [PubMed: 16981890]
- Hahn YK, Vo P, Fitting S, Block ML, Hauser KF, Knapp PE. beta-Chemokine production by neural and glial progenitor cells is enhanced by HIV-1 Tat: effects on microglial migration. *Journal of neurochemistry*. 2010; 114:97–109. [PubMed: 20403075]
- Hall J, Thomas KL, Everitt BJ. Rapid and selective induction of BDNF expression in the hippocampus during contextual learning. *Nature neuroscience*. 2000; 3:533–535.
- Hornig M, Weissenbock H, Horscroft N, Lipkin WI. An infection-based model of neurodevelopmental damage. *Proceedings of the National Academy of Sciences of the United States of America*. 1999; 96:12102–12107. [PubMed: 10518583]
- Hutchinson MR, Lewis SS, Coats BD, Rezvani N, Zhang Y, Wieseler JL, Somogyi AA, Yin H, Maier SF, Rice KC, Watkins LR. Possible involvement of toll-like receptor 4/myeloid differentiation factor-2 activity of opioid inactive isomers causes spinal proinflammation and related behavioral consequences. *Neuroscience*. 2010a; 167:880–893. [PubMed: 20178837]
- Hutchinson MR, Loram LC, Zhang Y, Shridhar M, Rezvani N, Berkelhammer D, Phipps S, Foster PS, Landgraf K, Falke JJ, Rice KC, Maier SF, Yin H, Watkins LR. Evidence that tricyclic small molecules may possess toll-like receptor and myeloid differentiation protein 2 activity. *Neuroscience*. 2010b; 168:551–563. [PubMed: 20381591]
- Hutchinson MR, Shavit Y, Grace PM, Rice KC, Maier SF, Watkins LR. Exploring the neuroimmunopharmacology of opioids: an integrative review of mechanisms of central immune signaling and their implications for opioid analgesia. *Pharmacological reviews*. 2011; 63:772–810. [PubMed: 21752874]
- Inoue K, Takano H, Sakurai M, Oda T, Tamura H, Yanagisawa R, Shimada A, Yoshikawa T. Pulmonary exposure to diesel exhaust particles enhances coagulatory disturbance with endothelial damage and systemic inflammation related to lung inflammation. *Experimental biology and medicine* (Maywood, N.J.). 2006a; 231:1626–1632.
- Inoue K, Takano H, Yanagisawa R, Hirano S, Ichinose T, Shimada A, Yoshikawa T. The role of toll-like receptor 4 in airway inflammation induced by diesel exhaust particles. *Archives of Toxicology*. 2006b; 80:275–279. [PubMed: 16254717]
- Lynch MA. Age-related neuroinflammatory changes negatively impact on neuronal function. *Frontiers in aging neuroscience*. 2010; 1:6. [PubMed: 20552057]
- Marin-Teva JL, Dusart I, Colin C, Gervais A, van Rooijen N, Mallat M. Microglia promote the death of developing Purkinje cells. *Neuron*. 2004; 41:535–547. [PubMed: 14980203]
- Marshall-Clarke S, Reen D, Tasker L, Hassan J. Neonatal immunity: how well has it grown up? *Immunology today*. 2000; 21:35–41. [PubMed: 10637557]
- Matzinger P. The danger model: a renewed sense of self. *Science*. 2002a; 296:301–305. [PubMed: 11951032]
- Matzinger P. An innate sense of danger. *Annals of the New York Academy of Sciences*. 2002b; 961:341–342. [PubMed: 12081934]
- McGuire W, Dyson L, Renfrew M. Maternal obesity: consequences for children, challenges for clinicians and carers. *Seminars in fetal & neonatal medicine*. 2010; 15:108–112. [PubMed: 19828390]

- McKimmie CS, Graham GJ. Astrocytes modulate the chemokine network in a pathogen-specific manner. *Biochemical and biophysical research communications*. 2010; 394:1006–1011. [PubMed: 20331977]
- Merrill JE. Tumor necrosis factor alpha, interleukin 1 and related cytokines in brain development: normal and pathological. *Developmental neuroscience*. 1992; 14:1–10. [PubMed: 1350976]
- Meyer U, Feldon J, Schedlowski M, Yee BK. Immunological stress at the maternal-foetal interface: a link between neurodevelopment and adult psychopathology. *Brain Behav Immun*. 2006; 20:378–388. [PubMed: 16378711]
- Milanski M, Degasperi G, Coope A, Morari J, Denis R, Cintra DE, Tsukumo DM, Anhe G, Amaral ME, Takahashi HK, Curi R, Oliveira HC, Carnevali JB, Bordin S, Saad MJ, Velloso LA. Saturated fatty acids produce an inflammatory response predominantly through the activation of TLR4 signaling in hypothalamus: implications for the pathogenesis of obesity. *The Journal of neuroscience: the official journal of the Society for Neuroscience*. 2009; 29:359–370. [PubMed: 19144836]
- Mortensen PB, Pedersen CB, Westergaard T, Wohlfahrt J, Ewald H, Mors O, Andersen PK, Melbye M. Effects of family history and place and season of birth on the risk of schizophrenia. *The New England journal of medicine*. 1999; 340:603–608. [PubMed: 10029644]
- Nelson KB, Willoughby RE. Infection, inflammation and the risk of cerebral palsy. *Current opinion in neurology*. 2000; 13:133–139. [PubMed: 10987569]
- Nolan Y, Maher FO, Martin DS, Clarke RM, Brady MT, Bolton AE, Mills KH, Lynch MA. Role of interleukin-4 in regulation of age-related inflammatory changes in the hippocampus. *The Journal of biological chemistry*. 2005; 280:9354–9362. [PubMed: 15615726]
- Osrin D, Vergnano S, Costello A. Serious bacterial infections in newborn infants in developing countries. *Current opinion in infectious diseases*. 2004; 17:217–224. [PubMed: 15166824]
- Owen D, Andrews MH, Matthews SG. Maternal adversity, glucocorticoids and programming of neuroendocrine function and behaviour. *Prenatal Programming of Behavior, Physiology and Cognition*. 2005; 29:209–226.
- Pang Y, Cai Z, Rhodes PG. Disturbance of oligodendrocyte development, hypomyelination and white matter injury in the neonatal rat brain after intracerebral injection of lipopolysaccharide. *Developmental Brain Research*. 2003; 140:205–214. [PubMed: 12586426]
- Patterson PH. Immune involvement in schizophrenia and autism: etiology, pathology and animal models. *Behavioural brain research*. 2009; 204:313–321. [PubMed: 19136031]
- Perry VH, Hume DA, Gordon S. Immunohistochemical localization of macrophages and microglia in the adult and developing mouse brain. *Neuroscience*. 1985; 15:313–326. [PubMed: 3895031]
- Rezaie P, Male D. Colonisation of the developing human brain and spinal cord by microglia: a review. *Microscopy research and technique*. 1999; 45:359–382. [PubMed: 10402264]
- Rezaie P, Trillo-Pazos G, Everall IP, Male DK. Expression of beta-chemokines and chemokine receptors in human fetal astrocyte and microglial co-cultures: potential role of chemokines in the developing CNS. *Glia*. 2002; 37:64–75. [PubMed: 11746784]
- Ross FM, Allan SM, Rothwell NJ, Verkhratsky A. A dual role for interleukin-1 in LTP in mouse hippocampal slices. *Journal of neuroimmunology*. 2003; 144:61–67. [PubMed: 14597099]
- Rudy JW, Huff NC, Matus-Amat P. Understanding contextual fear conditioning: insights from a two-process model. *Neurobiology of Cognition in Laboratory Animals: Challenges and Opportunities*. 2004; 28:675–685.
- Schaeffler A, Gross P, Buettner R, Bollheimer C, Buechler C, Neumeier M, Kopp A, Schoelmerich J, Falk W. Fatty acid-induced induction of Toll-like receptor-4/nuclear factor-kappaB pathway in adipocytes links nutritional signalling with innate immunity. *Immunology*. 2009; 126:233–245. [PubMed: 18624726]
- Schafer DP, Lehrman EK, Kautzman AG, Koyama R, Mardinly AR, Yamasaki R, Ransohoff RM, Greenberg ME, Barres BA, Stevens B. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron*. 2012; 74:691–705. [PubMed: 22632727]
- Schneider H, Pitossi F, Balschun D, Wagner A, del Rey A, Besedovsky HO. A neuromodulatory role of interleukin-1beta in the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America*. 1998; 95:7778–7783. [PubMed: 9636227]

- Schwarz JM, Bilbo SD. LPS elicits a much larger and broader inflammatory response than *Escherichia coli* infection within the hippocampus of neonatal rats. *Neuroscience letters*. 2011; 497:110–115. [PubMed: 21536105]
- Schwarz JM, Sholar PW, Bilbo SD. Sex differences in microglial colonization of the developing rat brain. *Journal of neurochemistry*. 2012; 120:948–963. [PubMed: 22182318]
- Shi L, Smith SE, Malkova N, Tse D, Su Y, Patterson PH. Activation of the maternal immune system alters cerebellar development in the offspring. *Brain, behavior, and immunity*. 2009; 23:116–123.
- Simmons R. Perinatal programming of obesity. *Seminars in perinatology*. 2008; 32:371–374. [PubMed: 18929161]
- Skogstrand K, Hougaard DM, Schendel DE, Bent NP, Svaerke C, Thorsen P. Association of preterm birth with sustained postnatal inflammatory response. *Obstetrics and gynecology*. 2008; 111:1118–1128. [PubMed: 18448744]
- Spulber S, Bartfai T, Schultzberg M. IL-1/IL-1ra balance in the brain revisited - evidence from transgenic mouse models. *Brain, behavior, and immunity*. 2009; 23:573–579.
- Stevens B, Allen NJ, Vazquez LE, Howell GR, Christopherson KS, Nouri N, Micheva KD, Mehalow AK, Huberman AD, Stafford B, Sher A, Litke AM, Lambris JD, Smith SJ, John SW, Barres BA. The classical complement cascade mediates CNS synapse elimination. *Cell*. 2007; 131:1164–1178. [PubMed: 18083105]
- Urakubo A, Jarskog LF, Lieberman JA, Gilmore JH. Prenatal exposure to maternal infection alters cytokine expression in the placenta, amniotic fluid, and fetal brain. *Schizophrenia research*. 2001; 47:27–36. [PubMed: 11163542]
- Wang CC, Wu CH, Shieh JY, Wen CY. Microglial distribution and apoptosis in fetal rat brain. *Brain Res Dev Brain Res*. 2002; 139:337–342.
- Williamson LL, Bilbo SD. Chemokines and the hippocampus: A new perspective on hippocampal plasticity and vulnerability. *Brain, behavior, and immunity*. 2013 in press.
- Williamson LL, Sholar PW, Mistry RS, Smith SH, Bilbo SD. Microglia and memory: modulation by early-life infection. *The Journal of neuroscience: the official journal of the Society for Neuroscience*. 2011; 31:15511–15521. [PubMed: 22031897]
- Xu J, Kaur C, Ling EA. Variation with age in the labelling of amoeboid microglial cells in rats following intraperitoneal or intravenous injection of a fluorescent dye. *Journal of anatomy*. 1993; 182(Pt 1):55–63. [PubMed: 8509300]

Highlights

- Microglia and many immune molecules are critical for normal brain development.
- Immune activation during brain development can impact behavior throughout life.
- The developmental immune history of an individual may predict risk of neuropsychiatric disorders.
- A model of neonatal infection may inform diverse environmental challenges which impact the developing immune system.