INFLAMMATION AND ACCUMULATING RISK FOR DEVELOPING PSYCHIATRIC DISORDERS

By

Kelli Marie Money

Dissertation

Submitted to the Faculty of the

Graduate School of Vanderbilt University

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

in

Neuroscience

December, 2016

Nashville, Tennessee

Approved:

Doug McMahon, Ph.D., chair

Bruce Carter, Ph.D.

Kevin Ess, M.D., Ph.D.

Brad Grueter, Ph.D.

Gregg Stanwood, Ph.D.

Karoly Mirnics, M.D., Ph.D.

ACKNOWLEDGEMENTS

I have been fortunate to have not one but two mentors instruct me. I am thankful to Gregg Stanwood for helping me form a solid foundation of scientific knowledge and technical skills. I am also fortunate that Karoly Mirnics was willing to take me into his fold with such short notice. I have learned so very much under Karoly's tutelage, and I will continue to enjoy working with him on the projects that we still have to complete.

I am indebted to my wonderfully supportive parents, sister, and fiancé. Without their patient listening and constant encouragement, the completion of this dissertation would have been much more difficult for all involved. To my two cats and two dogs, you are adorable, but you helped none. Literally, none whatsoever. Your constant attempts to distract me actually slowed me down some. All in all, I am blessed to have made it this far in my life and look forward to the excitement the future holds.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SUPPLEMENTARY TABLES

LIST OF SUPPLEMENTARY FIGURES

LIST OF ABBREVIATIONS

ACC .... anterior cingulate cortex

ALR...average log ratio

BAC ....bacterial artificial chromosome

BDNF..brain derived neurotrophic factor

CNTF ....ciliary neurotrophic factor

CSF .... colony stimulating factor

Ct ....detection cycle threshold

CTR.. control

CXCL... C-X-C chemokine ligand

CXCR... C-X-C chemokine receptor

D1R..dopamine D1 receptor

D1tom6 .... *Drd1a*-tdTomato transgenic mouse line 6

D2R...dopamine D2 receptor

eGFP .... enhanced green fluorescent protein

ERK..extracellular signal-regulated kinase

GABA .... γ-aminobutyric acid

GAD-67.. glutamic acid decarboxylase, 67 kD

GD... gestational day

GENSAT ....gene expression nervous system atlas

GPCR ....G protein coupled receptor

HDF .... human dermal fibroblast

HFM .... high fat fed poly(I:C) treated

HFS ....high fat fed saline treated

HLA .... human leukocyte antigen

HPA...hypothalamic-pituitary-adrenal

IFITM... interferon induced transmembrane protein

IL .... interleukin

IFN ....interferon

JAK .... Janus kinase

JNK .... c-Jun N-terminal kinase

LIF ....leukemia inhibitor factor

LPS .... lipopolysaccharide

MCP...monocyte chemoattractant protein

MDD .... major depressive disorder

MET .... hepatocyte growth factor receptor

MFC...medial frontal cortex

MHC .... major histocompatibility complex

MIA..maternal immune activation

MyD88..myeloid differentiation primary response 88

NFκB... nuclear factor kappa B

NGF...nerve growth factor

PND.. postnatal day

poly(I:C)...polyinosinic-polycytidylic acid

qPCR..quantitative polymerase chain reaction

RANTES .... regulated on activation, normal T cell expressed and secreted factor

CHAPTER I

INTRODUCTION

Psychiatric disorders affect a large portion of the world's population (Kessler et al., 2009). Those affected are left with a reduced quality of life, and society as a whole suffers due to the enormous cost of psychiatric disorders both from the illness itself and lost productivity of those individuals to society (Murray et al., 2012; Vos et al., 2012). To make the much needed progress in prevention and treatment, we must identify the biological and environmental factors underlying disorder pathogenesis and progression. Since psychiatric disorders are highly heritable, genetic factors are clearly involved. In fact, schizophrenia has an estimated heritability between 30 – 70% (Light et al., 2014; Sullivan et al., 2003; Wray and Gottesman, 2012). Interestingly, 60% of those with schizophrenia do not have a schizophrenic first- or second-degree relative (Gottesman and Erlenmeyer-Kimling, 2001), and monozygotic twin studies only approach 50% concordance- not the 100% concordance that would be expected if genetic factors were the sole etiology (McGue and Gottesman, 1991). Tsuang and colleagues have suggested that non-shared environment may account for most of the discordance in these monozygotic twin studies (Tsuang et al., 2001). In autism spectrum disorder, heritability estimates are around 50% (Sandin et al., 2014), and twin concordance is estimated to be between 40-98% (Kim and Leventhal, 2015; Tick et al., 2016). With regards to major depressive disorder (MDD), heritability from family pedigrees is estimated to be between 30 – 45% with ~40% concordance reported in monozygotic twin studies (Edvardsen et al., 2009; Fernandez-Pujals et al., 2015;

Kendler and Prescott, 1999; McGuffin et al., 1996; Wray and Gottesman, 2012). Therefore, genetic risk alone is insufficient to explain the development of psychiatric disorders.

Environment-associated risk factors both modify genetic risk and add to the accumulating risk by altering crucial events in brain development and function (Jaffee and Price, 2007; Klengel and Binder, 2015). Periods of important neurodevelopmental events (*in utero* and early childhood) are particularly sensitive to environmental perturbation (Johnson et al., 2015; Meredith, 2015; Roth and Sweatt, 2011). Both schizophrenia and autism have been associated with numerous *in utero* disruptions, including nutritional deprivation (Cannell, 2008; Grant and Soles, 2009; McGrath et al., 2003; McGrath and Tschan, 2004; McGrath et al., 2010; St Clair et al., 2005; Susser and Lin, 1992), prenatal infection (Atladóttir et al., 2010; Buka et al., 2001a), gestational diabetes (Cannon et al., 2002b; Gardener et al., 2009; Xiang et al., 2015), and perinatal complications (Langridge et al., 2013; Nicodemus et al., 2008). Early childhood environmental risk factors that increase risk for MDD include maltreatment, bullying, urban upbringing, psychosocial stress, and low socioeconomic status (Colman et al., 2012; Cummings et al., 2014; Gamez-Guadix et al., 2013), whereas cannabis use in adolescence has been associated with increased incidence of schizophrenia (French et al., 2015; Giordano et al., 2015).

Autism, schizophrenia, and MDD have distinct clinical presentations, which led to their separation into distinct disorders. However, the etiologies of these symptomatically distinct disorders share many commonalities. The NIMH recognizes this overlap, creating the research domain criteria matrix. The framework consists of 5 major neuropsychiatric domains (negative valence systems, positive valence systems, cognitive systems, systems for social processing, and

arousal/regulatory systems). Within each domain of the matrix, columns define the type of data (e.g. genes, circuits, behavior, self-report), and the rows define the construct (e.g. fear, reward valuation, loss). This allows data collected from multiple psychiatric disorders to be placed in one inclusive matrix (Insel et al., 2010; Kozak and Cuthbert, 2016). In addition, the Psychiatric Genomics Consortium has compiled genome-wide association studies across many countries and garnered significant support for the existence of at least some shared genetic risk factors amongst psychiatric disorders. This group has proposed that genetic risk variants aggregate in particular biologically-defined pathways, such as immune-related genes, that can be shared between psychiatric disorders albeit some disorders overlap considerably more than others, which is discussed in further detail below (Consortium, 2015; Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013; Lee et al., 2013). As noted above, shared psychiatric disorder etiology also includes environmental risk factors. The research in this document will focus particularly on inflammation-associated risk factors, the ability of inflammation to pathologically alter both the developing and adult brain, and how these alterations contribute to the pathogenesis and pathophysiology of psychiatric disorders.

Inflammatory mediators and the adult brain

Inflammation when appropriate is protective, but aberrant inflammation is not. Inflammation during critical developmental time windows can produce long-lasting changes in both immune and brain function (Coe and Lubach, 2003; Meyer et al., 2007). Our lab as well as many others have proposed that low grade chronic inflammation adds to risk for multiple psychiatric disorders, interacting with other environmental and genetic risk factors to increase

3

risk for specific disorders (Maes et al., 2009; Michel et al., 2012; Patterson, 2009). The Psychiatric Genomics Consortium has found immune-related genes like those associated with TGFβ signaling and B lymphocyte activation to be shared risk loci for schizophrenia, MDD, and bipolar disorder (Consortium, 2015). Furthermore, the Zhao lab found pleiotropy (one gene associated with multiple phenotypes) with some immune related genes in relationship to both psychiatric disorders and immune disorders, which could explain the increased co-occurrence of immune and psychiatric disorders such as rheumatoid arthritis and MDD, Crohn's disease and schizophrenia, and autoimmune thyroiditis and post-traumatic stress disorder (Covic et al., 2012; O'Donovan et al., 2015; Wang et al., 2015). Moreover, these genetic risk factors are aptly positioned to interact with inflammation-inducing environmental risk factors. Many environmental risk factors have the potential to alter the inflammatory state during critical developmental windows, including prenatal infection, gestational diabetes, maternal obesity, and nutritional deficiencies (Berk et al., 2013; Fagundes et al., 2013). Therefore, this document puts forth evidence that environmental risk factors that increase inflammation perturb gene expression patterns in the brain, which can both increase risk for psychiatric disorders and promote psychiatric disorder progression.

For many years, the brain was considered immune-privileged due to the impenetrable nature of the blood brain barrier to immune cells and the factors they release. We now know that the immune system is highly integrated with the nervous system, both peripherally and centrally. This is possible via multiple alternate modes of communication between the activated peripheral immune system and the brain (Dantzer, 2006; Rivest, 2003). In order to discuss the impact of inflammation on the brain both developmentally and during adulthood, the basic mechanisms of

inflammation and how they interact with an immune privileged site such as the brain must be understood.

The role of the nervous system in peripheral inflammation

The immune system defends against infection, toxins, and trauma and can be subdivided into two systems: innate and adaptive. Innate immunity consists of physical barriers (skin, mucosa, and cilia), chemical barriers (gastric acid, digestive enzymes, and lysozymes), and phagocytes (macrophages) (Janeway and Medzhitov, 2002). As part of the innate immune response, foreign antigens, such as dsRNA in viruses or lipopolysaccharide (LPS) in gram-negative bacterial membranes, are initially recognized by either the complement system or toll-like receptors (TLRs) (Janeway and Medzhitov, 2002; Schoneveld and Cidlowski, 2007). The complement systems recognizes and targets for degradation pathogen-associated molecular patterns (Sim and Tsiftsoglou, 2004). Recognition of pathogen-associated molecular patterns by TLRs leads to activation of nuclear factor kappa B (NFκB) and activation of the host's innate immune system through the induction of pro-inflammatory genes and the release of pro-inflammatory cytokines (De Nardo, 2015). In contrast, adaptive immunity is slower to develop and consists of the B- and T-lymphocyte mediated response that is specific to the foreign antigen, depending on gene rearrangements to generate new receptors for antigens.

The adaptive response depends on the presentation of antigen complexed to major histocompatibility complex (MHC) to CD4+ helper T lymphocytes, which is followed by differentiation and clonal expansion of B- and T-lymphocytes specific for that antigen as well as

5

the formation of memory cells to ensure a more effective and efficient response upon re-exposure (Coquet et al., 2015). This adaptive immune response is targeted to the type of pathogen encountered. Although there are additional T helper cell subsets, T helper cells generally differentiate into either T helper type 1 (Th1) cells or T helper type 2 (Th2) cells, depending on the cytokines present (Hirahara and Nakayama, 2016). Th1 differentiation is induced by IL12. Th1 cells produce IL2, IFNγ, and TNF and direct a cell-mediated antiviral response by activation of cytotoxic T cells, macrophages, and natural killer cells (Schoneveld and Cidlowski, 2007; Trinchieri, 1995). On the other hand, IL4 promotes Th2 differentiation, which leads to a humoral response against extracellular pathogens. These cells produce cytokines like IL6 and IL4, which activate mast cells and eosinophils, and promote B lymphocyte differentiation into antibody-secreting cells (Mosmann and Sad, 1996; Paul and Seder, 1994). Some groups greatly stress the importance of balanced Th1 and Th2 cell populations, with implications for a Th2 shift in multiple psychiatric disorders (Chiang et al., 2013; Cohly and Panja, 2005; Elenkov, 2004). Both normal and dysfunctional states are associated with alterations in the Th1/Th2 balance, such as autoimmune disease, asthma, pregnancy, and psychosocial stress (Kidd, 2003; Makhseed et al., 2001; Zhang et al., 2014). Yet others suggest that focusing too much on only Th1 and Th2 cell subsets is too simplistic, considering the emerging research on additional important T helper cell subsets (Hirahara and Nakayama, 2016; Zhang et al., 2014). Nevertheless, T helper cell subsets and the effector cells of the adaptive immune response are required for the host response against both intracellular and extracellular pathogens.

Cytokines are pleiotropic, often redundant, inflammatory mediators. Almost all cells are capable of producing cytokines although leukocytes are the main source (Dantzer, 2006; Janeway and Medzhitov, 2002; Pitossi et al., 1997). Cytokines can be categorized by their structure and predominant effect (anti-inflammatory, pro-inflammatory, or growth promoting). Interleukins (e.g. IL6, IL10) communicate between leukocytes; tumor necrosis factors (e.g. TNFα, TNFβ) initiate inflammation, cachexia, and necrosis; colony-stimulating factors (e.g. G-CSF, GMCSF) promote proliferation of target cell populations; and interferons (e.g. IFNα, IFNγ) interfere with viral replication (Aggarwal et al., 2012; Dantzer, 2007; Paul and Seder, 1994; Tanaka et al., 2014). However, this classification system is only based on the "major" cytokine function. Cytokines are capable of additional, quite diverse functions throughout the body (Dantzer, 2007; Moraga et al., 2014). In the brain, cytokines can regulate synapse formation/stabilization/pruning, cell division, and migration- all processes essential for proper development of the brain (Barnabe-Heider et al., 2005; Bauer et al., 2007; Deverman and Patterson, 2009; Fontaine et al., 2008; Galli et al., 2000; Lee et al., 2010a; Michaelson et al., 1996; Shimazaki et al., 2001; Stellwagen and Malenka, 2006). Of relevance to the research proposed in this document, the three main pro-inflammatory cytokines that orchestrate the innate immune response and are implicated in many psychiatric disorders are IL6, IL1β, and TNFα (Michel et al., 2012; Patterson, 2011). The role of these cytokines in the central response to immune stimuli is emphasized in the following discussion, which is not intended to undermine the importance of other cytokines in the holistic immune response but to maintain relevance of this discussion.

IL6 has had many names. In the 1980s, several assumedly distinct soluble proteins were discovered, with the ability to induce B lymphocyte differentiation (B cell stimulatory factor 2), promote acute phase protein synthesis in hepatocytes (hepatocyte-stimulating factor), enhance growth of plasma and myeloma fusion cells (hybridoma growth factor), and increase antiviral activity (IFN-β2) (Hirano et al., 1986; Kishimoto, 1985; Kishimoto, 1989). However, successful cDNA cloning showed them all to actually be IL6 (Kishimoto, 1989). IL6 also regulates serum iron and zinc and promotes platelet release from megakaryocytes, T helper cell type 17 (Th17) differentiation, and cytotoxic T cell differentiation (Ishibashi et al., 1989; Korn et al., 2009; Liuzzi et al., 2005; Nemeth et al., 2004; Okada et al., 1988). In target tissues, IL6 can initiate bone resorption, angiogenesis via VEGF production, and fibroblast proliferation (Grossman et al., 1989; Hashizume et al., 2009; Poli et al., 1994). IL6 is among the first cytokines produced in the immune response, and TNFα and IL1β can also induce IL6 synthesis (Tanaka et al., 2014). IL6 binds the IL6 receptor complexed with gp130, a signal transduction protein shared by all members of the IL6 family of cytokines including LIF and cardiotrophin-1 (Kishimoto et al., 1995). Intracellular signaling propagates through the JAK-STAT pathway, which promotes nuclear translocation of the pro-inflammatory transcription factor NFκB. IL6 signaling is dampened by sequestration via the soluble form of the IL6 receptor as well as induction of the negative feedback protein suppressor of cytokine signaling 3 (SOCS3) (Narazaki et al., 1993; Schmitz et al., 2000). IL6 plays a role both in the immune response and in normal brain development and function (Braida et al., 2004; Burns et al., 1993). It also is one of the most consistently implicated cytokines in psychiatric disorders (Sukoff Rizzo et al., 2012; Wei et al., 2011). The role of IL6 as well as IL1β and TNFα in brain development and function as well as psychiatric disorder pathology will be discussed in great detail below.

IL1 is a master regulator of inflammation. In the immune system, IL1 acts as a co-stimulatory molecule of T lymphocytes, induces the humoral Th2 immune response, and promotes Th17 cell differentiation (Dinarello, 2009; Johnson et al., 2005; Sutton et al., 2006). IL1 can also increase adhesion molecule expression on mesenchymal and endothelial cells, promote angiogenesis and tumor metastasis, stimulate myeloid differentiation of bone marrow progenitor cells, and potently regulate circulating levels of IL6 (Dinarello, 2007; Dinarello, 2009; Voronov et al., 2003). The IL1 family of cytokines includes the IL1α and IL1β isoforms, IL1 receptors 1 and 2, IL1 receptor accessory protein, and IL1 receptor antagonist. IL1α is expressed constitutively in most cell types whereas IL1β is mostly produced by inflammatory stimuli in immune cells (Berda-Haddad et al., 2011). IL1 biological activity is controlled by transcription and post-translational processing. IL1β transcription must be induced by an inflammatory signal such as pathogens, itself, or TNFα (Dinarello, 1987). IL1 is produced in an inert pro-peptide form that must be cleaved by enzymes such as caspase-1 or neutrophil serine proteases (Guma et al., 2009; Joosten et al., 2009). Upon binding of biologically active IL1, the IL1 receptor heterodimerizes, interacts with IL1 receptor accessory protein, and promotes pro-inflammatory signaling through a myeloid differentiation factor 88 (MyD88)-dependent pathway to activate NFκB and multiple stress-activated kinases like JNK and ERK (Palomo et al., 2015). The IL1 receptor antagonist is usually released by the same immune cells that release IL1, dampening excess IL1 signaling via competitive antagonism at the IL1 receptor (Dripps et al., 1991; Schreuder et al., 1997). IL1 receptor 2 is expressed on immune cells and cytokine targets like the brain. This deceptive receptor decreases IL1 signaling by acting as decoy receptor (Gabellec et al., 1996; McMahan et al., 1991). IL1 receptor 2 expression is induced by anti-

inflammatory factors whereas pro-inflammatory cytokines induce IL1 receptor 1 expression (Colotta et al., 1993; Martin et al., 2013; Orlando et al., 1997).

If IL1 is the master regulator of the immune response, then TNFs are the masters of death. TNFs received their name because they were first observed to induce tumor regression and are crucial immune regulators of cell death (Carswell et al., 1975). Since then, roles for TNFs have grown to include inflammation, apoptosis, cell proliferation, angiogenesis, and cell invasion (Aggarwal et al., 2012). The TNF superfamily of cytokines includes 19 ligands that bind to 29 separate receptors, but the research discussed here will focus on one of the first members identified, TNFα (Aggarwal et al., 2012; Clark, 2007). TNFα interacts with TNF receptors 1 and 2. TNF receptor 1 is ubiquitously expressed and contains an intracellular death domain, which recruits cell death-related proteins upon activation. On the other hand, TNF receptor 2 is specific to immune cells, endothelial cells, and neurons. Both TNF receptors interact with various TNF receptor-associated factors to signal (Arch et al., 1998). Similar to IL1, TNFα is pro-inflammatory partly through activation of NFκB and stress-activated kinases like JNK and ERK (Chen and Goeddel, 2002). Moreover, TNFα also functions to induce mitochondrial release of reactive oxygen species, cytochrome C, and Bax to promote apoptosis through caspase activation (Morgan and Liu, 2010). Interestingly, TNFα can induce either a proliferative or apoptotic response, depending on the intracellular adaptor proteins recruited (Aggarwal, 2003).

As highlighted above, IL6, IL1β, and TNFα can have broad impact on cellular function, but they also do not readily enter the brain. The majority of cytokines in the brain are produced

locally in neurons, astrocytes, microglia, and immune cells, and their receptors can be found on

most cell types within the brain (Dantzer, 2007; Szelényi, 2001). Peripheral cytokines must

utilize an indirect pathway to interact with central pathways necessary for the immune response.

This cytokine to brain communication is a gradual process that involves multiple immune to

brain communication pathways (Dantzer, 2006). Peripheral cytokines interact with the brain in

order to coordinate the host response to systemic infection (Borovikova et al., 2000). Yet since

cytokines and leukocytes cannot penetrate the blood brain barrier, the signal must reach the brain

through one of three indirect mechanisms: activation of perivascular cells at circumventricular

organs, direct entrance via saturable active transport, or stimulation of peripheral afferents

(Dantzer, 2007; Dantzer et al., 2007). Firstly, the lack of a functional blood brain barrier at

circumventricular organs as well as in the choroid plexus and leptomeninges allows for

activation of TLRs or cytokine receptors on endothelial cells, perivascular macrophages, and

microglia (Rivest, 2003). Activation of these immune sensing cells increases prostaglandin E2

synthesis and release, which diffuses throughout the brain parenchyma and induces an

inflammatory response. Secondly, active transport can occur for some cytokines with differing

transport rates among those cytokines allowed through (Banks, 2005; Pan et al., 2011). When

circulating cytokine levels are high, this mode of immune to brain communication seems to

predominate. However, inflammation is focused at the site of injury, which is often quite far

from the brain.

The third mechanism of immune-brain communication provides a more rapid pathway

utilizing neural afferents at the peripheral site of inflammation (Konsman et al., 2002). Cytokines

produced in inflamed tissues can activate afferent vagus nerve fibers at concentrations much

lower than those required to reach the brain via circulation. In fact, numerous studies have demonstrated that vagotomy blunts fever with low intra-abdominal doses of inflammatory stimuli, suggesting this pathway is crucial for immune to brain communication in early inflammation processes (Hansen et al., 2000a; Hansen et al., 2000b; Maier et al., 1998). These afferents activate neurons in the nucleus tractus solitaries, which inhibit vagal efferents to the viscera and digestive tract and/or relay this information to the hypothalamus, amygdala, and forebrain (Czura et al., 2007; Pavlov et al., 2003; Smith et al., 1998). Interestingly, vagal efferent fibers also provide feedback inhibition for peripheral inflammation. Stimulation of vagal efferents can inhibit TNF synthesis in the liver, spleen, and heart during infection or injury, likely by activation of α7 subunit containing cholinergic receptors on cytokine producing cells like tissue macrophages (Borovikova et al., 2000; Wang et al., 2003).

In a healthy brain, immunocompetent cells like microglia and perivascular and meningeal macrophages exist in an inactive state. The state of activation of these immune cells influences the severity of a transduced inflammatory response. Of importance to neurological and psychiatric disease, low grade chronic inflammation can lead to a "primed" state that increases the severity of response to local or systemic immune stimuli (Czura et al., 2007; Sibley et al., 1985). In the brain, immune cell activation and initiation of an inflammatory response occurs upon binding of cytokines or prostaglandin E2. Prostaglandin E2 is thought to be the primary mediator of translating inflammation from the periphery to the brain. Most cytokines in the brain are produced locally by activated microglia, astrocytes, and mast cells (Breder and Saper, 1996; Breder et al., 1993). Supporting the importance of prostaglandins, the inhibition of prostaglandin synthesis can prevent the manifestations of the brain response to inflammation like fever and

corticotrophin activation (Johnson and von Borell, 1994; Konsman et al., 2002; Stitt and Bernheim, 1985; Zhang et al., 2003).

The brain is a crucial site for the systemic immune response due to its control of fever, sickness behaviors, and the hypothalamic-pituitary-adrenal (HPA) axis (Harden et al., 2015). Fever is a brain-mediated component of the acute phase reaction and functions to produce an unfavorable environment for the growth of microbial pathogens. To induce fever, prostaglandin E2 binds to EP1 and EP3 receptors in the preoptic area, which leads to disinhibition of hypothalamic and brain stem nuclei that activate thermogenic brown adipose tissue and initiate vasoconstrictive heat conservation (Lazarus and Saper, 2007; Lu et al., 2001; Madden and Morrison, 2003; Madden and Morrison, 2004; Morrison, 2004). IL6 is thought to be the most important inflammatory mediator for inducing fever although the role of prostaglandins in the pyrogenic effects of IL6 is unclear (Kagiwada et al., 2004; Lenczowski et al., 1999). Even though considerable literature has suggested that IL1 can also induce fever in animal models, this effect is lost in IL6 knockout mice, supporting that IL6 is the primary pyrogen (Kagiwada et al., 2004; Li et al., 2003a).

On the other hand, sickness behaviors require modification of numerous central pathways and represent an overall change in the motivational state of the organism, allowing priorities to shift towards coping with infection and the immune response towards that infection. Sickness behaviors include decreased spontaneous activity, decreased sexual behaviors, anhedonia, apathy and lack of self-care, anorexia, generalized discomfort (malaise), fatigue, and sometimes confusion and hyperalgesia (Dantzer et al., 2007). Studies administering individual cytokines to

animal models have demonstrated that although redundancy exists for many cytokine functions, some of these sickness behaviors are strongly induced by particular cytokines (Kent et al., 1992; Zalcman et al., 1998). Decreased spontaneous activity, anorexia, apathy, and malaise are often observed together and are induced by IL1 and to a lesser extent TNFα (Bluthe et al., 1994; Kent et al., 1992). IL1, IL6, IL8, TNFα, or IFNα administration produces anorexia in rodents (Plata-Salaman et al., 1996; Reyes-Vazquez et al., 1994). And rodent sexual behavior is disrupted by IL1β and TNFα with effects more prominent in females than males (Avitsur et al., 1997; Avitsur and Yirmiya, 1999). Although these behavioral changes may appear harmful, Hart and other neuroimmunologists have strongly argued that these changes support and increase efficiency of the defensive metabolic and physiologic changes needed in the infected individual (Bazar et al., 2005; Harden et al., 2015; Hart, 1988).

Glucocorticoids are immunosuppressive steroid hormones released by the adrenal gland in response to stimulation of the HPA axis. Release of corticotrophin releasing factor from the hypothalamus stimulates adrenocorticotrophic hormone release by the pituitary into the blood (Schreiber et al., 1993). Adrenocorticotrophic hormone stimulates the adrenal gland to release glucocorticoids, which act on genes with glucocorticoid response elements to repress inflammatory pathways and induce a catabolic state (Christoffels et al., 2000; Scott et al., 1998). Glucocorticoids negatively regulate expression of adhesion molecules on endothelial cells, decreasing extravasation of leukocytes into inflamed tissue (Brostjan et al., 1997; Liden et al., 2000). Glucocorticoids also repress cytokine and cytokine receptor expression (Almawi et al., 1996; Amano et al., 1993; Paliogianni and Boumpas, 1995), upregulate anti-inflammatory genes (Ayroldi et al., 2001; Hayashi et al., 2004; Ray and Prefontaine, 1994), and shift the T helper

lymphocyte cytokine production from a Th1 (cytotoxic) to a Th2 (humoral) response (Elenkov et al., 1996). TNFα, IL1, and IL6 are the most potent cytokines for activating the HPA axis (Dunn, 1992; Lenczowski et al., 1999). HPA activation by cytokines and subsequent glucocorticoid release serves as a negative feedback mechanism to prevent excessive inflammation during the response to an immune stimulus.

Interaction between the brain and immune system during peripheral inflammation coordinate the host immune response (Hart, 1988). Due to the blood brain barrier, communication between immune cells and the brain must utilize restrictive, indirect pathways. These include activation of perivascular cells at circumventricular organs, active transport of select cytokines via saturable carrier proteins, or activation of vagal afferents by cytokines or other inflammatory mediators (Dantzer, 2006; Dantzer, 2007; Dantzer et al., 2007). Once immunocompetent cells in the brain parenchyma are activated, an inflammatory response is initiated, including prostaglandin E2 and cytokine release in the brain. These inflammatory mediators promote changes to assist and control the response to the peripheral immune stimulus (Besedovsky et al., 1991; Zhang et al., 2003). Concurrent HPA axis activation prevents excessive inflammation that can damage tissue (Amano et al., 1993; Ayroldi et al., 2001; Hayashi et al., 2004; Liden et al., 2000). Part of the central response to peripheral immune activation, induction of fever produces an environment unfavorable to microbial growth (Harden et al., 2015). In addition, adoption of sickness behaviors creates a metabolic and physiologic state thought to increase the efficiency and effectiveness of the host response to immune stimuli (Dantzer, 2006; Konsman et al., 2002). Yet the activation of a central response to immune stimuli by cytokines is only one of the physiologic roles of cytokines in the brain (Vitkovic et al., 2000). Equally

important, cytokines can influence normal neural activity and neurodevelopment (Deverman and Patterson, 2009).

Cytokines and neural plasticity

Considerable evidence suggests that cytokines participate in neural processes in the non-inflamed state (Vitkovic et al., 2000). Genes for cytokines and their receptors are expressed in a region- and cell-specific manner in the normal healthy brain (Breder et al., 1988; Pitossi et al., 1997; Takao et al., 1990; Tchelingerian et al., 1995). Impairment of cytokines or their receptors in the absence of an immune stimulus can alter behavior. For example, mice with impaired IL1 signaling have altered basal pain sensitivity and tolerance to morphine-induced analgesia (Shavit et al., 2005). IL6 knockout mice have altered sleep patterns, and both TNFα knockout mice and IL6 knockout mice have altered emotional behavior (Morrow and Opp, 2005; Yamada et al., 2000). With regards to learning and memory, IL1β has been shown to impair performance in hippocampal-dependent memory tasks at both high and low concentrations, suggesting a strict range at which IL1β can facilitate learning and memory processes (Banks et al., 2001; Yirmiya et al., 2002). With increasing age, increases in IL6 have been found to impair learning and memory (Heyser et al., 1997). Unlike the strict beneficial range seen with IL1β, loss of IL6 signaling appears beneficial to learning and memory processes. IL6 knockout mice actually have improved performance on complex spatial tasks like the radial arm maze (Braida et al., 2004; Heyser et al., 1997; Oitzl et al., 1993). Similar to IL6, TNFα does not appear to be required for normal learning and memory but can impair memory processes in an age- and dose-dependent manner (Aloe et

al., 1999; Fiore et al., 1996). Together, this data suggest that some normal behaviors do depend on basal cytokine levels to occur normally.

Both absence and excess of IL1β impair hippocampal LTP, suggesting disruptions in synaptic plasticity underlie the poor performance on hippocampal-dependent learning and memory tasks. Application of IL1β to rodent hippocampal slices *in vitro* has been reported to inhibit both LTP induction and maintenance (Katsuki et al., 1990; Murray and Lynch, 1998). Conversely, IL1β mRNA is increased during LTP (Schneider et al., 1998). The importance of IL1β in hippocampal synaptic plasticity is emphasized by the complete absence of LTP in hippocampal slices from IL1 receptor knockout mice (Avital et al., 2003). In agreement with the IL6- and TNFα-induced impairment in spatial learning and memory, LTP is also impaired by increasing concentrations of these cytokines (Bellinger et al., 1995; Tancredi et al., 1992). Surprisingly, Jankowsky and colleagues found IL6 gene expression is increased after LTP induction *in vivo*, which was later confirmed by the Besedovsky lab (Balschun et al., 2004; Jankowsky et al., 2000). The Besedovsky lab went on to demonstrate that anti-IL6 antibody administration following the *in vivo* induction of LTP resulted in longer maintenance, suggesting a role for IL6 in physiologic inhibition of LTP (Balschun et al., 2004). Together, this behavior and electrophysiologic evidence support a physiologic role for basal cytokine levels in learning and memory processes.

Cytokines are important for normal functioning in the adult brain just as the brain is important to mount an immune response. Vitkovic and his colleagues suggest that cytokines are capable of acting as neuromodulators in the adult brain in the non-inflamed state (Vitkovic et al.,

2000). The evidence shows an excess or lack of cytokines can alter animal behavior, learning and memory, and synaptic plasticity with IL1β likely playing a strong role (Avital et al., 2003; Yirmiya et al., 2002). Regardless of the label you give these functions, it is inarguable that cytokines have the ability to alter numerous pathways in the brain in addition to those that cause sickness behaviors and fever. This brings to question the consequences of aberrant inflammatory process on the adult brain. Low grade inflammation, which has been associated with several psychiatric disorders, has the potential to leave the brain in "primed", hyperresponsive state (Frank et al., 2015; Leboyer et al., 2016). Vulnerability to inflammation-inducing stimuli such as infections or psychosocial stress ensues, impacting brain function and possibly contributing both to psychiatric disorder development and progression.

The concurrent maturation of the developing brain and immune system

The prenatal environment primes the fetus for the outside world, and a prenatal environment discordant with the outside world forms a repertoire of inappropriate responses. This gives prenatal insults the potential for long-lasting consequences on both brain and immune system function. Our lab and others hypothesize that inflammation-inducing prenatal insults work through maternal, placental, and fetal disturbances to alter developmental trajectory of the brain and inappropriately prime the immune system to be hyperresponsive to the outside world (Horvath and Mirnics, 2014; Hsiao and Patterson, 2012; Michel et al., 2012; Millan et al., 2016). Later in this document, a detailed discussion of animal models and clinical studies will outline the likely role for inflammation in psychiatric disorder pathology. Here the maturation of the fetal immune and nervous systems and how they interact will be elaborated.

Fetal immunodevelopment

Maternal/fetal interaction at the decidua-placental junction was recognized as an immunological paradox decades ago (Medawar, 1953). Maternal leukocytes are in close proximity to fetal trophoblast cells with paternal alloantigens, and yet the maternal leukocytes do not react to non-self (La Rocca et al., 2014). Thus, a delicate balance of fetal immune tolerance, without overt maternal immunosuppression, is required for the survival and well-being of developing offspring (Jansson and Powell, 2007).

The placenta mediates interactions between the mother and fetus. It has both maternally (decidua)- and fetally-derived cells (Huppertz, 2008). The placenta maintains intrauterine homeostasis, provides nutrients and oxygen, removes waste, secretes hormones and growth factors, provides immunoprotection for the semi-allogenic fetus, and buffers deleterious substances from maternal circulation (Bronson and Bale, 2016; Jansson and Powell, 2007). The decidua, the most superficial compartment of the placenta, is densely packed with maternal leukocytes like uterine natural killer cells and macrophages (Matson and Caron, 2014). Maternal blood from spiral arteries and fetal blood from umbilical arteries exchange waste for nutrients and other vital blood components in the next deepest layer, the labyrinth zone (chorionic villous in humans). Layers of trophoblasts separate the fetal and maternal endothelial cells in the labyrinth zone to control this exchange and prevent direct blood contact (Hsiao and Patterson, 2012). The maternal decidua is responsible for systemic immunological tolerance of the developing embryo (Arck and Hecher, 2013). Fetal trophoblast cells ensure this function by fetal

microchimerism, trophoblast shedding to desensitize maternal circulation to paternal alloantigens, limited MHC expression on trophoblasts, and induction of a maternal Th1 to Th2 shift by secretion of factors like chorionic gonadotrophin and α-fetoprotein (Aluvihare et al., 2004; Makhseed et al., 2001). This complex balance is challenged even further by the need for vascular expansion, which is critical to ensure appropriate blood supply for the growing fetus. Fetal trophoblasts must invade the decidua and spiral arteries and coordinate with maternal leukocytes to induce vascular remodeling, increasing blood supply to the fetus (Harris et al., 2010; Hazan et al., 2010; Moffett and Loke, 2006). Any significant disruption in fetal immune tolerance can cause fetal rejection and/or impaired placental vascular expansion, which has devastating effects on neurodevelopment as well as pregnancy success as a whole.

The developmental course of the fetal immune system is far from a set course, greatly depending on the intrauterine environment to prepare for the outside world (Mandal et al., 2013). The fetal thymus, the site of T lymphocyte maturation, develops early in gestation concurrently with the emergence of T lymphocytes from the hematopoietic stems cells in the bone marrow (Haddad et al., 2006; Liu and Ellis, 2016). The thymus is a unique lymphoid organ in that it is actually more prominent at birth with size peaking at puberty then gradually declining (Kendall et al., 1980; Mackall and Gress, 1997). Fetal thymus maturation requires appropriate amounts of sex hormones and glucocorticoids with excess leading to involution and insufficient amounts accelerating development (Hendrickx et al., 1975; Seiki and Sakabe, 1997; Selye and Marion, 1955). Although adaptive immunity is immature at birth, some T lymphocyte differentiation and selection against self-recognizing lymphocytes occurs prenatally, which is critical for preventing autoimmune responses later in life (Bodey, 2002). By midgestation, human cord blood shows

evidence of allergen-activated lymphocytes, likely by small molecular weight substances from maternal circulation that cross the placenta. This prenatally primes the fetus to respond to expected allergens in the environment (Devereux et al., 2001; Loibichler et al., 2002).

At full-term birth, the neonatal immune system is characterized by a more established innate rather than adaptive immunity, reduced antigen-presenting capacity, low cytokine production and sensitivity, Th2 bias and impaired proliferative response of T lymphocytes, and a passive immunity from the mother (Adkins, 1999; Coe and Lubach, 2007). The maternal Th1 to Th2 shift confers this passive immunity during late pregnancy (Allansmith et al., 1968; Coe et al., 1994). Interestingly, the early neonatal period also requires significant immunosuppression to allow for bacterial colonization on mucosal linings like the gut. A unique CD4+ T suppressor lymphocyte is required to prevent hyperreactivity of the still developing and quite underprepared immune system (Coe et al., 1996; Papadogiannakis et al., 1990). Thus, immunodevelopment is heavily influenced by the intrauterine and early postnatal environment, creating specific time windows to alter immune functions that can affect many systems including the brain.

A disrupted intrauterine environment can have long lasting consequences on immune function. In both rodents and non-human primates, prenatal disruptions that change immunoreactivity (e.g. high levels of prenatal glucocorticoid exposure) alter postnatal gut bacterial colonization profiles, disrupting lymphoid tissue maturation and immune responses to gut-associated infection throughout the lifetime of the offspring (Bailey et al., 2004; Barreau et al., 2004; Cebra, 1999; Hooper and Gordon, 2001). In fact, Coe and colleagues have demonstrated long lasting immune changes in non-human primate offspring from both

glucocorticoid-treated and psychologically stressed mothers. At 1-3 years of age, the offspring have decreased cytokine production with LPS stimulation as well as glucocorticoid resistance and prolonged glucocorticoid secretion after exposure to social stressors (Coe et al., 2003; Coe et al., 2002; Coe and Lubach, 2000; Coe and Lubach, 2005). Likewise, maternal immune activation (MIA) induced with either LPS or poly(I:C) (polyinosinic:polycytidylic acid, a synthetic dsRNA that activates an antiviral immune response) during midgestation is associated with a pro-inflammatory phenotype in the offspring (Garay et al., 2013; Mandal et al., 2013; Onore et al., 2014). The Ponzio lab found poly(I:C) induced MIA to increase robustness of response to an innate immune activator, and this pro-inflammatory state was made more severe when dams had immunological memory (Mandal et al., 2013). Additional studies have demonstrated increased macrophage infiltration, microglial activation, and cytokine levels in the brains of MIA-exposed rodent offspring (Arsenault et al., 2014; Dada et al., 2014). Finally, perhaps one of the most dramatic impacts of the intrauterine environment on fetal immune development is preterm birth. This deprives the fetus of some passive immune transfer, forces the immature immune system to interact with the external world too early, and increases occurrence of allergies and asthma (Fergusson et al., 1997; Kent et al., 2016; Pekkanen et al., 2001).

Environmental risk factors like prenatal stress can synergistically add to the accumulating risk for pathological processes, which is particularly important for the research described in this document. When prenatal stressors are combined with a lack of the recommended supplementary iron intake for pregnancy, the immune deficits are amplified (Coe et al., 2007; Kochanowski and Sherman, 1985; Lubach and Coe, 2006). Iron is transferred to the infant in most mammalian species both via the placenta and breast milk. However, if inadequate quantities reach the

offspring *in utero,* iron obtained from breast milk will become insufficient within a few months after birth (Zetterstrom, 2004). Although it is generally accepted that the placental transfer of the products of maternal stress (i.e. glucocorticoids) influence the fetal immune system, Coe and colleagues demonstrated that other placentally transferred factors like micronutrients can also be disrupted by psychosocial stress. In their research with non-human primates, they found that infants prenatally stressed developed iron deficiency anemia soon after birth (Lubach and Coe, 2006). Iron is critical for the function of many systems, including the immune system. Therefore, it was not surprising that at 4-6 months of age, the primates developed impairments in innate immune functions like natural killer cell activity in addition to the previously noted impaired lymphocyte function (Coe et al., 2007; Lubach and Coe, 2006). Similarly, iron deficiency in rodent neonates is associated with decreased number of macrophages, natural killer cell activity, and antibody formation as well as an increased vulnerability to infection (Hallquist et al., 1992; Kochanowski and Sherman, 1985). It is worth noting that nutritional deficiencies can be more severe in litter-bearing species like rodents. Not only do these studies highlight that environmental risk factors can be additive, but they also demonstrate the importance of the prenatal period on development, with perturbations having consequences that last long past the initial insult.

Immunomodulation in neurodevelopment

Neurodevelopmental events are exquisitely sensitive to changes in the microenvironment in which they occur. Even minor deviations early in development have the potential to permanently alter neurodevelopmental trajectories. These deviations can be inherited or caused

by changes in the intrauterine or early postnatal environment. In general, the closer the insult is to the beginning of that neurodevelopmental event, the greater the deviation will be amplified over time (Zimmerman and Connors, 2010). Proper neurodevelopment requires a carefully orchestrated sequence of proliferation, migration, differentiation, formation of synaptic contacts, and pruning of inappropriate synaptic contacts and generally unnecessary neurons (Rubenstein, 2011; Schubert et al., 2015; Workman et al., 2013). Here, the focus will remain on understanding the events in neurodevelopment that may be disrupted by aberrant inflammation.

Cytokines are basally expressed throughout most of development and participate in neurodevelopmental processes. Beginning in early development, cytokines act as regulatory factors for the renewal of neural precursor cells (Bauer et al., 2007; Lee et al., 2010a; Pinto and Gotz, 2007). Stem cell self-renewal allows for the continued proliferation of neural precursors that differentiate into neurons and glia in the developing brain. The cytokines leukemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF) promote self-renewal of both human and rodent neural stem cells (Galli et al., 2000; Pagano et al., 2000; Pitman et al., 2004). During early stages of neural progenitor proliferation, CNTF prevents restriction of neural stem cells to a glial lineage (Shimazaki et al., 2001). Yet in later stages of neural progenitor proliferation, cytokines like LIF and cardiotrophin-1 promote gliogenesis *in vitro* (Barnabe-Heider et al., 2005; Chang et al., 2004). Neurogenesis occurs before gliogenesis, and these cytokines may be important in the timing of this developmental switch as well as promotion of astrocyte differentiation once gliogenesis begins (Bonaguidi et al., 2005; Johe et al., 1996; Rajan and McKay, 1998). Contrary to this theory, deletion of LIF or CNTF *in vivo* has little effect on astrogliogenesis (Bugga et al., 1998). Complicating the matter further, deletion of gp130, the

cytokine receptor used by these cytokines as well as others in the IL6 superfamily, causes

profound *in vivo* loss of astrocytes in late gestation, suggesting that there are likely more

members of the IL6 superfamily important for this function (Deverman and Patterson, 2009;

Koblar et al., 1998; Nakashima et al., 1999). In short, both neurogenesis and gliogenesis utilize

cytokines although there is potential for redundancy within the IL6 superfamily.

After neurons are produced, they must migrate from the proliferative zone to their mature

position in the brain. In the cortex, there are two types of migration: tangential migration of

interneurons from the basal forebrain to the cortex and radial migration of pyramidal neurons

(Metin et al., 2008). As neurons migrate, they are exposed to signals in their microenvironment

that affect their migration and eventual maturation (Marin, 2013). For this reason, even transient

delays in the migratory journey of neurons can alter mature function (Teixeira et al., 2014). Not

surprisingly, the chemoattractants of the immune system aid in neuronal migration. The

chemokine CXCL2 and its receptor CXCR4 are necessary for both tangential migration of

interneurons in the developing cortex and Purkinje cell migration in the cerebellum (Lazarini et

al., 2003; Stumm et al., 2003). Meningeal expression of CXCL12 affects the distribution of

Cajal-Retzius cells, a transient cell population located superficially in cortex that guides inside-

out laminar patterning (Borrell and Marin, 2006). In addition to migration, CXCL12/CXCR4

have also been shown to decrease the repellant effects of the axonal guidance cues slit2,

semaphorin 3A, and semaphorin 3C in primary neuronal cultures, which likely contributes to

temporal and spatial control of axonal paths *in vivo* (Chalasani et al., 2003). Although additional

chemokines may affect neuronal chemoattractive/repellent interactions, this field has been slow

moving due to the general promiscuity of chemokines and their receptors.

Cytokines also affect cell differentiation. Specifically, TGFβ has been shown to be required for differentiation of dopaminergic neurons in the ventral midbrain (Roussa et al., 2006). *In vivo* loss of both TGFβ2 and TGFβ3 isoforms leads to a dramatic reduction of ventral midbrain dopaminergic neurons, and excess TGFβ *in vitro* leads to ectopic tyrosine hydroxylase expression (Roussa et al., 2006). This relationship may be especially relevant to psychiatric disorders, considering the importance of dopamine and its cognate receptors for normal brain development and function as well as for psychiatric disorder pathological processes (Money and Stanwood, 2013; Spencer et al., 1998; Vuillermot et al., 2010). Importantly, we know that dopamine's effects on neurodevelopment and adult function are receptor subtype specific (Arnsten et al., 2012; D'Haenen H and Bossuyt, 1994; Money and Stanwood, 2013; Ross et al., 2015). In the **Appendix**, we evaluate one tool used to investigate the role of dopamine receptors in normal and pathologic brain processes, which we demonstrate useful in some brain regions for understanding the receptor subtype specificity of cytokine-associated (or other) perturbations in dopamine during development.

A significant delay exists between the end of migration and the beginning of synaptogenesis, which correlates with the appearance of astrocyte processes (Ullian et al., 2001). Astrocytes are necessary to release pro-synaptogenesis factors such as thrombospondins and cholesterol (Barres, 2008). Although numerous synaptic connections form, only those synapses that form strong, stable connections will survive. Cytokines moderate this process in both early and late forming neural circuits. In a particularly drastic example, *TGFβ2* knockout mice lack rhythmic respiratory activity and die at birth. Impaired respiration in this model occurs due to

presynaptic dysfunction in the central respiratory rhythm-generating network in the brainstem, not from neuromuscular dysfunction (Heupel et al., 2008). The widespread expression both prenatally and postnatally suggests TGFs may function similarly in other networks, but the lack of viable offspring makes this difficult to determine (Burns et al., 1993; Pelton et al., 1991). Furthermore, microglial produced TNFα can promote synaptic strength in hippocampal neurons by increasing expression of AMPA-type glutamate receptors likely through a β3 integrin-dependent mechanism (Beattie et al., 2002; Cingolani et al., 2008). The Malenka lab found glia are capable of sensing general synaptic activity and utilize TNFα to maintain homeostasis. In their studies, prolonged inhibition of spontaneous activity stimulates TNFα release from glia in hippocampal slice cultures. Conversely, increased activity related signals like glutamate decrease TNFα release (Stellwagen and Malenka, 2006). In agreement with these findings, TNFα loss abolishes the compensatory strengthening of the non-deprived eye in the monocular vision deprivation model (Kaneko et al., 2008). Thus, TNFα likely works to provide synaptic stability in experience-de pendent neural networks (Deverman and Patterson, 2009; Turrigiano and Nelson, 2004)

More neurons and synapses form during development than are needed. Pruning is required for formation of adult neural circuitry. As cell death and removal of cell debris are known immune functions, cytokines, to no surprise, participate in this neurodevelopmental process. Phagocytic microglia are present at the onset of neurogenesis (Alliot et al., 1999; Ashwell, 1991). Evidence from cerebellar slice cultures shows loss of microglia reduces both phagocytosis and death of cells, suggesting an active role for microglia in regulating neuronal cell death (Marin-Teva et al., 2004). Surprisingly, microglia can either promote cell death by

production of TNFα or cell survival with increases in local vascularization and/or secretion of neurotrophic M-CSF, NGF, or BDNF (Bessis et al., 2005; Checchin et al., 2006; Kim and de Vellis, 2005; Michaelson et al., 1996). In the cortex, IL9 is important to survive this pruning process, with exogenous IL9 reducing apoptotic markers in early postnatal mice *in vivo.* Both IL9 and the IL9 receptor are expressed by developing cortical neurons in the early postnatal mouse brain and act in an autocrine and paracrine fashion to support survival (Fontaine et al., 2008). With regards to synapse elimination, glial-induced neuronal expression of complement protein is critical. Stevens and colleagues found mice deficient in complement protein C1q or the downstream signaling component C3 have a profound lack of normal synapse elimination (Stevens et al., 2007). Their results show that C1q localizes to synapses, and in a process involving C3 likely serve as an elimination "tag" for microglia (Gasque et al., 2002; Stevens et al., 2007). Granted, the maintenance of some synapse elimination suggest a complement-independent pathway also exists.

Critical to the functioning of all neurons, oligodendrocyte survival is also regulated by cytokines. Oligodendrocytes migrate long distances from their points of origin, making connections with axons along the way both for trophic factor support and eventual myelination (Barres and Raff, 1994). These trophic factors include CNTF, LIF, IL6, and IL11 (Barres et al., 1993; Louis et al., 1993; Zhang et al., 2006). Far from trophically acting, IFNγ overexpression in either oligodendrocytes or astrocytes results in less brain myelination, especially in the cerebellum. This poor myelination is associated with disrupted cerebellar neuron migration and neurogenesis as well as cerebellar dysfunction (ataxia and tremors) (Corbin et al., 1996; LaFerla et al., 2000). IFNγ also induces oligodendrocyte death in culture (Andrews et al., 1998; Baerwald

and Popko, 1998). Importantly, oligodendrocyte dysfunctions have been observed in many neurologic and psychiatric brain disorders (Arion et al., 2007; Behrendt et al., 2013; Rajkowska et al., 2015; Saetre et al., 2007; Voineskos et al., 2013). Altogether, these results suggest that changes in IFNγ during periods of oligodendrocyte development have the capacity to severely alter circuit formation and contribute to pathology in neurological and psychiatric disorders.

Cytokines are intricately intertwined with neurodevelopmental processes but are not the only immune-related signal involved. This is best illustrated by MHC class I. A neurodevelopmental role for MHC I is particularly interesting considering the strong association between mutations in this gene locus and schizophrenia, which are discussed in further detail below (Sekar et al., 2016). The mechanism for the hypomyelination in IFNγ overexpressing animals is mostly mediated by MHC class I induction in oligodendrocytes. This induction leads to subsequent changes in gene expression and a shortage of oligodendrocytes, producing a hypomyelinating phenotype (Turnley et al., 1991a; Turnley et al., 1991b). MHC class I expression also serves as one of the complement-independent pathways for synapse elimination. Carla Shatz's lab elegantly delineated this function of MHC class I. They initially found neuronal MHC class I components to be repressed with inhibition of spontaneous activity, suggesting synapses that would be eliminated repress some MHC class I components (Corriveau et al., 1998; Penn et al., 1998; Shatz and Stryker, 1988; Sretavan et al., 1988). They went on to show that mice lacking the CD3ζ component of MHC class I have impaired synapse elimination. In these mice, retinal ganglion axons are inappropriately incorporated in excess in the lateral geniculate nucleus (Huh et al., 2000). Since neuronal MHC class I is expressed in many experience-dependent brain regions, they theorized effects could be found outside of the visual

system (Liu et al., 2013; van Leeuwen and Samelson, 1999; Zhang et al., 2015). And indeed, disruption of neuronal MHC class I enhanced LTP and blocked LTD in the adult hippocampus (Huh et al., 2000). Altogether, their results demonstrate a role for class I MHC in activity-dependent synaptic weakening and structural refinement in the developing and adult brain.

Even so, perhaps the most interesting interaction is the prolonged developmental time course shared by both the nervous and immune systems. In addition, neuro- and immune-development can be affected by the same perturbations, like premature birth and maternal infection, which disrupt development of both the immune and nervous systems and increase risk for brain and immune dysfunction later in life (Abdallah et al., 2012; Ashwood et al., 2011a; Atladóttir et al., 2010; Fergusson et al., 1997; Hsiao et al., 2012; Kent et al., 2016; Knud Larsen et al., 2010; Mandal et al., 2013; Mathiasen et al., 2011; Pekkanen et al., 2001; Singh et al., 2013). In fact, Anders and Kinney posit that protracted immune system development may mask latent immune vulnerability from prenatal or early childhood insults until immunosenescence, the degeneration of the immune system mainly due to T lymphocyte related changes from thymic involution at puberty (Anders and Kinney, 2015). They suggest this thymic protection from the altered immune system contributes to the protracted development of psychiatric disorders (Gui et al., 2012; Kinney et al., 2010).

Clinical evidence of inflammation-associated disruption of brain development and function

As the previous sections have amply discussed, the brain and immune system are intricately intertwined and highly dependent on each other for normal function. Yet interactions

between the brain and immune system are certainly not all positive. Excess inflammation leads to pathologic alterations of brain structure and function that contribute to psychiatric disorder development and progression later in life (Muller, 2014; Saetre et al., 2007). Both genetic and environmental risk factors contribute to an altered inflammatory state, working together to shape the disease-specific pathology of psychiatric disorders (Horvath and Mirnics, 2014). Here we will discuss the clinical studies that have addressed altered inflammation in psychiatric disorders like MDD, autism spectrum disorder, and schizophrenia. This will be followed by review of animal models of inflammation with an emphasis on environmentally derived inflammation as seen in obesity, gestational diabetes, and MIA.

Gene mutations and aberrant inflammation in psychiatric disorders

Although the focus of this document is on environmental sources of aberrant inflammation, genetic evidence also strongly supports a role for inflammation in psychiatric disorder pathology. Mutations in the immune system and inflammation response genes have been associated with multiple psychiatric disorders (Consortium, 2015; Michel et al., 2012). This is not surprising considering that disorders such as schizophrenia and autism are highly heritable and associated with increased inflammation and immune disturbances (Cohly and Panja, 2005; Leboyer et al., 2016; Sandin et al., 2014; Wray and Gottesman, 2012). These mutations alter the inflammatory state themselves as well as shape the response to environmental risk factors, such as prenatal infection and childhood trauma.

Schizophrenia is associated with polymorphisms in the MHC locus (Shi et al., 2009; Sullivan et al., 2008; Yamada et al., 2015; Yue et al., 2011). Although not all studies have found similar associations, a large meta-analysis found over 100 polymorphisms in the extended MHC locus related to schizophrenia (Consortium, 2011). Interestingly, the Lencz lab has found schizophrenia to be associated with an excess of runs of homozygosity, which are long, homozygous chromosomal segments found throughout the genome even in outbred individuals (Keller et al., 2012; Lencz et al., 2007). In multiple ethnically distinct, homogenous cohorts, MHC homozygosity was found to have significant excess of homozygosity with the strongest recessive effects near the *HLA-A* gene (Mukherjee et al., 2014). Importantly, MHC variation plays a key role in determining resistance or susceptibility to immune stimuli and autoimmune processes (Traherne, 2008). MHC proteins also serve as necessary negative regulators of synapse formation and plasticity in both the developing and adult brain, suggesting a potential for *HLA* gene mutations to contribute to schizophrenic pathology (Elmer and McAllister, 2012; Shatz, 2009). Supporting this potential, mutations in *HLA* genes have been associated with decreased thalamic volume, a structural imaging finding associated with schizophrenia (Brucato et al., 2015). In addition, Walters and colleagues found the schizophrenia-associated SNP (rs6904071) in the MHC region was reproducibly associated with delayed episodic memory in large German and Irish cohorts of patients and controls (Walters et al., 2013).

Genes involved in the innate immune response (e.g. cytokines and complement proteins) have also been associated with increased schizophrenia risk. Mutations in the genes encoding IL2 and IL4, which both promote Th2 differentiation, increase schizophrenia risk, although this result has not been repeated in some studies (Fila-Danilow et al., 2012; Frydecka et al., 2013;

Schwarz et al., 2001; Schwarz et al., 2006; Tatsumi et al., 1997; Watanabe et al., 2008). *IL6, IL10,* and *IL3* polymorphisms are also associated with schizophrenia risk (Almoguera et al., 2011; Bocchio Chiavetto et al., 2002; Chen et al., 2007; Paul-Samojedny et al., 2010). In a Japanese cohort, polymorphisms in the gene encoding NFκB associated with deficits in sensorimotor gating in schizophrenic male patients (Hashimoto et al., 2011). In addition, Sekar and colleagues found that schizophrenia-associated complement C4 alleles are associated with increased C4A expression in the schizophrenic brain. Interestingly, C4 is important for elimination of unnecessary synapses. C4 deficient mice have reduced synaptic complement deposition and synaptic pruning (Sekar et al., 2016). C4 promotes C3 activation, which targets synapses for elimination in the developing mouse brain (Stevens et al., 2007). Thus, excessive complement activity from increased C4A expression may contribute to the reduced synapse number observed in schizophrenia (Garey et al., 1998; Glantz and Lewis, 2000; Glausier and Lewis, 2013).

The evidence for immune-related gene polymorphisms in autism spectrum disorder is significant although somewhat less compelling than the strong *HLA* associations found in schizophrenia. Genome wide association studies of autism have not implicated immune function genes in autism risk (Anney et al., 2010; Ma et al., 2009; Weiss et al., 2009). Even so, single nucleotide polymorphisms in *HLA-A2* and *HLA-DR4* were associated with autism in hypothesis-driven genetic assessments (Guerini et al., 2011; Lee et al., 2006; Torres et al., 2006). In addition, complement C4B genetic deletions as well as decreased C4B protein have been reported by multiple groups as significantly increased in autism (Mostafa and Shehab, 2010; Odell et al., 2005; Warren et al., 1994; Warren et al., 1991). This suggests C4 may contribute to

a failure of synapse elimination in autism, which is the opposite of the C4-mediated excessive

synapse elimination proposed in schizophrenia. Furthermore, a genetic variant that disrupts MET

receptor tyrosine kinase transcription was found to be associated with autism by the Levitt lab

(Campbell et al., 2006). Not exclusive to the immune system, MET receptor tyrosine kinase

mediates hepatocyte growth factor/scatter factor signaling and is a pleiotropic signaling protein

in immune function as well neurodevelopment and gastrointestinal repair (Okunishi et al., 2005;

Powell et al., 2001; Tahara et al., 2003). Consequently, reduced hepatocyte growth factor/MET

signaling disrupts the tangential migration of interneurons in cortex and decreases granule cell

proliferation in the cerebellum (Ieraci et al., 2002; Powell et al., 2001). Both of these

abnormalities are also observed in autism, strongly implicating MET as a pathologic genetic

variant in autism (Palmen et al., 2004).

MDD has a somewhat different genetic story than autism and schizophrenia. With only

modest heritability confounded by environmental effects, it is not surprising that even meta-

analysis of genome wide association studies have found no significant mutations in MDD (Major

Depressive Disorder Working Group of the Psychiatric, 2013; Sullivan et al., 2000; Wray and

Gottesman, 2012). However, most believe MDD to be genetically heterogeneous as to the exact

mutation locus although still revolving around consistent pathways. Some groups focusing on

pathway analysis have found immune function pathway mutations to be enriched in MDD (Jia et

al., 2011; Kao et al., 2012; Song et al., 2013). Similar to autism, targeted genetic assessments

have also found associations between immune-related genes and MDD risk. These include

polymorphisms in *IKBKE*- a pro-inflammatory gene associated with NFκB nuclear translocation-

and *IL33* (Koido et al., 2010; Kudinova et al., 2016; Traks et al., 2015). Even so, it is possible

that with increased sample sizes and better defined subgroups of MDD that additional candidate risk genes will arise over time.

With regards to immune function-associated genetic risk for psychiatric disorders, the most interesting part is the overlap between disorders. The field has long believed that many psychiatric disorders share genetic etiology to some degree. Family studies have suggested that psychiatric disorders in general, not specific psychiatric diagnoses, run in large pedigrees, but frequently insufficient sample size has made the extent of these relationships unclear (Lichtenstein et al., 2010; Smoller and Finn, 2003). The Psychiatric Genomics Consortium has compiled copious amounts of genome-wide genotyping data in order to have the power to address shared genetics, focusing on autism, attention-deficit/hyperactivity disorder, MDD, bipolar disorder, and schizophrenia. Using common SNPs, they found genetic correlation to be high between schizophrenia and bipolar disorder and moderate between schizophrenia and bipolar disorder, bipolar disorder and MDD, and attention-deficit/hyperactivity disorder and MDD. A low genetic correlation was also found between schizophrenia and autism with no significant correlations with the negative control (Crohn's disease) (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013). When pathway analysis was applied, immune function pathway genes were associated with schizophrenia, bipolar disorder, and MDD. Although the *HLA* genes strongly associated with schizophrenia were excluded due to the high linkage disequilibrium at that locus, pathways such as TGFβ signaling and B and T lymphocyte activation were still significantly correlated between disorders. Interestingly, analysis of how these pathways are expressed revealed an enrichment for late prenatal and early postnatal

development, which suggests a period of time in which these genetics factors are modifiable in either a positive (prevention) or negative (environmental insult) way (Consortium, 2015).

Clinical evidence for aberrant inflammation in psychiatric disorder development

Evidence of actively altered inflammatory states in both studies of patients and post mortem analysis provide definitive evidence of the importance of inflammation in psychiatric disorders (Patterson, 2009). This includes observed changes in cytokines and acute phase proteins in patient serum, altered immune cell types and numbers in patient blood, *in vivo* and post mortem evidence of inflammation in the brain, and *ex vivo* models from patients demonstrating aberrant responses to inflammatory stimuli, which are all discussed below.

In 1977, the first study suggesting immune dysfunction in autism reported altered lymphocyte responsiveness to a mitogen in children with autism (Stubbs et al., 1977). Since then, reports of increased blood chemokines (e.g. eotaxin) and cytokines (e.g. IL6 and IL1β), presence of autoantibodies against brain proteins, increased total blood monocytes, reduced natural killer cell activity, lower CD4+/CD8+ T lymphocyte ratio, and many other immune disturbances have been documented in autistic patients (Ashwood et al., 2011a; Ashwood et al., 2011b; Ricci et al., 2013; Sweeten et al., 2003; Warren et al., 1987). Centrally, increased expression of immune-related genes has been found in frontal and temporal cortices in multiple autistic case-control cohorts (Garbett et al., 2008; Gupta et al., 2014; Voineagu et al., 2011). At the protein level, IFNγ, TNFα, IL6, and granulocyte-CSF are increased in post mortem frontal cortex (Singh, 1996). Increased microglial activation has also been observed cortically both *in vivo* via PET

imaging and in post mortem analysis (Ashwood and Wakefield, 2006; Okada et al., 2007). In one particularly interesting study, the Arking lab found that gene expression patterns corresponding to the M2 microglial activation state negatively correlated with a differentially expressed neuronal module (Gupta et al., 2014). They extrapolate this to mean that increased microglial activation occurs in concert with altered neuronal activity to create the autistic brain. Altogether, these data provide support for an immune dysregulation in autism although the source of that dysfunction remains somewhat unclear.

Peripheral markers of inflammation correlate with symptom severity in autism. In lymphoid cells isolated from autistic patients and non-affected siblings, Hu and colleagues found TNFα signaling gene expression changes correlated with diagnosis (Hu et al., 2009). When comparing monozygotic twins discordant in autism severity, the same group found TNFα signaling-related gene expression changes also correlated with symptom severity (Hu et al., 2006). Autistic children with low levels of the anti-inflammatory cytokine TGFβ have been reported to have lower adaptive behavior scores and more severe behavior symptoms (Ashwood et al., 2008). In a separate cohort, autistic children with the regressive form of autism had higher pro-inflammatory cytokine levels. Increasing cytokine levels correlated with more severe communication impairments and aberrant behaviors (Ashwood et al., 2011b). Thus, it appears that levels of both pro- and anti-inflammatory cytokines modify symptom severity in autism.

Schizophrenia shares many of the core immune dysfunctions seen in autism. Autoantibodies against the NMDA receptor have been detected in schizophrenia (Steiner et al., 2013; Vincent and Bien, 2008), and psychotic symptoms have been frequently reported in anti-

NMDA receptor encephalitis (Barry et al., 2011; Cleland et al., 2015; Kayser et al., 2013; Kramina et al., 2015). Peripheral blood mononuclear cell transcriptomics show increases in NFκB- and cytokine-related gene expression (Drexhage et al., 2010; Song et al., 2009). Schizophrenic patients also display a blunted Th1 and increased Th2 lymphocytic profile (Muller and Schwarz, 2006). A meta-analysis of 40 serum cytokine studies in schizophrenic patients found consistent increases in IL1β, IL6, and TGFβ, particularly during acute exacerbations. By contrast, IL12, IFNγ, TNFα, and soluble IL2 receptor were elevated independent of illness state, implicating cytokines as both markers of the disorder as well as acute exacerbations (Miller et al., 2011). Suggesting even more specificity, Kurian and colleagues identified blood gene expression changes associated particularly with symptomatic states with genes related to interleukin signaling correlating with hallucinations and delusions (Kurian et al., 2011). In fact, increases in pro-inflammatory cytokine expression in the frontal cortex correlated with both increased microglial activation and decreased parvalbumin and somatostatin transcript expression- interneuron subtype markers decreased in schizophrenic brains (Fillman et al., 2013). Many non-cytokine immune response transcripts are upregulated in schizophrenic brains as well. Particularly, interferon-induced transmembrane 2 and 3 proteins (IFITM2 and IFITM3) and alpha-1-antichymotrypsin (SERPINA3) are induced by cytokines and repeatedly found to be upregulated in schizophrenic prefrontal cortex (Arion et al., 2007; Fillman et al., 2013; Saetre et al., 2007). It is worth noting that gene expression profiling of the schizophrenic brain shows great diversity amongst patients. Over time, the field has categorized this diversity into five frequently overlapping sub-groups: synaptic, GABAergic, mitochondrial, oligodendrocyte, and immune disturbances (Horvath and Mirnics, 2014). Therefore, it is reasonable to conclude that at

least a subpopulation of schizophrenic patients have consistent immune dysfunction contributing to pathology.

Heightened inflammation in schizophrenia is associated both with disease pathogenesis and active pathologic processes. This second point is best demonstrated by measures of immune dysfunction before and after antipsychotic treatment regimens. A meta-analysis combining 23 studies, totaling 762 subjects, found antipsychotic treatment to significantly increase soluble IL2 receptor and decrease IL1β and IFNγ plasma levels in schizophrenic patients (Tourjman et al., 2013). Correspondingly, the Myint lab found that differences in LPS induced cytokine release in schizophrenic derived peripheral blood mononuclear cell cultures normalized with quetiapine and risperidone antipsychotic co-incubation, suggesting antipsychotics may directly dampen systemic inflammation in addition to their central effects (Krause et al., 2013). However, other studies have found only some antipsychotics, mainly atypicals, normalize serum cytokine levels, which is likely complicated by cytokine induction from metabolic side effects frequently experienced with antipsychotics (de Witte et al., 2014; Krause et al., 2013; Song et al., 2014). Although antipsychotics can either increase or decrease certain serum cytokine levels, the general trend is for symptom improvement to associate with normalization of cytokine levels, suggesting cytokine involvement in ongoing schizophrenia pathology.

The similar symptomology between cytokine induced sickness behavior and symptoms of MDD make this psychiatric disorder the least surprising to involve immune dysfunction. In fact, 20-82% of patients treated with pro-inflammatory cytokines develop MDD. For example, IFN treatment is often used to treat hepatitis C viral infection. In a study of 50 hepatitis C virus

infected patients treated with IFN, 82% developed severe depressive symptoms, usually within

the first week of the 48 week treatment regimen (Reichenberg et al., 2005). In this study and

others, MDD occurs most often in patients with a worse baseline score on depression scales,

suggesting this increased inflammation interacts with a predisposition to develop MDD in these

patients (Capuron et al., 2002; Krishnadas et al., 2011; Musselman et al., 2001; Reichenberg et

al., 2005). On the other hand, anti-inflammatory TNFα antagonists attenuate depressive

symptoms in psoriasis patients (Krishnan et al., 2007; Tyring et al., 2006). Furthermore, patients

with pro-inflammatory diseases have a higher incidence of MDD than the general population

(Dickens et al., 2002; Graff et al., 2009; Krishnadas et al., 2011; Lo Fermo et al., 2010).

Altogether, these observations clearly link heightened inflammatory states with MDD, likely

working with existing risk factors to increase incidence.

Similar to autism and schizophrenia, increased blood levels of acute phase proteins,

cytokines like IL6, IL1β, and TNFα, and chemokines have been reported in MDD patients (Dahl

et al., 2014; Dowlati et al., 2010; Erdem et al., 2011; Maes et al., 1997; Maes et al., 1993; Raison

et al., 2006). Decreased blood natural killler cells and soluble IL2 receptor is also observed in

MDD (Maes, 1995; Zorrilla et al., 2001). Yet MDD is also associated with an increased

CD4+/CD8+ T lympocyte ratio, the opposite of that observed in autism (Zorrilla et al., 2001).

Centrally, a PET imaging study by Setiawan and colleagues noted increased inflammation, with

activated microglia detected in prefrontal cortex, anterior cingulate cortex, and insula of MDD

patients (Setiawan et al., 2015). Differentially expressed genes in post mortem MDD brains also

heavily implicate ongoing inflammation, especially genes associated with IFNγ and chemokine

signaling (Kang et al., 2007; Lalovic et al., 2010). However, it must be acknowledged that not all

studies have found immune dysfunction in MDD, emphasizing that MDD, like other psychiatric

disorders, is a heterogenous disorder with at least a portion of patients having aberrant

inflammation (Rothermundt et al., 2001).

As observed with autism and schizophrenia, increased plasma cytokines correlate with

increased symptom severity in MDD (Howren et al., 2009; Maes et al., 2009; Yirmiya et al.,

2000). Importantly, cytokine levels also correlate with symptom improvement with successful

treatment. In a study by Dahl and colleagues, cytokine levels were tested in previously

medication-free patients before and after 12 weeks of treatment with antidepressants. They found

baseline elevations in IL1β, IL1 receptor antagonist, IL5, IL6, IL7, IL8, IL10, granulocyte-CSF,

and IFNγ in patients compared to controls. In patients with successful symptom improvement

after the 12 weeks, all but IL1β and IL5 levels were brought back to control levels (Dahl et al.,

2014). This strongly supports a role for increase cytokines in ongoing MDD progression.

Studies of inflammation's role in susceptibility and progression of MDD have taken

advantage of the systemic nature of MDD, utilizing peripherally derived patient models. Using

peripheral blood mononuclear cell cultures derived from patients, an altered response to *in vitro*

LPS exposure has been observed (Krause et al., 2012). Along the same lines, the Navarra lab

found blunted PGE$_2$ release but increase transcription of *IL1B* and *IL6* after 24 hour LPS

exposure, which was partially rescued in cell cultures derived from the same patients after eight

months of antidepressant treatment (Lisi et al., 2013). Similarly, normalization of IL6 signaling

as well as natural killer cell pathway gene expression patterns was observed by Jansen and

colleagues in peripheral blood measurements taken before and after 2 years of antidepressant

therapy (Jansen et al., 2016). This supports the treatment-induced serum cytokine normalization discussed above as well as the utility of peripheral models to study brain disorders. In fact, our lab and others have made use of cultured human dermal fibroblasts (HDFs) to study MDD. They are easily maintained, carry the same genetic variance as the brain, express many of the same receptors and signal transduction proteins as the brain, and provide a relevant system to interrogate immune stimulation effects on cellular function (Bahn and Chan, 2015; Manier et al., 2000; Shelton et al., 1996). MDD HDFs have decreased PKA activity and CREB phosphorylation as well as altered expression of immune, apoptosis, oxidative stress, and energy production pathways at baseline (Akin et al., 2005; Garbett et al., 2015a; Manier et al., 2000). In addition, IL1β treatment induces increased acute phase protein expression compared to control HDFs (Shelton et al., 2004). In total, the *in vitro* and *in vivo* human studies of MDD provide overwhelming support for increased inflammation's role both in susceptibility and symptom severity.

Environmental factors associated with psychiatric disorder risk in clinical studies

The presence of inflammation in psychiatric disorders is not debatable, as the above studies clearly demonstrate. For quite some time, our lab has been specifically interested in addressing the source of this inflammation. The genetic associations with immune function and inflammatory response genes do suggest at least some is derived from genetics, but the environmental contribution to increased inflammation is considerable (McOmish et al., 2014). Inflammation's role in psychiatric disorder risk factors is often underestimated. There are obvious sources of inflammation such as prenatal infection and autoimmune disease, and then

there are the more complex inflammatory risk factors such as early childhood trauma, chronic

psychosocial stress, obesity, and gestational diabetes, which can greatly perturb inflammatory

status (Berk et al., 2013). Importantly, epidemiologic evidence shows that these are

inflammation-associated risk factors for psychiatric disease, which is discussed below.

Since many psychiatric disorders are purported to have neurodevelopmental origins, a

considerable portion of literature has focused on the importance of prenatal and early postnatal

inflammation-associated events. It has long been known that *in utero* infections can alter the fetal

brain, ranging from mild behavioral irregularities to severe deficits. Active maternal infections

with herpes simplex virus, cytomegalovirus, rubella, and toxoplasmosis can lead to childhood

intellectual disability and learning difficulties (Hagberg and Kyllerman, 1983; Meyer, 1969).

Recent events make this association all the more important: Zika virus has emerged as the

newest viral threat to the developing fetus. The congenital syndrome characterized thus far

includes microcephaly, hyperreflexia, spasticity, facial disproportionality, and irritability

(Miranda-Filho Dde et al., 2016). Fortunately, most cases of *in utero* infection do not cause

severe malformations at birth, but that does not make them harmless. When the perfect storm of

genetic and environmental risk factors are combined with *in utero* infection, the developmental

trajectory of the fetus shifts towards an end result of psychiatric dysfunction.

The earliest studies of *in utero* infection and psychiatric risk observed increases in

schizophrenia diagnosis after influenza epidemics. This observation began with the 1957 A2

influenza pandemic. Groups in Finland, England, and Wales reported considerable increases in

schizophrenia risk in individuals born a few months after the pandemic peak (Mednick et al.,

1988; O'Callaghan et al., 1991). But others have been unable to replicate these results (Selten et al., 2010). Evaluation of schizophrenia risk associated with other influenza outbreaks is plagued with the same inconsistent study results. Many studies find a relationship between second trimester infections and schizophrenia diagnosis (Adams et al., 1993; Barr et al., 1990; Kendell and Kemp, 1989; Kunugi et al., 1995; Limosin et al., 2003; Sham et al., 1992; Takei et al., 1993; Takei et al., 1994) while many others find no significant associations (Grech et al., 1997; Kendell and Kemp, 1989; Mino et al., 2000b; Morgan et al., 1997; Susser et al., 1994; Takei et al., 1995). Similar mixed results have been found for diphtheria, pneumonia, measles, varicella, mumps, and even poliovirus (Cahill et al., 2002; O'Callaghan et al., 1994; Suvisaari et al., 1999; Torrey, 1988; Watson et al., 1984). Most of these ecological studies calculated schizophrenia risk based on birth cohorts, comparing those born a few months after a pandemic peak versus a cohort without a gestational influenza pandemic. Therefore, the true effects of infection were muddied by the large portion of the population that had gestational time overlapping with pandemics but were not actually exposed (Brown and Derkits, 2010).

Once *in utero* infection is actually confirmed in the "exposed" cohort, the results become more definitive. Using well-characterized birth cohorts with biological specimens or medical record infection diagnosis, the Brown lab published a series of nested case-control and birth cohort analysis studies, finding strong associations between schizophrenia diagnosis and maternal elevated cytokines, viral infection, upper respiratory infection, and/or genitourinary infections (Babulas et al., 2006; Brown et al., 2004a; Brown et al., 2001; Brown et al., 2004b; Brown et al., 2005). Subsequently, other serological or record-based birth cohort studies replicated these results with similarly less discrepancies than ecological studies (Buka et al.,

2008; Buka et al., 2001a; Buka et al., 2001b; Clarke et al., 2009; Mortensen et al., 2007;

Sorensen et al., 2009). Yet the crux of the matter is how these infections interact with the fetus.

To this end, the Karlsson lab studied dried neonatal blood samples from 199 psychosis cases and

525 matched controls, measuring toxoplasmosis, cytomegalovirus, and herpes simplex virus

targeted IgG and accompanying acute phase proteins. Interestingly, risk of psychosis-related

disorders only increased in neonates with antiviral IgG AND low pro-inflammatory acute phase

protein levels, supporting disrupted development of the fetal immune system could participate in

modulating the long-lasting effects of prenatal infection (Blomstrom et al., 2015).

Similar to schizophrenia, autism has been associated with *in utero* infections for decades.

Congenital rubella infection was one of the first known causes of autism (Chess, 1971). In

addition to rubella, other viral pathogens like cytomegalovirus as well as bacterial infections,

especially in the second trimester, have been associated with later autism diagnosis (Atladóttir et

al., 2010; Chess, 1977; Libbey et al., 2005; Sweeten et al., 2004; Yamashita et al., 2003; Zerbo et

al., 2015). Specifically, there are two large, well-characterized birth cohorts that provide the

strongest link between prenatal infection and autism risk. Using the Danish medical birth register

and associated medical records, Altadóttir and colleagues were able to dissect both the nature

and timing of infection and its interaction with autism risk in a birth cohort of 1,612,342 children

(10,133 diagnosed with autism) born from 1980 to 2005. No association between autism

diagnosis and all maternal infections at any point in pregnancy was found. However,

stratification by trimester revealed a significant effect of infection requiring hospitalization in the

second trimester, with adjusted hazards ratio of 1.3 (CI: 1.01 – 1.67) (Atladóttir et al., 2010). In

the second seminal study, *in utero* infection and autism diagnosis were examined in 2,371,403

individuals (24,414 autism cases) from the Swedish-register based birth cohort born 1987 –
2007. After adjusting for co-variates such as parental age and birth year, infection requiring
hospitalization at any point in pregnancy was associated with an up to 30% increase in autism
risk. The odds ratio was slightly higher for viral pathogens and infections in the upper respiratory
tract (Lee et al., 2015). Clearly, bacterial and viral infections during pregnancy are associated
with later autism diagnosis.

Cytokines and chemokines measured in maternal serum and amniotic fluid midgestation
provide evidence for the "smoking gun" with regards to infection's role in autism pathogenesis.
In the Danish birth register cohort discussed above, elevation of the chemokine MCP1 in
amniotic fluid was associated with autism risk (Abdallah et al., 2012). Increased IFNγ, TNF, IL4,
IL10, and IL5 during midgestation may also increase autism risk up to 50% (Abdallah et al.,
2013; Goines et al., 2011). Interestingly, exclusion of cases and controls with documented *in
utero* exposure to maternal infection or autoimmune disease had no effect on the significant
correlation between amniotic fluid cytokines and autism (Abdallah et al., 2013). This illuminates
a point for later discussion: non-infection insults modifying *in utero* inflammation.

The literature on *in utero* infections and later MDD diagnosis is really limited when
compared to schizophrenia and autism. However, there is evidence, albeit quite dated, of
increased MDD diagnosis when gestational time overlapped with the 1957 influenza pandemic.
In Finland, Machón and colleagues reported an increased diagnosis of affective disorders
compared to a control birth cohort from 6 years prior to the pandemic (Machón et al., 1997).
Using maternal report and psychiatric hospital admission data to estimate exposure and later

diagnosis, Cannon found an increased relative risk (OR = 1.59, CI: 1.15 – 2.19) for depressive

disorder with maternal influenza infection (Cannon et al., 1996). However, as seems to be the

trend with ecological data, there is also data showing no association of *in utero* viral infection

and depression (Mino et al., 2000a; Pang et al., 2009). As a result, there is neither strong

evidence for or against an association between *in utero* infection and MDD risk in the offspring,

but perhaps appropriately powered future studies will provide clarification on this matter.

In utero exposure to maternal autoimmune diseases has been associated with autism and

schizophrenia risk, but no significant evaluation of maternal autoimmune disorders and MDD

has been performed thus far. Maternal history of celiac disease, type 1 diabetes, or rheumatoid

arthritis increases autism risk (Atladottir et al., 2009), whereas type 1 diabetes and thyrotoxicosis

have an increased frequency in mothers of schizophrenic patients (Gilvarry et al., 1996;

MacSweeney et al., 1978). In addition, familial clustering of autoimmune disorders is

considerably higher in relatives of autistic children (21% vs. 4%) (Comi et al., 1999). This also

holds true for schizophrenia: parents and siblings of schizophrenic patients have a significantly

higher frequency of autoimmune disorders (OR = 6.1, 95% CI: 2.3 – 16.5), especially type 1

diabetes (OR = 9.65, 95% CI: 1.3 – 429.2) (Wright et al., 1996). Other than causing a prolonged

pro-inflammatory state, it is currently unclear how these autoimmune disorders disrupt prenatal

neurodevelopmental processes to predispose to psychiatric disorders.

Maternal *in utero* psychosocial stress alters the inflammatory state the developing brain

and immune system are exposed to. Chronic stress leads to HPA axis hyperactivity followed by

insensitivity, elevated inflammatory markers, and heightened expression of NFκB-regulated

transcripts (Miller et al., 2008). As highlighted above by the landmark prenatal stress studies by Lubach and Coe, *in utero* exposure to high levels of glucocorticoids, even acutely, can permanently change the developing brain and immune system, especially with regards to how the offspring will respond to stressors in the environment (Coe and Lubach, 2005). A study of female children whose mothers experienced major negative life events during pregnancy supports the human relevance for Lubach and Coe's findings. Peripheral blood mononuclear cells isolated from prenatally stressed women had a Th2 bias (increased IL4 relative to IFNγ) as well as elevated IL6 and IL10 production after *in vitro* T cell stimulation, demonstrating altered immune function associated with prenatal psychosocial stress (Entringer et al., 2008).

Gestational psychosocial stress also increases risk for psychiatric disorders. Schizophrenia risk is increased with multiple forms of maternal psychosocial stress during gestation: bereavement, natural disaster, war, and not wanting the pregnancy (Brown, 2002; Huttunen and Niskanen, 1978; Markham and Koenig, 2011; Spauwen et al., 2004; Sullivan, 2005; van Os and Selten, 1998). Similarly, autism is diagnosed at higher frequency in individuals with gestational time overlapping with natural disasters like the Quebec ice storm or devastating hurricanes in Louisiana, with maximal impact suggested at midgestation (Beversdorf et al., 2005; Kinney et al., 2008; Walder et al., 2014). Although mothers of autistic children experience increased familial discord during gestation, maternal bereavement has not been associated with autism risk (Li et al., 2009; Rai et al., 2012; Ward, 1990). Few studies have evaluated the effects of prenatal stress on MDD, but severe maternal stress such as earthquakes and famine have been linked with increased MDD in offspring (Brown et al., 2000; Watson et al., 1999). Most of these studies simply measured increased incidence of diagnosis in populations that experience some

major stressor or with self-reported maternal stress, which limit the interpretations that can be made from this data. However, there is at least an increased incidence of events that increase psychosocial stress, which is known to alter maternal inflammation and immune function.

Severe stress in early life can be equally devastating to the developing brain and immune system. Analogous to prenatal stress, early life stress over activates the sympathetic nervous system and HPA axis, leading to prolonged and excessive inflammation (Fagundes et al., 2013; Kaiser and Sachser, 2005). Studies show altered immune function over the lifetime of individuals exposed to severe early childhood abuse, trauma, neglect, or psychosocial stress. These effects persist decades after the stressful event and include increased inflammatory response to acute stressors and basal elevation of C reactive protein, cytokine and chemokine levels, and NFκB activity (Carpenter et al., 2010; Danese et al., 2011; Danese et al., 2007; Kiecolt-Glaser et al., 2011; Pace et al., 2012; Slopen et al., 2010; Taylor et al., 2006). Interestingly, Danese and colleagues found that C reactive protein was only significantly higher in adolescents when they were both maltreated and depressed but not in depressed or maltreated alone groups. This suggests that childhood maltreatment interacts with genetic and/or other environmental risk factors to promote an underlying dysfunction (Danese et al., 2011; Fagundes et al., 2013). Early adversity has also been linked to altered development in brain regions associated with the HPA axis, autonomic control, and central response to inflammatory stimuli (Chiang et al., 2015). Most importantly, early childhood trauma has been strongly linked to increased risk for autism (Kerns et al., 2015; Roberts et al., 2015), MDD (Hayashi et al., 2015; Hovens et al., 2015; Lu et al., 2013), and schizophrenia (Baudin et al., 2016; Carr et al., 2013; Chae et al., 2015). The effects of childhood maltreatment can also be modified by both genetic

49

and other environmental risk factors to produce more severe disorder phenotypes (Aas et al., 2014; Fisher et al., 2013; Green et al., 2014; Toda et al., 2016). Altogether, both prenatal and early childhood severe stress have a lasting negative impact on brain and immune function.

Maternal obesity, excessive gestational weight gain, and metabolic disorders disrupt behavior and physiology of offspring (Maftei et al., 2015; Olson et al., 2009; Stice et al., 2011). Adipose tissue consists mostly of adipocytes and macrophages, which are capable of secreting bioactive lipids, cytokines, and leptin (satiety inducing, pro-inflammatory adipokine). In addition to the pro-inflammatory nature of adipocyte secretions, poorly controlled obesity and diabetes create a harmful feedforward loop: increased adiposity and hyperglycemia cause increased mitochondrial flux, which increases oxidative stress, causes additional increased inflammation, and further worsens glycemic control (Guest et al., 2007). Clinical studies suggest even higher inflammation when obese women are pregnant. This includes increased C reactive protein, IL6, IL8, IL1β, and MCP1 at midgestation with most no longer present at birth when compared to non-obese pregnant women (Christian and Porter, 2014; Friis et al., 2013). In addition, placenta from obese mothers show reduced vascular function as well as activation of pathways associated with increased oxidative stress and inflammation (Jansson et al., 2013; Stewart et al., 2007). A small cohort study also found maternal body mass index to positively correlate with increased maternal insulin, leptin, MCP1, and TNFα as well as increased fetal leptin. Increased maternal BMI was also associated with placental phosphorylation of NFκB signaling partners and IL1β levels, implicating both maternal and placental dysfunction in fetal effects (Aye et al., 2014; Martino et al., 2016). With such increased inflammation, it is not surprising that increased pre-pregnancy BMI and excessive gestational weight gain have been linked to increased risk for

autism (Bilder et al., 2013; Dodds et al., 2011; Gardner et al., 2015; Moss and Chugani, 2014; Reynolds et al., 2014), schizophrenia (Kawai et al., 2004; Schaefer et al., 2000), and MDD (Colman et al., 2012; Jones et al., 1998; Van Lieshout et al., 2013).

Maternal obesity often coincides with either type 2 or gestational diabetes (Martin et al., 2015; Whiteman et al., 2015). Actually, 1 in 10 pregnancies are complicated by glucose intolerance (DeSisto et al., 2014; Perkins et al., 2007). Gestational onset diabetes typically occurs in the second trimester from a combination of increased insulin resistance and inadequate pancreatic β cell compensation (Georgieff, 2006). Both gestational diabetes and type 2 diabetes increase risk for macrosomia, hypoglycemia, and hyperinsulinemia from islet cell hyperplasia at birth (Frias et al., 2007; Perkins et al., 2007). Chronic fetal hypoxia, polycythemia, brain-iron deficiency, and respiratory distress syndrome can also occur when the placenta is unable to provide the increased oxygen delivery required for the increased metabolic rate (Georgieff, 2006). As is the theme of the risk factors discussed, gestational diabetes is associated with dysregulation of inflammatory pathways in addition to the more obvious metabolic issues (Abell et al., 2015). Similar to and sometimes in conjunction with maternal obesity, gestational diabetes leads to an increased production of reactive oxygen species by hyperglycemia-induced increased electron transport in mitochondria (Nishikawa et al., 2000). Gestational diabetes has also been associated with increased leptin, IL6, and TNFα as well as decreased adiponectin (insulin-sensitizing adipokine) levels midgestation (Gao et al., 2008; Kautzky-Willer et al., 2001; Kinalski et al., 2005; Nergiz et al., 2014; Ortega-Senovilla et al., 2011; Park et al., 2013b; Vitoratos et al., 2001). In addition, placenta from gestationally diabetic mothers show pro-inflammatory DNA methylation and gene expression patterns, indicating a potential role for

placental inflammation in fetal disruption (Enquobahrie et al., 2009; Rong et al., 2015). Altogether, gestational diabetes is a maternal and placental pro-inflammatory state (Fasshauer et al., 2014; Jawerbaum and Gonzalez, 2006).

Accumulating evidence indicates diabetes during gestation is harmful to neurodevelopment. Adolescents with gestationally diabetic mothers have impaired motor proficiency and intellectual performance as well as increased inattention/hyperactivity, with severity usually correlating with lack of glycemic control (Ornoy et al., 1998; Ornoy et al., 2001; Ratzon et al., 2000; Rizzo et al., 1995; Silverman et al., 1998; Torres-Espinola et al., 2015). With regards to autism and maternal diabetes, a meta-analysis performed by Xu and colleagues found significant pooled odds ratios of 1.48 (95% CI: 1.25 – 1.75) and 1.72 (95% CI: 1.24-2.41) for 3 cohort and 9 case-control studies, respectively (Xu et al., 2014). A large retrospective cohort study of 322,323 singleton children born 1995-2009 at Kaiser's California hospitals confirmed this finding (Xiang et al., 2015). Furthermore, schizophrenia risk has been documented as increased with diabetes during gestation, with a meta-analysis by Mary Cannon finding a significant odds ratio of 7.76 (95% CI: 1.37 – 43.9) (Cannon et al., 2002a; Van Lieshout and Voruganti, 2008). Similarly, a study of 1051 offspring from 315 Finnish families found a 1.66-fold increase risk for developing schizophrenia with gestational diabetes exposure (Wegelius et al., 2011). To date, no studies have interrogated a role for diabetes during gestation in relative risk for offspring MDD. However, psychoneuroimmunology is a constantly evolving field. As more data suggests many inflammatory risk factors are shared between psychiatric disorders, it is highly likely that new associations will arise, possibly even increased MDD risk in children from gestational diabetic mothers.

In summation, clinical data overwhelming implicate inflammation-associated risk factors in promotion of psychiatric disorder susceptibility. From associated gene mutations in the MHC region to the higher frequency of psychiatric disorders with increased prenatal and early childhood inflammation, inflammation is involved in the pathogenesis of psychiatric disorders, especially schizophrenia, autism, and MDD (Berk et al., 2013; Patterson, 2011; Sekar et al., 2016). Clinical data may identify the factors involved, but preclinical data define the mechanism of inflammatory factors in pathology. These clinical findings have been used by our lab and others to focus studies on the most relevant models of inflammation, allowing us to delineate how inflammation pathologically alters neurodevelopment to lead to dysfunction later in life.

Inflammation-associated disruption of brain development and function in animal models

Environmental and genetic animal models of inflammation have distinct, psychiatrically relevant phenotypes. Each model sheds light on the role of that individual perturbation on brain and immune dysfunction. Preclinical models allow us to test hypotheses and mechanisms that present far too much risk to the developing fetus for evaluation in clinical studies. Although there are many more models of environmentally- and genetically-induced excess inflammation than are discussed in this document, we will focus on rodent models with the most relevance to the clinical risk factors discussed above. These include genetic models of excess cytokine signaling as well as environmental models of MIA, stress, obesity, and gestational diabetes.

Genetic induction of excess inflammation

Mouse models of transgenic overexpression of cytokines or deletion of their antagonists show the cytokine-specific consequences of chronic inflammation. Although these mutations are not seen in psychiatric disorders, these models provide insight into the potential roles of individual cytokines in pathological processes. This informs associations between neuropathology and immune dysfunction, shaping our interpretations into strong, evidence-based hypotheses.

Astrocyte-specific IL1β conditional overexpressing mice were developed by the O'Banion lab in 2007. Once the transgene was activated, these mice displayed months of astrocytic and microglial activation and an upregulation of pro-inflammatory cytokines (Shaftel et al., 2007b). In later studies, they confined the chronic overexpression to the hippocampus. Blood-brain barrier leakage, increased expression of MCP1 and adhesion molecules, and parenchymal infiltration of leukocytes was observed with no overt neuronal degeneration. A chronic increase in neutrophil numbers in the hippocampus was associated with increased chemokines like KC (Shaftel et al., 2007a). Many of the neuroinflammatory effects were attributable to increased prostaglandin E2 synthesis (Matousek et al., 2010). These mice also display hyperactivity and hippocampal-dependent memory deficits with hippocampal-independent fear memory remaining intact. In addition, reduced hippocampal volume, a structural MRI finding reported in both schizophrenia and MDD, was found after 6 but not 3 months of overexpression (Hein et al., 2012). Overall, this mouse model shows an IL1β-specific pattern of inflammation dependent on prostaglandin synthesis, which results in cognitive deficits and hippocampal volume loss.

Two lines of TNFα overexpressing mice were developed by the Kollias lab, one targeted to neurons and one to astrocytes. Unlike IL1β overexpressing mice, these lines were cell-specific but constitutively, not conditionally, expressed. Between postnatal weeks 3-8, all transgenic mice developed an inflammatory demyelinating disease, manifesting as ataxia, paresis, seizures, and eventual death. Both the brain and meninges had significant T lymphocyte infiltration, astrocytic and microglial activation, and focal demyelination (Probert et al., 1995). They were able to perform behavioral assessment of adolescent mice to evaluate the effects of this heightened inflammation on brain function. These adolescent mice displayed increased anxiety in the light/dark box and novel object exploration tasks, slower acquisition of passive avoidance behaviors in the hot-plate task, and impaired exploratory behavior in the hole-board task. Plus, these behaviors were particularly perturbed in more stressful conditions (Fiore et al., 1998; Fiore et al., 1996). In the astrocyte-specific TNFα overexpressing mice, the same inflammatory demyelinating disease followed by early death was observed (Akassoglou et al., 1997). The Campbell lab also established an astrocyte-specific TNFα overexpressing mouse line, which displayed a similar neurodegenerative phenotype. Surprisingly, they observed increased cell apoptosis only around leukocyte infiltrates in symptomatic mice, suggesting that TNFα indirectly induced apoptosis *in vivo* through recruited leukocytes. These mice also displayed increased expression of chemokines (e.g. RANTES, MCP1), adhesion molecules (e.g. VCAM), metalloproteases, and other cytokines (IL1) (Campbell et al., 1998). The severe neurodegeneration of TNFα overexpressing mice somewhat limits the interpretations that can be made in the context of psychiatric disorder pathology. However, these mouse models do display

an inflammatory profile and dysfunction distinct from IL1β, suggesting potential unique contributions of TNFα to structural and functional perturbations seen with chronic inflammation.

Neuron-specific IL6 overexpressing mice develop reactive astrocytosis and activated microglia but no overt pathology or early death (Fattori et al., 1995). These mice to have an exaggerated production TNFα, IL1β, and endogenous IL6 in the brain after intracerebroventricular injection of LPS as well as higher sensitivity to lower LPS doses as compared to wildtype mice (Di Santo et al., 1996). On the other hand, astrocyte-specific IL6 overexpressing mice have mild tremor, ataxia, leukocyte infiltration, blood-brain barrier breakdown, and overt neurodegeneration, a much more severe phenotype. Increased IL1, TNFα, RANTES, adhesion molecules, acute phase proteins, and complement C3 are also detected in the brain of astrocyte-specific IL6 overexpressing mice (Campbell et al., 2010; Chiang et al., 1994). In the hippocampus, these mice have dendritic vacuolization and reduced branching of dendritic spines in CA1 neurons (Campbell et al., 1993; Campbell et al., 1998; Chiang et al., 1994). In addition, hippocampal EEG shows anomalous paroxysmal discharges and suppressed theta rhythm (Steffensen et al., 1994). Likewise, impairment in hippocampal LTP, adult neurogenesis, HPA hyper-reactivity to stress, and learning deficits have been observed, supporting a prominent role for IL6 in psychiatric pathology especially in the hippocampus (Bellinger et al., 1995; Heyser et al., 1997; Raber et al., 1997; Vallieres et al., 2002).

Environmentally induced excess inflammation

Transgenic overexpression of cytokines induces brain dysfunction relevant to psychiatric disorder pathogenesis, but rarely is a single inflammatory marker increased by the clinical factors discussed above (i.e. *in utero* infection, maternal stress and obesity, and gestational diabetes). Environmental risk factors that induce inflammation are complex, having unique profiles of teratogenic inflammatory and non-inflammatory processes (Berk et al., 2013; Croonenberghs et al., 2002; Maes et al., 2009). Although single factor perturbation models (e.g. IL6 overexpressing mouse) can identify the role of certain cytokines, the best model for understanding inflammation's role in psychiatric disorder pathology is to mimic the environmental perturbation in a reliable, reproducible manner. Fortunately for the psychoneuroimmunology community, well-characterized models of environmental risk factors have been produced that mimic both the acute condition experienced by the mother as well as the altered neurodevelopmental processes likely occurring in the offspring. These include viral and bacterial infection mimetics, acute and chronic stress paradigms like social defeat, nutrient balanced high fat diet-induced obesity, and both diet- and drug-induced models of gestational diabetes (Brunton and Russell, 2010; Chandna et al., 2015; Garbett et al., 2012; Salbaum and Kappen, 2012). In this section, these models and their relevant findings will be reviewed.

In utero infection is a risk factor for several psychiatric disorders, with influenza being the most studied association (O'Callaghan et al., 1991; Simanek and Meier, 2015; Zerbo et al., 2015). Indeed, animal models of *in utero* influenza infection produce offspring with abnormal developmental trajectories, leading to impaired corticogenesis, brain atrophy, reduced hippocampal volume, decreased expression of GABAergic interneuron subpopulation markers, and abnormal behaviors responsive to antipsychotic treatment (Fatemi et al., 1999; Fatemi et al.,

2008; Kneeland and Fatemi, 2013; Moreno et al., 2011). However, review of epidemiologic studies shows numerous viral and bacterial pathogens are implicated in neurodevelopmental disruptions, questioning the importance of the pathogen itself (Atladóttir et al., 2010; Babulas et al., 2006; Blomstrom et al., 2015; Brown and Derkits, 2010). In fact, influenza virus is not detectable in the fetus after maternal infection, implicating the maternal immune response to the pathogen as the true insult (Shi et al., 2003; Shi et al., 2005). This has led to the use of bacterial (LPS) and viral (poly(I:C)) mimetics, stimulating maternal immune activation in the absence of live pathogens. These mimetics have proven to produce a more consistent maternal response and much of the prenatal infection literature has utilized these models (Meyer and Feldon, 2012).

Poly(I:C) is a synthetic double stranded RNA that activates an acute but significant antiviral immune response. Double stranded RNA is recognized by TLR3 as a viral replication intermediate, leading to an innate immune response that includes NFκB activation and pro-inflammatory cytokine expression (Meyer, 2014; Meyer and Feldon, 2012). Consistent with viral infection, poly(I:C) induces acute fever, sickness behavior, and weight loss that resolves in 24-48 hours (Patterson, 2009). Comparable to clinical *in utero* infection data discussed above, poly(I:C) has the greatest effect midgestation, with most studies inducing sickness between gestational day (GD) 6 and 14 in rodents (Meyer et al., 2006; Meyer et al., 2007). Thus, poly(I:C) effectively produces an antiviral response that mimics the intrauterine environment of *in utero* viral infection and has become a valuable tool in psychoneuroimmunology research.

The acute, pro-inflammatory changes induced by poly(I:C) affect the inflammatory state and developmental processes in the fetal brain. In the presence of poly(I:C), neurogenesis and

neuronal migration experience transient delays at 1-2 days post-injection that "catch up" within a couple days (De Miranda et al., 2010; Soumiya et al., 2011b). Even though this disruption is transient, the developing brain relies on precise timing of developmental events. Changing the time and rate at which neurons are proliferating and migrating can completely change the factors these neurons are exposed to as well as their final position and fate in the brain (Garey, 2010; Metin et al., 2008). A second study by Soumiya and colleagues clearly demonstrates the potential harm of transient delay. Offspring exposed midgestation to poly(I:C) have histologically normal appearing cortical laminar organization. However, staining for layer specific pyramidal cell markers shows a 15-25% decrease in layer II-IV markers (Cux1 and Brn1) at postnatal day (PND) 10. They also demonstrated a decrease in axosomatic synapses, which are typically inhibitory input, and an increase in spines in layer III pyramidal cells, suggesting an increase in the excitatory to inhibitory signaling ratio (Soumiya et al., 2011a). At the same time, subcortical structures are also altered by poly(I:C). Paul Patterson's lab found cholinergic signaling in the fetal basal forebrain to be altered (Pratt et al., 2013), whereas Urs Meyer demonstrated increased midbrain dopaminergic cell populations at mid- to late-gestation (Meyer et al., 2008; Vuillermot et al., 2010).

Studies from our lab and others have measured poly(I:C) induced changes in the fetal brain more globally. For example, Khalil and colleagues found poly(I:C) treatment altered developmentally important protein levels across the whole brain, including GluN2B, doublecortin, and the netrin 1 receptor (Khalil et al., 2013). A collaborative project between our lab and Paul Patterson's lab characterized the expanse of poly(I:C) induced gene expression changes and specifically identified IL6 as the main instigator of poly(I:C) induced fetal

disruption. Midgestation treatment with either live influenza virus, poly(I:C), or recombinant IL6 induced similar, profound gene expression changes in the fetal brain. Interestingly, the expression profile suggests induction of a neuroprotective response with the unintended consequence of disrupting neurodevelopmental processes, which is in line with the reported transience of developmental disruptions observed by Soumiya and others above (Garbett et al., 2012).

Prenatal poly(I:C) treatment studies have demonstrated molecular, structural, and behavioral abnormalities reminiscent of psychiatric disorder pathology in the offspring. For example, exposure to poly(I:C) *in utero* has been associated with regionally specific increases in dopamine and decreases in serotonin in the adult brain (Winter et al., 2009). On a more global scale, proteomic analysis of adolescent and adult offspring has implicated AKT, GSK3β, synaptic proteins, myelination proteins, and heat shock proteins amongst many others to be disrupted by prenatal poly(I:C) exposure (Deng et al., 2011; Forrest et al., 2012; Khalil et al., 2013; Makinodan et al., 2008; Willi et al., 2013). Interestingly, GSK3β expression is also altered by antipsychotics (Alimohamad et al., 2005; Freyberg et al., 2010), and treatment with a selective GSK3β inhibitor rescued working memory deficits and hyperresponsiveness to amphetamine in adults exposed prenatally to poly(I:C) (Willi et al., 2013). In addition to these impairments, behavioral dysfunctions have been reported from early postnatal period to adulthood. As neonates, offspring have impaired ultrasonic vocalizations and delayed development of righting, geotaxis, and grasping reflexes (Arsenault et al., 2014; Malkova et al., 2012). During adolescence but not adulthood, poly(I:C) associated impairments in cerebellar architecture co-occur with decreased social preferences as well as poor motor coordination in the

rotarod and ladder rung tests (Aavani et al., 2015). Pineda and colleagues also observed

decreased sociability in adolescents in addition to an increased hippocampal excitability and

accelerated kindling rate (Pineda et al., 2013). Together, these studies support an alteration in

developmental trajectory, with unique alterations observable as the offspring mature.

Consistent with many psychiatric disorders, several of the behavioral disruptions

observed with poly(I:C) induced MIA are observed in adulthood. These include deficiencies in

emotional and social communication, sensorimotor gating, and working memory in addition to

increased anxiety, depressive, and repetitive behaviors (Connor et al., 2012; Khan et al., 2014;

Malkova et al., 2012; Shi et al., 2003; Soumiya et al., 2011a; Yee et al., 2012). Significantly,

many of these behaviors have been associated with brain pathology in MIA offspring. A study by

Coiro and colleagues found decreased number and turnover rates of cortical dendritic spines in

both adolescent and adult MIA offspring. This spine decrease was associated with increased

repetitive behaviors as well as with increased excitatory and decreased inhibitory signaling.

Coiro concluded that the repetitive behaviors resulted from an excitation/inhibition imbalance in

the cortex, which are both observed in autism (Coiro et al., 2015; Patterson, 2009). In addition,

the hippocampus, which is of considerable relevance to many psychiatric disorders, is

particularly affected in MIA offspring. Decreased adult neurogenesis and hippocampal volume,

impaired LTP and paired-pulse facilitation, and deficient hippocampal dependent learning and

memory are seen in adult MIA offspring (Khan et al., 2014; Zhang and van Praag, 2015). Most

importantly, blocking NFκB or IL6 signaling during the acute response to MIA ameliorates the

epileptic phenotype, PPI and learning and memory deficits, altered exploratory/social behavior,

and disrupted gene expression patterns observed in adult MIA offspring (Pineda et al., 2013;

Smith et al., 2007; Song et al., 2011). Thus, MIA adult offspring display both cortical and

hippocampal associated molecular and behavioral dysfunction that is strongly tied to MIA-

induced increases in IL6 and NFκB.

Prenatal poly(I:C) treatment has long lasting effects on immune development as well as

the brain, which likely exacerbates the neurodevelopmental disruptions. From the acute *in utero*

immune response to adulthood, cytokine levels in the brain of the offspring continue to change.

High levels of many inflammatory markers are acutely increased in the fetal brain, including

IL1β, IL7, IL13, and MCP1 (Arrode-Bruses and Bruses, 2012). Garay and colleagues measured

longitudinal inflammatory marker changes in frontal cortex, cingulate cortex, and the

hippocampus. They observed a general pattern of increased cytokines and chemokines at birth,

which decrease in adolescence then only to peak again in early adulthood (Garay et al., 2013).

Interestingly, these MIA-induced central immune changes are limited, with differences rarely

observed in microglial activation, blood-brain barrier integrity, or leukocyte infiltration in the

adult brain (Garay et al., 2013; Smolders et al., 2015). In addition, offspring present with a

generalized, pro-inflammatory state peripherally. Adult MIA offspring show exaggerated cellular

activation and cytokine production after immune stimuli like the TLR2 agonist zymosan (Mandal

et al., 2013). Furthermore, bone marrow macrophages from 10 week old MIA offspring produce

increased chemokines and cytokines with *in vitro* LPS stimulation (Onore et al., 2014). In

summation, MIA induces central and peripheral immune changes that last long after the acute

insult, potentially contributing to the continually evolving brain dysfunction observed throughout

the postnatal period in MIA offspring.

LPS is a widely used bacterial infection mimetic, which induces an innate immune response via TLR4 activation (Akira and Takeda, 2004). The maternal immune response is very similar to that of poly(I:C) with a few notable dissimilarities. Poly(I:C) induces higher IFN expression whereas LPS more effectively stimulates TNFα expression, leading to more severe maternal fever and sickness behaviors as well as fetal neurotoxic effects with LPS (Clark, 2007; Hopwood et al., 2009; Kimura et al., 1994). With regards to differences observed in offspring, prenatal LPS actually decreases the number of midbrain dopaminergic neurons unlike the increased number observed in prenatal poly(I:C) offspring (Carvey et al., 2003; Vuillermot et al., 2010). Yet on the whole, many behavioral abnormalities observed with prenatal LPS exposure are similar to that seen with poly(I:C). These include deficits in sensorimotor gating, increased sensitivity to amphetamine-induced locomotion, increased anxiety and depressive behaviors, and impaired social interactions and learning (Borrell et al., 2002; Enayati et al., 2012; Fortier et al., 2004; Romero et al., 2006). There are also common brain pathologies reported with prenatal LPS and poly(I:C) exposure, including reduced adult hippocampal neurogenesis and GluN1 expression (Escobar et al., 2011; Lin and Wang, 2014). Both poly(I:C) and LPS induced MIA produce brain pathology and altered behaviors in the offspring associated with psychiatric disorders, serving as reliable, reproducible animal models to study the effects of prenatal infection on psychiatric disorder pathogenesis.

One of the less obvious inflammatory factors (prenatal stress) ramps up both pro-inflammatory factors and anti-inflammatory HPA axis activity in rodents (Merlot et al., 2008). The increase in pro-inflammatory cytokine production is likely at least somewhat related to the decrease in maternal progesterone, increasing Th1 cytokine (i.e. TNFα, IFNγ, and IL12)

production in the placenta (Blois et al., 2004; Joachim et al., 2003). In addition, HPA axis activation increases maternal glucocorticoids, which readily cross the placenta when in excess. Glucocorticoid excess alters fetal immune and HPA axis development, and both immunosuppressive glucocorticoid excess and increased cytokine levels during pregnancy alter fetal neurodevelopment, making prenatal stress a uniquely effective disruption (Merlot et al., 2008). Altogether, this produces a phenotype in the offspring of heightened HPA and immune stress reactivity, altered brain structure, and disrupted learning and emotional behaviors, all relevant features to psychiatric dysfunction (Merlot et al., 2008).

Prenatally stressed rodents are hyperreactive to stressful situations. Acute stress induces greater, more prolonged HPA axis activity in prenatally stressed offspring. This prolonged response is theorized to result from a lack of negative feedback due to decreased glucocorticoid receptor expression in in the hippocampus (Brunton and Russell, 2010; Henry et al., 1994). Maternal restraint stress increases CD8+ T lymphocytes and natural killer cells as well as IFNγ expression in adult offspring (Vanbesien-Mailliot et al., 2007). In addition, prenatally stressed offspring display a heightened basal inflammatory state in the brain as well as increased hippocampal cytokine expression and activation of microglia and astrocytes with immune stimuli (Diz-Chaves et al., 2013; Szczesny et al., 2014). Contrary to this data, there are also reports of immunosuppression in prenatally stressed offspring, suggesting variable effects on immune status possibly due to gestational timing, stress model, and species (Merlot et al., 2008).

The immunologic effects of prenatal stress on the offspring may be variable, but molecular and cellular changes in the brain as well as behavioral disruptions have been

repeatedly reported in prenatally stressed offspring. Anxiogenic behavior in adult offspring from prenatally stressed dams has been reported in the elevated plus maze and light-dark box (Brunton and Russell, 2010; Fride and Weinstock, 1988; Richardson et al., 2006). Interestingly, depressive behaviors are also increased with prenatal stress exposure (Mueller and Bale, 2008; Szymanska et al., 2009). Spatial learning and memory as well as working memory task deficits have also been observed (Gue et al., 2004; Lemaire et al., 2000; Markham et al., 2010). Yet sensorimotor gating deficits are only found in prenatal stress exposure models in which the dam cannot habituate to the stressor, emphasizing that different forms of prenatal stress can produce distinct behavioral phenotypes (Koenig et al., 2005; Lehmann et al., 2000). Electrophysiologically, increased excitatory/inhibitory ratio within the hippocampus, a dysfunction relevant to both schizophrenia and autism, occurs in prenatally stressed offspring, indicated by altered expression and activation of NMDA receptors (Jia et al., 2010; Yaka et al., 2007). These observations are paired with decreased dendritic spine density as well as a diminution in LTP. Furthermore, adult neurogenesis and BDNF expression are decreased in the hippocampus of prenatally stressed offspring, which is highly relevant to MDD pathology (Lemaire et al., 2000; Neeley et al., 2011; Yaka et al., 2007). With regards to dopamine, there is an over-activation of midbrain dopamine systems and enhancement of psychostimulant-induced locomotion in prenatally stressed offspring (Fride and Weinstock, 1988; Koenig et al., 2005; Silvagni et al., 2008). However, the dopaminergic changes in prenatally stressed rodents are intriguingly complicated. Carboni and colleagues observed decreased amphetamine-induced dopamine release in the medial frontal cortex of adolescent rats, suggestive of a hypodopaminergic cortical state like that observed in schizophrenia (Carboni et al., 2010; Markham and Koenig, 2011). Taken together, these data describe a molecular, structural, behavioral, and immunological impaired phenotype associated

with prenatal stress exposure, likely attributable to increased inflammation and glucocorticoids *in utero.*

Obesity is a low-grade inflammatory state (Friis et al., 2013). Excessive weight gain leads to adipocyte hyperplasia, which also leads to increased secretion of pro-inflammatory adipokines and cytokines. Consequently, macrophage and T lymphocyte populations in adipose tissue increase, gut permeability decreases, and the gut microbiome are altered with accumulation of adipose tissue (Castanon et al., 2015). The clinical studies discussed above implicate high pre-pregnancy BMI and excessive gestational weight gain in psychiatric disorder risk (Bilder et al., 2013; Kawai et al., 2004; Olson et al., 2009). The biological factors mediating risk to the developing fetus include excessive nutrients (e.g. glucose), hormones (e.g. insulin, leptin), and inflammatory markers (e.g. cytokines) (Rivera et al., 2015). In preclinical studies, animal models of maternal obesity due to high-fat diet concur with clinical data, suggesting increased risk for dysfunction in numerous domains, including neurodevelopment (Kang et al., 2014; Sasaki et al., 2013).

High fat diet produces obesity in rodents. Most researchers utilize a 32% to 60% fat by calorie food, which also includes sufficient vitamins and nutrients, for 4-20 weeks, producing a steady increase in weight over time without overt nutritional deficiencies (Pasek and Gannon, 2013). The offspring from high fat fed dams have significant molecular and behavioral alterations suggestive of increased psychiatric disorder risk. Behaviorally, offspring of obese dams demonstrate increased depressive and anxiety-like behaviors, decreased sociability, hyperactivity, and impaired cognition (Bilbo and Tsang, 2010; Can et al., 2012; Kang et al.,

2014; Sasaki et al., 2013; Tozuka et al., 2010). Scarily, high fat diet exposure during lactation alone is sufficient to alter depressive and anxiety-like behaviors, suggesting many points of vulnerability to high fat diet (Sasaki et al., 2013). With regards to ongoing inflammation, adult female offspring from high fat fed dams have chronic increases in inflammation, indicated by increased IL1β and TNFα as well as microglial activation (Kang et al., 2014). Interestingly, the hippocampus, like with many other models of prenatal inflammation, is disrupted by maternal obesity. In a study from the Wada lab, dams fed a 32% high fat diet from 6 weeks prior to mating through lactation had excessive weight gain as well as increased triglycerides and free fatty acids. At weaning, the offspring had increased hippocampal protein oxidation paired with decreased BDNF expression. Hippocampal pyramidal cell dendrites displayed decreased branching, and hippocampal-dependent spatial memory was impaired in these offspring (Tozuka et al., 2010). Increased hippocampal inflammation in adult offspring was also observed by Bilbo and Tsang, highly implicating obesity and maternal high fat diet in hippocampal dysfunction (Bilbo and Tsang, 2010). In total, maternal high fat diet and obesity produce offspring with heightened brain inflammation, disrupted hippocampal development, and an anxiogenic, depressive, cognitively impaired behavioral phenotype that supports a role of maternal obesity in neurodevelopmental disruption.

It is difficult to separate rodent models of maternal obesity from models of gestational diabetes. Maternal obesity is a state of metabolic dysfunction and excessive inflammation, both of which contribute to insulin resistance, diabetes, and further inflammation (Abell et al., 2015). This creates a vicious cycle with metabolic dysfunction and excessive inflammation promoting each other. Not surprisingly, hyperglycemia, hyperinsulinemia, and hyperleptinemia are

associated with rodent models of obesity, which greatly increase risk for gestational diabetes (Gallou-Kabani et al., 2007; Rivera et al., 2015). In fact, leptin insensitivity has been reported after as few as 16 days of high fat (45% by calorie) diet while mild glucose intolerance has been observed after only 28 days of high fat (60% by calorie) diet in mice (Golson et al., 2010; Van Heek et al., 1997). This makes high fat diet a physiologically relevant, effective model for studying the effect of gestational diabetes on offspring development.

In the context of predisposing factors such as maternal obesity and unhealthy diet, gestational diabetes often inevitably develops (Martin et al., 2015; Whiteman et al., 2015). Increased maternal glucose and free fatty acid production to accommodate the developing fetus combined with heightened inflammation produce insulin insensitivity. Most pregnant females are able to compensate with pancreatic beta cell hyperplasia and hypertrophy and increased insulin production, leading to a hyperinsulinemic, normoglycemic state (Pasek and Gannon, 2013). However, when the factors propagating insulin resistance cannot be overcome by maternal compensation, gestational diabetes develops- a hyperinsulinemic, hyperglycemic state that often resolves postpartum (Georgieff, 2006). For the developing fetus, this translates into increases in both inflammatory (e.g. cytokines) and metabolic (e.g. insulin, leptin, glucose, free fatty acids) factors that either act at the placenta or freely cross the placenta to disrupt normal development (Silverman et al., 1998).

There are many rodent models of gestational diabetes. Many studies of disrupted neurodevelopment utilize drug-induced (streptozotocin- STZ) or genetic models of diabetes during gestation. STZ destroys pancreatic beta cells, producing a type 1 diabetic phenotype

(López-Soldado and Herrera, 2003). Genetic models that inhibit maternal beta cell compensation do reliably produce gestational diabetes, but these mutations are rarely if ever found in gestational diabetic women (Pasek and Gannon, 2013). Even though high fat diet induction of gestational diabetes is by far the most clinically relevant model of gestational diabetes, results from other models are still critical for understanding the effects of maternal hyperglycemia on the developing fetus.

Offspring of gestational diabetic mice show increased inflammation and altered neurodevelopment. During late gestation and early postnatal periods, there are varying reports of increased or decreased offspring size, with STZ decreasing or increasing size and high fat generally increasing size (Cerf et al., 2010; Jones et al., 2009; Li et al., 2013; Plagemann et al., 1998a; Tang et al., 2015; Tozuka et al., 2009). Adult offspring, especially females, often present with increased body weight, hyperinsulinemia, hyperglycemia, hypertension, and higher serum levels of triglycerides and C reactive protein (Elahi et al., 2009; Liang et al., 2009; Plagemann et al., 1998b). With regards to neurodevelopmental effects, heightened brain inflammation, disrupted neurocircuitry, and altered metabolic signaling have been observed. Reports of increased neural tube defects and cranial malformations have been reported, but these studies utilized either high dose STZ or longer than typical (15 weeks) high fat diet regimens prior to mating (Jiang et al., 2008; Lian et al., 2004; Liao et al., 2004; Tang et al., 2015). Interestingly, multiple groups report increased expression and/or activity of cytokines, NFκB, and proteins related to hypoxic response and oxidative stress (Chandna et al., 2015; Lian et al., 2004; Melo et al., 2014; Tang et al., 2015; Wu et al., 2015). Of particular interest, both the fetal brain and the placenta show high levels of RAGE, MCP1, and NFκB protein (Chandna et al., 2015; Li et al.,

2013; Tang et al., 2015). Evidence also suggests the placenta is hypoxic, cellularly disrupted, and inflamed with compromised clearance of protein and glucose (Jones et al., 2009; Li et al., 2013; Liang et al., 2010). Thus, the placenta likely plays a considerable role in the effects of gestational diabetes on the fetal brain.

Considering the metabolic dysfunction in these models, it is not surprising that the brain of offspring has altered metabolic pathways. For example, GLUT2, insulin-like growth factor 1 receptor, and insulin receptor expression are increased in early postnatal offspring (Aerts and Van Assche, 2001; Cerf et al., 2010). Due to the importance of the hypothalamus in feeding behavior, its disruption in gestational diabetic offspring has been particularly well characterized. Norepinephrine is increased whereas the ratio of norepinephrine to dopamine is decreased in the offspring's hypothalamus, likely associated with the increase in tyrosine hydroxylase expressing cells in the paraventricular nucleus of the hypothalamus (Plagemann et al., 1998a). In addition, some hypothalamic neuron subpopulations fail to make appropriate connections with their targets, and the arcuate nucleus has increased numbers of NPY expressing neurons, demonstrating clear disruption of hypothalamic circuitry in offspring of gestational diabetic dams (Plagemann et al., 1998b; Vogt et al., 2014). These maldeveloped systems most likely contribute to the metabolic phenotype observed in the offspring.

Disruption of hippocampal development occurs with gestational diabetes exposure. Inflammation in the hippocampus is persistently increased in offspring, evident by increases in RAGE, NFκB, protein oxidation, and lipid peroxidation (Chandna et al., 2015; Tozuka et al., 2010; Tozuka et al., 2009). In addition, decreased hippocampal pyramidal cell branching,

decreased numbers of hippocampal pyramidal cells, and impaired performance in the Barnes maze spatial task suggest impaired hippocampal function (Golalipour et al., 2012; Tozuka et al., 2010). In a study by Chandna and colleagues, increased excitability and a more hyperpolarized resting potential was observed in primary hippocampal cultures. They also found gestational diabetic offspring have sensorimotor gating deficits, increased novelty seeking, and difficulty changing strategies in the reversal learning task, which are together highly suggestive of an abnormal phenotype relevant to schizophrenia and autism (Chandna et al., 2015). Of greatest importance, studies that controlled hyperglycemia with insulin or metformin treatment observed significant attenuation of these phenotypes, demonstrating that the severity of these neurodevelopmental defects is not set in stone but can be identified and addressed (Chandna et al., 2015; Jing et al., 2014; Wu et al., 2015).

The environmental risk factors for psychiatric disorders that produce excessive inflammation are numerous, complex, and frequently occur in the general population (Berk et al., 2013; Chiang et al., 2015; Muller, 2014; Slavich and Irwin, 2014). This presents a daunting task for researchers who seek to understand how these *in utero* and early childhood risk factors impact the developing brain and immune system. However, many groups have painstakingly characterized the perturbed state in preclinical models of inflammatory risk factors, allowing for a deeper understanding of the dysfunctions they create as well as the specific factors associated with those dysfunctions (Meyer, 2014; Tozuka et al., 2010; Tozuka et al., 2009; Voorhees et al., 2013). Yet it is so easy for researchers to forget that rarely do these risk factors occur in isolation and almost never do psychiatric disorders develop without additional accumulated risk. Combining multiple, commonly co-occurring genetic and/or environmental risk factors brings

these models of inflammatory risk factors closer to a truly clinically relevant scenario (Wermter et al., 2010). Some groups have already recognized this crucial point. For example, human tissue cultures with intact baseline genetic mutations can demonstrate the effects of environmental exposure in the context of a patient's associated genetic background, which is the basis behind studies of patient peripheral blood mononuclear cell and dermal fibroblast cultures (Czarny et al., 2015; Lisi et al., 2013; Manier et al., 2000). With regards to animal models, groups have observed peripubertal stress combined with *in utero* poly(I:C) exposure causes more severe effects as well as effects at lower poly(I:C) concentrations. The phenotypes found to be more affected include dopaminergic circuitry, central inflammation, and behaviors relevant to psychiatric disorders such as sensorimotor gating, anxiety, and psychostimulant response (Deslauriers et al., 2013; Giovanoli et al., 2016; Giovanoli et al., 2013). The results of these studies should direct the field's attention to the importance of accumulated risk in both clinical and preclinical models of psychiatric disorder risk factors. A shift towards combined risk factors will help our lab and others to better understand inflammation in both psychiatric disorder susceptibility and continued progression/severity.

Hypotheses

The immune system and brain are intricately intertwined in both development and adult function. Unfortunately, this relationship can lead to dysfunction when balance in either system is disturbed. Psychoneuroimmunologists have long believed that altered or inappropriately timed immune function and inflammation can disrupt neurodevelopment and predispose to psychiatric disorders, a belief strongly grounded in clinical and preclinical data (Cohly and Panja, 2005;

Golan et al., 2005; Kneeland and Fatemi, 2013; Leboyer et al., 2016; Van Lieshout and Voruganti, 2008). This inflammation does not have to be chronic nor excessive to predispose to psychiatric disorders but depends greatly on the genetic and other environmental factors it is combined with (Berk et al., 2013; Derry et al., 2015). In most cases, clinical data implicate increased inflammation and altered immune function in individuals with psychiatric disorders like MDD, autism, and schizophrenia (Maes et al., 2009; Patterson, 2009). Genetic studies associate mutations within the MHC locus (*HLA* genes) with increased frequency of autism and schizophrenia diagnosis (Lee et al., 2013; Mukherjee et al., 2014; Torres et al., 2006). Furthermore, epidemiologic studies link *in utero* and early childhood environmental risk factors associated with increased inflammation to MDD, autism, and schizophrenia risk (Maes et al., 2009; Michel et al., 2012). Along the same lines, animal models of prenatal and early postnatal inflammation identify vulnerable brain regions (e.g. hippocampus) and developmental processes affected by these risk factors (Diz-Chaves et al., 2013; Fatemi et al., 2008; Moreno et al., 2011; Szczesny et al., 2014). Lastly, animal models of combined inflammation-associated risk factors demonstrate that these frequently co-occurring risk factors can be modified to create unique phenotypes, emphasizing the importance of accumulated risk in advancing our understanding of inflammation and psychiatric disorders (Deslauriers et al., 2013; Giovanoli et al., 2016).

While each of the discussed clinical and preclinical models are valuable targets of study, our lab has chosen to focus on accumulated risk. We seek to uncover the role of inflammation in both psychiatric disorder pathogenesis and ongoing disease processes, especially in models of combined genetic and/or environmental risk factors. **Our central hypothesis is that the impact of inflammation-associated psychiatric disorder risk factors is modified by altered genetic**

or environmental context. In this document, we apply this central hypothesis to both gene x environment interactions as well as environment x environment interactions. More specifically, we proposed that gene x environment interactions in MDD uncover an altered gene expression phenotype that is driven by IL6. We were led to this hypothesis by the development of MDD in only a subpopulation of those treated with high doses of exogenous cytokines, suggesting interaction with some other predisposing factor- like a higher risk genetic profile- mediates the effects of cytokines on MDD development (Bonaccorso et al., 2001; Reichenberg et al., 2005). We further hypothesized IL6 would likely produce this novel phenotype due to evidence of chronic alterations of IL6 in MDD and the significant brain structure/function perturbations attributable to IL6 in preclinical studies (Lisi et al., 2013; Maes et al., 1997; Maes et al., 1995; Pace et al., 2006; Voorhees et al., 2013).

With regards to environment x environment interaction, we proposed that gestational diabetes modifies MIA-induced relative gene expression changes in the fetal brain. We hypothesized that these modified gene expression changes are both transient in nature and associated with both increases in cytokines and adipokines. We based these hypotheses on several pieces of data. First, both MIA and gestational diabetes increase inflammation (Abell et al., 2015; Deverman and Patterson, 2009). Second, both perturbations have their peak influence midgestation (Buka et al., 2001a; Xiang et al., 2015). Third, both perturbations can alter cytokine levels, such as IL6, that can cross the placenta and affect the developing fetal brain (Hsiao and Patterson, 2011; Hsiao and Patterson, 2012; Li et al., 2013). And lastly, both gestational diabetes and MIA have been linked to increased occurrence of autism and schizophrenia in birth cohort studies (Atladóttir et al., 2010; Cannon et al., 2002a; Lee et al., 2015; Van Lieshout and

Voruganti, 2008; Xiang et al., 2015). Further understanding of inflammation in these domains focuses attention on when and what inflammatory factors should be addressed to prevent and/or lessen severity of psychiatric disorders.

Experiments in Chapters 2 and 3 will utilize two distinct but equally important models to address these hypotheses. Chapter 2 investigates the role of cumulative genetic predisposition in the response to excessive inflammation. HDFs derived from control and MDD patients are used to investigate the role of genetic predisposition in gene expression response to exogenous cytokines, allowing us to study the role of genetic changes in environmental increases in inflammatory markers. This data furthers our knowledge of how gene x environment interactions can contribute to both susceptibility and severity of MDD as well as other psychiatric disorders.

In Chapter 3, rodent models of diet-induced gestational diabetes and maternal immune activation via poly(I:C) are combined to investigate the significance of accumulated risk with these two inflammation-inducing psychiatric disorder risk factors. Characterization of the maternal and intrauterine environment highlight the unique fetal exposure created by this combination whereas fetal brain gene expression changes demonstrate a distinct transcriptomic response to these accumulating risk factors. The importance of these findings and their relationship to the groundwork laid by the studies reviewed above are discussed in great detail in Chapter 4.

CHAPTER II

AN ALTERED PERIPHERAL IL6 RESPONSE IN MAJOR DEPRESSIVE DISORDER

Kelli M. Money, Zita Olah, Zeljka Korade, Krassimira A. Garbett, Richard C. Shelton, and

Karoly Mirnics

Introduction

It is generally accepted that low grade, chronic inflammation contributes to pathology in at least a subset of MDD patients. However, not every individual with chronic inflammation develops depression. As suggested by studies of depressive symptom development with IFN treatment, chronic inflammation likely interacts with additional risk factors (e.g. genetic or epigenetic encoded risk) to produce a depressive phenotype (Reichenberg et al., 2005). To test the hypothesis that MDD patient genetics modify response to inflammation, we acutely treated MDD patient- and control-derived HDFs with cytokine (IL6, IL1β, or TNFα) or vehicle. RNA isolated from HDFs was then synthesized into cDNA, and quantitative PCR was used to determine the relative expression of several groups of inflammation-responsive genes.

Methods

Patient population

This study was approved by the Vanderbilt University Institutional Review Board. The diagnosis of each patient was set as current major depressive episode according to the Structured Clinical Interview for DSM-IV-TR (First et al., 2002). Exclusion criteria included other primary DSM-IV-TR (Association, 2000) diagnoses, a history of bipolar disorder or non-mood psychotic disorder, known systemic inflammatory disorder or unstable medical condition- any condition that would preclude the biopsy (e.g., hemophilia) (Garbett et al., 2015b; Manier et al., 2000). Written informed consent was obtained from all the participants. HDFs were generated from samples taken from seven female MDD patients and seven age-matched female controls (CTR). Demographic data are presented in **Supplementary Table 1**.

Harvesting and culturing HDFs

Harvesting procedures and culturing methods have been described previously (Garbett et al., 2015a). The HDF cultures underwent 5-10 passages in full Dulbecco`s Modified Eagle`s medium. The medium contained 250 mM glucose, 1mM sodium pyruvate, 2mM L-glutamine, antibiotic/antimycotic solution, and 10% fetal bovine serum. Cultures were kept on 37°C at 5% CO_2 in 100 mm, non-coated plastic flasks with $0.75\text{-}1 \times 10^6$ starting density. Culture maintenance was performed as previously described (Garbett et al., 2015b; Manier et al., 2000).

Cytokine exposure and RNA collection/isolation

Briefly, when HDF cultures reached 12-16 million cells/sample, they were subcultured and plated onto 60 mm non-coated plastic plates with a starting cell density of $0.7\text{-}1.2\text{x}10^6$. Treatment was always performed a day after the subculturing, after an overnight adherence. To eliminate the effects of cytokines that might be present in the serum-containing media, all cytokine stimulations were performed in serum-free media. All the cytokine and vehicle treatments lasted for 8 hours in 6 mL Dulbecco`s Modified Eagle`s medium containing 250 mM glucose and 1mM sodium pyruvate, which was supplemented with 2mM L-glutamine and antibiotic/antimycotic solution. Treatment conditions were either 20 ng/mL IL6, 30 ng/mL IL1β, 20 ng/mL TNFα, or media with no cytokine added. Dose was based on concentrations used in previously published cell culture studies (Guo et al., 2006; Liu et al., 2011b; Niemand et al., 2003; van Zuiden et al., 2012). After the treatment, samples were washed twice with 5 mL ice-cold PBS then collected in 1.5 ml Trizol and stored -20°C for 24 hours until RNA was isolated, following the protocol given by the manufacturer. For each sample and treatment, RNA was isolated from ~1,000,000 cells.

cDNA synthesis and quantitative Real Time-PCR

The concentration of isolated RNA was measured by Nanodrop. Agilent 2100 Bioanalyzer was used to determine the quality of the samples. Total RNA integrity for RNA samples was between 9.5 and 10. From 1000 ng of the previously isolated RNA, cDNA was generated with random primers using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The primers used for quantitative Real Time PCR (qPCR) are shown in **Supplementary Table 2**. Primer3 (http://biotools.umassmed.edu/bioapps/primer3_www.cgi)

was used to design primers. Each primer pair was run in at least triplicate for each sample. No

reverse transcriptase cDNA reactions were used to verify the lack of genomic DNA

contamination in each RNA sample. The cycle at which the PCR amplification product reached

the detection threshold (Ct) was obtained for each gene. Scaled Ct (normalized to *PGK1*) and

ΔΔCt (normalized to *PGK1* expression and vehicle condition) were calculated for each sample as

previously published (Arion et al., 2007; Garbett et al., 2012; Kalman et al., 2014).

Statistical approaches

Average expression values for each group (MDD and CTR) were calculated for each

gene tested. Gene expression difference between groups was considered significant at $p < 0.05$ in

a two-tailed, unpaired student t test. Difference in gene expression between treatment conditions

within a group was considered significant at $p < 0.05$ in a two-tailed, paired student t test.

Results

After 8 hours of vehicle treatment, there was also no difference in baseline gene

expression between MDD and CTR in any of the 46 target genes tested (**Figure 1,**

Supplementary Table 3). The target gene set focused on aberrant cellular functions associated

with inflammation in the context of MDD and included genes that play a role in tryptophan

metabolism, lipid metabolism, mitochondrial function, and oxidative stress response. The target

genes were chosen based on pathways found to be disrupted in multiple blood and brain MDD

studies (Czarny et al., 2015; Lalovic et al., 2010; Quak et al., 2014; Shelton et al., 2011;

Spinazzola and Zeviani, 2009), and gene sets were grouped together based on the functional

roles of the target gene products. Importantly, all these genes were expressed in MDD and CTR

HDFs at baseline, providing additional evidence that HDFs express genes relevant to cellular

dysfunction observed in neuropsychiatric disease as seen in previous studies (Garbett et al.,

2015b; Manier et al., 2000; Shelton et al., 2004).
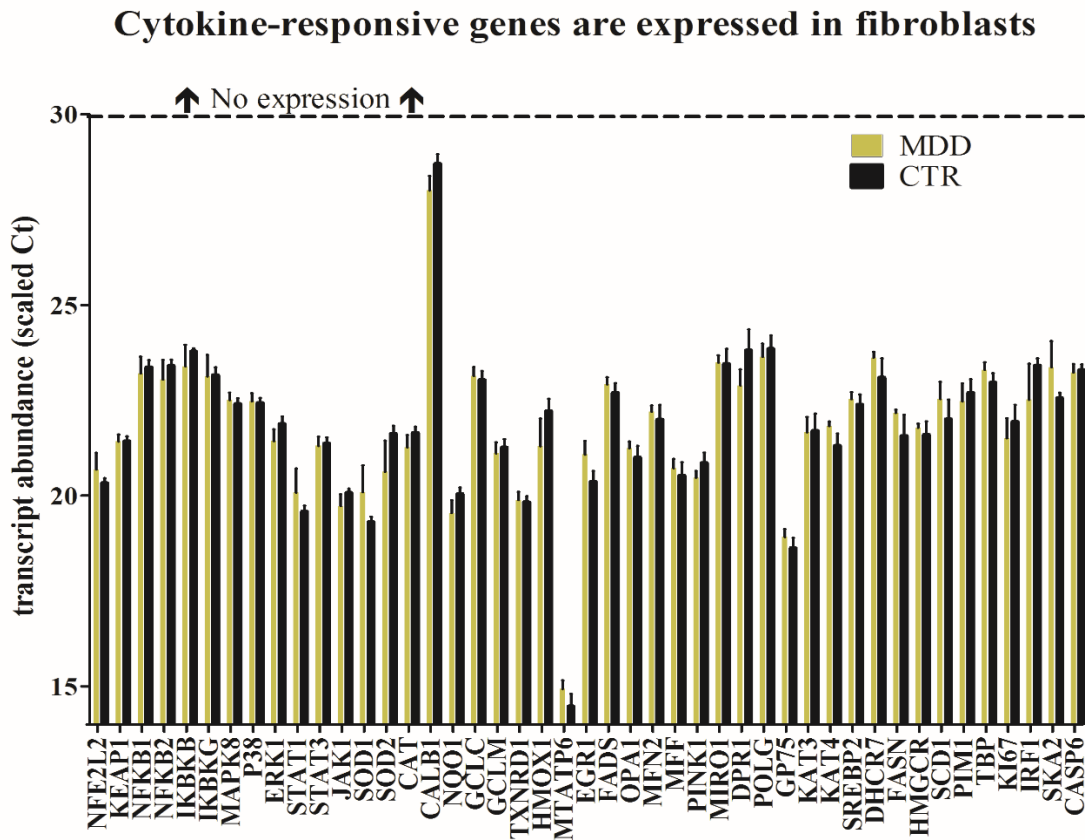
Figure 1. Both MDD and CTR HDFs express inflammation-responsive genes at baseline.
Gene expression was assessed by qPCR. MDD and CTR HDFs treated with vehicle express
cytokine-responsive genes. At baseline, MDD and CTR HDFs had comparable expression. X-
axis shows the tested gene transcripts whereas Y-axis shows data as a scaled qPCR threshold
cycle (Ct). Smaller Ct values indicate higher abundance of transcripts. Ct values >30 (dashed
line) indicate unexpressed transcripts. Bars denote standard error of means (SEM).

In a within subject comparison, both MDD and CTR HDFs displayed altered expression of the genes tested in response to cytokine stimulation with a unique set of changes observed for IL1β, TNFα, and IL6 (**Table 1**). To IL1β and TNFα stimulation, both MDD and CTR HDFs responded with considerable induction of immune and oxidative stress response genes such as *NFKB1*, *SOD2*, and *IRF1*. IL1β stimulation produced increased repression of the gene encoding early growth response 1 in both MDD and CTR HDFs compared to other cytokine stimulation conditions (**Figure 2, Supplementary Table 4**) whereas TNFα caused a greater induction of *PIM1* in both MDD and CTR HDFs (**Figure 3, Supplementary Table 4**). TNFα also produced a subtle repression of *SKA2* in CTR HDFs that was not altered in MDD HDFs.

Table 1. MDD and CTR HDF responses to cytokine stimulation in comparison to vehicle treatment

WITHIN SUBJECT COMPARISON OF CYTOKINE RESPONSE BY GENE SET						
	MDD (p-value)			CTR (p-value)		
	IL6	IL1β	TNFα	IL6	IL1β	TNFα
All gene sets	*0.143*	*0.174*	*0.013*	*0.000*	*0.430*	*0.265*
Oxidative stress	*0.011*	*0.015*	*0.008*	*0.000*	*0.079*	*0.078*
Mitochondrial function	*0.522*	*0.052*	*0.329*	*0.000*	*0.094*	*0.031*
Tryptophan/lipid metabolism	*0.037*	*0.072*	*0.988*	*0.004*	*0.040*	*0.074*
Proliferation/apoptosis/immune response	*0.272*	*0.604*	*0.362*	*0.003*	*0.555*	*0.394*

A paired student t test was used to compare the expression of the indicated gene sets between vehicle and each cytokine treatment condition for MDD and CTR HDFs. At least one gene set was altered by each cytokine stimulation compared to baseline expression for both MDD and CTR (p < 0.05 shaded).
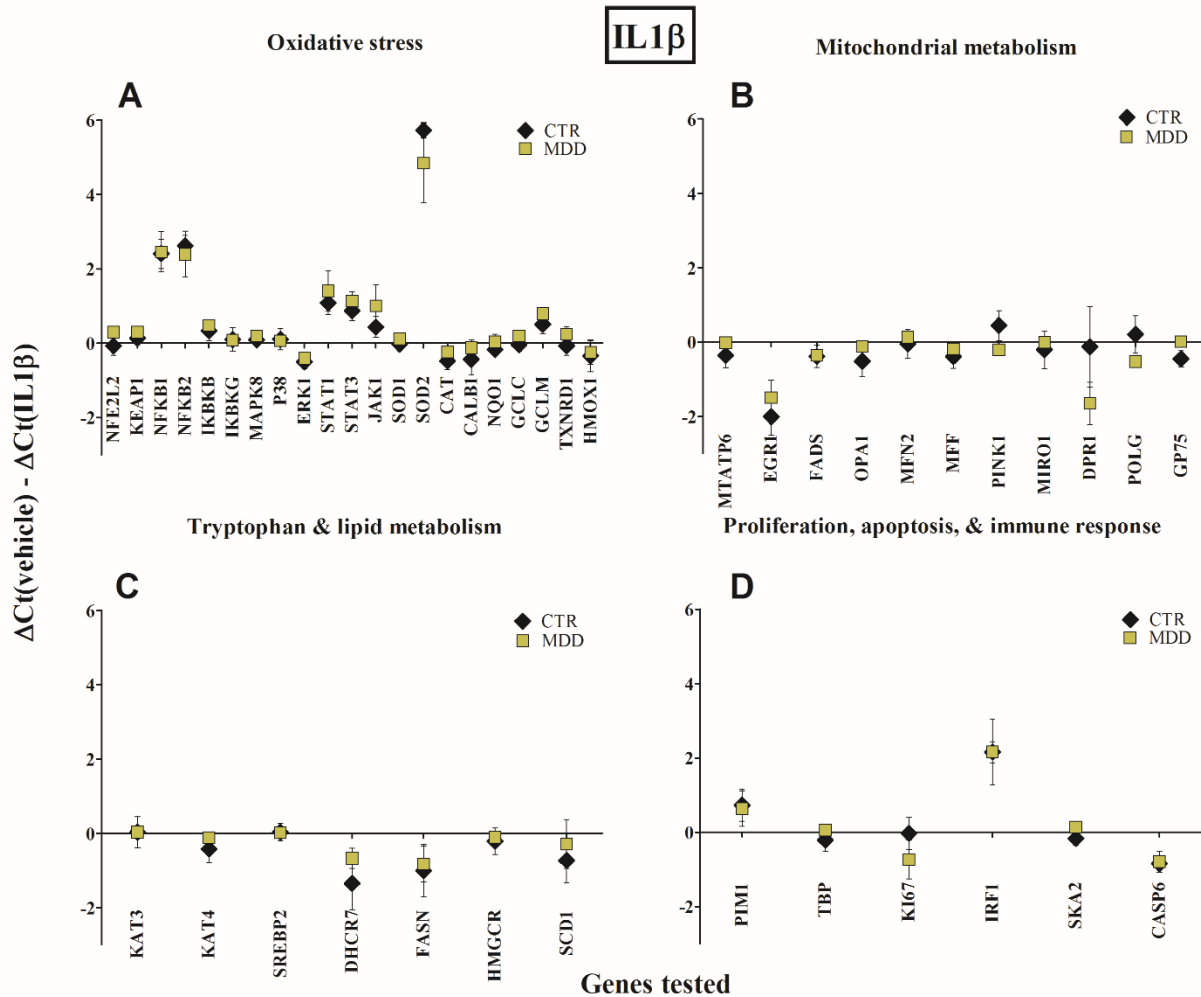
Figure 2. MDD and CTR HDFs respond to IL1β stimulation, but with no differences across diagnosis observed in magnitude of response. IL1β stimulation changed the relative expression of the cytokine-responsive genes tested in MDD and CTR HDFs, demonstrating the ability of HDFs to be stimulated by cytokines. This response was not significantly different for the oxidative stress (A), mitochondrial metabolism (B), tryptophan & lipid metabolism (C), or proliferation, apoptosis, & immune response (D) related gene sets. X-axis shows the tested gene transcripts whereas Y-axis denotes data as the normalized expression difference between cytokine and vehicle-treated conditions (ΔΔCt). A ΔΔCt value of 0 indicates that the gene was expressed at the same relative level with vehicle or cytokine treatment, while values < 0 indicates repression and > 0 indicates transcript induction by treatment. An absolute ΔΔCt value on 1 corresponds to a 2-fold change. Error bars denote SEM.
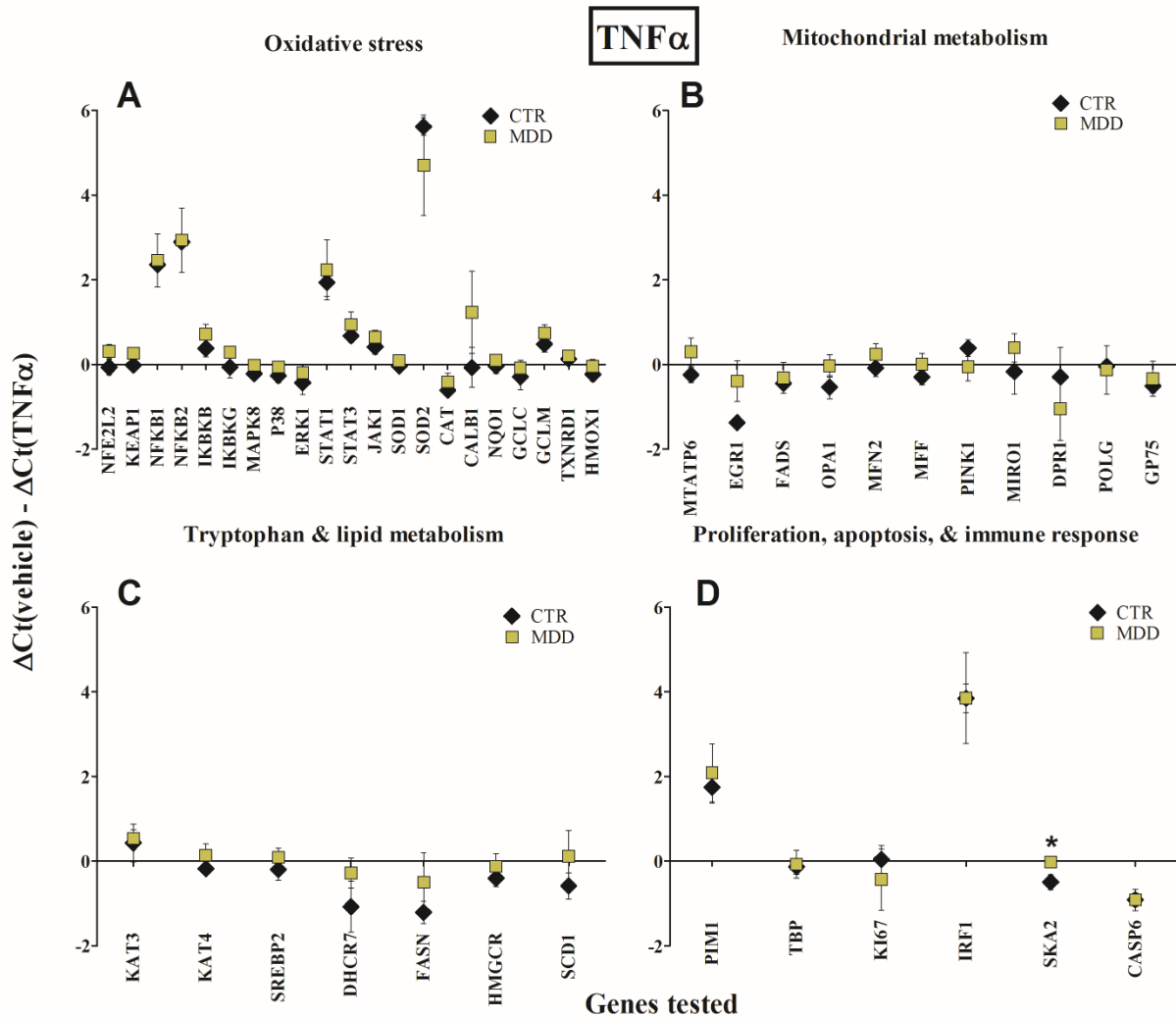
Figure 3. **MDD and CTR HDFs have a comparable response across diagnosis to TNFα stimulation.** Figure layout is identical to that of Figure 2. TNFα stimulation changed the relative expression of the cytokine-responsive genes tested in MDD and CTR HDFs. The response was not significantly different by unpaired student t-test between MDD and CTR fibroblasts in the oxidative stress (**A**), mitochondrial metabolism (**B**), or tryptophan & lipid metabolism (**C**) related gene sets. (**D**) A significant difference ($p < 0.05$, *) was observed with *SKA2* expression, being repressed in CTR HDFs but unchanged in MDD HDFs with TNFα stimulation. Error bars denote SEM.

Treatment with IL1 β or TNFα did not produce any discernable differences in the relative expression level of the tested genes between CTR and MDD HDFs. On the contrary, IL6

stimulation resulted in a differential MDD response compared to CTR (**Table 2**). In general,

expression of the tested genes was reduced in CTR HDFs treated with IL6 compared to vehicle.

In sharp contrast to CTR, the expression of the tested genes remained mainly unchanged in MDD

HDFs with the exception of *NFKB1* and *SOD2*, which were changed in the opposite direction

compared to CTR. (**Figure 4, Supplementary Table 4**). In other words, on a transcriptional

level, MDD HDFs did respond differently to IL6 stimulation compared to CTR HDFs, and we

interpret these data as a diminished IL6 response in MDD.

Table 2. Comparison of response to cytokine stimulation between MDD and CTR

ACROSS DIAGNOSIS COMPARISON OF CYTOKINE RESPONSE BY GENE SET			
	MDD vs. CTR (p-value)		
	IL6	**IL1β**	**TNFα**
All gene sets	*0.000*	*0.729*	*0.357*
Oxidative stress	*0.000*	*0.742*	*0.539*
Mitochondrial function	*0.002*	*0.835*	*0.267*
Tryptophan/lipid metabolism	*0.000*	*0.330*	*0.090*
Proliferation/apoptosis/immune response	*0.032*	*0.967*	*0.997*

An unpaired student t test was used to compare the relative expression difference from baseline of the indicated gene set between MDD and CTR with p < 0.05 considered significance (shaded). When comparing all tested genes or each gene set, only the relative gene expression response to IL6 was significantly different between MDD and CTR.
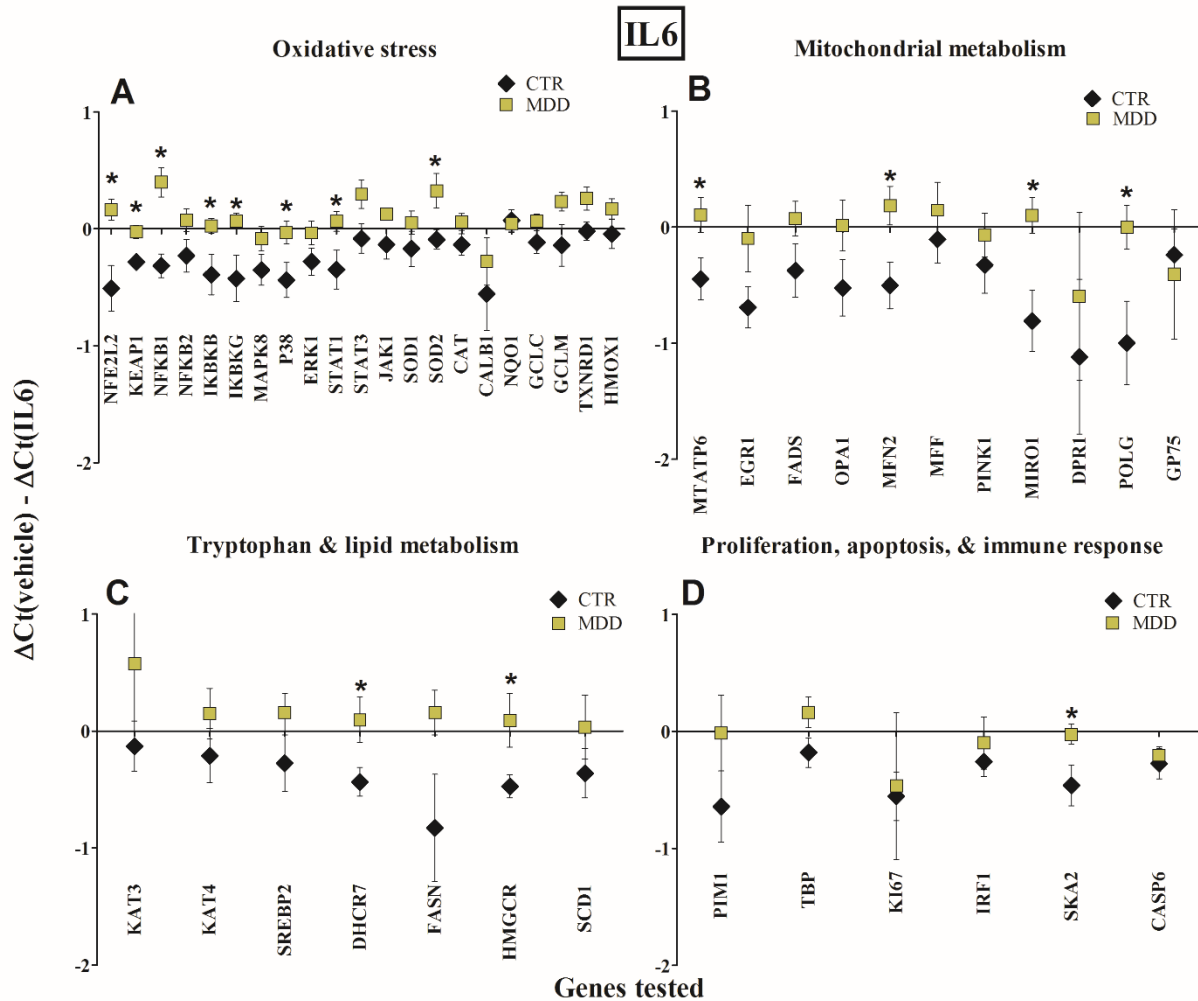
Figure 4. **MDD and CTR HDFs show a different gene expression response to IL6 stimulation.** Figure layout is identical to that of Figures 2-3. Several gene transcripts showed a differential response to IL6 stimulation between MDD and CTR HDFs (**A-D**). Error bars denote SEM; * denotes statistical significance ($p < 0.05$) by unpaired student t test.

In MDD HDFs, all of the tested gene sets demonstrated differential relative expression in response to IL6. The differentially changed oxidative stress related mRNA transcripts included *NFKB1, IKBKB, IKBKG, NFE2L2, KEAP1, SOD2, STAT1*, and *P38*. *NFKB1, IKBKG,* and *IKBKB* gene products all promote signaling of the transcription factor NFκB (Liu et al., 2012; Tak and Firestein, 2001). *NFE2L2* and *KEAP1* encode the transcription factor Nrf2 and its

cytoplasmic repressor, respectively; and *STAT1* and *P38* produce stress related signaling proteins whereas Mn-dependent superoxide dismutase (*SOD2*) neutralizes oxidants (Bresciani et al., 2015; Levy and Darnell, 2002; Zarubin and Han, 2005). From the mitochondrial gene set, several genes were strongly repressed in CTR, but not in MDD HDFs. These were genes crucial for ATP synthesis (*MTATP6*), mitochondrial fission and transport (*MFN2, MIRO1*), and mtDNA synthesis (*POLG*) (Al-Damluji, 2004; Degos et al., 2014; Hayashi et al., 2009; Las and Shirihai, 2014). Similar differences were observed in the lipid metabolism-related genes *DHCR7* and *HMGCR*, both crucial in cholesterol metabolism (Sharpe and Brown, 2013). The IL6 induced expression level of target genes important for tryptophan breakdown was not different in MDD compared to CTR HDFs. Spindle and kinetochore association complex subunit 2 (*SKA2*) was found to be differentially regulated in response to IL6 in MDD compared to CTR HDFs.

Due to the substantial alteration in relative gene expression changes observed with IL6 stimulation, next we measured the levels of the IL6 receptor (*IL6R*), its signal transducer gp130 (*IL6ST*), and suppressor of cytokine signaling 3 (*SOCS3*) (**Figure 5**) (Babon et al., 2014; Fischer et al., 2004). Interestingly, we found that the relative gene expression of *IL6R* was higher not only at baseline, but also after IL6 stimulation in MDD HDFs compared to CTR. Additionally, *SOCS3* was considerably induced in response to IL6 stimulation in MDD HDFs whereas no change from baseline expression was observed in CTR HDFs. This pattern of expression suggests an increase capacity of MDD HDFs to respond to IL6 stimulation as well as an active dampening of IL6 signaling by induction of *SOCS3*.

A IL6 signaling-related genes expressed in fibroblasts

B Relative gene expression change with IL6

C

ACROSS DIAGNOSIS COMPARISON of EXPRESSION of IL6 SIGNALING-RELATED GENES								
	Baseline nCt			IL-6 nCT			ΔΔCt		
gene	MDD	CTR	p-value	MDD	CTR	p-value	MDD	CTR	p-value
IL6R	26.36 ± 0.32	27.61 ± 0.48	*0.041*	25.73 ± 0.27	26.90 ± 0.42	*0.031*	0.13 ± 0.18	0.34 ± 0.33	*0.550*
IL6ST	20.46 ± 0.46	20.86 ± 0.47	*0.525*	19.79 ± 0.32	20.62 ± 0.36	*0.085*	0.05 ± 0.28	0.62 ± 0.33	*0.184*
SOCS3	25.08 ± 0.44	24.62 ± 0.27	*0.355*	23.43 ± 0.50	23.98 ± 0.18	*0.308*	-0.94 ± 0.21	0.07 ± 0.25	*0.007*

Figure 5. **MDD HDFs have increased *IL6R* and *SOCS3* expression.** (**A**) MDD HDFs had a significantly higher relative gene expression of *IL6R* (IL6 receptor) than CTR HDFs with both vehicle and IL6 exposure. (**B**) IL6 stimulation resulted in a strong, MDD-specific induction in *SOCS3* levels, while the change in expression of *IL6R* and *IL6ST* were not significantly different between the MDD and CTR HDFs. Error bars denote SEM; * denotes significant response by unpaired student t test between the CTR and MDD HDF responses. Values with SEM and associated p-values are listed in **C**.

CHAPTER III

GESTATIONAL DIABETES MODIFIES MIA INDUCED GENE EXPRESSION IN THE FETAL BRAIN

Kelli M. Money, Theresa Barke, David M. Aronoff, Krassimira A. Garbett, and Karoly Mirnics

Introduction

Psychiatric disorders are not purely genetic in nature. Environmentally sourced risk plays an important role. Previous work has shown that *in utero* disturbances lead to long term consequences on brain and behavior (Hamlyn et al., 2013; Patterson, 2009; Rapoport et al., 2012). However, most studies have focused on a single environmental perturbation or a combination of gene x environment, neglecting potential environment x environment interactions. Our lab is interested in understanding the interaction of environmental risk factors particularly during gestation, a highly sensitive period for both the developing immune and nervous systems. To this degree, we combined two commonly co-occurring environmental insults: gestational diabetes and maternal infection- mimicked here with poly(I:C) treatment. Both are well documented to affect brain development and are suggested to increase psychiatric disorder risk (Abell et al., 2015; Mandal et al., 2013; Meyer, 2014; Patterson, 2011; Salbaum and Kappen, 2012; Torres-Espinola et al., 2015). Currently, it is unknown how these inflammation-inducing psychiatric disorder risk factors interact. To address this, we begin by characterizing our two models: poly(I:C) induced MIA and high fat diet induced gestational diabetes. We then

compare gene expression changes in the fetal brain produced by MIA alone or high fat diet alone as well as interrogate how high fat diet induced gestational diabetes alters the gene expression response to MIA.

Methods

Animal procedures

All animal procedures were approved by the Vanderbilt Animal Care and Use Committee. Mice were housed in ventilated cages under standard laboratory conditions and allowed *ad libitum* access to food and water. Female and male C57Bl/6J mice were obtained from The Jackson Laboratory (Bar Harbor, ME, USA) for timed pregnant breeding. Control fed female mice received standard chow throughout the experiment (5LOD, Lab Diet, St. Louis, MO, USA). To induce gestational diabetes, females received a 60% calories by fat diet (58Y1, Test Diet, St. Louis, MO, USA) from 4 weeks of age until 10 weeks of age, continuing on the high fat diet throughout pregnancy. At 10 weeks of age, mice were mated overnight, and the presence of a vaginal plug the following morning marked gestational day 0.5 (GD0.5). Pregnant females were left undisturbed except for cage changes at GD9.5 and weight measurements. Weight was recorded on GD0.5, GD9.5, GD12.5, and GD16.5, depending on age of sacrifice.

High fat and control females were assigned to either saline or MIA (poly(I:C) treatment). This 2 x 2 design created 4 groups: control fed saline (SAL), high fat fed saline (HFS), control fed poly(I:C) (MIA), and high fat fed poly(I:C) (HFM). At GD12.5, pregnant females were injected intraperitoneally with either sterile saline or 20 mg/kg poly(I:C) potassium salt (Sigma

Aldrich, St. Louis, MO, USA) in sterile saline, which was based on the weight of the poly(I:C) itself and not the total weight including the potassium salts. Pregnant mice were sacrificed at two time points: 3 hours after injection at GD12.5 or 4 days after injection at GD16.5. The inclusion of two time points allowed us to investigate both the transient heightened poly(I:C) response in the fetal brain as well as the changes observable after the immune stimulus has passed. Maternal serum and embryonic brains were obtained from each pregnant female. Maternal serum was utilized for cytokine, adipokine, and non-fasting insulin measurement with the assistance of the Vanderbilt Hormone core via Luminex multiplex assays. Brains were flash frozen for later RNA isolation, and tail snips were taken from each embryo for sex genotyping. Each group at each time point (4 groups, 2 time points) contained 9 pregnant females with the exception of GD12.5 MIA that only contained 8 pregnant females, making a total of 71 females.

Glucose tolerance testing and body composition analysis

A separate cohort from the females above of control fed and high fat fed females were bred at 10 weeks of age as described above for glucose tolerance testing (n= 6 per group). At GD12.5, pregnant control fed and high fat fed females were fasted for 6 hours (7 AM – 1 PM). Body composition was then measured via whole body NMR (Minispec Model mq7.5, Bruker Instruments) with the assistance of the Vanderbilt Mouse Metabolic Phenotyping Center. All blood samples for glucose tolerance testing were obtained from tail snips. At 1 PM, fasting glucose and insulin samples were taken followed by intraperitoneal injection of 2g dextrose/kg body weight. Utilizing an Aviva Accu-Chek glucometer, blood glucose measurements were taken at 10, 20, 30, 45, 60, 75, 90, and 120 minutes post-injection. Blood samples were collected for insulin measurement at 10, 30, 60, 90, and 120 minutes post-injection and were processed by

the Vanderbilt hormone core. Dams were sacrificed after testing was complete to confirm pregnancy/gestational age. To evaluate the effects of time on high fat diet alone, one 10-12 week old non-pregnant control group (n=8) was utilized as the control for non-pregnant high fat females age 10 weeks (n=7) and age 12 weeks (n=8).

Sex genotyping

Sex genotyping was performed on each embryo to ensure each group had litters with the same proportion of males: females, which is greatly relevant due to the proposed male bias of both gestational diabetes and MIA on offspring (Dada et al. 2014, Sasaki et al. 2013). Digested tail samples from each embryo were utilized for sex determination. Forward (5'-CGCTGCCAAATTCTTTGG - 3') and reverse (5'- TGAAGCTTTTGGCTTTGAG - 3') primers for SmcXY locus were utilized (Jacobs et al., 2009). Thermocycler conditions started with 93°C for 2 minutes; followed by 30 cycles of 93°C for 30 seconds, 58°C for 45 seconds, and 72°C for 45 seconds; and ended with 72°C for 10 minutes. Female embryos produced only an upper band at 330 bp while male embryos had both an upper (330 bp) and lower (290 bp) band. Percentage of the litter that was male was calculated for each dam and compared across groups.

Luminex analysis

Immediately after sacrifice, a syringe was utilized to collect blood directly from the heart, which is accomplished by puncturing the skin and pericardial cavity directly via syringe insertion under the ribcage. Approximately 0.5 to 1 mL of blood was collected from each pregnant dam. After the blood was allowed to clot at room temperature for 30 minutes, the samples were spun at 8,000 rpm for 10 minutes at 4°C. The supernatant was transferred to a new sterile tube and

spun again at 8,000 rpm for 10 minutes at 4°C in order to remove any residual red blood cells. The mostly acellular supernatant (serum) was transferred to a new tube and kept at -80°C until submitted for Luminex analysis of cytokine, chemokine, adipokine, and insulin levels.

Luminex analysis utilizes a multiplexed assay, using x-map technology via the MagPix system. The reactants are attached to the surfaces of tiny fluorescent microspheres. Each set of microspheres carries a unique biological reagent distinguishable by internal dye ratios. Identification of an analyte is based upon specific fluorescent emission spectra of the bead associated with the analyte. Two LEDs with high speed digital signal processors and computer algorithms distinguish which analyte is being carried on each microsphere while quantifying the reaction based on fluorescent reporters signals. This allows for analysis of multiple analytes from a single aliquot of sample. Luminex analysis was performed by the Vanderbilt hormone core (http://hormone.mc.vanderbilt.edu), which is funded in part by NIH grants (DK059637 and DK020593). The Luminex multiplex panels (EMD Millipore, Darmstadt, Germany) used for analysis of the maternal serum were the mouse metabolic hormone panel (insulin and leptin – Cat# MMHMAG-44K), mouse adiponectin (Cat # MADPNMAG-70K-01), and mouse cytokine/chemokine panel 14 plex (eotaxin, IFNγ, IL1α, IL1β, IL4, IL6, IL10, IL12p40, IL13, IL17, KC, MCP1, RANTES, and TNFα- Cat# MCYTOMAG-70K).

RNA isolation and Nanostring gene expression analysis

The right hemisphere from each embryo, which included both telencephalon and diencephalon, was utilized for RNA isolation. RNA was isolated in 1 mL Trizol, following the protocol given by the manufacturer. Samples then underwent a Qiagen RNeasy clean-up,

following the instructions provided by the manufacturer. RNA concentration was measured with a Thermo Scientific Nanodrop 2000. Equal amounts of RNA (1250 ng) from each embryo were combined to create a pool litter sample. These pooled samples again underwent a Qiagen RNeasy clean-up to ensure absence of contaminants. Thermo Scientific Nanodrop 2000 was utilized to measure the final concentration and ensure purity (260/280 values above 2 and 260/230 values above 1.5). Agilent 2100 Bioanalyzer was utilized to ensure RNA integrity, with all RNA integrity numbers falling between 9.5 and 10.

150 ng of purified RNA from each pooled litter sample was used for Nanostring nCounter analysis (http://www.nanostring.com/applications/technology). Two panels (mouse inflammation V2 panel- 254 genes- and a custom inflammation and neurodevelopment 46 gene panel) were utilized to determine gene expression. Nanostring multiplexed gene expression measurement technology utilizes a digital color-coded reporter tag to identity and count expression of genes, with the capacity to detect more than 800 genes in a single lane. RNA from each sample was hybridized with the identifying reporter probe as well as capture probes, which allow for immobilization of the complex for data collection, at 65°C for 24 hours. The hybridized samples were then placed in the Nanostring nCounter prep station, where the complexes formed in each sample were aligned and immobilized in the nCounter cartridge, which was then read on the Nanostring nCounter digital analyzer. The raw "counts"- the number of times the reporter probe was detected in each sample- was normalized to positive and negative spike ins as well as 5 housekeeping genes (*Cltc, Gapdh, Gusb, Hprt,* and *Tubb5*). To reduce noise, a baseline of 10 counts was set, and all counts below 10 were raised to 10 for the purpose of analysis. Importantly, any changes made statistically significant by this manipulation were

excluded from significant results, allowing us to decrease noise without artificially increasing significance due to reduced variability. Normalized counts were then log2 transformed. To investigate the effects of high fat diet or MIA alone, HFS and MIA were each compared to SAL. To investigate the effect of MIA in the context of high fat diet, HFM was compared to HFS. To uncover differences in the MIA induced changes in the context of high fat diet induced gestational diabetes compared to in the context of control diet, dMIA [each MIA log2(counts) – the average SAL log2(counts)] and dHFM [each HFM log(2counts) – the average HFS log2(counts)] was calculated and then compared.

Statistical Approaches

To investigate the effects of diet and treatment exposure on litter statistics, a two-way ANOVA with Bonferonni post hoc analysis was used, with $p<0.05$ considered significant. Otherwise, an unpaired student t-test with Welch's correction was utilized to compare values between two groups, with again $p<0.05$ considered significant. With regards to gene expression data, criteria for significance included >10% change (>0.138 ALR), significance by unpaired student t-test with Welch's correction, and not artificially created by the raised baseline. These group comparisons also utilized unpaired student t-test with Welch's correction with $p<0.05$ considered significant.

Results

8 to 9 pregnant dams per group (SAL, MIA, HFS, HFM) per time point (GD12.5, GD16.5) were given either standard chow or high fat diet to induce gestational diabetes in

combination with GD12.5 saline or poly(I:C) treatment. Illness produced by poly(I:C) treatment causes a transient increase in cytokine production, accompanied by anorexia, fever, and sickness behaviors that usually resolves in 24-48 hours. Poly(I:C) or saline treated dams sacrificed at GD16.5 were weighed 1 day after exposure. The poly(I:C) treated dams either lost weight or failed to gain weight compared to saline treated dams (SAL = 0.60 ± 0.33, MIA = -0.83 ± 0.34; HFS = 1.33 ± 0.25, HFM = -0.67 ± 0.31). As determined by a two-way ANOVA with a significant p-value<0.05, neither diet nor treatment altered the average number of embryos per litter nor the male: female ratio (**Supplementary Table 5**). At GD16.5, poly(I:C) treatment produced a significantly decreased crown-rump length compared to saline treated litters (two-way ANOVA, $p<0.05$), but Bonferroni post-hoc analysis demonstrated this to be only true for control fed mice ($t = 2.775$, $p<0.05$).

Although the high fat fed dams were on a 60% by calorie high fat diet for 6 weeks prior to mating and throughout pregnancy, these were not obese mice. Weight was only significantly different across the entire cohort used for embryonic brain collection at GD9.5 (control diet = 24.6 g ± 0.3, high fat diet = 25.8 g ± 0.4, $p<0.05$) with significance determined by unpaired student t test with Welch's correction. Even so, this high fat model successfully produced a gestationally diabetic phenotype. To demonstrate this, a separate cohort of timed pregnant control- and high fat-fed dams (n = 6 per group) were utilized for glucose tolerance testing. Compared to control diet fed dams, high fat fed dams had increased body weight and percent fat mass at GD12.5 (**Figure 6A/B**). In addition, high fat GD12.5 dams had both impaired fasting glucose and fasting insulin after a 6 hour fast (**Figure 6C/D**). When challenged with 2 g/kg dextrose after a 6 hour fast, high fat GD12.5 dams displayed marked glucose intolerance and

exaggerated glucose-induced insulin secretion that remains high even at 2 hours post-injection

(**Figure 6 E/F/G**). This demonstrates a considerable glucose intolerance with likely insulin

insensitivity/resistance, although insulin insensitivity would require additional evaluation to truly
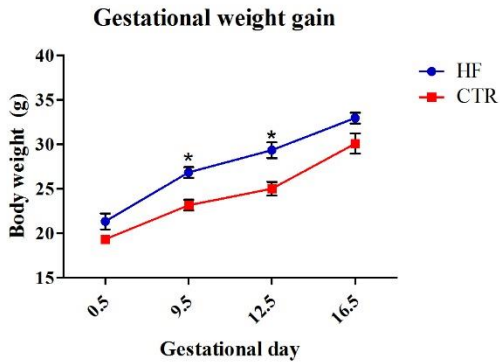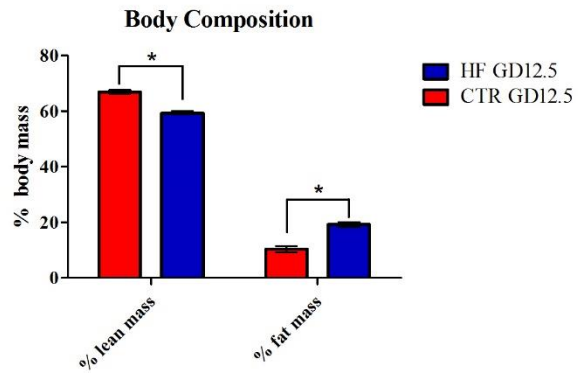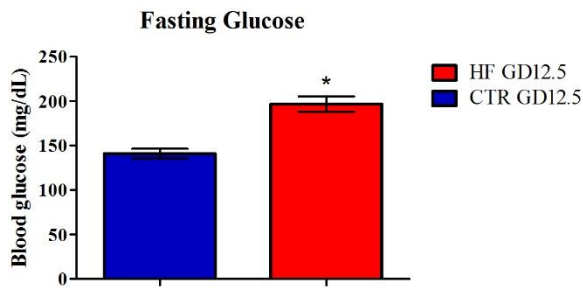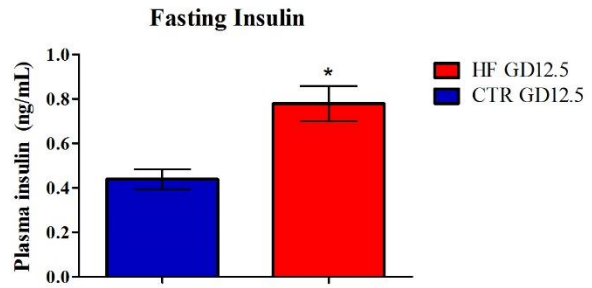
confirm (Ayala et al., 2010).
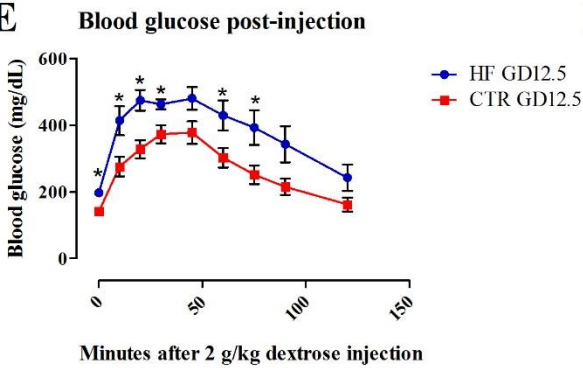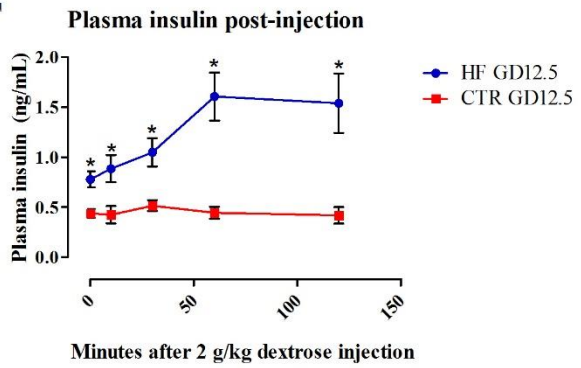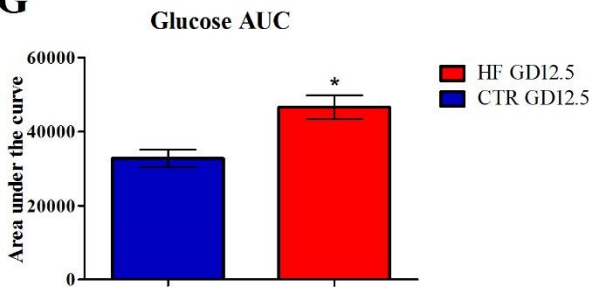
Figure 6. High fat diet produces a diabetic phenotype midgestation. Body composition, 6 hour fasting glucose and insulin, and glucose tolerance measurements were performed on a

cohort of high fat and control fed GD12.5 pregnant dams (n=6 per group). (**A**) High fat fed dams showed significant increases in body weight at GD9.5 and GD12.5. (**B**) Whole body NMR at GD12.5 showed significant changes in % body mass in high fat dams with increased % fat mass and decreased % lean body mass. (**C-D**) 6 hour fasting blood glucose and serum insulin were both significantly increased in high fat dams. (**E-G**) After a 6 hour fast, dams were injected intraperitoneally with 2 g dextrose/kg body weight. High fat fed dams demonstrated a higher peak and prolonged increase in blood glucose with significant differences from control dams observable at 0 10, 20, 30, 60, and 75 minutes post-injection, which is supported by a significantly increased area under the curve. A significant heightened insulin response that was maintained up until the 2 hour end point was also observed in high fat dams. Error bars represent SEM. Significance of $p<0.05$ is indicated by * and determined by unpaired student t-test with Welch's correction in all assays, n=6 dams per group.

To investigate whether this diabetic phenotype was truly gestation-dependent, two cohorts of high fat fed dams age-matched for GD0.5 and GD12.5 were used. A single non-pregnant 10-12 week old control fed dam cohort was used as the control group for both the 10 week (GD0.5) and 12 week (GD12.5) old high fat fed dam cohorts. If a diabetic phenotype was due to diet alone at the initiation of gestation or at the time point measured as GD12.5, then this would be a model of type 2 diabetes, not gestational diabetes. Supporting a gestationally diabetic model, high fat fed GD0.5 dams do not show significant differences in body weight, body composition (% lean or fat mass), or fasting glucose or insulin (**Supplementary Figure 1 A-D**). When challenged with 2 g/kg dextrose after a 6 hour fast, high fat GD0.5 dams also do not show significant differences at any time point in blood glucose compared to controls (**Supplementary Figure 1 E-F**). This suggests that high fat dams do not have a diabetic phenotype at conception. In addition, GD12.5 age-matched, non-pregnant high fat fed dams did not display a diabetic phenotype although they did show increased body weight and a trend towards impaired glucose tolerance (**Supplementary Figure 2**). Although eventually glucose intolerance would develop

with high fat diet, the increased glucose production and insulin insensitivity induced by pregnancy likely produces this phenotype at an earlier time point (Abell et al., 2015; Gallou-Kabani et al., 2007). Altogether, this data suggests this model is a relevant model for gestational diabetes.

To demonstrate the induction of an immune response in the pregnant dams exposed to poly(I:C), maternal cytokines and chemokines were measured at the time of sacrifice (either 3 hours after poly(I:C) exposure on GD12.5 or 4 days after poly(I:C) exposure on GD16.5). 3 hours post exposure, MIA exposed dams in both the control and high fat diet induced gestational diabetes contexts demonstrated increases in eotaxin, IL1β, IL6, IL10, IL12p40, IL17, KC, MCP1, and RANTES (**Figure 7**). It must be noted that many exceeded the range of the assay for MCP1 and IL6 or fell below the detectable range in saline treated conditions and were therefore analyzed as the highest detectable or lowest detectable value. Since MCP1 levels were the most consistently upregulated, any poly(I:C) treated dams sacrificed at 3 hours post exposure that were below 2 standard deviation from the MCP1 mean were excluded from the study as non-responders or poor injections. Importantly, maternal serum increases in all of these cytokines/chemokines have been observed by others during the acute response to poly(I:C) (Arrode-Bruses and Bruses, 2012; Mandal et al., 2013). No significant changes were observed with IL1α, IL4, or IL13 (data not shown). Not surprisingly, by GD16.5, only IL13 was significantly increased by MIA, and this was only observed in the control condition (**Supplemental Figure 3**). Between 1 – 8 days post injection, most studies observe subtle or no increase in cytokines or chemokines in maternal serum, which is expected with the time-limited maternal response to poly(I:C) (Arrode-Bruses and Bruses, 2012; Mandal et al., 2013; Meyer et

al., 2008). Altogether, our results support that poly(I:C) at GD12.5 produces an acute, but strong maternal increase in multiple cytokines and chemokines, including IL6, that is no longer present 4 days post injection at GD16.5. We believe this pro-inflammatory cytokine environment is the most likely driver of fetal brain changes attributable to MIA.
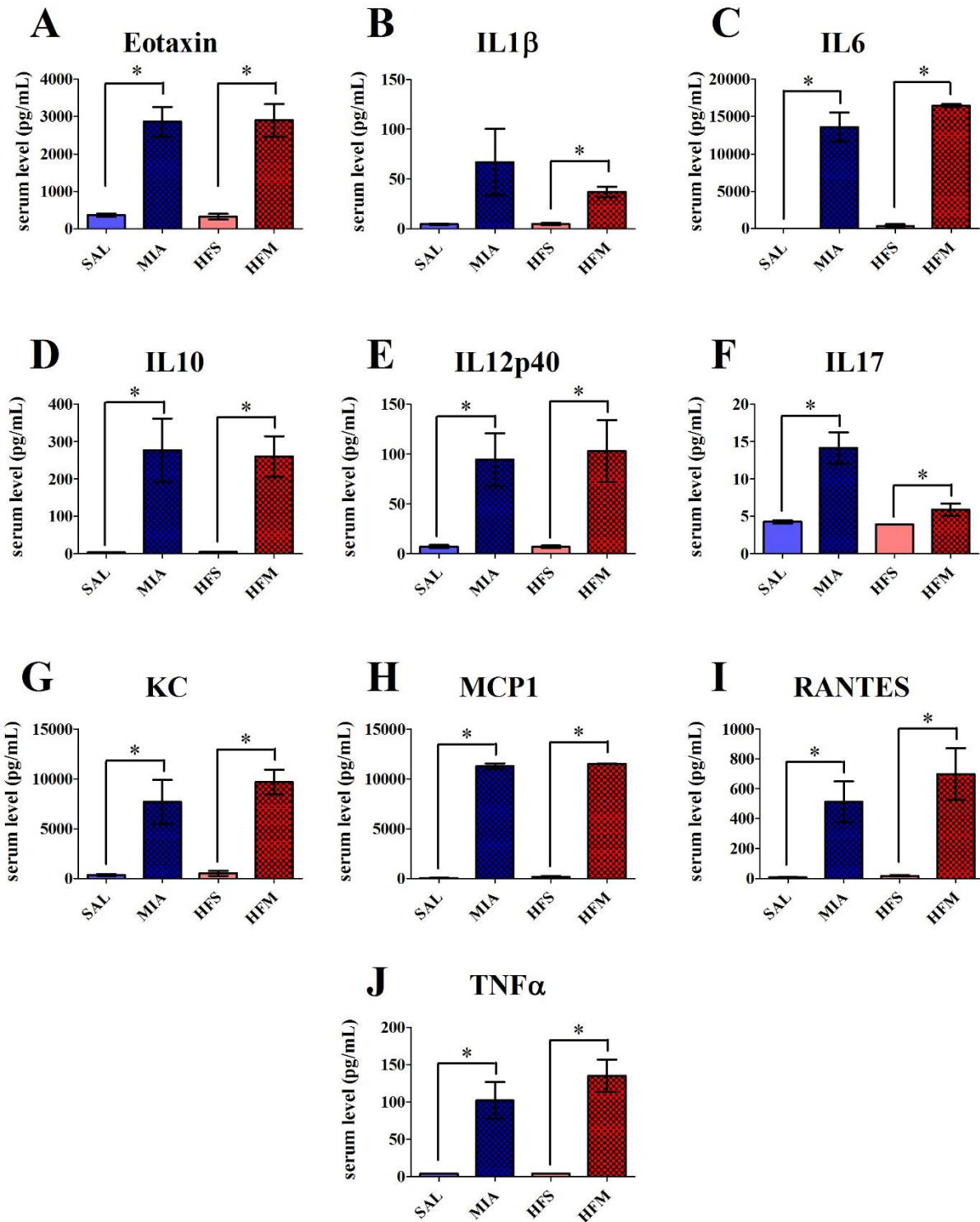
Figure 7. MIA increases maternal serum levels of chemokines and cytokines. Maternal serum collected 3 hours post injection on GD12.5 was analyzed for chemokine and cytokine levels via Luminex multiplex assay. (**A-J**) Significant increases were observed with MIA in both

the context of control diet and high fat diet in all shown but IL1β, which demonstrated a significant increase with MIA exposure only in the context of high fat diet induced gestational diabetes. Error bars represent SEM. Significance of p<0.05 is indicated by * and determined by unpaired student t-test with Welch's correction in all assays. Serum was collected from dams whose embryos were utilized for Nanostring gene expression analysis (SAL n=9, MIA n=8, HFS n=9, HFM n=9).

Maternal serum levels of metabolic hormones were found to be altered by both high fat diet induced gestational diabetes and MIA at GD12.5. Adiponectin was found to be significantly decreased by high fat diet induced gestational diabetes alone and trended towards a decrease with MIA exposure alone, but adiponectin levels did not appear to be altered further by MIA exposure in the context of high fat diet induced gestational diabetes (**Figure 8A**). Adiponectin is usually thought to dampen excessive inflammation but can also participate in the immune response, making alterations in this hormone highly dependent on the context in which they occur (Chandran et al., 2003; Cheng et al., 2012; Wilk et al., 2011). In addition, non-fasting serum insulin levels trended toward an increase with high fat diet induced gestational diabetes alone, but the combination of high fat diet and MIA exposure produced a considerable significant increase in serum insulin levels not seen with MIA exposure in the control context (**Figure 8B**). Interestingly, the immune-activating, satiety-inducing hormone leptin was significantly increased by high fat diet induced gestational diabetes and further increased by combination of high fat diet induced gestational diabetes and MIA exposure (**Figure 8C**). Increase serum leptin and insulin as well as leptin and insulin insensitivity have been reported in adult mice fed a high fat diet (Gallou-Kabani et al., 2007; Van Heek et al., 1997). At GD16.5, reduced adiponectin and increased leptin levels were still observable with high fat diet induced gestational diabetes. In addition, a significant increase in adiponectin levels induced by MIA in the context of high fat

diet induced gestational diabetes was observed, which was not seen at GD12.5 (**Supplemental**

Figure 3). Although the effects of high fat diet induced gestational diabetes on metabolic

hormones appears to remain consistent from GD12.5 to GD16.5, the interaction of MIA and

gestational diabetes appears to evolve from the acute immune response to post "infection".

Taken together, the maternal serum metabolic hormone levels likely promote a pro-inflammatory

phenotype in high fat diet induced gestational diabetes, which is exacerbated when combined

with MIA. Thus, the HFM dams have a pro-inflammatory profile of cytokines, chemokines, and

metabolic hormones.
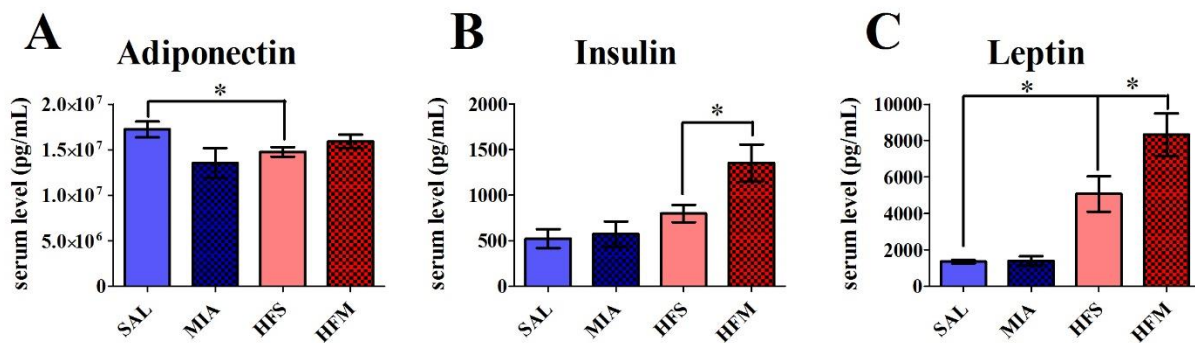
Figure 8. High fat diet induced gestational diabetes alters non-fasting levels of metabolic hormones. Maternal serum collected 3 hours post injection on GD12.5 was analyzed for non-fasting adipokine and insulin levels via Luminex multiplex assay. (**A**) The anti-inflammatory metabolic hormone adiponectin is significantly decreased by high fat diet induced gestational diabetes, with a trend towards a decrease with MIA. (**B**) Non-fasting serum insulin levels are not quite significantly increased with high fat diet induced gestational diabetes alone, but MIA in the context of high fat diet does produce a significant increase. (**C**) The pro-inflammatory satiety hormone leptin is significantly increased by high fat diet induced gestational diabetes. Leptin levels are also significantly increased by MIA in the context of high fat diet induced gestational diabetes. Error bars represent SEM. Significance of $p<0.05$ is indicated by * and determined by unpaired student t-test with Welch's correction in all assays. Serum was collected from dams

whose embryos were utilized for Nanostring gene expression analysis (SAL n=9, MIA n=8, HFS n=9, HFM n=9).

Both high fat-induced gestational diabetes and MIA results in altered gene expression in the fetal brain at GD12.5, with brains collected 3 hours after poly(I:C) treatment during the acute immune response. We evaluated many genes with a predominant inflammation function and a small number of neurodevelopment genes as well as the major neuronal glucose transporters. When comparing gene expression changes significantly different between SAL and MIA, HFS, or HFM in a supervised hierarchical clustering analysis, each diet x treatment group clearly aggregates together (**Figure 9**, **Supplemental table 6**). Thus, we clearly see group-specific gene expression profiles.
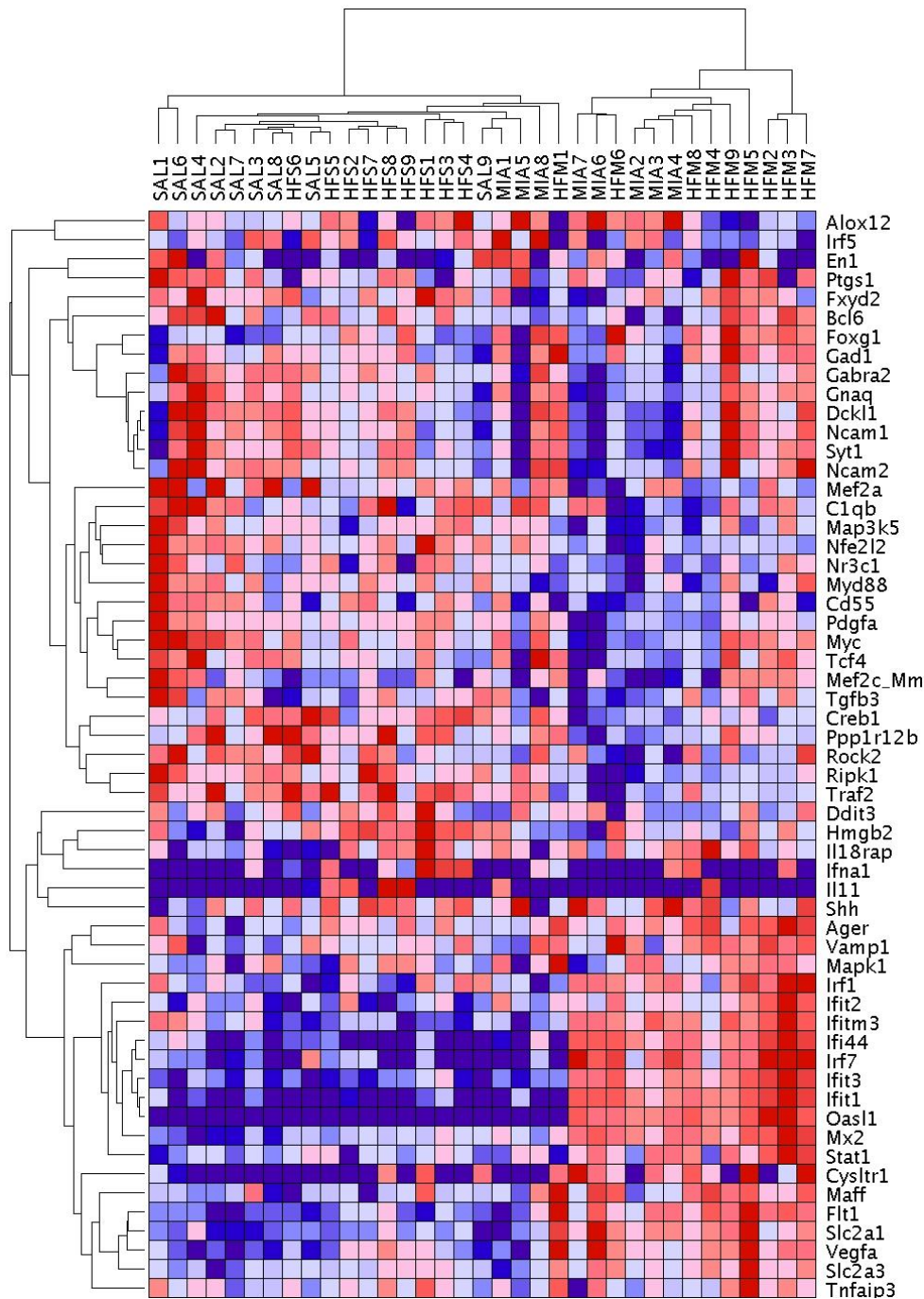
Figure 9. Both high fat diet and MIA alter gene expression at GD12.5. Genes that demonstrated a significant change in expression in either HFM, MIA, or HFS when compared to SAL (57 genes) were utilized for supervised hierarchical clustering analysis. Each diet x treatment group aggregates fairly well together, demonstrating distinct gene expression changes in each group. Significance was determined by unpaired student t-test with Welch's correction and p<0.05. Values are in **Supplementary table 6.**

High fat diet induced gestational diabetes alone displayed significant differences in gene expression compared to SAL, demonstrating mixed changes. Neurodevelopment genes involved in patterning were both induced (*Shh*) and repressed (*En1*) by HFS (Chiang et al., 1996; Sadler et al., 1995). Likewise, inflammation-associated genes were also both induced (*Hmgb2, Ifna1, Il11, Mx2*) and repressed (*Ifitm3, Mef2a, Ptgs1*), with these genes functioning in IFN/antiviral response, growth, cell cycle regulation, and apoptosis (Liu et al., 2011a; McKinsey et al., 2002; Yanai et al., 2009). Moreover, HFS increased *Ddit3* and *Vegfa* expression, which are both associated with a hypoxic environment (**Figure 10**) (Paschen et al., 1998; Pearce et al., 2011).
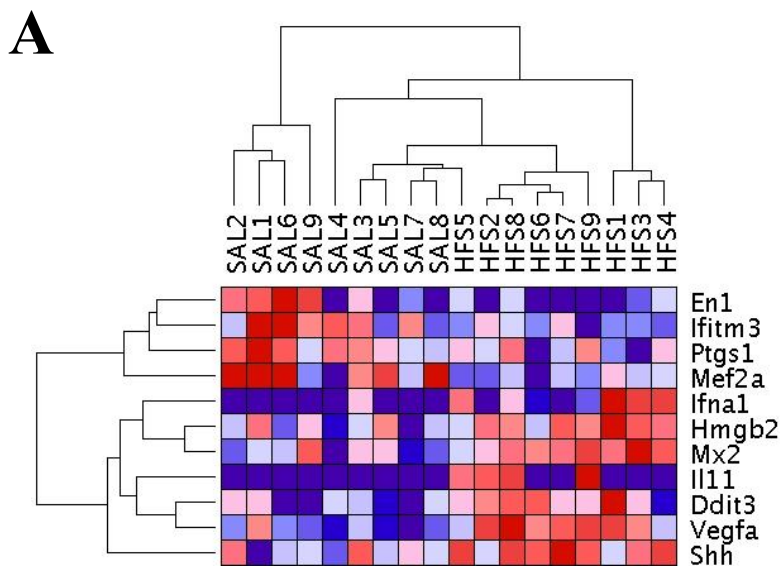
B

Gene	SAL		HFS			
	log$_2$(counts)	SEM	log$_2$(counts)	ALR (HFS-SAL)	SEM	p-value
Ddit3	9.69	0.03	9.84	0.14	0.05	*0.017*
En1	3.97	0.23	3.46	-0.51	0.06	*0.049*
Hmgb2	9.92	0.05	10.11	0.19	0.04	*0.009*
Ifitm3	8.41	0.10	8.15	-0.26	0.05	*0.027*
Ifna1	3.36	0.04	3.86	0.51	0.20	*0.026*
Il11	3.32	0.00	3.56	0.23	0.10	*0.043*
Mef2a	9.38	0.07	9.19	-0.19	0.03	*0.027*
Mx2	4.29	0.17	4.93	0.64	0.09	*0.004*
Ptgs1	6.28	0.06	6.07	-0.21	0.07	*0.031*
Shh	10.71	0.07	10.90	0.19	0.03	*0.024*
Vegfa	11.20	0.03	11.42	0.22	0.04	*0.000*

Figure 10. High fat diet induced gestational diabetes alters expression of neurodevelopment and inflammation genes at GD12.5. Genes that demonstrated a significant change in expression in HFS litters when compared to SAL (11 genes) were utilized for supervised hierarchical clustering analysis. (**A**) HFS exposed litters cluster together and display both induction and repression of neurodevelopment and inflammation genes. Significance was determined by unpaired student t-test with Welch's correction and p<0.05. Values are listed in **B**.

Our lab has exquisitely characterized the gene expression changes in the fetal brain 3 hours after maternal poly(I:C) treatment at GD12.5, demonstrating alterations in many inflammation responsive genes (Garbett et al., 2012). Here again, in a more limited gene expression analysis, we demonstrate disruption of inflammation responsive gene expression with MIA alone compared to SAL. Supervised hierarchical clustering analysis utilizing the significantly changed genes between MIA and SAL, like that seen with HFS and SAL, demonstrates distinct changes with poly(I:C) treatment (**Figure 11**). We see both induction and repression of inflammation associated genes by MIA. All of these induced genes participate in in antiviral/IFN response (*Ifi44, Ifit3, Mx2, Oasl1*) with the exception of *Alox12,* which produces an

enzyme that catalyzes stereo-specific lipid peroxidation (Dobrian et al., 2011; Liu et al., 2011a; Zhou et al., 2013). Those repressed participate in cell cycle regulation/apoptosis (*Bcl6, Cd55, Map3k5, Mef2a, Mef2c, Myc, Ripk1, Tcf4, Traf2*), cell growth (*Pdgfa, Rock2, Tgfb3*), intracellular signaling pathways associated with both anti- and pro-inflammatory pathways (*Creb1, Fxyd2, Gnaq, Myd88, Nfe2l2, Nr3c1, Ppp1r12b*) (Barton and Medzhitov, 2003; Demoulin and Essaghir, 2014; McKinsey et al., 2002; Spellman et al., 1998). Yet, distinct from the gene expression profile of fetal brains from gestationally diabetic dams, neurodevelopment genes affected all are involved in neuronal migration and were only repressed with MIA (*Dckl1, Gabra2, Ncam1, Ncam2, Syt1*) (Marin, 2013; Valiente and Marin, 2010). Furthermore, the constitutively expressed glucose transporter GLUT1 (*Slc2a1*) and hypoxia-associated genes (*Flt1, Vegfa*) were induced by MIA, suggesting insufficient oxygen and nutrients reaching the fetal brain (Pearce et al., 2011; Salihagić-Kadić et al., 2006).

A
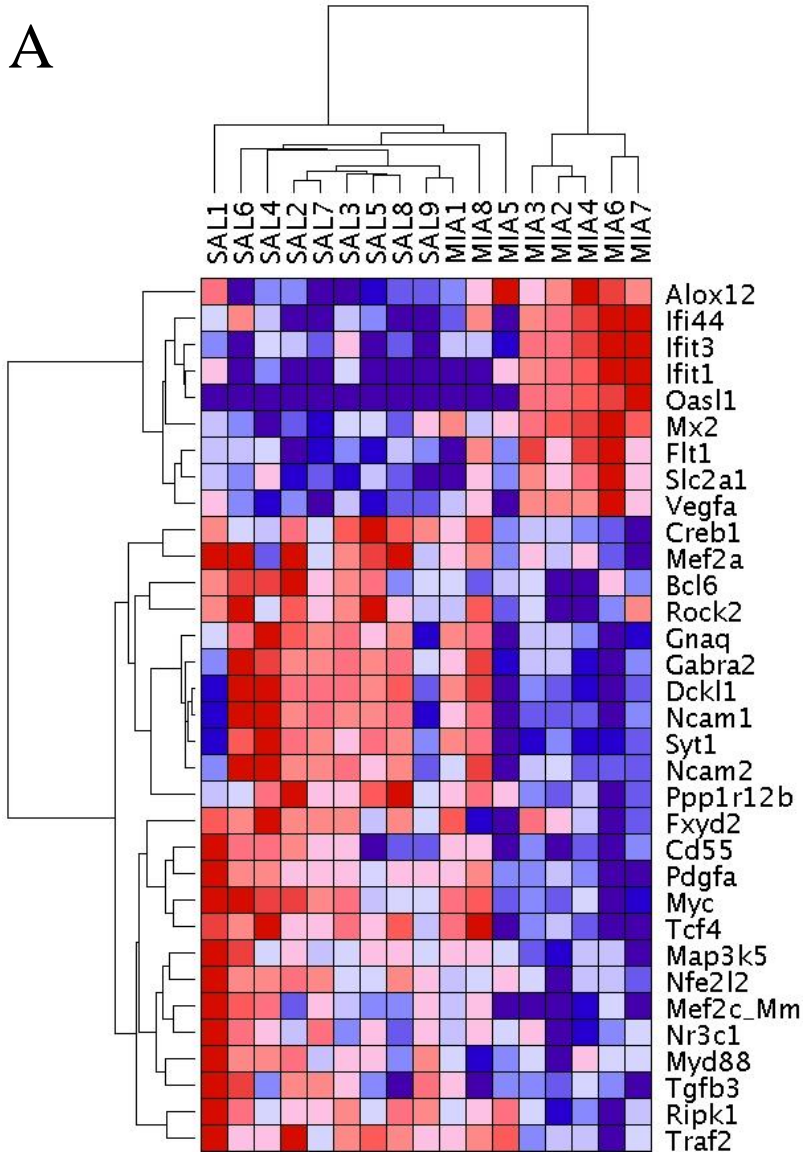
B

Gene	SAL		MIA			
	log$_2$(counts)	SEM	log$_2$(counts)	ALR (MIA-SAL)	SEM	p-value
Alox12	4.37	0.08	4.89	0.52	0.12	0.003
Bcl6	4.89	0.13	4.22	-0.67	0.12	0.001
Cd55	6.90	0.12	6.63	-0.27	0.05	0.049
Creb1	7.64	0.06	7.34	-0.30	0.09	0.014
Dckl1	12.19	0.09	11.87	-0.32	0.10	0.024
Flt1	8.84	0.02	9.04	0.20	0.07	0.025
Fxyd2	7.09	0.06	6.83	-0.26	0.10	0.048
Gabra2	9.42	0.09	9.01	-0.41	0.13	0.018
Gnaq	12.67	0.04	12.52	-0.15	0.04	0.028
Ifi44	3.82	0.19	4.94	1.12	0.35	0.017
Ifit1	3.54	0.12	4.61	1.07	0.34	0.017
Ifit3	4.66	0.14	5.77	1.11	0.32	0.010
Map3k5	6.93	0.13	6.58	-0.34	0.08	0.033
Mef2a	9.38	0.07	9.16	-0.23	0.07	0.031
Mef2c_Mm	8.94	0.09	8.71	-0.23	0.03	0.026
Mx2	4.29	0.17	5.54	1.25	0.21	0.000
Myc	9.85	0.07	9.53	-0.32	0.09	0.010
Myd88	7.52	0.06	7.32	-0.20	0.04	0.016
Ncam1	13.75	0.08	13.49	-0.26	0.07	0.020
Ncam2	9.41	0.08	9.17	-0.24	0.09	0.048
Nfe2l2	10.52	0.04	10.34	-0.18	0.04	0.006
Nr3c1	8.80	0.07	8.63	-0.17	0.03	0.047
Oasl1	3.32	0.00	4.39	1.07	0.34	0.016
Pdgfa	9.60	0.09	9.27	-0.33	0.07	0.010
Ppp1r12b	9.07	0.05	8.88	-0.19	0.05	0.016
Ripk1	8.28	0.04	8.07	-0.21	0.06	0.014
Rock2	9.88	0.04	9.73	-0.15	0.05	0.026
Slc2a1	11.23	0.02	11.37	0.14	0.06	0.046
Syt1	9.55	0.09	9.26	-0.29	0.09	0.025
Tcf4	13.84	0.04	13.65	-0.19	0.07	0.040
Tgfb3	8.24	0.17	7.80	-0.45	0.08	0.028
Traf2	9.62	0.03	9.46	-0.15	0.06	0.040
Vegfa	11.20	0.03	11.44	0.24	0.10	0.033

Figure 11. GD12.5 MIA exposure represses neurodevelopment genes and produced mixed expression changes in inflammation genes. Genes that demonstrated a significant change in expression in MIA litters when compared to SAL (33 genes) were utilized for supervised clustering analysis. (**A**) Even with a somewhat heterogenous response, MIA exposed litters

aggregate together. Litters exposed to MIA display both induction and repression of inflammation associated genes and significant repression of several crucial neurodevelopment genes. Significance was determined by unpaired student t-test with Welch's correction and p<0.05. Values are listed in **B**.

Similarly, poly(I:C) treatment in the context of high fat diet induced gestational diabetes produces marked induction of many inflammation and neurodevelopment genes (**Figure 12**). HFM both induced (*Foxg1, Gad1, Mbp, Vamp1*) and repressed (*Fgf8, Pax5*) neurodevelopment genes involved in patterning, migration, and oligodendrocyte development (Danesin and Houart, 2012; Marin, 2013; Ohtsuka et al., 2013; Ozgen et al., 2014; Smith et al., 2011; Toma et al., 2014; Walshe and Mason, 2003). Inflammation genes induced by HFM are involved in antiviral/IFN response (*Ifi44, Ifit1, Ifit2, Ifit3, Ifitm3, Irf1, Irf7, Maff, Mx2, Oasl1*), the generalized innate immune response (*Ager, Ccl21a, Csf1, Il1r1*), cell growth (*Tgfb2*), and apoptosis (*Mef2d*) (Barton and Medzhitov, 2003; Liu et al., 2011a; McKinsey et al., 2002; Zhou et al., 2013). Interestingly, antiviral/IFN response (*Irf3, Irf5*), apoptosis/cell cycle regulation (*Ddit3, Hmgb2, Ripk1, Tradd, Traf2*), and cell growth (*Pdgfa*) also had genes repressed by HFM (Demoulin and Essaghir, 2014; Liu et al., 2011a; Spellman et al., 1998). HFM also repressed genes that are critical for intracellular signaling pathways associated with pro- and anti-inflammatory processes (*Creb1, Jun, Nfe2l2*) (Bryan et al., 2013; Janeway and Medzhitov, 2002). Moreover, both the constitutively expressed GLUT1 and high affinity neuronal specific GLUT3 genes (*Slc2a1, Slc2a3*) as well as hypoxia-associated genes (*Flt1, Vegfa*) were induced in HFM compared to HFS.

A
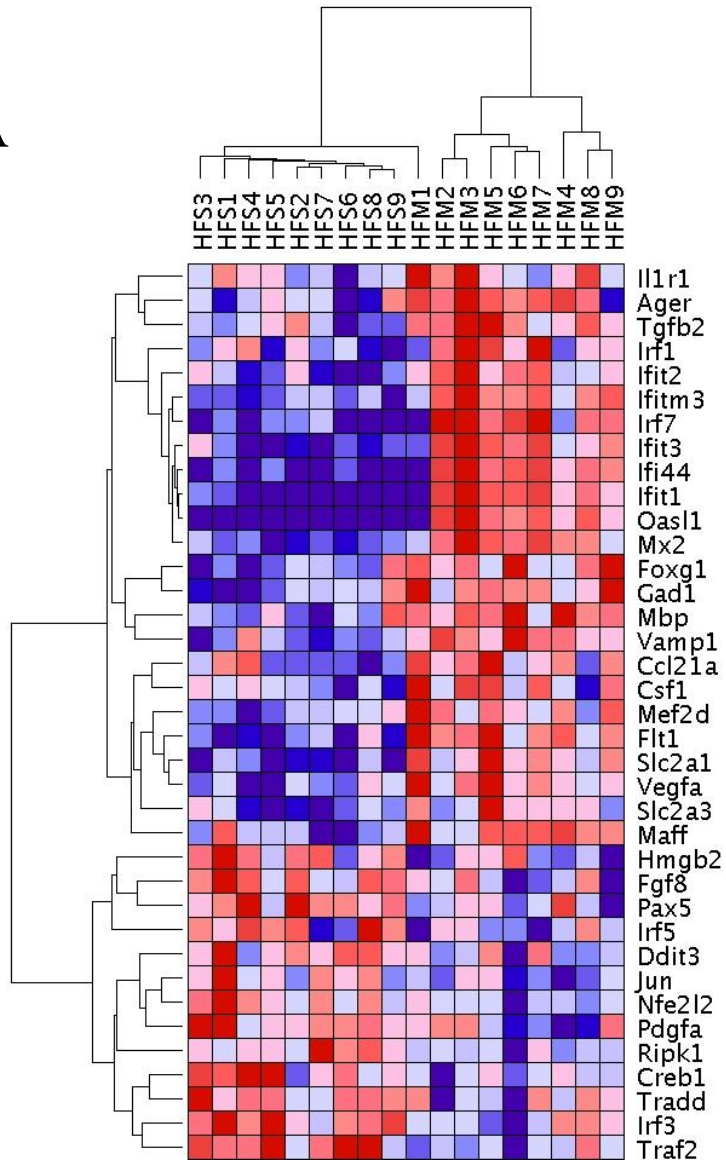
B

Gene	HFS		HFM			
	log$_2$(counts)	SEM	log$_2$(counts)	ALR (HFM-HFS)	SEM	p-value
Ager	7.38	0.07	7.64	0.27	0.07	0.008
Ccl21a	7.81	0.09	8.13	0.31	0.10	0.025
Creb1	7.66	0.06	7.45	-0.21	0.05	0.013
Csf1	6.34	0.04	6.49	0.15	0.06	0.031
Ddit3	9.84	0.05	9.69	-0.14	0.05	0.036
Fgf8	7.39	0.10	6.95	-0.44	0.14	0.014
Flt1	8.92	0.03	9.24	0.33	0.05	0.000
Foxg1	10.85	0.04	11.07	0.22	0.05	0.002
Gad1	9.77	0.06	10.07	0.31	0.06	0.002
Hmgb2	10.11	0.04	9.97	-0.14	0.04	0.015
Ifi44	3.54	0.12	5.70	2.16	0.40	0.000
Ifit1	3.47	0.09	5.63	2.16	0.41	0.000
Ifit2	8.81	0.05	9.18	0.36	0.09	0.002
Ifit3	4.93	0.16	6.79	1.86	0.38	0.001
Ifitm3	8.15	0.05	8.79	0.64	0.12	0.000
Il1r1	5.92	0.09	6.19	0.26	0.09	0.042
Irf1	7.01	0.07	7.28	0.27	0.10	0.041
Irf3	6.37	0.05	6.14	-0.23	0.08	0.024
Irf5	4.82	0.07	4.64	-0.17	0.04	0.040
Irf7	3.64	0.13	5.22	1.59	0.35	0.001
Jun	11.90	0.07	11.73	-0.17	0.03	0.040
Maff	6.21	0.11	6.69	0.48	0.07	0.001
Mbp	6.35	0.06	6.63	0.28	0.04	0.002
Mef2d	8.81	0.03	8.97	0.16	0.04	0.005
Mx2	4.93	0.09	6.18	1.25	0.21	0.000
Nfe2l2	10.44	0.06	10.26	-0.18	0.04	0.013
Oasl1	3.32	0.00	5.30	1.97	0.36	0.000
Pax5	4.86	0.13	4.30	-0.56	0.19	0.020
Pdgfa	9.50	0.03	9.37	-0.14	0.05	0.024
Ripk1	8.25	0.05	8.06	-0.19	0.04	0.005
Slc2a1	11.28	0.01	11.46	0.18	0.05	0.004
Slc2a3	9.73	0.02	9.90	0.17	0.07	0.042
Tgfb2	9.30	0.05	9.58	0.28	0.05	0.001
Tradd	5.93	0.07	5.60	-0.34	0.13	0.034
Traf2	9.67	0.04	9.44	-0.22	0.05	0.002
Vamp1	7.89	0.03	8.09	0.21	0.03	0.000
Vegfa	11.42	0.04	11.69	0.27	0.07	0.003

Figure 12. GD12.5 MIA exposure in the context of high fat diet induced gestational diabetes alters both neurodevelopment and inflammation genes. Genes that demonstrated a significant

change in expression in HFM litters when compared to HFS (37 genes) were utilized for supervised clustering analysis. (**A**) Litters exposed to MIA display both induction and repression of inflammation and neurodevelopment associated genes. Significance was determined by unpaired student t-test with Welch's correction and p<0.05. Values are listed in **B**.

However, one of the more important questions is how does MIA interact with high fat diet induced gestational diabetes? To answer this question, we compared the relative gene expression changes induced by MIA in the context of high fat diet to those induced by MIA in the context of control diet. This was accomplished by substracting the average SAL $\log_2$(counts) for each gene from each MIA $\log_2$(counts), creating a "dMIA" value. The same operation was performed for HFM (dHFM = HFM $\log_2$(counts) – average HFS $\log_2$(counts)). Interestingly, several genes were found to be significantly different when comparing the change induced by MIA in high fat to that with control diet, leaning towards higher relative expression of many inflammation and neurodevelopment genes in dHFM (**Figure 13**). In this data, some trends stand out. First, MIA combined with gestational diabetes led to induction of some neurodevelopment associated genes (e.g. *Gad1, Mbp*) not seen with MIA alone. Second, MIA combined with gestational diabetes produced stronger induction of some antiviral response genes (*Ifit2, Ifitm3*) than MIA alone. Third, MIA alone produced signficantly stronger repression of many inflammation (*Bcl6, Gnaq, Mef2c, Myc, Tc4, Tgfb3*) and neurodevelopment (*Dckl1, Gabra2, Ncam1, Ncam2, Syt1*) genes than MIA combined with gestational diabetes. Altogether, it is clear that the effects of MIA on fetal brain gene expression are modified by high fat diet, demonstrating that high fat diet induced gestational diabetes co-occurring with maternal infection is not simply the sum of its parts but creates a novel phenotype worth investigating.

A

B
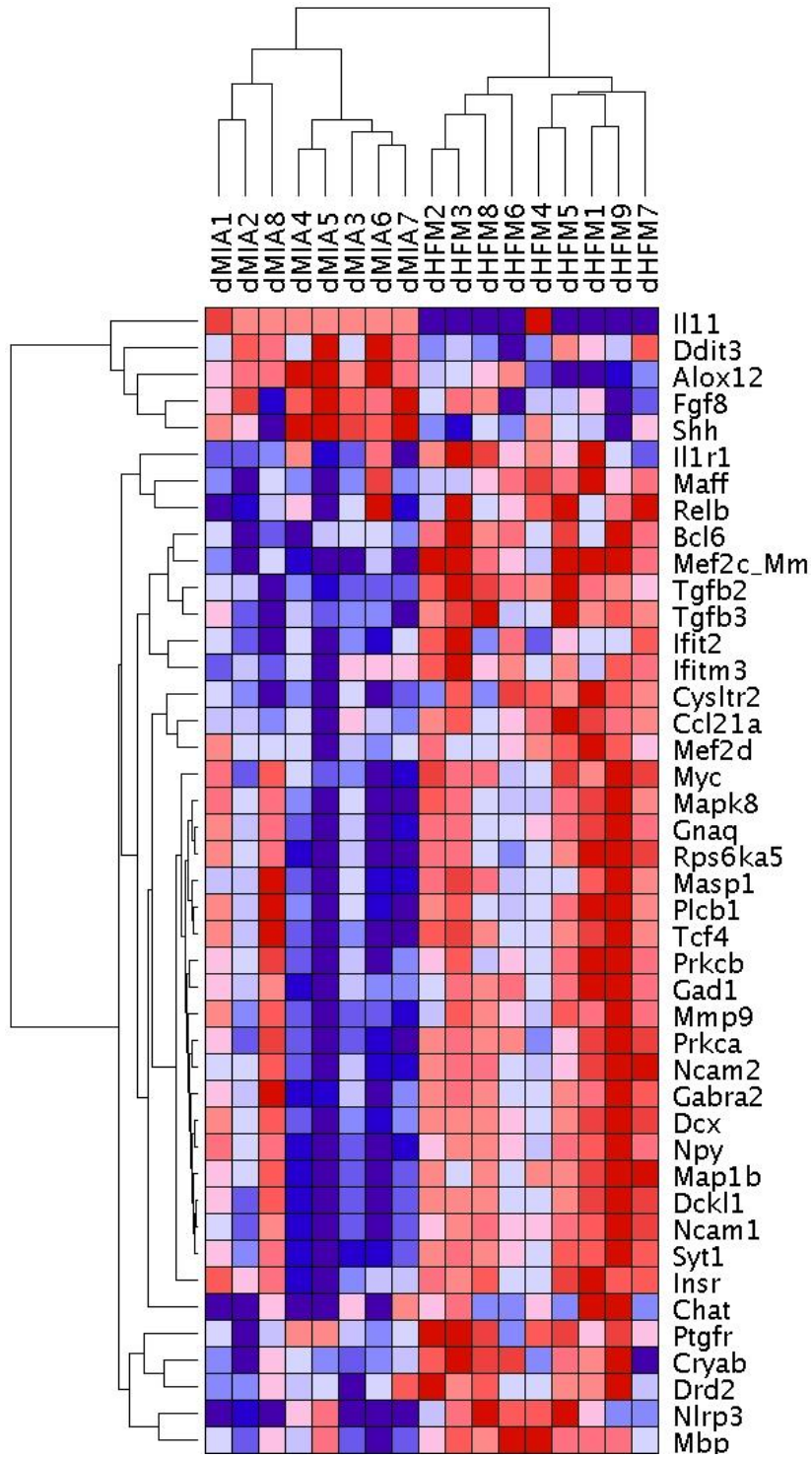
Gene	dMIA		dHFM			
	MIA- Avg SAL	SEM	HFM- Avg HFS	SEM	dALR (dHFM-dMIA)	p-value
Alox12	0.52	0.12	-0.48	0.18	-1.00	0.000
Bcl6	-0.67	0.12	0.08	0.11	0.75	0.000
Ccl21a	-0.37	0.12	0.31	0.10	0.68	0.001
Chat	-0.23	0.11	0.22	0.11	0.45	0.047
Cryab	-0.21	0.10	0.34	0.09	0.56	0.010
Cysltr2	-0.20	0.04	0.14	0.07	0.34	0.001
Dckl1	-0.32	0.10	0.08	0.09	0.40	0.004
Dcx	-0.20	0.08	0.07	0.07	0.27	0.008
Ddit3	0.02	0.04	-0.14	0.05	-0.17	0.011
Drd2	-0.31	0.12	0.02	0.12	0.33	0.041
Fgf8	0.06	0.15	-0.44	0.14	-0.50	0.021
Gabra2	-0.41	0.13	-0.04	0.12	0.37	0.025
Gad1	-0.11	0.10	0.31	0.09	0.42	0.002
Gnaq	-0.15	0.04	0.03	0.03	0.18	0.005
Ifit2	0.09	0.04	0.36	0.09	0.27	0.012
Ifitm3	0.13	0.13	0.64	0.12	0.51	0.008
Il11	0.03	0.03	-0.17	0.07	-0.20	0.017
Il1r1	-0.10	0.08	0.26	0.09	0.37	0.009
Insr	-0.07	0.05	0.07	0.05	0.14	0.025
Maff	0.14	0.10	0.48	0.07	0.34	0.015
Map1b	-0.11	0.04	0.04	0.04	0.15	0.004
Mapk8	-0.13	0.06	0.03	0.04	0.16	0.036
Masp1	-0.15	0.07	0.08	0.05	0.23	0.016
Mbp	0.00	0.07	0.28	0.07	0.28	0.005
Mef2c_Mm	-0.23	0.03	0.11	0.05	0.34	0.000
Mef2d	-0.05	0.06	0.16	0.04	0.21	0.013
Mmp9	-0.40	0.17	0.21	0.13	0.61	0.012
Myc	-0.32	0.09	0.01	0.05	0.33	0.007
Ncam1	-0.26	0.07	0.11	0.06	0.38	0.000
Ncam2	-0.24	0.09	0.09	0.08	0.33	0.006
Nlrp3	-0.24	0.11	0.30	0.13	0.53	0.006
Npy	-0.32	0.16	0.18	0.15	0.50	0.012
Plcb1	-0.19	0.09	0.05	0.06	0.24	0.036
Prkca	-0.26	0.10	0.12	0.07	0.37	0.011
Prkcb	-0.13	0.06	0.05	0.04	0.18	0.019
Ptgfr	-0.50	0.19	0.15	0.16	0.65	0.019
Relb	-0.09	0.05	0.07	0.04	0.16	0.026
Rps6ka5	-0.17	0.07	0.07	0.06	0.24	0.012
Shh	0.17	0.09	-0.07	0.09	-0.24	0.031
Syt1	-0.29	0.09	0.08	0.08	0.37	0.002
Tcf4	-0.19	0.07	0.05	0.04	0.24	0.013
Tgfb2	-0.16	0.05	0.28	0.05	0.45	0.000
Tgfb3	-0.45	0.08	0.11	0.09	0.56	0.000

116

Figure 13. High fat diet exposure alters MIA induced changes in gene expression. (**A**) Genes found to be significantly different between MIA exposure with the control diet (dMIA = MIA – average SAL) and MIA exposure with high fat diet (dHFM = HFM – average HFS) were utilized for supervised clustering analysis (43 genes). Significance was determined by unpaired student t-test with Welch's correction and p<0.05. Values are listed in **B.** MIA exposure with high fat diet shows significant differences in both inflammation and neurodevelopment gene expression changes compared to MIA exposure with control diet. These differences range from a lack of change in genes altered in the context of one diet but not the other to exacerbated increases or decreases in gene expression. Altogether, high fat diet induced gestational diabetes modifies the MIA produced relative gene expression changes observed in the fetal brain.

At GD16.5, much less changes in relative gene expression are detected, which should be expected since the acute immune response induced by poly(I:C) exposure passes after 24 – 48 hours. We only observe a few genes altered by MIA at GD16.5 in either control (*Itgb2, Mef2c*) or high fat diet induced gestational diabetes (*Hras1, Ltb4r2, Tlr2*) (**Figure 14A**). Since the insult produced by high fat diet induced gestational diabetes is still occurring at GD16.5, we are not surprised to see several relative gene expression changes produced by high fat diet alone at GD16.5, including induction of neurodevelopment genes (*Bdnf, Ncam2*), both repression (*Ccr1, Cd163, Itgb2, Ptgs1*) and induction (*Hras1, Ptger3*) of inflammation genes, and continued induction of the hypoxia associated gene *Vegfa* (**Figure 14B**). This supports continued effects of high fat diet induced gestational diabetes on the fetal brain. When comparing the impact of high fat diet induced gestational diabetes on MIA effects, several significantly changed genes are found. This increase in differences not seen when comparing MIA to SAL or HFM to HFS can be explained by the nature of those changes. Many changes detected are subtle induction in one condition accompanied by a subtle repression in the other, which reaches the required 10% change without necessarily being a change noted in the previous tables. The directionality of

change is mixed at GD16.5 with some inflammation genes slightly repressed by MIA with high

fat diet and slightly induced by MIA in control fed dams (e.g. *Tnfaip3* and *Ager)* whereas other

inflammation genes are slightly induced by MIA in high fat fed dams (e.g. *Ptgs1* and *Csf1*) and

slightly repressed by MIA in control dams (**Figure 15**). However, gene expression changes can

lead to induction and repression of other genes, working in cascades. We must emphasize that

there may be gene expression changes caused by the GD12.5 MIA exposure that are not included

in the gene list measured at GD16.5. Although subtle, this evolving phenotype reflects ongoing

changes in neurodevelopment and immune response in the brain of the developing fetus.

A

Gene	SAL		HFS			
	Log_2(counts)	SEM	Log_2(counts)	ALR (HFS- SAL)	SEM	p-value
Bdnf	7.51	0.05	7.67	0.16	0.06	*0.045*
Ccr1	4.41	0.10	3.99	-0.41	0.16	*0.035*
Cd163	5.69	0.10	5.35	-0.33	0.05	*0.009*
Hras1	6.99	0.04	7.24	0.24	0.04	*0.001*
Itgb2	5.29	0.08	4.72	-0.56	0.19	*0.014*
Ncam2	10.99	0.03	11.16	0.17	0.03	*0.001*
Ptger3	7.35	0.07	7.56	0.21	0.06	*0.033*
Ptgs1	6.54	0.05	6.35	-0.19	0.06	*0.019*
Vegfa	11.49	0.03	11.68	0.19	0.05	*0.005*

Gene	SAL		MIA			
	Log_2(counts)	SEM	Log_2(counts)	ALR (MIA- SAL)	SEM	p-value
Itgb2	5.29	0.08	5.05	-0.24	0.08	0.046
Mef2c Mm	11.38	0.05	11.23	-0.16	0.03	0.009

Gene	HFS		HFM			
	Log_2(counts)	SEM	Log_2(counts)	ALR (HFM-HFS)	SEM	p-value
Hras1	7.24	0.04	7.07	-0.16	0.06	0.039
Ltb4r2	3.81	0.11	4.09	0.28	0.07	0.038
Tlr2	4.73	0.12	5.22	0.49	0.15	0.018

B
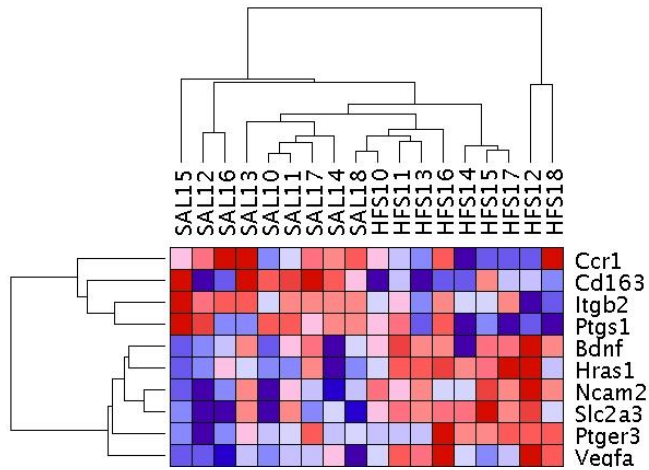
Figure 14. High fat diet induced gestational diabetes and GD12.5 MIA exposure still show altered gene expression patterns at GD16.5. Significant differences in relative gene expression between SAL and MIA, SAL and HFS, and HFS and HFM are listed in **A.** Genes significantly changed by high fat diet exposure compared to SAL were used for a supervised hierarchical clustering analysis (10 genes), shown in **B**. Significance was determined by unpaired student t-test with Welch's correction and p<0.05.

A

B
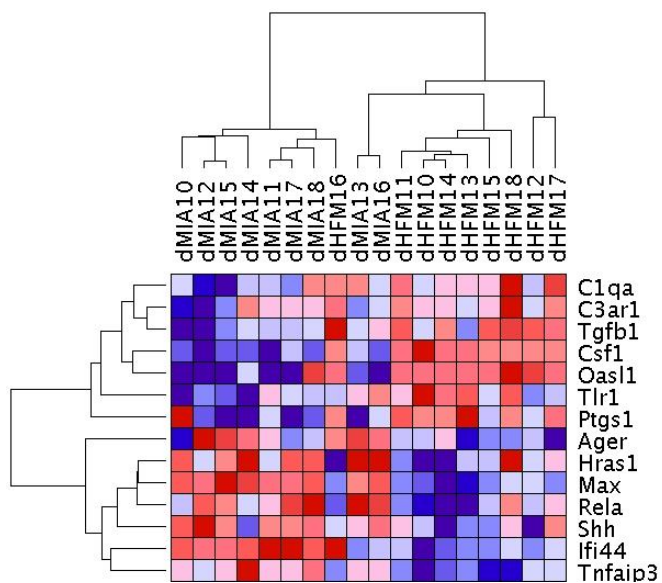
Gene	dMIA		dHFM			
	MIA- Avg SAL	SEM	HFM- Avg HFS	SEM	dALR (dHFM-dMIA)	p-value
Ager	0.11	0.09	-0.13	0.05	-0.24	*0.029*
C1qa	-0.07	0.04	0.09	0.04	0.15	*0.016*
C3ar1	-0.10	0.06	0.10	0.05	0.20	*0.020*
Csf1	-0.29	0.03	0.10	0.04	0.39	*0.000*
Hras1	0.07	0.04	-0.16	0.06	-0.23	*0.005*
Ifi44	0.54	0.21	-0.36	0.25	-0.90	*0.010*
Max	0.18	0.02	-0.03	0.02	-0.21	*0.000*
Oasl1	-0.28	0.06	0.03	0.02	0.31	*0.000*
Ptgs1	-0.06	0.06	0.14	0.04	0.20	*0.009*
Rela	0.14	0.03	-0.02	0.03	-0.16	*0.001*
Tgfb1	-0.11	0.03	0.08	0.04	0.19	*0.001*
Tlr1	-0.21	0.14	0.31	0.19	0.52	*0.036*
Tnfaip3	0.15	0.08	-0.11	0.03	-0.26	*0.009*
Shh	0.10	0.04	-0.04	0.04	-0.15	*0.007*

Figure 15. High fat diet induced gestational diabetes combined with GD12.5 MIA exposure also continues to show altered gene expression patterns at GD16.5. Genes found to be significantly different between MIA exposure with the control diet (dMIA = MIA – average SAL) and MIA exposure with high fat diet (dHFM = HFM – average HFS) were utilized for supervised hierarchical clustering analysis (14 genes), shown in **A** with values in **B**. Significance was determined by unpaired student t-test with Welch's correction and p<0.05. Although less prominent than at GD12.5, MIA exposure with high fat diet continues to show significant differences in both inflammation and neurodevelopment gene expression changes compared to MIA exposure with control diet. These differences range from a lack of change in genes altered in the context of one diet but not the other as well as exacerbated increases or decreases in gene expression. This demonstrates MIA exposure in the context of high fat diet induced gestational diabetes continues to alter gene expression patterns days after the immune stimulus has passed.

Taken together, the altered relative gene expression patterns observed with each perturbation or combination of perturbations suggests that neurodevelopment is being affected. Although more prominent during the heightened cytokine exposure observed with poly(I:C) at

GD12.5, both induction and repression of neurodevelopment and inflammation associated genes with the likely involvement of hypoxia can be observed in HFS, MIA, and HFM. The fetal brain gene expression response to MIA exposure both acutely and after the cytokine surge has passed is unique in the context of high fat diet-induced gestational diabetes, suggesting an interaction between these two inflammation-associated psychiatric disorder risk factors.

CHAPTER IV

DISCUSSION

Aberrant inflammation can predispose to psychiatric disorders (Haroon et al., 2012; Kneeland and Fatemi, 2013; Leboyer et al., 2016; Muller, 2014). Altered inflammatory states and/or responses can be attributed to both genetic and environmental causes (Dodds et al., 2011; Tsuang et al., 2001). For quite some time, we have known that many different insults increase inflammation and risk for later development of psychiatric disorders, and the field has made great strides in understanding the impact of individual genetic and environmental factors on the developing and adult brain (Lemaire et al., 2000; Meyer and Feldon, 2012; Niwa et al., 2010). Yet these insults can and do co-occur. Here we investigated the interface between gene x environment and environment x environment interactions. We found that in both of these types of interactions a novel phenotype can be uncovered. In this section, each interaction will be discussed in detail separately, beginning with the gene x environment interactions investigated in Chapter 2. Then, the implications of the data presented in this document will be discussed and placed into the context of what we know and what future studies may lie ahead.

Inflammation-associated gene x environment interactions in MDD HDFs

For Chapter 2, we very carefully chose the psychiatric disorder, model system, and inflammatory stimuli to best address the complexity of gene x environment interactions in a way that could be broadly applied to other psychiatric disorders. We elected to treat MDD patient-

and control-derived HDFs with vehicle or cytokine (IL1β, TNFα, or IL6), evaluating the transcriptomic response to inflammatory stimuli in the context of genetic predisposition for MDD. Although heterogenous, MDD has been demonstrated to have a genetic component, especially among women (Bierut et al., 1999; Derry et al., 2015), and numerous clinical observations show heightened inflammation in predisposed individuals instigates a depressive phenotype (Dantzer, 2006; Kiecolt-Glaser et al., 2015).

HDFs provided the simplest system in which we could investigate the transcriptional response to inflammatory stimuli in the context of a genetic predisposition to MDD. HDFs are easy to maintain, carry the same genetic variance as other tissues, including brain, and provide a reliable, relevant system to interrogate the effects of different conditions and/or treatments on cellular function in systemic diseases (Bahn and Chan, 2015; Manier et al., 2000). Unlike peripheral blood mononuclear cell cultures, the use of HDFs after several passages negates the majority of effects that arise from medications and/or humoral factors, as only a small portion of the cells in culture are actually from the original biopsy sample when the experiment is performed. HDFs express many of the same receptors and signal transduction proteins as neural tissue, and the signal transduction changes observed in HDFs might facilitate our understanding of signaling changes in the brain (Shelton et al., 1996). This has provided a much needed model for our laboratory and others to study various brain disorders, including Fragile X disorder (Kumari et al., 2014), Huntington's disease (Fernandez-Estevez et al., 2014; Jia et al., 2015), bipolar disorder (Gaspar et al., 2014; McCarthy et al., 2013), and MDD (Akin et al., 2005; Garbett et al., 2015a; Shelton et al., 1996).

MDD patients have been reported to display a myriad of immune- and inflammation-associated changes (Diniz et al., 2010; Erdem et al., 2011; Maes et al., 1997; Savitz et al., 2015). Although there are many paths to a heightened inflammatory state, increased cytokine levels has been consistently seen in a many patients with psychiatric disorders, including MDD. The specific cytokines profile may vary from patient to patient, but most patients that have elevated cytokine levels show changes in at least one of the major pro-inflammatory cytokines (IL1β, TNFα, and IL6) (Dowlati et al., 2010; Lu et al., 2013; Maes et al., 1997; Muller, 2014; Rothermundt et al., 2001). Thus, we chose to treat MDD patient- and control-derived HDFs with each of these cytokines, knowing that all three cyokines may not interact with the MDD patient genome in the same way.

Utilizing this model, our results demonstrate that 1) MDD and CTR HDFs expressed cytokine-responsive genes, 2) MDD and CTR HDFs responded to IL1β, IL6, and TNFα stimulation, and this response was unique for the various cytokines tested, 3) MDD HDFs had an altered response specifically to IL6, but not to IL1β and TNFα, and 4) the differential IL6 response between the MDD and CTR HDFs might be due to active dampening of IL6 signaling by induction of *SOCS3*. Additionally, this is the first demonstration of the utility of HDFs to understand the effects of IL6 on a molecular level in MDD patients.

We used HDFs to test gene expression changes in 46 genes with a variety of cellular functions in response to several cytokines. With IL6 stimulation, we observed a unique gene expression response in MDD, including genes encoding proteins related to crucial stress response transcription factors (*STAT1*, *P38*, NFκB subunit- *NFKB1*, IKK subunits- *IKBKB &*

IKBKG, Nrf2- *NFE2L2*, and *KEAP1*) (Bryan et al., 2013; Levy and Darnell, 2002; Tak and Firestein, 2001; Zarubin and Han, 2005). The JAK/STAT pathway is the main signaling cascade activated by IL6 with STAT1/3 producing the first wave of gene expression changes (Heinrich et al., 2003). NFκB interacts both synergistically and competitively with STAT transcriptional regulation to promote inflammation response gene expression (Grivennikov and Karin, 2010; Heinrich et al., 2003). Moreover, P38 MAP kinase produces NFκB activation and induction of transcripts crucial for regulating numerous inflammatory diseases (Badger et al., 1998; Guan et al., 1998; Hollenbach et al., 2004; Perregaux et al., 1995; Zarubin and Han, 2005). The transcription factor Nrf2 can also be activated by P38 MAP kinase through the release from its cytoplasmic repressor Keap1. Nrf2 regulates genes with antioxidant response elements and is strictly part of the defensive response to oxidative stress (Bakunina et al., 2015). In addition, SOD2 (Mn-dependent superoxide dismutase), a target gene of NFκB and Nrf2, was induced with IL6. Our results suggest that MDD fibroblasts have a diminished IL6 response with the exception of *NFKB1* and *SOD2* induction. Our findings, in concordance with previous studies in MDD patients, support a role for NFκB expression in MDD pathogenesis (Lukic et al., 2014; Pace et al., 2006).

The genes demonstrating an altered relative expression in response to IL6 stimulation in MDD HDFs also included transcripts crucial for cellular metabolism. The altered genes involved in mitochondrial function (*POLG, MIRO1, MFN2,* and *MTATP6*) and lipid metabolism (*HMGCR* and *DHCR7*) suggest IL6 normally represses genes necessary for metabolic processes (Al-Damluji, 2004; Cyster et al., 2014; Graham and Allen, 2015; Ness, 2015). Interestingly, altered mitochondrial function has been associated with disturbed cholesterol metabolism (Allen

and Graham, 2012), and both of these processes appear to play a role in MDD pathophysiology (Zorumski et al., 2013). Altered cholesterol biosynthesis may even exacerbate inflammation in MDD by causing increased release of pro-inflammatory cytokines and altering NFκB signaling (Andres et al., 2012; Ghisletti et al., 2007; Lawlor and Vince, 2014; Shibata and Glass, 2010; Traversari and Russo, 2012). In addition, we found *SKA2* to be subtly less repressed in response to IL6 stimulation in MDD, which may reflect inefficiency in the anti-proliferative response to IL6 (Hanisch et al., 2006). Altogether our results suggest a lack of repression of growth and energy production processes in the MDD HDF IL6 response.

It was perhaps not surprising that the gene encoding the IL6 receptor was expressed at a higher level both at baseline and after IL6 stimulation in MDD. IL6 signals through an IL6 receptor/gp130 complex that phosphorylates JAK and causes subsequent phosphorylation/activation of STAT transcription factors as well as MAPK and ERK signaling pathways (Babon et al., 2014). Suppressor of cytokine signaling 3 (*SOCS3*) transcription is induced by STAT3 and binds both the IL6 receptor/gp130 complex and JAK to inhibit further signaling and to mark both JAK and the receptor for degradation. SOCS3 inhibition of IL6 signaling often returns signaling induced by IL6 to baseline (Babon et al., 2012; Kershaw et al., 2013). *SOCS3* was found to be significantly induced in MDD but not in CTR in response to IL6. This finding in combination with the increased baseline expression of the IL6 receptor transcript suggests an increased capacity to respond to IL6 with a concomitant increase in feedback inhibition by SOCS3. Importantly, IL6-mediated SOCS3 dampening was not observed in CTR HDFs.

It is notable that we observe a blunted response in MDD fibroblasts to IL6 exposure. Yet, previous findings suggests that increased IL6 signaling produces depressive symptoms (Sukoff Rizzo et al., 2012). While this might appear contradictory at first glance, these data are not mutually exclusive: our findings might be related to an adaptive suppression feedback mechanism which prevents excessive stimulation in response to elevated IL6 levels. Additionally, we must emphasize that the molecular phenotype is only revealed after a challenge with cytokines. Simply measuring basal levels of mRNA transcripts cannot reveal the phenotype we describe here. Yet our study is not without limitations. We selected a limited gene set, used 3 cytokines for stimulation, and tested a single response time, leaving considerable room for follow-up studies.

Our goal is that some of these follow-up studies expand our findings to other genetic predispositions and additional environmental insults. We utilized MDD and CTR HDF treatment with cytokines to demonstrate gene x environment interactions. However, since other psychiatric disorders like schizophrenia and autism are also associated with low grade chronic inflammation, including altered serum cytokine profiles, it is likely that HDFs derived from these patients may also have an altered response to exogenous cytokines like IL6 (Miller et al., 2011; Okada et al., 2007; Ricci et al., 2013). Furthermore, similar experiments could also be performed with either more broad inducers of generalized inflammation such as reactive oxygen species or more specific inducers like NFκB activators (Czarny et al., 2015; Manuvakhova et al., 2011). As new combinations of genetic predispositions with inflammatory stimuli are studied, we hypothesize that novel, disorder-specific transcriptomic responses will be observed, uncovering more disrupted molecular phenotypes.

In summary, we observed MDD and control HDFs to have a divergent response to IL6 stimulation. Here we were able to identify oxidative stress and cellular metabolism related genes as differentially expressed in MDD patient HDFs compared to CTR only with IL6 stimulation and not with IL1β or TNFα. The mostly unaltered relative gene expression with IL6 stimulation in MDD as well as the increased relative baseline expression of *IL6R* and IL6 stimulated induction of *SOCS3* provides strong evidence that there is an aberrant inflammatory response in MDD. We believe this requirement of combined genetic x environmental context to reveal the disrupted transcriptomic response may also occur with other psychiatric disorders and with other inflammatory stimuli.

Inflammation-associated environment x environment interactions: the combined effect of gestational diabetes and MIA on gene expression patterns in the fetal brain

In Chapter 3, we sought to investigate the impact of environment x environment interactions on the fetal brain. To accomplish this, we combined two environmental insults that commonly occur during gestation, induce inflammation, and are associated with increased risk for psychiatric disorders later in life- gestational diabetes and maternal infection (Atladóttir et al., 2010; Brown et al., 2004a; Gardener et al., 2009; Xiang et al., 2015; Yamashita et al., 2003). We chose high fat diet to induce gestational diabetes (Pasek and Gannon, 2013). The presence of impaired glucose tolerance, fasting glucose, and fasting insulin in our data set demonstrates that 60% fat by calorie diet from 6 weeks prior to mating through pregnancy is sufficient to produce a gestationally diabetic phenotype. To model maternal infection, we utilized the viral mimetic

poly(I:C). In our cohort, poly(I:C) exposure induced weight loss 24 hours post-injection, which

has also been noted in previous studies (Missault et al., 2014; Smith et al., 2007).

To better characterize these models, we measured maternal serum cytokine, chemokine,

adipokines, and insulin levels both during the acute immune response at GD12.5 and four days

after MIA exposure at GD16.5. At GD12.5, we observed maternal serum increases in several

cytokine and chemokines 3 hours after poly(I:C) exposure, clearly demonstrating an immune

response in the pregnant dam as has also been noted by others (Arrode-Bruses and Bruses, 2012;

Connor et al., 2012; Forrest et al., 2012; Khan et al., 2014; Mandal et al., 2013; Song et al.,

2011). This MIA-induced cytokine/chemokine increase was similar in control fed and high fat

fed conditions, suggesting that high fat diet induced gestational diabetes does not appear to

modify the cytokine/chemokine response observed. Although no cytokine increase reached

significance with high fat diet induced gestational diabetes alone, we did observe significantly

increased leptin and decreased adiponectin levels, with a trend towards increased insulin with

high fat diet induced gestational diabetes alone. This metabolic profile is expected with a

diabetic phenotype, attributable due to leptin resistance, increased fat mass, hyperglycemia,

and/or insulin resistance (Fasshauer et al., 2014; Gallou-Kabani et al., 2007; Kautzky-Willer et

al., 2001; Van Heek et al., 1997; Vitoratos et al., 2001). Most interesting, we found that the acute

response to MIA in the context of high fat diet induced gestational diabetes produced a further

increase in leptin and insulin levels. The pro-inflammatory cytokine leptin has been reported to

be increased by infection in diabetic patients, although we are unaware of any observations in

gestationally diabetic patients (Gómez-Ambrosi et al., 2004; Zhang et al., 2013). In addition, a

heightened inflammatory state can increase insulin resistance, likely promoting the heightened

insulin levels when high fat diet induced gestational diabetes is combined with MIA (Anderson et al., 2007; Chen et al., 2015a).

Although the pro-inflammatory adipokine leptin has been reported to be increased during infection, adiponectin is thought to be lowered or unchanged in diabetic patients during an infection (Chandran et al., 2003; Doruk et al., 2014; Gómez-Ambrosi et al., 2004; Pala et al., 2015; Zhang et al., 2013). However, we find that at GD16.5, four days after exposure to MIA, that adiponectin levels are significantly higher in the HFM group when compared to HFS. In other words, an increase in adiponectin with MIA exposure is observed only in the context of high fat diet induced gestational diabetes and only after the acute immune response has passed. Interestingly, the research community has not come to an agreement on whether adiponectin is pro-inflammatory, anti-inflammatory, or if it just depends on the context (Fantuzzi, 2008). For example, i*n vitro,* adiponectin initiates pro-inflammatory gene expression in activated T cells and macrophages but, on the other hand, can also suppress antigen-specific T cell expansion (Cheng et al., 2012; Wilk et al., 2011). We do know that adiponectin increases insulin sensitivity, is usually reported as decreased in diabetes, and is suppressed by pro-inflammatory factors like TNFα (Swarbrick and Havel, 2008; Tsuchihashi et al., 2006; Yokota et al., 2000). We hypothesize that the GD16.5 maternal serum changes represent a remnant of anti-inflammatory response induced to dampen and/or terminate the transient heightened inflammatory state produced by MIA exposure. This is supported by the MIA induced increase in the anti-inflammatory cytokine IL13 in control dams at GD16.5 (Wynn, 2003). Taken together, the maternal serum data supports that 1) MIA produces a transiently heightened inflammatory state in the dam, 2) high fat diet induces adipokines changes consistent with a diabetic phenotype, and

3) MIA and high fat diet interact to produce an even higher increase in pro-inflammatory leptin levels. This soundly demonstrates that our models of MIA and gestational diabetes are pro-inflammatory as well as provides novel insight into the interaction at the maternal level of MIA and gestational diabetes.

Utilizing these models to understand gene expression changes in the fetal brain, our results demonstrate that 1) high fat diet induced gestational diabetes alone alters expression of neuronal patterning, IFN response, growth, cell cycle regulation, and apoptosis genes in the fetal brain, 2) MIA alters expression of many genes both alone (antiviral/IFN response, lipid peroxidation, cell cycle regulation/apoptosis, cell growth, inflammation-associated intracellular signaling, hypoxia, and neuronal migration) and in the context of high fat diet induced gestational diabetes (antiviral/IFN response, innate immune response, cell cycle regulation/apoptosis/growth, inflammation-associated intracellular signaling, neuronal patterning/migration/myelination, hypoxia, and glucose transport), 3) high fat diet induced gestational diabetes modifies the MIA produced relative gene expression changes observed in the fetal brain with a general trend towards stronger induction of inflammation and neurodevelopment associated genes, and 4) the majority of relative gene expression changes are only transiently observed during the acute immune response to MIA at GD12.5 and not at GD16.5. Additionally, this study pioneers investigation of the environment x environment interactions of gestational diabetes and MIA.

Our gestational diabetic rodent model disrupts gene expression patterns in fetal brains at GD12.5. Neurodevelopmentally, altered expression of sonic hedgehog and engrailed 1 indicates there may be a deficit in regional patterning of the brain (Chiang et al., 1996; Sadler et al., 1995). Several IFN response, cell growth, apoptosis, and cell cycle related genes were also affected in the HFS group. It must be emphasized that many of these genes are classified as having a predominant inflammation function, but many also participate in neurodevelopmental processes as well. For example, *Hmgb2* produces a chromatin structural protein thought to regulate neural stem cell maintenance (Abraham et al., 2014). Changes in expression level of neurodevelopmentally active gene products can disrupt the fetal brain, which we find equally important to the associated inflammation response functions. Angiogenic and hypoxic response genes (*Ddit3, Vegfa*) being induced in HFS is highly suggestive of a hypoxic intrauterine environment. A hypoxic intrauterine environment has been suggested by both clinical and preclinical gestational diabetes studies although the presence of hypoxia in the fetal brain has been unclear (Escobar et al., 2013; Li et al., 2013). Our results strongly suggest that gestational diabetes does alter expression of neurodevelopmentally relevant genes and can produce hypoxia in the fetal brain. These observations together provide potential mechanisms by which gestational diabetes increases risk for psychiatric disorders.

It is no surprise that MIA altered relative gene expression 3 hours after poly(I:C) injection on GD12.5. The gene expression profile observed when comparing MIA to SAL litters is distinct from that produced by HFS. The inflammation associated genes that were induced strongly participate in antiviral processes whereas many of the genes repressed produce proteins important for cell cycle regulation, apoptosis, cell growth, and inflammation-associated

intracellular signaling (Barton and Medzhitov, 2003; Liu et al., 2011a; McKinsey et al., 2002; Spellman et al., 1998; Yanai et al., 2009; Zhou et al., 2013). It appears as though the fetal brain is producing the perceived necessary antiviral response while minimizing generalized inflammation and cell proliferation/growth. Distinct from the gene expression profile of fetal brains from gestationally diabetic dams, neurodevelopment genes are only repressed with MIA, and all of these genes play some role in neuronal migration, which has been reported as disrupted by MIA (Fatemi et al., 1999; Soumiya et al., 2011b). Furthermore, the constitutively expressed glucose transporter GLUT1 (*Slc2a1*) and hypoxia-associated genes (*Flt1, Vegfa*) are induced by MIA, suggesting insufficient oxygen and nutrients are reaching the fetal brain (Bastian et al., 2015). Altogether, this data suggests that although the fetal brain is attempting to minimize the effects of the mounted antiviral response, neurodevelopment is at least transiently disrupted, which is in agreement with our previous MIA work (Garbett et al., 2012). In addition, either due to the metabolic demands for mounting this response or the direct effects of MIA on the placental/maternal circulation, some degree of hypoxia is occurring in the fetal brain.

MIA in the context of high fat induced gestational diabetes also alters relative gene expression. With regards to neurodevelopment associated genes, *Foxg1* induction and *Fgf8* and *Pax5* repression support deficient regional patterning in the HFM fetal brains (Danesin and Houart, 2012; Ohtsuka et al., 2013; Walshe and Mason, 2003), and *Gad1* and *Vamp1* induction likely promotes and potentially accelerates neuronal migration (Marin, 2013). At this point in development, myelin basic protein is more likely involved in oligodendrocyte proliferation/differentiation rather than myelination (Spassky et al., 1998; Takebayashi and Ikenaka, 2015). Although loss clearly hinders oligodendrocyte development, the increased *Mbp*

expression found in HFM fetal brains may also disrupt oligodendrocyte proliferation and differentiation (Ozgen et al., 2014; Smith et al., 2011). Similar to MIA alone, the inflammation genes induced were mostly those critical for an antiviral response (Barton and Medzhitov, 2003; Liu et al., 2011a; Zhou et al., 2013). Unlike MIA alone, genes encoding generalized innate immune response proteins, TGFβ2, and apoptosis-regulating proteins were also induced. This suggests that the MIA induced transcriptional response in the context of gestational diabetes may be less "contained", although many inflammation associated genes were also found to be repressed.

Interestingly, *Tgfb2* and *Pdgfa* are both "inflammation" associated genes affected by MIA in gestational diabetes, but all also have the known neurodevelopmental roles as well (Funa and Sasahara, 2014; Lu et al., 2005). The observed induction of *Ager* may indicate disrupted hippocampal development. Chandna and colleagues previously reported increased RAGE (the protein produced by *Ager*) in STZ-induced gestational diabetes, which was associated with abnormal hippocampal development (Chandna et al., 2015). Moreover, both the constitutively expressed GLUT1 and high affinity neuronal specific GLUT3 genes (*Slc2a1, Slc2a3*) as well as hypoxia-associated genes (*Flt1, Vegfa*) were induced in HFM compared to HFS. Having already demonstrated that high fat diet dams acquire glucose intolerance and hyperglycemia, the glucose transporters are likely induced both due to increased metabolic need from heightened inflammation and increased availability of glucose for uptake (Baumann et al., 2002; Jansson et al., 1999). Altogether, HFM fetal brain gene expression profiles demonstrate a strong antiviral and inflammation response although there are clear attempts to repress inflammation signaling. There is also evidence for altered neurodevelopment associated genes, indicating a potential for

altered regional patterning, accelerated neuronal migration, and disrupted oligodendrocyte proliferation/differentiation. HFM fetal brains also have evidence of hypoxia that is likely driven by processes associated with both MIA and gestational diabetes.

One of the more important questions we sought to answer was if gestational diabetes interacts with MIA to produce a novel phenotype. We found many genes were differentially affected by MIA in the context of high fat diet versus control diet, suggesting an interaction between high fat diet induced gestational diabetes and MIA impacted the fetal brain gene expression profile. In this data, the genes changed most strongly by MIA within the control or gestational diabetes contexts (*Ifi44, Ifit1, Ifit3, Mx2, Oasl1*) were not different in this comparison, suggesting that the induction of these antiviral response genes are independent the context in which they occur and are primarily driven by MIA. Of those genes discussed above that were changed by MIA in one context and not the other, trends do arise. MIA in the context of gestational diabetes leads to stronger induction of genes associated with neuronal migration, oligodendrocyte proliferation/differentiation, and cell growth/proliferation in addition to *Il1r1*. Yet MIA WITHOUT gestational diabetes more strongly represses genes associated with apoptosis and inflammation-associated intracellular signaling. We interpret these general trends to demonstrate that MIA in the context of gestational diabetes disrupts distinct neurodevelopmental processes and produces an exaggerated inflammation-associated transcriptional response compared to MIA alone.

Lastly, we demonstrated that the effects produced by MIA are mostly transient. But transiently changed does not mean transiently important. When the precise timing or the extracellular environment in which neurodevelopmental events take place are disrupted, the trajectory of those neurons/brain regions affected can be permanently shifted (Millan et al., 2016; Ross et al., 2015). With regards to our transient changes, we only observe a few inflammation associated genes altered by MIA at GD16.5 in either control (*Itgb2, Mef2c*) or high fat diet induced gestational diabetes (*Hras1, Ltb4r2, Tlr2*). There are many genes differentially affected to some degree between dHFM and dMIA, but these changes are much more subtle than those observed at GD12.5, suggesting that the effects of MIA are still present but minor at GD16.5. We believe although subtle changes linger, that neurodevelopmental programs resume when the immune stimulus has passed. These results support this interpretation, demonstrating that almost all substantial gene expression changes induced by MIA are no longer present 4 days after the immune insult.

Since the insult produced by high fat diet induced gestational diabetes is still occurring at GD16.5, we see several relative gene expression changes produced by high fat diet alone at GD16.5, including induction of neurodevelopment genes that promote growth, differentiation, and migration (*Bdnf, Ncam2*). We also observed mixed changes in neurodevelopmentally active inflammation associated genes such as those producing the chemokine C-C receptor 1 and integrin β2 (Marin, 2013; Metin et al., 2008). Interestingly, *Vegfa* induction is still observable at GD16.5 in HFS fetal brains. This supports continued effects of high fat diet induced gestational diabetes on the fetal brain, which includes a continued hypoxic state. These gestational diabetes associated gene expression changes both support observations from previous studies as well as

add new molecular pathways that may be altered by gestational diabetes (Li et al., 2013; Tang et al., 2015). These findings provide novel targets for paths between gestational diabetes and neuropathology that predisposes to psychiatric disorder development later in life.

In this study, we identify molecular targets in the transcriptome arising from individual and combined environmental insults, focusing strongly on how co-occurring environmental insults can modify the transcriptional changes. Modeling potentially co-occurring environmental insults is important. It can inform our understanding of environment x environment interactions. Our two environmental insults modeled here (gestational diabetes and maternal infection) both increase inflammation. This produce a pro-inflammatory intrauterine environment, altering neurodevelopmental processes and increasing risk for psychiatric disorders (**Figure 16**) (Brown, 2012; Chess, 1971; Gardener et al., 2009; Wegelius et al., 2011; Xiang et al., 2015). It is true that neither of these insults are specific to a particular disorder, but they do generally predispose to brain disorders, with disorder specification dependent on the genetic context (Horvath and Mirnics, 2014). This work demonstrates the importance of understanding the complexity of these generally predisposing insults so that the knowledge gained can be applied to both environment x environment and gene x environment interactions.
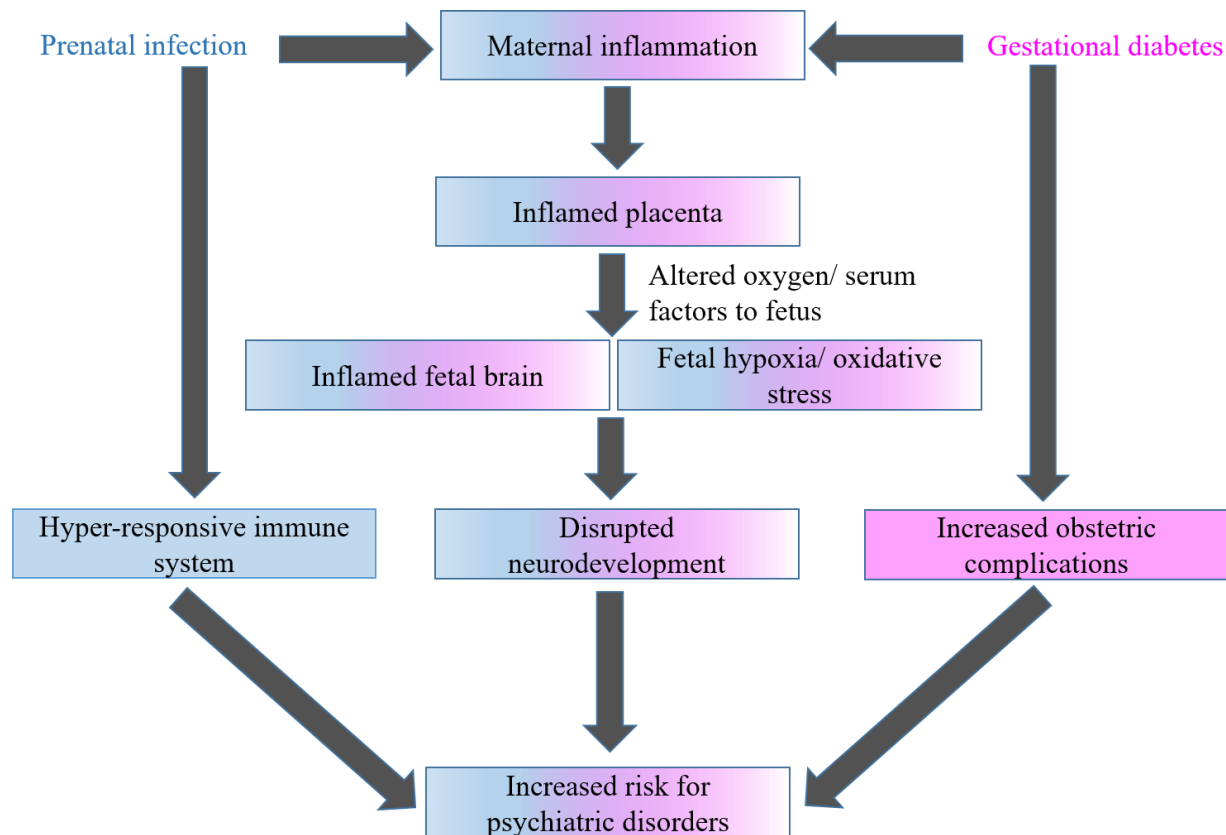
Figure 16. Inflammation from MIA & GDM increases psychiatric disorder risk. This diagram outlines the path from prenatal infection & gestational diabetes to increased risk of psychiatric disoders suggested by our data and studies of GDM or MIA alone. Both prenatal infection and gestational diabetes increase maternal inflammation. In addition to the passage of some pro-inflammatory factors through the placenta to the fetus, maternal inflammation also alters the oxygen/nutrients, placental hormones, and placenta-secreted cytokines transferred to the fetus. A pro-inflammatory intrauterine environment produces fetal brain inflammation, hypoxia, and increased oxidative stress, which disrupts neurodevelopment. This disrupted neurodevelopment combined with MIA-associated hyperresponsive immunity and GDM-associated obstetric complications increases risk for psychiatric disorders later in life.

Implications of interaction between inflammatory risk factors for psychiatric disorders

The research in this document highlights the importance of the environmental and/or genetic context in which an environmental insult occurs. We definitively demonstrate that the transciptomic effects of an environmental insult are unique to the context in which it occurs. Other than the highly detailed, model-specific conclusions discussed above, what does this really mean for the field going forward? In brief, as we have always known clinically but often had to ignore preclinically, it's just complicated. Modeling a single insult that leads to heightened inflammation in a controlled context will only inform one possible path that insult can take. To be clear, we, by no means, are discounting the value of single insult studies. Our lab has published many studies investigating a single genetic or environmental context, including psychiatric disorder-associated transcriptomics in post mortem brains and HDFs (Arion et al., 2007; Garbett et al., 2008; Garbett et al., 2015a; Hashimoto et al., 2008; Mirnics et al., 2000; Shelton et al., 2011), molecular and behavioral phenotypes of schizophrenia-related GABAergic knockdown mouse models (Brown et al., 2014; Brown et al., 2015; Garbett et al., 2010; Schmidt et al., 2014; Schmidt and Mirnics, 2012), and even transcriptional and behavioral changes associated with models of MIA (Garbett et al., 2012; Smith et al., 2007). The results in this document in their simplest terms show that the specific changes, at least with regards to the transcriptome, are highly dependent on at least some contextual components.

It is difficult to predict which inflammation-association psychiatric disorder risk factors will interact. There are many discussed in this document (e.g. psychosocial stress, obesity,

gestational diabetes, maternal infection). It has already been demonstrated that combined MIA and peripubertal stress interact to produce a novel adult behavioral and neurochemical phenotype (Deslauriers et al., 2013; Giovanoli et al., 2013). Thus, it has been established to at least some degree that genetic context and heightened IL6, peripubertal stress and midgestation MIA, and gestational diabetes and midgestation MIA do interact. But even within these established interactions, there are so many variables left unknown.

Many variables affect the impact of environmental risk factors, including the timing, duration, type, and strength of insult as well as the genetic/environmentally altered predisposition in which that insult occurs. Timing matters greatly. Exposures often have peak effect during certain windows of time like the limited gestational period prenatal cocaine exposure disrupts neurodevelopment or the lack of poly(I:C) induced effects very late in gestation (Meyer et al., 2007; Stanwood et al., 2001). With regards to type, it has already been discussed that each form of infection mimetic is subtly different with LPS exposure leading to a similar but slightly different neurodevelopmental deficit than poly(I:C) exposure (Arsenault et al., 2014; Meyer, 2014). Both duration and strength alterations can drastically impact the severity of the phenotype produced, with gestational diabetes models with multi-day, high dose STZ capable of producing quite severe maternal and fetal phenotypes (Jiang et al., 2008; Lian et al., 2004; Liao et al., 2004). We recognize the importance of each of these variables. In these studies, we were most interested in the context, or predisposition (i.e. MDD patient genetic background or gestational diabetes), in which our insult (i.e. exogenous cytokine treatment or the inflammatory state produced by MIA) occurred. Certainly, follow-up experiments could provide novel information by focusing on the other variables or utilizing alterative models. Clearly, it would be interesting

to see if our models corresponds with these others. We found the intersections between our chosen models appealed to us as the most reliable and clinically relevant, and our research now has opened up the possibility of so many more questions.

With these variables, many questions come to mind with regards to interacting inflammation-associated risk factors. For example, the primary genetic susceptibility locus for schizophrenia is the MHC region (Brucato et al., 2015; Mukherjee et al., 2014; Sekar et al., 2016; Walters et al., 2013; Yamada et al., 2015). Since appropriate immune responses are needed to maintain homeostasis of pro- and anti-inflammatory factors in normal and immune-stimulated states, could MHC region mutations amplify or attenuate the biological effects of environmental insults in a way to further increase risk for psychiatric disorders? Additionally, the combination of peripubertal chronic unpredictable stress and midgestation MIA clearly demonstrate an important interaction that exacerbates adult schizophrenia-like behaviors in mice (Giovanoli et al., 2013), but this shows the effects of one type of stressor at one of many times psychological stress can increase risk for psychiatric disorders. What if the timing of psychological stress combined with midgestation MIA was altered? Prenatal stress (e.g. maternal bereavement, war, natural disasters, familial discord) has all also been associated with increased psychiatric disorder risk (Huttunen and Niskanen, 1978; Kinney et al., 2008; Li et al., 2009; van Os and Selten, 1998; Walder et al., 2014; Ward, 1990; Watson et al., 1999). Would prenatal stress show the same effect, no effect, or a novel phenotype altogether? Would social defeat stress have a different impact than the unpredictable chronic stress models utilized by the Grignon and Meyer labs?

Any one insult has the potential to be altered by any or all of the above described variables. The insults chosen for our studies are no different. There are multiple methods for generating gestational diabetes (e.g. high fat diet and streptozotosin) and MIA (e.g. influenza, poly(I:C), and LPS) (Fortier et al., 2004; Garbett et al., 2012; Golson et al., 2010; López-Soldado and Herrera, 2003). There are also inflammatory stimuli in addition to IL6 that may produce a novel transcriptional phenotype in psychiatric disorder patient-derived HDFs, including chemokines and other cytokines. Timing, duration, and strength could also be varied and may attenuate, exacerbate, or have no effect on the phenotypes we observe in this document. We hope future studies by our lab and others will investigate some of these variables to better understand the dynamics of gene x environment and environment x environment interactions demonstrated here.

An important point raised by this research is the notion that the environmental context in which an insult occurs may be just as important to the impact on the developing brain as genetic predisposition. The response to MIA observed in the context of gestational diabetes demonstrates that like genetic predisposition, environmental context can shape the gene transcription response to environmental insults. This may be due to epigenetic changes induced by gestational diabetes on the fetal genome or by interaction of the ongoing transcriptional response to the disrupted metabolism and inflammation caused by gestational diabetes with MIA. Whatever the mechanism, the environmental context in which insults occur could be very important to better defining the culmination of factors that lead to psychiatric disorder development. These questions have been difficult to answer in past clinical studies because of the enormous sample sizes required to power studies of accumulated risk (Caspi and Moffitt, 2006; Nicodemus et al.,

2008). With increasing shifts towards electronic medical record databases that can be mined for trends, we hope soon that these questions will be addressed, providing clinical input to the advancement of the complex, interacting nature of accumulated psychiatric disorder risk.

Our demonstration that genetic predisposition can shape the effects of environmental insults is not conceptually novel. Utilizing HDFs derived from a similar cohort of MDD patients and matched controls, we demonstrated an effect of genetic predisposition in the context of metabolic stressors (Garbett et al., 2015b; Kalman et al., 2014). Something that is pioneering in the altered transcriptional response to IL6 in MDD HDFs is the notion that this effect is likely cyclical. We hypothesize that chronic systemic elevations of inflammatory stimuli (e.g. IL6) produced either by environmental or genetic perturbations produce long lasting genetically encoded changes in inflammatory response genes, possibly in an attempt to protect against low grade chronic inflammation, which in turn alters response to inflammatory stimuli. But what if this cyclical nature of genetic and environmental perturbations progressively leading to increased pathological changes is the norm and not the exception? For example, autism has been associated with rare and common variants of *MET* (Campbell et al., 2007; Campbell et al., 2006; Sousa et al., 2009). But *MET* gene mutations have also been associated with gestational diabetes, which also increases the risk for autism (Alvarez-Perez et al., 2014; Demirci et al., 2012; Mellado-Gil et al., 2011; Ornoy et al., 2015; Xiang et al., 2015). Although preclinical studies suggest MET tyrosine kinase dysfunction alters cortical development, what if *MET* mutations also impact autism risk through gestational diabetes (Campbell et al., 2007; Powell et al., 2003; Powell et al., 2001)? The inflammation produced by gestational diabetes may then worsen the immune-associated changes seen in mothers with *MET* mutations and their children that also carry the

mutation (Heuer et al., 2011). As we learn more about the potential impact of gene variants associated with psychiatric disorders, these potential cyclical interactions may be found to be more common than we thought.

In addition to the broader applicability of the general conclusions of this data, there are also important themes in the specific instigators and molecular pathways altered by the gene x environment and environment x environment interactions studied here. In particular, the research in this document demonstrates the importance of cytokines on brain development and adult function, with an emphasis on IL6. In addition, certain inflammation associated pathways are strongly involved in the transcriptional response to the herein studied inflammatory stimuli, particularly IL6 signaling and hypoxic response genes.

With the various roles they play both centrally and peripherally, cytokines are the most likely instigators when it comes to inflammation-associated psychiatric disorder pathology. Most cytokines exist at some basal level with dramatically heightened states produced by inflammation-induced leukocyte secretion (Pitossi et al., 1997). Both normal and pathologic processes utilize cytokine signaling. Cytokines function in the developing and adult brain as inflammatory mediators, neuromodulators, and neurodevelopmental regulators (Besedovsky et al., 1991; Deverman and Patterson, 2009; Konsman et al., 2002; Schneider et al., 1998). In the systemic immune response, cytokines interact with the brain to control fever, sickness behaviors, and immunoregulation via HPA axis activation (Harden et al., 2015). Developmentally, cytokines, chemokines, and complement proteins participate in self-renewal of neural stem cells (Bauer et al., 2007; Galli et al., 2000), gliogenesis (Barnabe-Heider et al., 2005), neuronal

144

migration (Lazarini et al., 2003; Stumm et al., 2003), synaptogenesis (Heupel et al., 2008; Kaneko et al., 2008), oligodendrocyte development (Barres et al., 1993; Corbin et al., 1996; Louis et al., 1993), and elimination of unnecessary synapses/neurons (Fontaine et al., 2008; Stevens et al., 2007). And in the adult brain, IL1β, TNFα, and IL6 have been implicated in pain sensitivity, sleep patterns, emotional behavior, and learning and memory (Heyser et al., 1997; Morrow and Opp, 2005; Shavit et al., 2005; Yamada et al., 2000; Yirmiya et al., 2002). The vast array of normal and immune stimulus-related roles played by cytokines opens the door to a multitude of potential brain dysfunctions when cytokine levels are altered. Thus, our finding that cytokines, especially IL6, are strong drivers of inflammation-associated transcriptome changes was, if anything, expected.

In particular, chronic low grade inflammation, which is found in gestationally diabetic mothers as well as in many patients with psychiatric disorders, has the potential to produce a "primed" state, altering and potentially exacerbating the response to additional inflammatory stimuli (Abell et al., 2015; Frank et al., 2015; Leboyer et al., 2016). Chronic low grade inflammation can increase microglial activation, disrupt the blood brain barrier, and alter gut permeability, leading to increased gastrointestinal microbial products in systemic circulation and thus further heightened inflammation (Bloomfield et al., 2016; Severance et al., 2012). Environmental stressors at multiple points in development- midgestation maternal infection, perinatal hypoxia, early childhood trauma, or adolescent cannabis use- are often considered co-factors or triggers for this "primed" state, which may have arose from genetic or genetic and environmental insults (Maes et al., 2009; Millan et al., 2016). In these studies, we find that "priming" either through gestational diabetes or genetically encoded predisposition in MDD

leads to an altered response to a secondary insult, either MIA or individual exogenous cytokines, respectively.

In both of the secondary insults used in this research, IL6 is the predominant cytokine inducing dysfunction. In the central immune response, IL6 is potently induces anorexia, fever, and HPA axis activation (Kagiwada et al., 2004; Lenczowski et al., 1999; Li et al., 2003a; Plata-Salaman et al., 1996). Peripherally, IL6 induces antiviral activity, acute phase protein synthesis, and angiogenesis via VEGF production (Hashizume et al., 2009; Kishimoto, 1989). IL6 is among the first cytokines produced in the immune response, and its synthesis is also induced by TNFα and IL1β (Tanaka et al., 2014). In addition, heightened levels of IL6 have been associated with impairments in learning and memory (Heyser et al., 1997), whereas members of the IL6 superfamily have been implicated in neurogenesis, gliogenesis, and oligodendrocyte development (Barres et al., 1993; Bonaguidi et al., 2005; Galli et al., 2000; Pitman et al., 2004; Rajan and McKay, 1998). This combined with the strong association observed between increased frequency of psychiatric disorders and heightened IL6 levels at multiple timepoints suggests IL6 has a central role in inflammation-induced psychiatric disorder pathogenesis (Ashwood et al., 2011b; Howren et al., 2009; Maes et al., 1995; Miller et al., 2011; Sukoff Rizzo et al., 2012).

Strong evidence exists for a primary role for IL6 in the aberrant inflammatory profile of MDD. Chronic stress-induced depressive symptoms in animal models have frequently been associated with increases in serum, CSF, and brain concentrations of IL6 (LeMay et al., 1990; Monje et al., 2011; Sukoff Rizzo et al., 2012; Voorhees et al., 2013). Successful amelioration of these depressive symptoms with antidepressants or IL6 receptor antibodies are accompanied by

normalization of IL6 levels, reminiscent of that observed in human studies (Hodes et al., 2014; Reus et al., 2015). Concordantly, IL6 knockout mice are resistant to develop depressive symptoms in models of chronic stress (Chourbaji et al., 2006), and blockade of IL6 receptor trans-signaling decreases the induction of depressive symptoms by IL6 (Sukoff Rizzo et al., 2012). In line with animal studies, MDD patient studies also find consistent increased concentrations of IL6 (Dowlati et al., 2010; Maes et al., 1993; Moieni et al., 2015; Quak et al., 2014). These IL6 elevations in MDD correlate with factors associated with depression, such as acute stress and childhood trauma (Carpenter et al., 2010). Furthermore, IL6 has the capacity to alter processes associated with depression such as increasing tryptophan catabolism and stimulating the HPA axis (Besedovsky et al., 1991; Fujigaki et al., 2006; Li et al., 2003b; Raison et al., 2009). Interestingly, the MDD HDF IL6 response we observed in Chapter 2 is unlikely to be influenced by most humoral (i.e. elevated serum IL6), medication, and epigenetic effects due to the minimum 5-10 culture passages after the initial biopsy culture before use in our experiments. We believe our data primarily represents a genetically encoded disruption interacting with heightened levels of IL6 as opposed to a transient adaptation. It is possible that this genetic disruption may even be due to chronic elevation of IL6 levels in MDD, a cyclical phenomena as described above. When taken together with the previously discussed studies, it appears that MDD is characterized by both a chronic elevation of IL6 and a genetically encoded, summative altered IL6 responsiveness. Therefore, we propose that the association of increased IL6 and an aberrant IL6 response in MDD might represent a molecular end-point of genetic predisposition to MDD and is in agreement with previously reported data (Su et al., 2009).

IL6 is not only the instigator but also is the altered molecular pathway mediating the differences observed in MDD HDF transcriptional response. In other words, not only is IL6 the only cytokine that uncovered a distinct transcriptional response in MDD HDFs, but *SOCS3* induction, which acts directly on the IL6 receptor complex to inhibit IL6 downstream signaling, likely produces the blunted IL6 response observed in MDD HDFs (Kershaw et al., 2013; Schmitz et al., 2000). SOCS3 does not alter the magniture or time of initiation of IL6 signal induction but prevents the persistence of downstream STAT1/3 signaling (Croker et al., 2012). SOCS3 is the primary regulator of IL6 signaling, but also can inhibit other inflammatory mediators such as LIF, G-CSF, and leptin (Croker et al., 2004; Kievit et al., 2006; Mori et al., 2004; Roberts et al., 2001). Even so, SOCS3 has a 10X higher affinity for the IL6 receptor than the leptin receptor (De Souza et al., 2002). Based on what is known about SOCS3 signaling and receptor affinity, the Nicola lab predicts that SOCS3 inhibits cytokines with SOCS3 binding sites at physiological levels (e.g. IL6 receptor, leptin receptor) but when overexpressed is capable of inhibiting any ligand that uses JAK1, JAK2, or TYK2 to mediate their signaling effects (Babon et al., 2014). This could include numerous other cytokines, including other ILs and IFNs, as well as erythropoetin signaling, which is critical for red blood cell production (Neubauer et al., 1998; Parganas et al., 1998; Rodig et al., 1998). The observed exaggerated *SOCS3* induction produced in an attempt to suppress IL6 effects could very well be suppressing basal signaling of many other needed pathways. Interestingly, *SOCS3* has also been observed to be strongly induced in IL6-treated primary cortical cultures derived from fetal brains exposed to poly(I:C) midgestation (Connor et al., 2012). Since our study and others have shown IL6 to be markedly increased by poly(I:C) exposure, this most likely primes the fetal brain to overexpress *SOCS3* in response to further challenge with IL6, in a similar fashion as seen in our MDD HDF responses (Arrode-

Bruses and Bruses, 2012; Khan et al., 2014; Mandal et al., 2013). This heightened *SOCS3* response may disrupt JAK1/JAK2/TYK2 utilizing signaling pathways necessary for neurodevelopmental processes, potentially adding continued pathological disruption when IL6 levels are increased post poly(I:C) treatment.

In addition, our lab has also demonstrated IL6 to be the main mediator of MIA's effects on the fetal brain, which makes IL6 the most likely inducer of psychiatric disorder related pathology stemming from maternal infection. Two collaborative studies between our lab and Paul Patterson's lab demonstrated that IL6 is the main contributor to both fetal transcriptional response and adult altered brain transcriptomics and behavior (Garbett et al., 2012; Smith et al., 2007). In our first study, we found a single IL6 injection midgestation was sufficient to produce prepulse inhibition and latent inhibition behavioral deficits in adult offspring. Furthermore, IL6 neutralizing antibodies co-administered with midgestation poly(I:C) or poly(I:C) treatment in IL6 knockout mice was sufficient to prevent prepulse inhibition, social interaction, and exploration behavioral phenotypes as well as most adult transcriptomic differences observed with poly(I:C) treatment (Smith et al., 2007). In the second study, we observed strong, common relative gene expression changes with GD9.5 influenza infection and GD12.5 poly(I:C) or IL6 exposure, suggesting IL6 likely drives fetal transcriptional changes as well (Garbett et al., 2012). In the data presented in Chapter 3, IL6 is the probable driver of HFS vs. HFM and SAL vs. MIA associated gene expression changes observed at GD12.5. Since IL6 is capable of producing a strong antiviral and angiogenic response, this fits well with the target genes we see altered (Hashizume et al., 2009; Kishimoto, 1989).

Of the transcriptional effects produced by gestational diabetes and MIA in Chapter 3, the hypoxic response caused by both insults is particularly interesting. Decreases in arterial O_2 tension results in a hypoxic state. During acute hypoxic episodes, the fetus compensates by vasodilation and redistribution of cardiac output to more vital organs like the metabolically demanding developing brain (Salihagić-Kadić et al., 2006). More chronic hypoxic processes can be caused by altered states such as placental insufficiency or maternal inflammation/infection and require additional compensations (Rees et al., 2008). These additional compensations include remodeling the vasculature in the fetal brain. Endothelial and smooth muscle cells within fetal cerebral arteries become enlarged, but these thickened vessels also have a decreased capacity to respond to endogenous vasodilators, leading to less control of vessel flow (Nauli et al., 2005; Pearce et al., 2009; Williams and Pearce, 2006). Interestingly, we observed induction of *Vegfa* and/or *Flt1*, which encodes VEGF receptor 1, with high fat diet induced gestational diabetes at both GD12.5 and GD16.5 and with MIA during the acute immune response. VEGF is known to increase with hypoxia and mediates vascular remodeling that occurs with chronic hypoxia, which is the likely cause and effect of *Vegfa* and *Flt1* induction observed in the fetal brains from our gestational diabetes and MIA models (Nilsson et al., 2004; Osada-Oka et al., 2008; Pearce et al., 2011).

Beyond the pathological state of hypoxia, VEGFA and its predominant receptors (VEGFR1 and VEGFR2) are required for normal vasculogenesis and neurodevelopment. In the developing brain, VEGFA is synthesized by neuronal precursors, forming a chemoattractive gradient that recruits developing endothelial cells to form vessels (Breier et al., 1992). In addition, VEGFA has been found to have nonvascular neurotrophic functions. VEGFA *in vitro*

promotes neuron survival and neurite outgrowth as well as protects cultured hippocampal neurons from hypoxia and excitotoxicity (Jin et al., 2000; Matsuzaki et al., 2001; Sondell et al., 1999; Sondell et al., 2000). VEGFA can also enhance neurogenesis, guide neuronal migration, and potentially serves as a chemoattractive axonal guidance cue (Carmeliet, 2003; Hashimoto et al., 2006; Jin et al., 2002; Schwarz et al., 2004). Thus, enhanced VEGFA signaling during development, especially when prolonged, directly affects vascular remodeling and neurodevelopment.

Importantly, hypoxic response and IL6 signaling pathways are altered in patients with psychiatric disorders (Caspi and Moffitt, 2006; Hayes et al., 2014; Naik et al., 2011; Sasayama et al., 2011; Schmidt-Kastner et al., 2012; Schmidt-Kastner et al., 2006; Sellmann et al., 2014; Shibata et al., 2013; Tartter et al., 2015). Our results support that the dysfunction in these pathways can be induced by environmental insults in addition to gene mutations. We believe these pathways to be important targets for both the production of psychiatric disorder pathology as well as its continued progression, lending themselves as viable targets for therapeutic correction.

In summary, we have shown that both gene x environment and environment x environment interactions alter the transcriptional response to inflammatory stimuli. The altered transcriptional response observed in Chapters 2 and 3 include genes implicated in psychiatric disorders, demonstrating that the clinical observation of these changes may be the result of accumulated risk factors. However, it is wise to not over-interpret these results. Many of the changes observed were subtle and require validation in a well-powered, clinically relevant

context. We strongly believe the data presented in this document pioneers the characterization of

the transcriptomic interaction of psychiatric disorder risk factors, providing a platform for study

of many other potentially interacting risk factors.

Supplementary Table 1. Participant demographic information

Skin biopsies from seven MDD female and seven CTR female participants were used to generate the human dermal fibroblast cultures for this study. Groups were matched by age (MDD age = 38.56 ± 3.89, CTR age = 41.18 ± 3.26). Due to the small number of African American participants, we were unable to match by race or test for race-specific effects.

Sample #	Group	Age	Gender	Race
104	CTR	48	female	Caucasian
123	CTR	40	female	Caucasian
139	CTR	32	female	African American
145	CTR	52	female	Caucasian
147	CTR	51	female	African American
159	CTR	44	female	Caucasian
193	CTR	34	female	African American
108	MDD	52	female	Caucasian
122	MDD	37	female	Caucasian
135	MDD	23	female	African American
174	MDD	23	female	Caucasian
185	MDD	43	female	Caucasian
187	MDD	53	female	Caucasian
198	MDD	45	female	Caucasian

Supplementary Table 2. qPCR primers

The table below contains the NCBI reference sequence ID for the mRNA sequence used to generate the forward and reverse primers for the genes listed below. The expected product length as well as the protein encoded by each gene is also included.

NCBI Reference Sequence ID	gene	protein product	type	sequence (5'-3')	product length
PRIMERS FOR GENES TESTED					
Mitochondrial function					
NM_001686.3	*MTATP6*	mitochondrial ATP synthase	forward	CTTTAGAGCGGGCACAGTGA	76
			reverse	GGGGTGTAGGTGTGCCTTCT	
NM_001964.2	*EGR1*	early growth response 1	forward	GCCATAGGAGAGGAGGGTTC	92
			reverse	GTTGGCCAATAGACCTTCCA	
NM_025207.4	*FADS*	flavin adenine dinucleotide synthetase 1	forward	TTGAGCCAAGACCAAGTCCT	98
			reverse	CACTGTGTGTCCCTTTGTGG	
NM_015560.2	*OPA1*	mitochondrial dynamin-like GTPase	forward	AGGTTGCTTGGGAGACCCTA	70
			reverse	TCATGCTCTTTCCCTTTCGGT	
NM_014874.3	*MFN2*	mitofusin 2	forward	CTGTCTGGGACCTTTGCTCA	71
			reverse	GCAATTTCCTGCTCCAGGTTC	
NM_001277061.1	*MFF*	mitochonrial fission factor	forward	AACCCCTGGCACTGAAAACA	120
			reverse	TGCCAACTGCTCGGATTTCT	
NM_032409.2	*PINK1*	PTEN induced putative kinase 1	forward	ACCTTTGCCCCTAACACGAG	105
			reverse	AACTGAACGTGCTGACCCAT	
NM_0010333568.2	*MIRO1*	mitochondrial Rho GTPase 1	forward	GAGTCCACTCCGTGCGT	74
			reverse	CAGGATCCGCACGTCTTTCT	
NM_012062.4	*DRP1*	dynamin-1 like	forward	ATTGCTGGTATGAAATGACACTAAA	78
			reverse	CTTTTGTCTCCCTTTCTCTGGG	
NM_002693.2	*POLG*	mitochondrial DNA polymerase catalytic subunit	forward	GCTGGTGGAAGAGCGTTACT	129
			reverse	TTGTGCCCCACCACTAACTG	
NM_000550.2	*GP75*	glycoprotein 75	forward	AGGACGTGAGCAGCAGATTG	113
			reverse	CCTTCTTTCGCCGGTCTTCT	

			Tryptophan metabolism		
NM_00100 8661.2	KAT3	kynurenine aminotransfer ase 3	forward	ACTATCAGCCATCCCCGTTTC	82
			reverse	AATGAAGCAAAAACGCACAA ACT	
NM_00208 0.3	KAT4	kynurenine aminotransfer ase 4	forward	TGTGGTGTGCAGCCTCTCAT	105
			reverse	AAGCCTGAACCCAGCTAGCA	
			Lipid metabolism		
NM_00459 9.3	SREBP2	sterol regulator element binding transcrption factor 2	forward	ATCGCTCCTCCATCAATGAC	91
			reverse	TTCCTCAGAACGCCAGACTT	
NM_00136 0.2	DHCR7	7-dehydrocholes terol reductase	forward	ACCATTGACATCTGCCATGA	89
			reverse	ACCCTGCAGCGTGTAAAGAT	
NM_00410 4.4	FASN	fatty acid synthetase	forward	GCCCAAGGAGGATGGTCT	108
			reverse	GAGCTCTGCACGGAGTTGA	
NM_00085 9.2	HMGCR	3-hydroxy-3-methylglutary l-CoA reductase	forward	AGGACTGGATGAAAATGTGT	95
			reverse	CTTGTTCAATATCCATGCTGA TC	
NM_00506 3.4	SCD1	stearoyal-CoA desaturase	forward	TTGGAGAAGCGGTGGATAAC	108
			reverse	AAAAATCCCACCCAATCACA	
			Oxidative stress response		
NM_00616 4.4	NFE2L2	nuclear factor erythroid 2 related factor 2 (Nrf2)	forward	AGTGGATCTGCCAACTACTCC	109
			reverse	TGTCATCTACAAACGGGAATG	
NM_20350 0.1	KEAP1	kelch-like ECH-associated protein 1 (Keap1)	forward	AGAGACGTGGACTTTCGTAGC	132
			reverse	TCGTAACACTCCACACTGTCC	
NM_00399 8.3	NFKB1	nuclear factor-κ-B (NF-κB) P50 subunit	forward	GAAACGTCAGAAGCTCATGC	124
			reverse	TGGACCTGTACTTCCAGTGC	
NM_00107 7494.3	NFKB2	nuclear factor-κ-B (NF-κB) P52 subunit	forward	CATTGAGGTTCGGTTCTATGA G	89
			reverse	TGGCATACTGTTTATGCACAT C	

			forward	ATAGCATGAATGCCTCTCGAC	
NM_00119 0720.2	*IKBKB*	inhibitor of NF-κB kinase subunit beta	reverse	TGCAGAGGTTATGTGCTTCAG	129
NM_00109 9857.2	*IKBKG*	inhibitor of NF-κB kinase subunit gamma	forward	GGATATCTACAAGGCGGACTT C	120
			reverse	GCCTTCAGTTTGCTGTACTCC	
NM_00275 0.3	*MAPK8*	stress-activated protein kinase (JNK1)	forward	TATAGGCTCAGGAGCTCAAG G	97
			reverse	ATTCTGAAATGGTCGGCTTAG	
NM_00131 5.2	*P38*	P38 MAP kinase	forward	CCAAGGTGTTTCCATTTCTCA	95
			reverse	CTCCTTCCTTCTTGCTCCAGT	
NM_00274 6.2	*ERK1*	extracellular signal-related kinase 1	forward	GTACTATGACCCGACGGATGA G	113
			reverse	GTGCTGTCTCCTGGAAGATGA	
NM_00731 5.3	*STAT1*	signal transducer and activator of transcription 1	forward	CGGCACCTGCAATTGAAAGA AC	93
			reverse	CCAAACCAGGCTGGCACAATT	
NM_13927 6.2	*STAT3*	signal transducer and activator of transcription 3	forward	CCTGGTGTCTCCACTGGTCT	109
			reverse	GCTACCTGGTCAGCTTCAG	
NM_00222 7.2	*JAK1*	Janus kinase 1	forward	CATCATGGAATTTCTGCCTTC	135
			reverse	TATTGCCGAGAACCCAAATAG	
NM_00045 4.4	*SOD1*	Cu/Zn-dependent superoxide dismutase	forward	CAATTTCGAGCAGAAGGAAA G	116
			reverse	AGCCTGCTGTATTATCTCCAA AC	
NM_00063 6.2	*SOD2*	Mn-dependent superoxide dismutase	forward	AGATAGCTCTTCAGCCTGCAC	134
			reverse	AAAGTCACGTTTGATGGCTTC	
NM_00175 2.3	*CAT*	catalase	forward	GAGGCAGTTTATTGCAAATTC C	105
			reverse	GATGCCATAGTCAGGATCTTC C	
NM_00492 9.3	*CALB1*	calbindin	forward	CTGTGCGAGAAGAATAAACA GG	126
			reverse	CCCAGCACAGAGAATAAGAG C	
NM_00090 3.2	*NQO1*	NADPH:quin one	forward	ATTCTGCATTTCTGTGGCTTC	131
			reverse	GTCTCATCCCAAATATTCTCC	

		oxidoreductase 1		AG	
NM_001498.3	*GCLC*	glutamate-cysteine ligase catalytic subunit	forward	TTTATCGCAAACCATCCTGAC	124
			reverse	ATCCAAGTAACTCTGGGCATTC	
NM_002061.3	*GCLM*	glutamate-cysteine ligase modifier subunit	forward	TTGGAACAGCTGTATCAGTGG	139
			reverse	TTGGATCATTGTGAGTCAACAG	
NM_003330.3	*TXNRD1*	thioredoxin reductase 1	forward	TGAACACATGGAAGAACATGG	125
			reverse	TTTCCTCACTATTGGTGGACTG	
NM_002133.2	*HMOX1*	hemeoxygenase 1	forward	GCAACAAAGTGCAAGATTCTG	114
			reverse	CCACCAGAAAGCTGAGTGTAAG	
Other (proliferation, apoptosis, immune response)					
NM_002648.3	*PIM1*	proto-oncogene serine/threonine-protein kinase	forward	GCGCGGCGATATCATCA	104
			reverse	GGGGCATTGCTGACTGTGTA	
NM_003194.4	*TBP*	TATA box binding protein	forward	TATAATCCCAAGCGGTTTGC	90
			reverse	GCACACCATTTTCCCAGAAC	
NM_002417.4	*KI67*	marker of proliferation Ki67	forward	CAGACTCCATGTGCCTGAGA	107
			reverse	TGAACACCTCTTGACACTCC	
NM_002198.2	*IRF1*	interferon regulator factor 1	forward	AGGACATCATGAAGCTCTTGG	137
			reverse	AATTTCTGGCTCCTCCTTACAG	
NM_182620.3	*SKA2*	spindle and kinetichore associated complex subunit 2	forward	AGAGAGTAAGAGCCGCATTTG	102
			reverse	TCTTTAGTCAGTGGTGACAGCTC	
NM_001226.3	*CASP6*	caspase 6	forward	TGCGCAGATAGAGACAATCTTAC	129
			reverse	ATCTGCGTGGCTAACAGTTG	
IL6 receptor and its signaling partners					
NM_00056	*IL6R*	IL6 receptor	forward	TTCTGAGGTTCAAGAAGACGT	131

5.3				G	
			reverse	GATGAGAGGAACAAGCACTGG	
NM_00218 4.3	*IL6ST*	gp130	forward	AGACACCAAGTTCCGTCAGTC	123
			reverse	AATACCATCACCGCCATCTAC	
NM_00395 5.4	*SOCS3*	suppressor of cytokine signaling 3	forward	GGAGGAGACGGGACATCTTT	115
			reverse	TGGGACAGGGAGCATTTAAG	

Supplementary Table 3. Baseline gene expression in MDD and CTR HDFs

Baseline gene expression was expressed as a scaled Ct with standard error of the mean. All cytokine responsive genes tested were expressed at a similar level at baseline in both MDD and CTR HDFs. Using an unpaired student t test, no significant differences were observed between MDD and CTR in any of the tested genes ($p > 0.05$).

BASELINE GENE EXPRESSION (scaled Ct)		
gene	MDD	CTR
Mitochondrial function		
MTATP6	14.94 ± 0.22	14.47 ± 0.33
EGR1	21.09 ± 0.35	20.37 ± 0.28
FADS	22.92 ± 0.18	22.70 ± 0.25
OPA1	21.25 ± 0.18	21.00 ± 0.20
MFN2	22.21 ± 0.16	22.00 ± 0.29
MFF	20.73 ± 0.24	20.53 ± 0.35
PINK1	20.47 ± 0.18	20.86 ± 0.27
MIRO1	23.49 ± 0.19	23.45 ± 0.40
DRP1	22.90 ± 0.41	23.81 ± 0.55
POLG	23.65 ± 0.34	23.86 ± 0.35
GP75	18.92 ± 0.20	18.62 ± 0.27
Tryptophan metabolism		
KAT3	21.67 ± 0.40	21.71 ± 0.44
KAT4	21.83 ± 0.11	21.31 ± 0.32
Lipid metabolism		
SREBP2	22.54 ± 0.18	22.39 ± 0.26
DHCR7	23.62 ± 0.15	23.10 ± 0.50
FASN	22.18 ± 0.09	21.57 ± 0.55
HMGCR	21.79 ± 0.10	21.60 ± 0.35
SCD1	22.54 ± 0.44	22.01 ± 0.51
Oxidative stress response		
NFE2L2	20.69 ± 0.44	20.33 ± 0.13
KEAP1	21.42 ± 0.18	21.43 ± 0.13
NFKB1	23.21 ± 0.44	23.37 ± 0.19
NFKB2	23.05 ± 0.51	23.41 ± 0.16
IKBKB	23.40 ± 0.56	23.78 ± 0.08
IKBKG	23.13 ± 0.56	23.16 ± 0.21
MAPK8	22.51 ± 0.20	22.41 ± 0.15
P38	22.48 ± 0.21	22.43 ± 0.13

ERK1	21.43 ± 0.30	21.88 ± 0.19
STAT1	20.08 ± 0.62	19.59 ± 0.16
STAT3	21.31 ± 0.24	21.37 ± 0.16
JAK1	19.74 ± 0.30	20.08 ± 0.11
SOD1	20.09 ± 0.70	19.32 ± 0.14
SOD2	20.63 ± 0.81	21.63 ± 0.21
CAT	21.27 ± 0.32	21.65 ± 0.16
CALB1	28.01 ± 0.38	28.70 ± 0.25
NQO1	19.55 ± 0.34	20.05 ± 0.17
GCLC	23.15 ± 0.23	23.04 ± 0.24
GCLM	21.12 ± 0.29	21.27 ± 0.22
TXNRD1	19.89 ± 0.21	19.83 ± 0.15
HMOX1	21.30 ± 0.73	22.22 ± 0.32
Other (proliferation, apoptosis, immune response, biomarkers)		
PIM1	22.48 ± 0.47	22.70 ± 0.36
TBP	23.30 ± 0.20	22.97 ± 0.24
KI67	21.51 ± 0.52	21.94 ± 0.45
IRF1	22.52 ± 0.94	23.41 ± 0.19
SKA2	23.37 ± 0.68	22.56 ± 0.14
CASP6	23.23 ± 0.23	23.29 ± 0.15

Supplementary Table 4. Relative gene expression differences after cytokine treatment in MDD and CTR HDFs

Relative gene expression is presented as ΔΔCt with standard error of the mean, which compares baseline response to the vehicle-treated condition. An unpaired student t test was used to determine significance between relative gene expression changes between MDD and CTR for each gene in each treatment condition ($p < 0.05$).

Relative gene expression [ΔCt (vehicle) - ΔCt(cytokine treated) (ΔΔCt)]									
Mitochondrial function									
	IL1β			**TNFα**			**IL6**		
gene	**MDD**	**CTR**	**p-value**	**MDD**	**CTR**	**p-value**	**MDD**	**CTR**	**p-value**
MTATP6	-0.02 ± 0.08	-0.36 ± 0.33	0.329	0.30 ± 0.32	-0.25 ± 0.18	0.163	0.11 ± 0.15	-0.45 ± 0.18	0.039
EGR1	-1.50 ± 0.47	-2.01 ± 0.50	0.469	-0.39 ± 0.48	-1.37 ± 0.14	0.075	-0.10 ± 0.28	-0.69 ± 0.18	0.102
FADS	-0.36 ± 0.15	-0.39 ± 0.30	0.930	-0.32 ± 0.37	-0.45 ± 0.10	0.741	0.07 ± 0.15	-0.37 ± 0.23	0.128
OPA1	-0.12 ± 0.10	-0.51 ± 0.41	0.372	-0.03 ± 0.26	-0.54 ± 0.27	0.210	0.02 ± 0.22	-0.52 ± 0.24	0.127
MFN2	0.12 ± 0.13	-0.05 ± 0.39	0.684	0.24 ± 0.26	-0.08 ± 0.21	0.351	0.19 ± 0.17	-0.50 ± 0.20	0.021
MFF	-0.19 ± 0.14	-0.39 ± 0.31	0.567	0.01 ± 0.25	-0.30 ± 0.18	0.351	0.15 ± 0.24	-0.10 ± 0.21	0.446
PINK1	-0.22 ± 0.14	0.44 ± 0.40	0.145	-0.06 ± 0.34	0.39 ± 0.20	0.282	-0.07 ± 0.19	-0.33 ± 0.24	0.415
MIRO1	-0.01 ± 0.12	-0.21 ± 0.51	0.713	0.39 ± 0.33	-0.16 ± 0.53	0.391	0.10 ± 0.16	-0.81 ± 0.26	0.012
DRP1	-1.65 ± 0.58	-0.12 ± 1.08	0.237	-1.04 ± 0.75	-0.29 ± 0.70	0.480	-0.60 ± 0.73	-1.12 ± 0.67	0.606
POLG	-0.52 ± 0.16	0.21 ± 0.50	0.189	-0.13 ± 0.58	-0.05 ± 0.15	0.893	-0.00 ± 0.19	-1.00 ± 0.36	0.026
GP75	0.01 ± 0.15	-0.45 ± 0.21	0.103	-0.34 ± 0.41	-0.51 ± 0.16	0.705	-0.41 ± 0.56	-0.24 ± 0.22	0.784
Tryptophan metabolism									
KAT3	0.03 ± 0.12	0.03 ± 0.41	0.997	0.54 ± 0.21	0.43 ± 0.45	0.834	0.57 ± 0.67	-0.13 ± 0.21	0.335
KAT4	-0.11 ± 0.15	-0.42 ± 0.36	0.447	0.14 ± 0.27	-0.18 ± 0.12	0.293	0.15 ± 0.21	-0.21 ± 0.23	0.276
Lipid metabolism									

SREBP2	0.02 ± 0.15	0.04 ± 0.23	*0.952*	0.09 ± 0.22	-0.20 ± 0.25	*0.416*	0.16 ± 0.16	-0.27 ± 0.24	*0.162*
DHCR7	-0.67 ± 0.27	-1.35 ± 0.71	*0.393*	-0.28 ± 0.35	-1.07 ± 0.60	*0.276*	0.10 ± 0.20	-0.43 ± 0.21	*0.040*
FASN	-0.82 ± 0.48	-1.00 ± 0.71	*0.837*	-0.50 ± 0.69	-1.21 ± 0.26	*0.356*	0.16 ± 0.19	-0.83 ± 0.46	*0.070*
HMGCR	-0.11 ± 0.09	-0.21 ± 0.35	*0.779*	-0.12 ± 0.30	-0.40 ± 0.20	*0.449*	0.09 ± 0.23	-0.47 ± 0.10	*0.043*
SCD1	-0.29 ± 0.66	-0.73 ± 0.60	*0.627*	0.12 ± 0.60	-0.59 ± 0.31	*0.318*	0.03 ± 0.27	-0.36 ± 0.21	*0.277*
Oxidative stress response									
NFE2L2	0.29 ± 0.08	-0.07 ± 0.26	*0.198*	0.31 ± 0.17	-0.07 ± 0.18	*0.156*	0.16 ± 0.09	-0.51 ± 0.19	*0.009*
KEAP1	0.30 ± 0.12	0.13 ± 0.18	*0.466*	0.26 ± 0.17	-0.02 ± 0.12	*0.073*	-0.03 ± 0.05	-0.28 ± 0.04	*0.003*
NFKB1	2.46 ± 0.54	2.41 ± 0.40	*0.945*	2.46 ± 0.63	2.36 ± 0.13	*0.879*	0.40 ± 0.13	-0.31 ± 0.10	*0.001*
NFKB2	2.40 ± 0.61	2.61 ± 0.28	*0.752*	2.93 ± 0.76	2.89 ± 0.11	*0.960*	0.07 ± 0.10	-0.23 ± 0.14	*0.101*
IKBKB	0.48 ± 0.17	0.33 ± 0.27	*0.657*	0.71 ± 0.24	0.38 ± 0.20	*0.312*	0.03 ± 0.06	-0.39 ± 0.17	*0.042*
IKBKG	0.08 ± 0.07	0.10 ± 0.32	*0.956*	0.29 ± 0.13	-0.06 ± 0.25	*0.236*	0.07 ± 0.06	-0.42 ± 0.20	*0.036*
MAPK8	0.19 ± 0.09	0.09 ± 0.14	*0.566*	-0.02 ± 0.07	-0.22 ± 0.11	*0.148*	-0.08 ± 0.11	-0.35 ± 0.13	*0.142*
P38	0.06 ± 0.07	0.10 ± 0.29	*0.891*	-0.06 ± 0.11	-0.27 ± 0.16	*0.320*	-0.03 ± 0.10	-0.44 ± 0.15	*0.043*
ERK1	-0.40 ± 0.13	-0.59 ± 0.19	*0.425*	-0.20 ± 0.12	-0.43 ± 0.28	*0.449*	-0.03 ± 0.10	-0.28 ± 0.12	*0.136*
STAT1	1.40 ± 0.55	1.09 ± 0.31	*0.624*	2.24 ± 0.71	1.94 ± 0.33	*0.707*	0.06 ± 0.09	-0.35 ± 0.17	*0.047*
STAT3	1.14 ± 0.24	0.87 ± 0.27	*0.480*	0.95 ± 0.30	0.68 ± 0.16	*0.444*	0.30 ± 0.12	-0.08 ± 0.13	*0.051*
JAK1	1.00 ± 0.56	0.43 ± 0.28	*0.383*	0.64 ± 0.16	0.41 ± 0.17	*0.343*	0.13 ± 0.04	-0.13 ± 0.13	*0.078*
SOD1	0.12 ± 0.14	-0.03 ± 0.18	*0.534*	0.09 ± 0.08	-0.04 ± 0.12	*0.375*	0.06 ± 0.10	-0.17 ± 0.15	*0.237*
SOD2	4.84 ± 0.07	5.73 ± 0.21	*0.434*	4.71 ± 1.18	5.62 ± 0.21	*0.462*	0.33 ± 0.15	-0.09 ± 0.08	*0.031*
CAT	-0.24 ± 0.25	-0.48 ± 0.23	*0.498*	-0.42 ± 0.21	-0.61 ± 0.10	*0.429*	0.06 ± 0.07	-0.13 ± 0.09	*0.124*
CALB1	-0.13 ± 0.22	-0.44 ± 0.42	*0.533*	1.23 ± 0.97	-0.07 ± 0.47	*0.249*	-0.28 ± 0.20	-0.56 ± 0.31	*0.465*
NQO1	0.03 ± 0.21	-0.18 ± 0.16	*0.437*	0.10 ± 0.08	-0.03 ± 0.18	*0.504*	0.05 ± 0.07	0.07 ± 0.09	*0.823*

GCLC	0.19 ± 0.15	-0.04 ± 0.17	0.318	-0.08 ± 0.18	-0.29 ± 0.31	0.575	0.07 ± 0.06	-0.11 ± 0.10	0.127
GCLM	0.79 ± 0.16	0.50 ± 0.25	0.351	0.74 ± 0.20	0.48 ± 0.20	0.375	0.24 ± 0.08	-0.14 ± 0.18	0.079
TXNRD1	0.23 ± 0.21	-0.07 ± 0.26	0.374	0.20 ± 0.14	0.13 ± 0.13	0.689	0.26 ± 0.10	-0.02 ± 0.08	0.051
HMOX1	-0.25 ± 0.31	-0.34 ± 0.43	0.877	-0.04 ± 0.16	-0.23 ± 0.17	0.434	0.17 ± 0.09	-0.04 ± 0.13	0.198
Other (proliferation, apoptosis, immune response, biomarkers)									
PIM1	0.64 ± 0.47	0.73 ± 0.43	0.887	2.09 ± 0.68	1.75 ± 0.37	0.670	-0.01 ± 0.32	-0.64 ± 0.30	0.183
TBP	0.06 ± 0.07	-0.21 ± 0.29	0.388	-0.07 ± 0.33	-0.13 ± 0.18	0.881	0.16 ± 0.13	-0.18 ± 0.13	0.083
KI67	-0.73 ± 0.52	-0.03 ± 0.43	0.317	-0.43 ± 0.73	0.04 ± 0.33	0.562	-0.47 ± 0.63	-0.55 ± 0.21	0.896
IRF1	2.16 ± 0.88	2.16 ± 0.29	0.994	3.85 ± 1.08	3.85 ± 0.34	0.998	-0.10 ± 0.22	-0.26 ± 0.12	0.540
SKA2	0.14 ± 0.10	-0.17 ± 0.18	0.160	-0.02 ± 0.08	-0.49 ± 0.18	0.028	-0.02 ± 0.09	-0.46 ± 0.17	0.043
CASP6	-0.79 ± 0.28	-0.85 ± 0.15	0.867	-0.91 ± 0.26	-0.91 ± 0.16	0.993	-0.20 ± 0.07	-0.27 ± 0.13	0.640

Supplementary Table 5. Litter statistics

35 GD12.5 pregnant dams and 36 GD16.5 pregnant dams were sacrificed, and the embryos collected. Average litter size, average number of resorptions and mal-developed pups (runts, severe hemorrhage or hypoxia) per litter, average ratio of male:female embryos, and average embryo crown-rump length are listed with SEM in the table below.

Diet	Exposure	GD age	Number of litters	litter size	resorptions	mal-developed embryos	male : female ratio	Crown – rump length
CTR	SAL	12.5	9	7.0 ± 0.4	1.3 ± 0.7	0.2 ± 0.2	0.6 ± 0.1	8.6 ± 0.1
CTR	MIA	12.5	8	7.8 ± 1.0	1.6 ± 0.6	0.3 ± 0.3	0.6 ± 0.1	8.3 ± 0.1
HF	SAL	12.5	9	8.0 ± 0.5	1.1 ± 0.6	0.2 ± 0.2	0.5 ± 0.1	8.4 ± 0.1
HF	MIA	12.5	9	7.4 ± 0.4	2.0 ± 0.8	0.1 ± 0.1	0.5 ± 0.0	8.4 ± 0.1
CTR	SAL	16.5	9	6.8 ± 0.8	1.8 ± 0.8	0.3 ± 0.2	0.5 ± 0.1	15.5 ± 0.2
CTR	MIA	16.5	9	7.3 ± 0.4	1.4 ± 0.4	0.1 ± 0.1	0.6 ± 0.1	14.8 ± 0.1
HF	SAL	16.5	9	7.8 ± 0.6	0.9 ± 0.3	0.1 ± 0.1	0.7 ± 0.1	15.1 ± 0.2
HF	MIA	16.5	9	6.7 ± 0.7	2.4 ± 0.6	0.2 ± 0.2	0.6 ± 0.1	14.9 ± 0.2
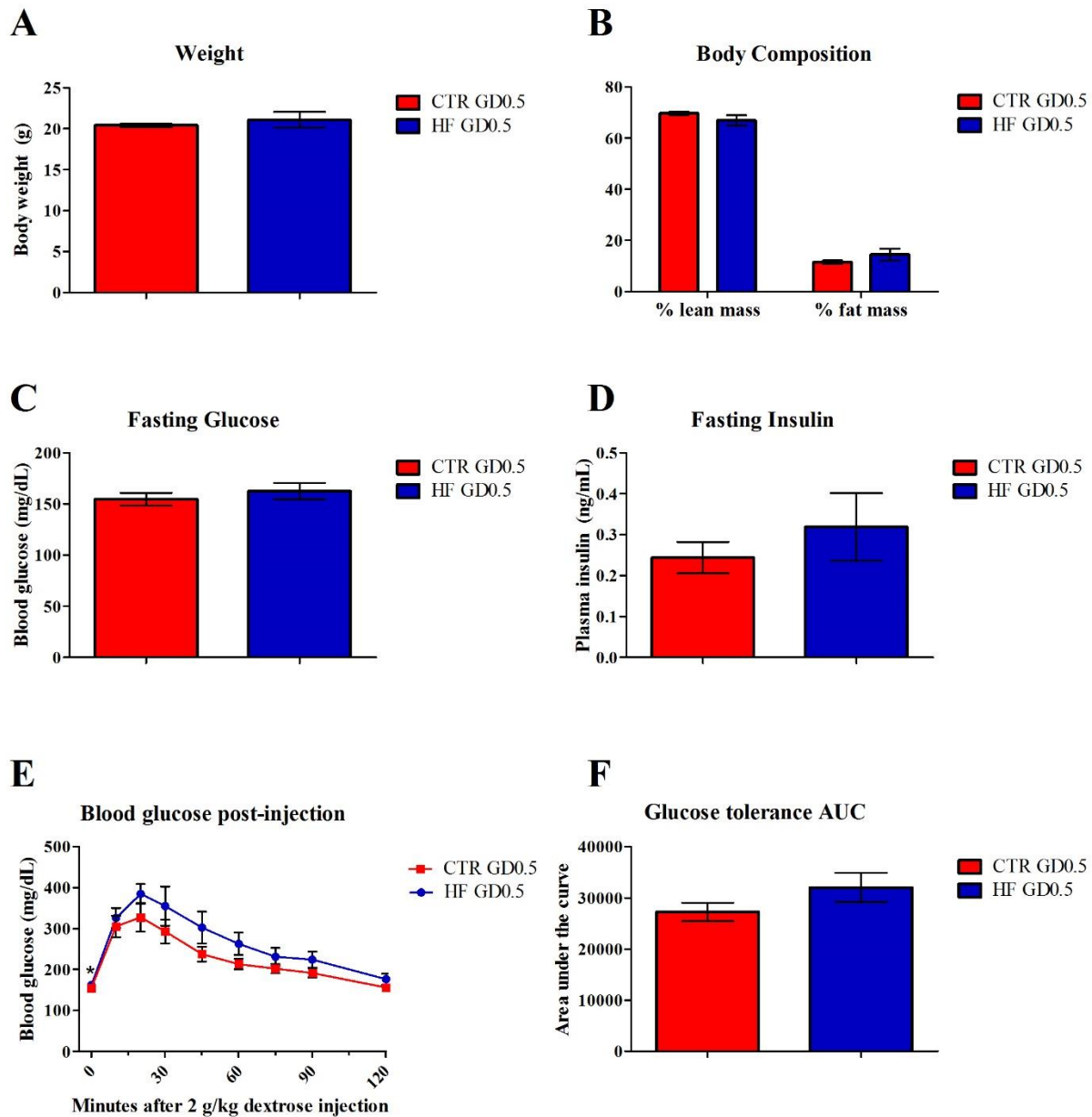
Supplementary Figure 1. High fat diet does not produce a diabetic phenotype at GD0.5.
Body composition, 6 hour fasting glucose and insulin, and glucose tolerance measurements were performed on a cohort of high fat and control fed GD0.5 pregnant dams (n=8 per group). (**A**) High fat fed dams did not show significant increases in body weight. (**B**) Whole body NMR also did not show significant changes in % body mass. (**C-D**) Neither 6 hour fasting blood glucose or serum insulin were significantly altered in high fat dams. (**E-F**) After a 6 hour fast, dams were injected intraperitoneally with 2 g dextrose/kg body weight. No significant difference in blood glucose between control and high fat dams was observable at 0 10, 20, 30, 60, and 75 minutes

post-injection. Error bars represent SEM. Significance of p<0.05 is indicated by * and determined by unpaired student t-test with Welch's correction in all assays, n=6 dams per group.
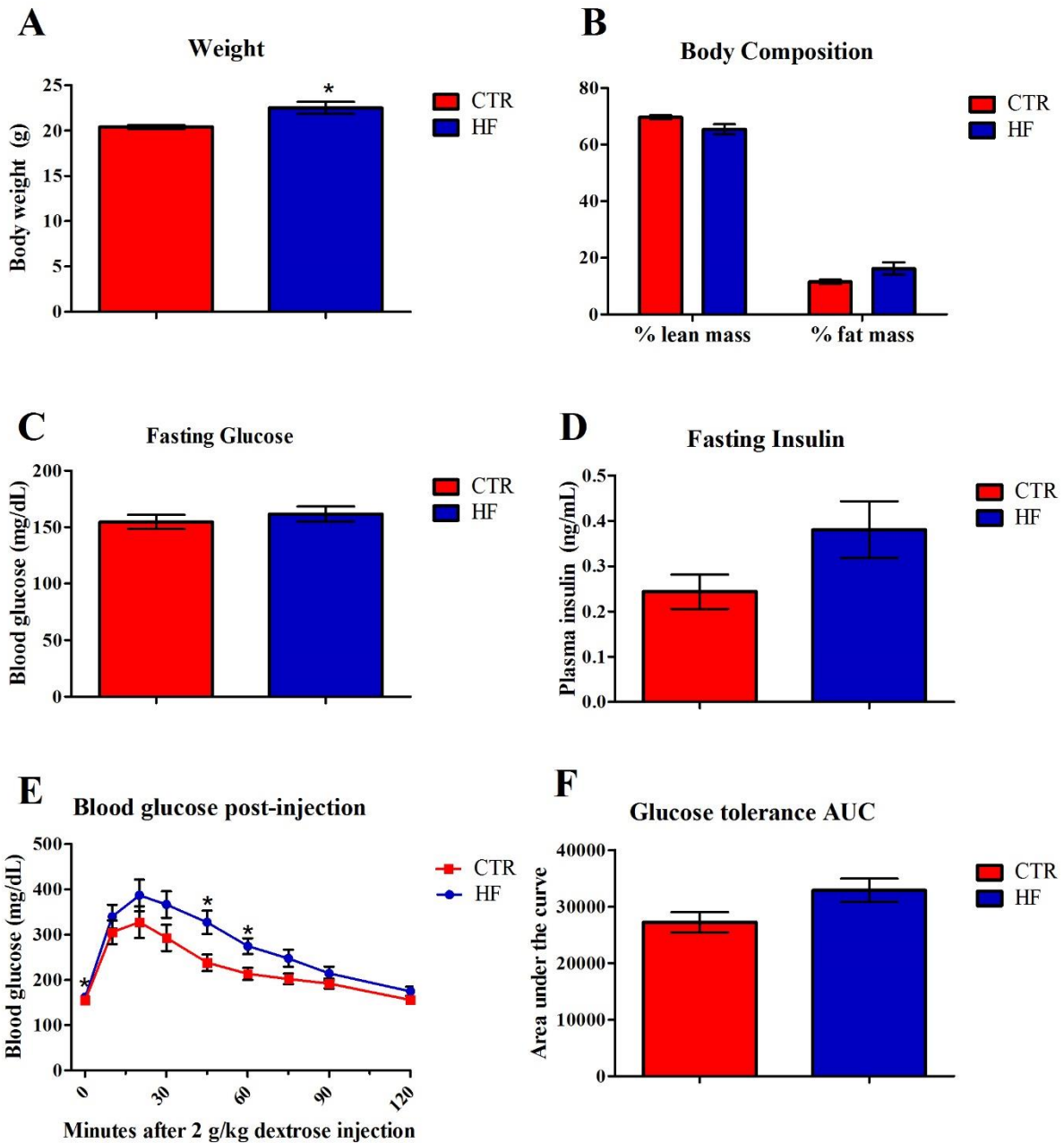
Supplementary Figure 2. 8 weeks of high fat diet produces increased body weight and a trend towards altered glucose tolerance. Body composition, 6 hour fasting glucose and insulin, and glucose tolerance measurements were performed on a cohort of high fat and control fed non-pregnant dams (n=8 per group). The purpose of this experiment was to determine if the GD12.5 high fat dams were glucose intolerant due to pregnancy + 8 weeks of high fat diet or if 8 weeks of high fat diet alone was sufficient to induce glucose intolerance. (**A-B**) High fat dams had a

significantly increased body weight but not altered body composition. (**C-D**) Fasting glucose and insulin after a 6 hour fast were NOT impaired in HF dams, unlike that seen in the pregnant cohort. (**E-F**) When challenged with 2 g/kg dextrose after a 6 hour fast, HF dams show significant differences at two time points (45 and 60 minutes post-injection), and there is a strong trend although not significant of a significantly increased area under the curve (best measure of glucose intolerance). Error bars represent SEM. Significance of $p<0.05$ indicated by * and determined by unpaired student t-test, n=8 for HF and CTR.
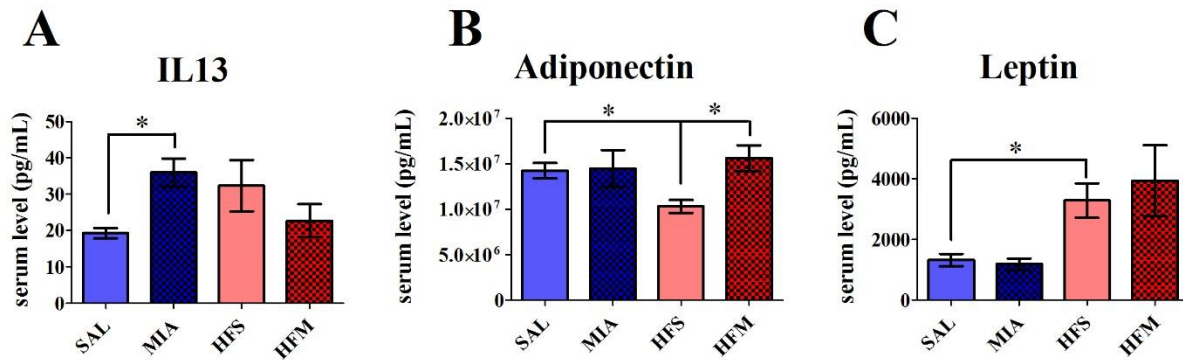
Supplementary Figure 3. High fat diet induced gestational diabetes and MIA continue to alter serum factor levels at GD16.5. Maternal serum collected at GD16.5- 4 days after poly(I:C) exposure- was analyzed for chemokine, cytokine, and metabolic hormone levels via Luminex multiplex assay. Only those serum factors that demonstrated a significant difference in at least one comparison (SAL vs. MIA, SAL vs. HFS, or HFS vs. HFM) are shown. (**A**) The only cytokine/chemokine significantly increased by MIA at GD16.5 was IL13, which actually was not significantly different at GD12.5. This was only observed in the control context and not in the context of high fat diet induced gestational diabetes. (**B**) The anti-inflammatory metabolic hormone adiponectin remained significantly decreased by high fat diet induced gestational diabetes at GD16.5, but was also increased by MIA in the context of high fat diet induced gestational diabetes, bringing the HFM group to a similar level as MIA and SAL. (**C**) The pro-inflammatory satiety hormone leptin also remained significantly increased by high fat diet induced gestational diabetes, but the further increase by MIA only in the context of high fat diet induced gestational diabetes was no longer observable. Error bars represent SEM. Significance of $p<0.05$ is indicated by * and determined by unpaired student t-test with Welch's correction in all assays. Serum was collected at GD16.5, four days after poly(I:C) exposure, from dams whose embryos were utilized for Nanostring gene expression analysis (SAL n=9, MIA n=9, HFS n=9, HFM n=9).

Supplementary Table 6. Both high fat diet and MIA alter gene expression at GD12.5.

Genes listed demonstrated a significant change in expression in either HFM, MIA, or HFS when compared to SAL (57 genes). The $\log_2$(counts) values, which are listed with SEM, for each group was utilized for a supervised hierarchical clustering analysis shown in **Figure 7**. Significance was determined by unpaired student t-test with Welch's correction and $p<0.05$ (significant values are shaded).

	SAL	HFS			MIA			HFM		
Gene	$\log_2$ (counts)	$\log_2$ (counts)	ALR (HFS-SAL)	p-value	$\log_2$ (counts)	ALR (MIA-SAL)	p-value	$\log_2$ (counts)	ALR (HFM-SAL)	p-value
Ager	7.21 ± 0.11	7.38 ± 0.07	0.17	0.182	7.41 ± 0.04	0.21	0.088	7.64 ± 0.07	0.44	0.003
Alox12	4.37 ± 0.08	4.43 ± 0.21	0.06	0.778	4.89 ± 0.12	0.52	0.003	3.96 ± 0.18	-0.42	0.045, ns
Bcl6	4.89 ± 0.13	4.73 ± 0.09	-0.16	0.302	4.22 ± 0.12	-0.67	0.001	4.81 ± 0.11	-0.08	0.637
C1qb	9.05 ± 0.04	8.99 ± 0.05	-0.06	0.303	8.97 ± 0.05	-0.08	0.242	8.91 ± 0.05	-0.14	0.030
Cd55	6.90 ± 0.12	6.78 ± 0.06	-0.13	0.342	6.63 ± 0.05	-0.27	0.049	6.62 ± 0.07	-0.29	0.051
Creb1	7.64 ± 0.06	7.66 ± 0.06	0.02	0.793	7.34 ± 0.09	-0.30	0.014	7.45 ± 0.05	-0.19	0.025
Cysltr1	3.45 ± 0.09	3.50 ± 0.13	0.06	0.718	3.73 ± 0.18	0.28	0.188	3.90 ± 0.17	0.45	0.030
Dckl1	12.19 ± 0.09	12.11 ± 0.05	-0.08	0.45	11.87 ± 0.10	-0.32	0.024	12.19 ± 0.07	0.00	0.994
Ddit3	9.69 ± 0.03	9.84 ± 0.05	0.14	0.017	9.71 ± 0.04	0.02	0.631	9.69 ± 0.05	0.00	0.988
En1	3.97 ±	3.46 ±	-0.51	0.049	3.92 ±	-0.05	0.855	3.71 ±	-0.27	0.360

	0.23	0.06			0.21			0.19	
Flt1	8.84 ± 0.02	8.92 ± 0.03	0.08	0.051	9.04 ± 0.07	0.20	0.025	9.24 ± 0.05	0.41	0.000
Foxg1	10.75 ± 0.03	10.85 ± 0.04	0.11	0.052	10.83 ± 0.08	0.09	0.311	11.07 ± 0.05	0.33	0.000
Fxyd2	7.09 ± 0.06	7.15 ± 0.06	0.06	0.509	6.83 ± 0.10	-0.26	0.048	7.07 ± 0.06	-0.03	0.730
Gabra2	9.42 ± 0.09	9.30 ± 0.04	-0.11	0.243	9.01 ± 0.13	-0.41	0.018	9.26 ± 0.07	-0.16	0.162
Gad1	9.74 ± 0.09	9.77 ± 0.06	0.02	0.837	9.64 ± 0.10	-0.11	0.396	10.07 ± 0.06	0.33	0.006
Gnaq	12.67 ± 0.04	12.62 ± 0.02	-0.05	0.295	12.52 ± 0.04	-0.15	0.028	12.65 ± 0.03	-0.02	0.722
Hmgb2	9.92 ± 0.05	10.11 ± 0.04	0.19	0.009	9.88 ± 0.05	-0.03	0.636	9.97 ± 0.04	0.05	0.464
Ifi44	3.82 ± 0.19	3.54 ± 0.12	-0.28	0.197	4.94 ± 0.35	1.12	0.017	5.70 ± 0.40	1.88	0.001
Ifit1	3.54 ± 0.12	3.47 ± 0.09	-0.07	0.627	4.61 ± 0.34	1.07	0.017	5.63 ± 0.41	2.09	0.001
Ifit2	8.86 ± 0.04	8.81 ± 0.05	-0.04	0.483	8.95 ± 0.04	0.09	0.074	9.18 ± 0.09	0.32	0.004
Ifit3	4.66 ± 0.14	4.93 ± 0.16	0.27	0.197	5.77 ± 0.32	1.11	0.010	6.79 ± 0.38	2.13	0.000
Ifitm3	8.41 ± 0.10	8.15 ± 0.05	-0.26	0.027	8.54 ± 0.13	0.13	0.410	8.79 ± 0.12	0.38	0.017
Ifna1	3.36 ± 0.04	3.86 ± 0.20	0.51	0.026	3.44 ± 0.08	0.09	0.350	3.54 ± 0.13	0.18	0.191
Il11	3.32 ± 0.00	3.56 ± 0.10	0.23	0.043	3.35 ± 0.03	0.03	0.374	3.39 ± 0.07	0.06	0.358
Il18rap	3.79 ±	4.15 ±	0.36	0.102	4.02 ±	0.23	0.161	4.30 ±	0.51	0.002

	0.10	0.19			0.12			0.11		
Irf1	7.01 ± 0.08	7.01 ± 0.07	0.00	0.973	7.14 ± 0.09	0.13	0.270	7.28 ± 0.10	0.27	0.040
Irf5	4.86 ± 0.08	4.82 ± 0.07	-0.05	0.637	4.92 ± 0.11	0.06	0.685	4.64 ± 0.04	-0.22	0.019
Irf7	3.78 ± 0.16	3.64 ± 0.13	-0.14	0.478	4.62 ± 0.36	0.84	0.060	5.22 ± 0.35	1.45	0.002
Maff	6.21 ± 0.07	6.21 ± 0.11	0.00	0.985	6.35 ± 0.10	0.14	0.258	6.69 ± 0.07	0.48	0.000
Map3k5	6.93 ± 0.13	6.76 ± 0.07	-0.17	0.238	6.58 ± 0.08	-0.34	0.033	6.61 ± 0.08	-0.32	0.040
Mapk1	11.22 ± 0.04	11.26 ± 0.04	0.04	0.416	11.26 ± 0.05	0.04	0.514	11.37 ± 0.04	0.15	0.009
Mef2a	9.38 ± 0.07	9.19 ± 0.03	-0.19	0.027	9.16 ± 0.07	-0.23	0.031	9.12 ± 0.05	-0.26	0.007
Mef2c_Mm	8.94 ± 0.09	8.76 ± 0.04	-0.18	0.074	8.71 ± 0.03	-0.23	0.026	8.87 ± 0.05	-0.06	0.500
Mx2	4.29 ± 0.17	4.93 ± 0.09	0.64	0.004	5.54 ± 0.21	1.25	0.000	6.18 ± 0.21	1.88	0.000
Myc	9.85 ± 0.07	9.72 ± 0.03	-0.13	0.081	9.53 ± 0.09	-0.32	0.010	9.72 ± 0.05	-0.13	0.140
Myd88	7.52 ± 0.06	7.40 ± 0.03	-0.12	0.097	7.32 ± 0.04	-0.20	0.016	7.34 ± 0.06	-0.19	0.043
Ncam1	13.75 ± 0.08	13.71 ± 0.04	-0.05	0.603	13.49 ± 0.07	-0.26	0.020	13.82 ± 0.05	0.07	0.483
Ncam2	9.41 ± 0.08	9.37 ± 0.02	-0.04	0.646	9.17 ± 0.09	-0.24	0.048	9.46 ± 0.06	0.06	0.560
Nfe2l2	10.52 ± 0.04	10.44 ± 0.06	-0.08	0.238	10.34 ± 0.04	-0.18	0.006	10.26 ± 0.04	-0.26	0.000
Nr3c1	8.80 ±	8.70 ±	-0.10	0.261	8.63 ±	-0.17	0.047	8.65 ±	-0.15	0.073

	0.07	0.06			0.03			0.04		
Oasl1	3.32 ± 0.00	3.32 ± 0.00	0.00	1.000	4.39 ± 0.34	1.07	0.016	5.30 ± 0.36	1.97	0.000
Pdgfa	9.60 ± 0.09	9.50 ± 0.03	-0.09	0.299	9.27 ± 0.07	-0.33	0.010	9.37 ± 0.05	-0.23	0.026
Ppp1r12b	9.07 ± 0.05	9.08 ± 0.04	0.01	0.877	8.88 ± 0.05	-0.19	0.016	9.01 ± 0.03	-0.06	0.255
Ptgs1	6.28 ± 0.06	6.07 ± 0.07	-0.21	0.031	6.16 ± 0.07	-0.11	0.199	6.15 ± 0.09	-0.13	0.236
Ripk1	8.28 ± 0.04	8.25 ± 0.05	-0.03	0.648	8.07 ± 0.06	-0.21	0.014	8.06 ± 0.04	-0.22	0.001
Rock2	9.88 ± 0.04	9.81 ± 0.03	-0.07	0.180	9.73 ± 0.05	-0.15	0.026	9.78 ± 0.04	-0.10	0.077
Shh	10.71 ± 0.07	10.90 ± 0.03	0.19	0.024	10.88 ± 0.09	0.17	0.143	10.83 ± 0.05	0.12	0.180
Slc2a1	11.23 ± 0.02	11.28 ± 0.01	0.05	0.040, ns	11.37 ± 0.06	0.14	0.046	11.46 ± 0.05	0.23	0.001
Slc2a3	9.61 ± 0.04	9.73 ± 0.02	0.12	0.021, ns	9.70 ± 0.06	0.10	0.201	9.90 ± 0.07	0.29	0.003
Stat1	9.31 ± 0.03	9.34 ± 0.03	0.03	0.457	9.43 ± 0.05	0.12	0.046, ns	9.50 ± 0.07	0.19	0.025
Syt1	9.55 ± 0.09	9.53 ± 0.06	-0.02	0.864	9.26 ± 0.09	-0.29	0.025	9.61 ± 0.06	0.07	0.517
Tcf4	13.84 ± 0.04	13.75 ± 0.03	-0.09	0.095	13.65 ± 0.07	-0.19	0.040	13.80 ± 0.04	-0.04	0.519
Tgfb3	8.24 ± 0.17	7.95 ± 0.06	-0.29	0.119	7.80 ± 0.08	-0.45	0.028	8.07 ± 0.09	-0.18	0.349
Tnfaip3	7.33 ± 0.06	7.46 ± 0.05	0.13	0.107	7.29 ± 0.09	-0.04	0.733	7.58 ± 0.09	0.25	0.036
Traf2	9.62 ±	9.67 ±	0.05	0.326	9.46 ±	-0.15	0.040	9.44 ±	-0.18	0.008

	0.03	0.04			0.06			0.05		
Vamp1	7.78 ± 0.08	7.89 ± 0.03	0.11	*0.233*	7.81 ± 0.08	0.03	*0.765*	8.09 ± 0.03	0.31	*0.004*
Vegfa	11.20 ± 0.03	11.42 ± 0.04	0.22	*0.000*	11.44 ± 0.10	0.24	*0.033*	11.69 ± 0.07	0.49	*0.000*

APPENDIX

**INCOMPLETE MARKING OF CEREBRAL CORTICAL INTERNEURON
EXPRESSION PATTERNS IN DOPAMINE D1 RECEPTOR BAC TRANSGENIC MICE**

Kelli M. Money, Yuanhu Jin, Vanessa G. Fuentes, Lisa R. Anderson, and Gregg D. Stanwood

Introduction

The use of bacteria artificial chromosome (BAC) transgenic reporter mice allows for the identification of cell populations not easily labeled otherwise. In this regard, BAC transgenic mice typically carry large DNA clones containing 100-200 Kb of genetic code that include complete regulatory sequences for the gene of interest and thus are able to achieve expression patterns that mimic those of endogenous genes (Gong et al., 2003; Heintz, 2004). This is particularly useful for target genes where selective antibodies have not been developed for their protein products, or in cases where expression levels are low and expressed in punctate patterns, like G protein-coupled receptors (GPCRs).

Specifically, the GENSAT (Gene Expression Nervous System Atlas) project developed *Drd1a*-EGFP and *Drd2*-EGFP transgenic mice that express enhanced green fluorescent protein (EGFP) in neurons containing dopamine D1 (D1R) or D2 (D2R) receptors, respectively. These lines eased the identification of the two subpopulations of medium spiny neurons (MSNs) in the dorsal striatum. MSNs of the direct pathway mainly express D1R and send monosynaptic projections directly to the substantia nigra, while those of the indirect pathway mainly express D2R and send projections to the same regions via the globus pallidus and the subthalamic

nucleus. Using *Drd1a* and *Drd2*-eGFP BAC transgenic mice, several studies have demonstrated

cell-specific morphological and cell-membrane properties that distinguish each population of

MSNs, as well as cell-specific synaptic plasticity and changes induced by cocaine and other

psychostimulants (Ade et al., 2011; Day et al., 2008; Gangarossa et al., 2013a; Gangarossa et al.,

2013b; Gangarossa et al., 2012; Gertler et al., 2008; Kreitzer and Malenka, 2008; Nelson et al.,

2012; Shen et al., 2008; Shuen et al., 2008; Tian et al., 2010; Valjent et al., 2009). Moreover,

additional lines of *Drd1*a reporter mice using tdTomato-based red fluorescent protein allow for

visualization of both D1 receptor and D2 receptor populations when combined with the *Drd2*-

eGFP mouse line (Ade et al., 2011; Gong et al., 2003; Shuen et al., 2008; Thibault et al., 2013).

Thus, *Drd1a*-tdTomato/*Drd2*-eGFP hemizygous mice provide opportunity to study the

electrophysiological and anatomical properties of both pathways simultaneously. Using BAC

transgenic reporter mice, the field has identified unique synaptic properties and pharmacologic

responsiveness of D1 and D2 receptor expressing neuronal populations in both the striatum and

the nucleus accumbens (Ade et al., 2011; Day et al., 2006; Kreitzer and Malenka, 2007). In

addition, utilization of acute or chronic cocaine administration in these lines show specific roles

for D1 and D2 receptor-expressing neuronal populations in addiction (Gerfen and Surmeier,

2011; Huang et al., 2015; Park et al., 2013a; Smith et al., 2013). With immunohistochemical

amplification of the fluorescent reporters of D1 and D2 receptors, our lab and others have

characterized the neuronal populations containing D1 and D2 receptors within several regions in

both normal and pathological states, including psychiatric disorders (Gangarossa et al., 2013a;

Graham et al., 2014; Thibault et al., 2013; Thompson et al., 2010). D1 and D2 receptor BAC

transgenic lines in combination with techniques such as calcium imaging are now being used

provide cell type-specificity to functional data (Luo et al., 2011). However, only a few studies

have utilized lines such as the *Drd1a*–tdTomato line 6 (D1tom6) in regions where dopamine receptor expression is considerably lower such as medial frontal cortex.

In comparison to the striatum, the medial frontal cortex (MFC) contains low levels of D1 and D2 receptors, which have been observed in both GABAergic and glutamatergic neurons (Araki et al., 2007; Santana et al., 2009). Previous studies in rodents have suggested that D1 receptors are found in multiple populations of cortical neurons, including glutamatergic pyramidal cells and parvalbumin-, calbindin-, and calretinin-expressing GABAergic interneurons (Gaspar et al., 1995; Le Moine and Gaspar, 1998; Santana et al., 2009). We explored the *Drd1a* expression patterns suggested by the *Drd1a*-eGFP and *Drd1a*-tdTomato BAC transgenic lines. We found that the expression patterns in these lines well mimic endogenous *Drd1a* transcript and/or protein in striatal MSNs and cortical projection neurons, but that the reporter lines significantly under-report Drd1 expression in parvalbumin- and calbindin- expressing GABAergic interneurons.

Thus while the reporter lines continue to be powerful tools to study the localization and connectivity of some D1R-expressing neurons, one cannot assume that the expression patterns are totally congruent with endogenous expression patterns. Similar issues have been recently raised for transgenic lines in other systems (Chen et al., 2015b).

Mice

D1tom6 BAC transgenic mice on a C57Bl/6J background were generous provided by the Calakos laboratory (Ade et al., 2011). D1eGFP BAC transgenic were obtained from the MMRRC (Gong et al., 2003) and backcrossed to C57Bl/6J for 9+ generations. The 5HT3a receptor-eGFP BAC transgenic mice on a Swiss Webster background were provided by the Southard-Smith lab at Vanderbilt. These mice were crossed to D1tom6 mice to produce D1tom6 hemizygous/5HT3a receptor-eGFP hemizygous offspring. Only single hemizygous offspring were used from all the transgenic lines. Mice were housed in a temperature- and humidity-controlled AAALAC-approved facility that is maintained on a 12 hour light/12 hour dark cycle. Mice had *ad libitum* access to rodent chow and water.

Genotyping

Primers for the *Drd1a*-tdTomato transgene included the forward primer (5'- CTT CTG AGG CGG AAA GAA CC-3') and reverse primer (5'- TTT CTG ATT GAG AGC ATT CG -3'). Generic eGFP transgene primers were used for both D1eGFP and 5HT3a receptor-eGFP genotyping [forward primer (5'- CCT ACG GCG TGC AGT GCT TCA GC -3') and reverse primer (5'- CGG CGA GCT GCA CGC TGC GTC CTC -3')]. The same control primers targeting the dopamine transporter- forward primer (5'- CCC GTC TAC CCA TGA GTA AAA -3') and reverse primer (5'- CTC CAC CTT CCT AGC ACT AAC -3')- were used for all reactions. The same PCR thermocycler protocol was also used for all reactions and is as follows:

94°C for 3 minutes then 94°C for 30 seconds, 61°C for 45 seconds, and 72°C for 45 seconds that is repeated for 30 cycles, which is followed by 72°C for 10 minutes and held at 4°C.

Immunohistochemistry

Adult (postnatal day 60) mice were anesthetized with sodium pentobarbital and transcardially perfused with 4% paraformaldehyde (n= 4-5 per line). Brains were then removed and post-fixed at 4°C overnight. Cryoprotection was performed using a series of sucrose solutions in PBS (10%, 20%, and 30%). Series of coronal sections were cut on a freezing microtome at 40 □m and stored at -20°C. Sections were treated with Tris-glycine (0.1 M glycine, pH 7.4) to reduce non-specific labeling, blocked in 4% Blotto (Nestle Carnation dry milk, Glendale, CA, USA) in PBS (pH 7.5), and incubated for 72 h with the appropriate primary antibody using minor variants of previously published protocols (Graham et al., 2014; Stanwood et al., 2009; Stanwood et al., 2005). Primary antibodies included rabbit anti-DSRed (Clontech, 632496, 1:1000), chicken anti-GFP (Life technologies, A10262, 1:1000), mouse anti-NeuN (Millipore, MAB 377, 1:100), mouse anti-glutamic acid decarboxylase 67 (GAD-67) (Millipore, MAB5406, 1:500), mouse anti-calretinin (Swant, 6B3, 1:500), mouse anti-calbindin (Swant, 300, 1:2000), or mouse anti-parvalbumin (Sigma, P3088, 1:200). Following several washes in blocking buffer, the sections were incubated for 1 h at room temperature with corresponding fluorescent secondary antibodies in blocking buffer. GAD-67, GFP, and calbindin staining required tyramide signal amplification. Images of ventral and dorsal anterior cingulate cortex as well as lateral septum, dorsal hippocampus, and dorsal striatum were captured at 20X on a Zeiss Axioimager epifluorescent microscope or Zeiss PASCAL confocal microscope. Image quantification for both presumed D1 receptor expression and the other protein of interest was

performed in anterior cingulate cortex images using ImageJ (Schneider et al., 2012). For colocalization, the cells that expressed both the D1 receptor and the other protein of interest were counted using the free software GIMP (www.gimp.org).

RNAScope in situ hybridization

To examine co-localization of Drd1a, parvalbumin, and calbindin mRNA transcript, RNA in situ hybridization was performed using the RNAscope® Multiplex Fluorescent Assay version 2.5 (Advanced Cell Diagnostics, Inc., Hayward, CA) (Wang et al., 2012). Twenty micron brain sections were cut using a Leica CM1850 Cryostat (Leica Biosystems, Wetzlar, Germany) and applied to Fisherbrand Superfrost Plus slides (Thermo Fisher Scientific, Waltham, MA). Sections were fixed with 4% paraformaldehyde, dehydrated with EtOH and pretreated with protease at room temperature. Sections were then incubated for 2 hours at 40°C with high humidity using a HybEZ Oven (Advanced Cell Diagnostics, Inc., Hayward, CA) with mRNA probes designed and produced by Advanced Cell Diagnostics targeting the D1R, parvalbumin or calbindin, and a positive control probe detecting the housekeeping gene, *Ppib*. Following incubation with target probes, a series of hybridization and signal amplifications steps were completed at 40°C with high humidity using the HybEZ Oven with thorough washing in between each step at room temperature using 1X Wash Buffer supplied by ACD Bio. Sections were then stained with DAPI and slides were mounted using ProLong Gold Antifade reagent (Life Technologies, Carlsbad, CA).

A minimum of three sections from each animal (n = 4-5) were quantified. Statistical differences between ventral and dorsal MFC were determined using an unpaired two-tailed student t-test (p< 0.05).

Results

At the regional level, the distribution and number of cells expressing the tdTomato reporter in D1tom6 mice is as expected (**Appendix Figure 1**). We examined these patterns in more detail with colocalization approaches in the medial frontal cortex (MFC), focusing on the ACC. Using the neuronal marker NeuN, we first determined the number of neurons within the ACC that express the tdTomato reporter in D1tom6 mice (**Appendix Figure 2**). Expectedly, we observed that 22% of NeuN-positive cells in the ACC express the *Drd1a* tdTomato reporter (**Appendix Figure 2, Appendix Table 1**), with a significantly higher proportion (p value = *0.012*) in ventral ACC (25%) than in dorsal regions (19%). The majority of these cells are morphologically similar to glutamatergic pyramidal neurons. Somewhat surprisingly, ~15% of the tdTomato+ cells were NeuN negative, although that may be due to poor NeuN staining in some neurons and not necessarily indicate glial expression of the D1 receptor.
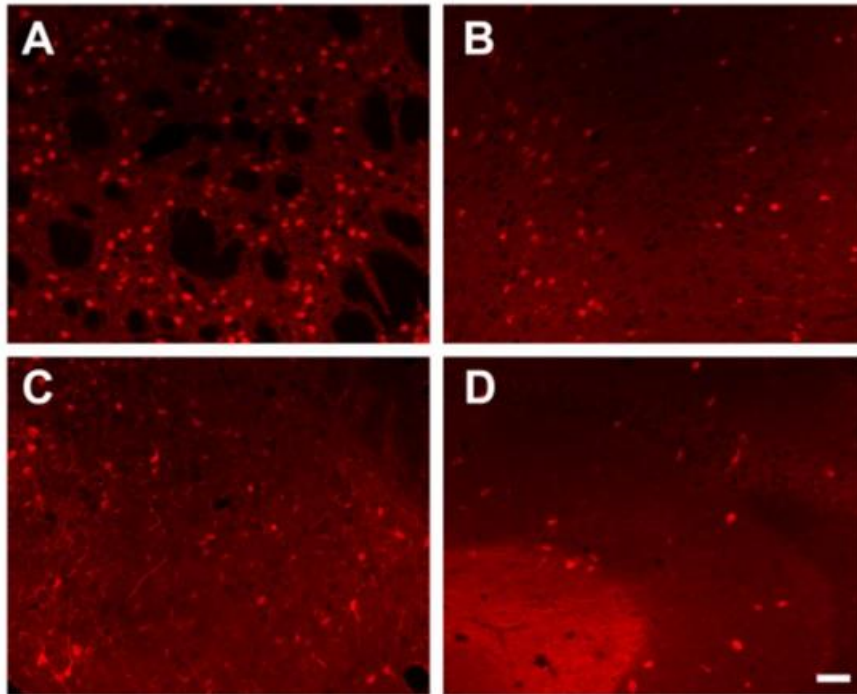
Appendix Figure 1. Anti-dsRed immunostaining in D1tom6 BAC transgenic mice reveal expected regional expression patterns of Drd1+ neurons in the striatum (**A**), ACC (**B**), lateral septum (**C**) and dorsal hippocampus (**D**). Scale bar = 100 µm.
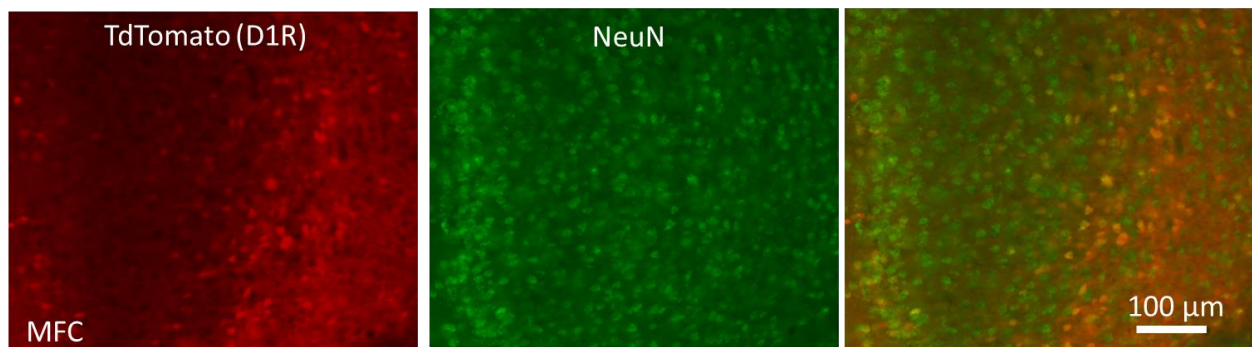

Appendix Figure 2. Anti-dsRed (**A**, red) and anti-NeuN double labeling (**B**, green) in D1tom6 BAC transgenic mice reveals extensive colocalization. The overlay is presented in panel **C**. Scale bar = 100 µm.

Appendix Table 1. Colocalization of Neuronal marker NeuN with tdTomato reporter in P60 D1tom6 mice.

Colocalization of Neuron marker with tdTomato reporter					
	tdTom/mm^2	NeuN/mm^2	both/mm^2	% of NeuN that are both	% of tdTom that are both
all ACC	309.3 ± 18.18	1195 ± 64.52	261.6 ± 20.82	21.9	84.4
Ventral ACC (Cg2)	361.7 ± 13.57	1294 ± 85.73	319.7 ± 14.79	24.8	88.4
Dorsal ACC (Cg1)	257.0 ± 24.20	1095 ± 48.02	203.5 ± 27.05	18.6	78.7

Previous studies have indicated presence of *Drd1a* mRNA not just in glutamatergic neurons in the frontal cortex, but also in up to 38% of GABAergic interneurons (Santana et al., 2009). We thus next performed colocalization with antisera against GAD-67 in the ACC, a selective marker for GABAergic neurons (**Appendix Figure 3A, Appendix Table 2**). Despite very strong and sensitive reporter staining (using immunohistochemical amplification), we found that only ~18% of GAD-67+ cells in the ACC of D1tom6 mice expressed the tdTomato transgene (**Appendix Table 2**).
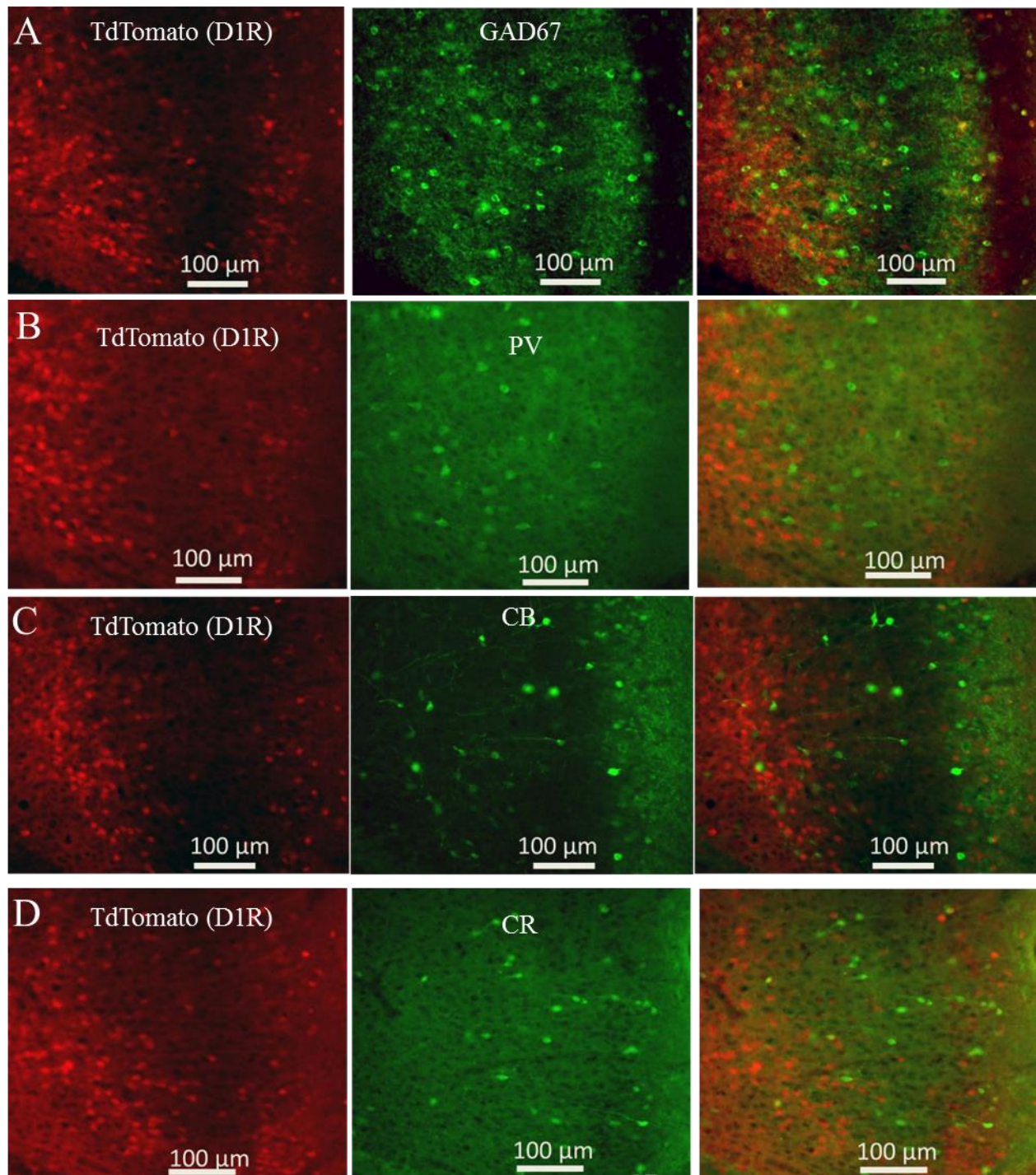
Appendix Figure 3. D1 reporter (red) is expressed in fewer than predicted GABAergic interneurons in the ACC. Anti-dsRed immunostaining (red) is in all panels and accompanied by anti-GAD-67 (A), parvalbumin (B), calbindin (C) and calretinin (D). Note the relatively modest

colocalization of the tdTomato reporter with GAD-67 and calretinin, and the almost none with parvalbumin and calbindin. Scale bar = 100 μm.

Appendix Table 2. Colocalization of GABAergic cell type markers with the tdTomato reporter in P60 D1tom6 mice.

Colocalization of GABAergic cell type markers with tdTomato reporter					
	tdTom/mm^2	GAD67/mm^2	both/mm^2	% of GAD67 that are both	% of tdTom that are both
all ACC	349.2 ± 16.62	263.4 ± 11.03	47.02 ± 2.73	17.8	13.5
Ventral ACC (Cg2)	412.4 ± 19.10	264.3 ± 10.83	53.69 ± 2.95	20.3	13.1
Dorsal ACC (Cg1)	268.4 ± 10.80	262.6 ± 13.10	38.59 ± 2.79	14.7	14.4
	tdTom/mm^2	Parv/mm^2	both/mm^2	% of parv that are both	% of tdTom that are both
all ACC	396.9 ± 37.84	110.6 ± 4.93	0.80 ± 0.18	0.72	0.20
Ventral ACC (Cg2)	454.2 ± 38.33	101.4 ± 3.20	0.62 ± 0.07	0.62	0.14
Dorsal ACC (Cg1)	341.0 ± 38.01	119.3 ± 8.34	0.96 ± 0.41	0.78	0.26
	tdTom/mm^2	CalR/mm^2	both/mm^2	% of CalR that are both	% of tdTom that are both
all ACC	360.7 ± 16.82	97.6 ± 7.51	28.3 ± 2.05	29.0	7.85
Ventral ACC (Cg2)	438.5 ± 26.94	109.5 ± 9.39	30.7 ± 2.62	28.1	7.01
Dorsal ACC (Cg1)	277.0 ± 17.71	86.8 ± 5.08	25.8 ± 2.63	29.6	9.34
	tdTom/mm^2	CalB/mm^2	both/mm^2	% of CalB that are both	% of tdTom that are both
all ACC	352.5 ± 11.90	87.4 ± 1.98	0.59 ± 0.17	0.67	0.16
Ventral ACC (Cg2)	413.1 ± 12.07	97.2 ± 3.05	0.83 ± 0.19	0.85	0.20

|				
Dorsal ACC (Cg1)	269.7 ± 12.23	71.4 ± 3.38	0.24 ± 0.14	0.33	0.09

To evaluate the discrepancy in GABAergic interneuron labeling further, we investigated the specific interneuron subpopulations previously identified to express D1 receptor transcript (Le Moine and Gaspar, 1998; Santana et al., 2009). In the MFC, parvalbumin-expressing interneurons have been observed to be the interneuron subpopulation with the largest proportion of D1 receptor expressing-cells in both rodents (~50%) and primates (~98%) (Le Moine and Gaspar, 1998; Muly et al., 1998). However, we observed almost no colocalization of the D1 receptor reporter tdTomato with parvalbumin (<1%, **Appendix Figure 3B** and **Appendix Table 2**). To ensure that this was not unique to the D1tom6 line, we also examined this in the D1eGFP reporter line created by GENSAT (Heintz, 2004), and the earlier D1tom5 line (data not shown)(Shuen et al., 2008), and again observed almost no colocalization between eGFP and parvalbumin.

This discrepancy between reporter expression and previous in situ data suggests that either the inaccurate reporter expression or problems in the sensitivity and/or specificity of previous *in situ* hybridization experiments. We therefore set up an RNAscope assay to detect *Drd1a* transcript at even very low levels in the adult mouse cerebral cortex. We observed robust colocalization of *Drd1a* and *Pvalb* (**Appendix Figure 4**). Quantification following confocal microscopy demonstrated that 59.5% ± 2.54% of *Pvalb* + cells in the ACC also expressed *Drd1a* (n=5), supporting previous estimates and suggesting that the discrepancy lies in the reporter expression (Le Moine and Gaspar, 1998).
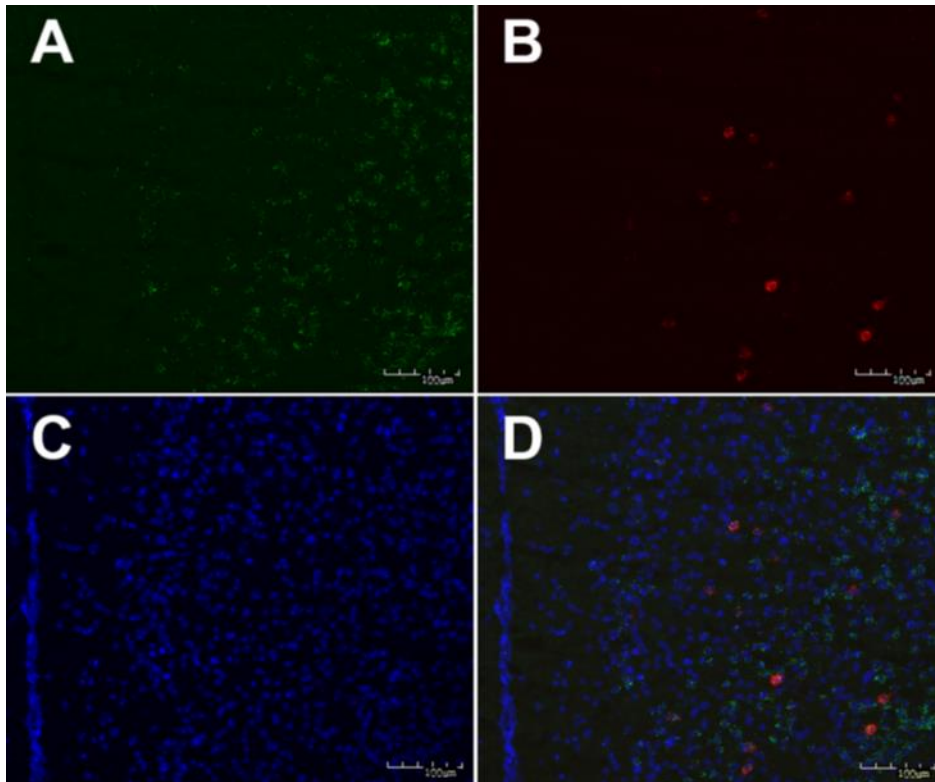
Appendix Figure 4. RNAScope detection of D1 receptor transcript (A, green), parvalbumin transcript (B, red), and DAPI-staining of nuclei (C, blue). Overlay in panel D demonstrates substantial expression of *Drd1* transcript with *Pvalb*. (See text for quantification.)

We observed a similar issue in the reporter mice for calbindin-positive interneurons. Previous data shows that 15-20% of calbindin-positive interneurons express the D1 receptor (Le Moine and Gaspar, 1998). However, we observed minimal colocalization of calbindin and tdTomato in the D1tom6 mice (<1%, **Appendix Figure 3C** and **Appendix Table 2**). Again in sharp contrast to this finding, analyses of double-label RNAScope labeling of transcripts found a much higher degree of colocalization, with 29.9% ± 1.44% (n=5) of *Calb1+* cells expressing detectable *Drd1a* transcript (**Appendix Figure 5**).
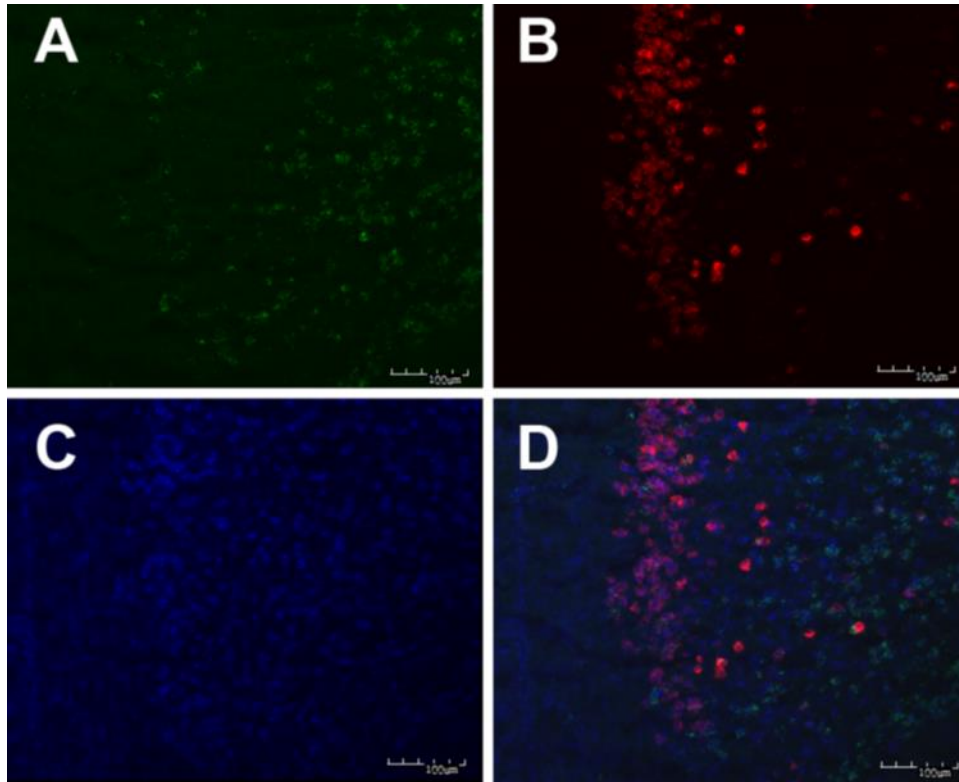
Appendix Figure 5. RNAScope detection of D1 receptor transcript (A, green), calbindin transcript (B, red), and DAPI-staining of nuclei (C, blue). Overlay in panel D demonstrates moderate co-expression of *Drd1* transcript with *Calb1*. (See text for quantification.) Note the tight layering of layer III pyramidal cells that also express moderate calbindin levels, these cells were not included in the analyses.

Another substantial population of GABAergic interneurons in the cerebral cortex express calretinin. Although to our knowledge D1 receptor expression in calretinin-expressing interneurons in the rodent ACC has not been previously quantified, studies in nonhuman primates demonstrate some colocalization (Muly et al., 1998). We found 29% of calretinin-expressing interneurons also expressed tdTomato in the reporter mice, suggesting that this population of interneurons correctly expresses the transgene (**Appendix Figure 3D** and **Appendix Table 2**).

The summation of parvalbumin-, calbindin-, and calretinin-expressing interneurons that are tdTomato-positive only accounts for ~60% of the GABAergic interneurons that are tdTomato-positive. We next sought to identify other GABAergic interneuron subpopulations that could account for some of the remaining interneurons. 5HT3a receptor-expressing interneurons are a subpopulation that overlap slightly with both calretinin- and calbindin- but not parvalbumin-expressing interneurons (Lee et al., 2010b; Taniguchi, 2014). We observed modest expression of 5HT3a receptor-eGFP reporter line of the eGFP-positive cells that overlapped with the tdTomato-positive (data not shown). By staining for calretinin in the blue channel, we were able to demonstrate that at least some of this 5HT3a receptor-eGFP/tdTomato expressing population does not express calretinin and, therefore, is one of the populations not accounted for by the quantifications in **Appendix Table 2**.

Discussion

In this simple study, we identified GABAergic interneuron subpopulations in the frontal cortex to assess whether they are appropriately labeled by the tdTomato reporter transgene in the D1tom6 BAC transgenic mouse line (Ade et al., 2011). Approximately 22% of MFC neurons expressed the *Drd1a* reporter in this study, similar to previous estimates (Zhang et al., 2010). The majority of these appeared to be excitatory pyramidal neurons based on size, laminar organization and shape. Our larger purpose in using the line, however, was to examine the role of dopamine receptors in the differentiation and function of cortical interneurons. We were therefore surprised to find only a modest percentage of GAD-67-immunoreactive cells expressing the tdTomato transgene (~18%). We note that this was observed in multiple

transgenic lines (D1tom6, D1tom5, D1eGFP), and following antibody amplification of transgene (data not shown) (Heintz, 2004; Shuen et al., 2008).

Although some GABAergic interneurons were identifiable by the reporter (i.e., calretinin, 5-HT3A), the two main GABAergic subpopulations that have been previously identified to contain D1 receptors in rodent MFC (parvalbumin- and calbindin-expressing) only very minimally colocalized with tdTomato (Le Moine and Gaspar, 1998). RNAScope fluorescent *in situ* hybridization for the D1 receptor and either parvalbumin or calbindin demonstrated significant colocalization of *Drd1a* with these interneuron markers (~60% and 30%, respectively).

Our findings thus suggest that although BAC transgenic mice used as reporters for *Drd1a* are very useful for some studies, they are imperfect. This is not surprising given the incomplete promoter elements that were introduced to these lines. These mice have been used fairly extensively to label neuronal populations in the striatum and nucleus accumbens, and previous studies have verified correct transgene expression patterns in these brain regions (Ade et al., 2011; Curran and Watson, 1995; Gangarossa et al., 2013b; Lu et al., 1998). We also examined these patterns qualitatively in our study and observed fidelity of the reporter in these regions (data not shown). Expression of the transgene by cortical pyramidal neurons also appears intact and reliable (Seong and Carter, 2012; Zhang et al., 2010). It is specifically the parvalbumin- and calbindin-containing cortical interneurons that are impacted by the false negative reporting.

Our study provides an important reminder that we should always carefully assess expression patterns in mouse transgenic reporter lines. While these specific lines continue to be powerful tools to study the localization and connectivity of some D1R-expressing neurons, one cannot assume that the expression patterns are totally congruent with endogenous expression patterns. Similar issues have been recently raised in other systems (Chen et al., 2015b). Finally, we note that RNAscope provides an extremely sensitive, selective and important assay system in which to ask these questions at the transcript level, but improved reporter mice or protein localization techniques will be needed to allow investigators to unambiguously differentiate between *Drd1a*-expressing and non-expressing neurons, at least within the cerebral cortex.

REFERENCES

Aas, M., Haukvik, U. K., Djurovic, S., Tesli, M., Athanasiu, L., Bjella, T., . . . Melle, I., 2014. Interplay between childhood trauma and BDNF val66met variants on blood BDNF mRNA levels and on hippocampus subfields volumes in schizophrenia spectrum and bipolar disorders. J Psychiatr Res. 59**,** 14-21.

Aavani, T., Rana, S. A., Hawkes, R., Pittman, Q. J., 2015. Maternal Immune Activation Produces Cerebellar Hyperplasia and Alterations in Motor and Social Behaviors in Male and Female Mice. Cerebellum. 14**,** 491-505.

Abdallah, M. W., Larsen, N., Grove, J., Bonefeld-Jorgensen, E. C., Norgaard-Pedersen, B., Hougaard, D. M., Mortensen, E. L., 2013. Neonatal chemokine levels and risk of autism spectrum disorders: findings from a Danish historic birth cohort follow-up study. Cytokine. 61**,** 370-6.

Abdallah, M. W., Larsen, N., Grove, J., Norgaard-Pedersen, B., Thorsen, P., Mortensen, E. L., Hougaard, D. M., 2012. Amniotic fluid chemokines and autism spectrum disorders: an exploratory study utilizing a Danish Historic Birth Cohort. Brain Behav Immun. 26**,** 170-6.

Abell, S. K., De Courten, B., Boyle, J. A., Teede, H. J., 2015. Inflammatory and Other Biomarkers: Role in Pathophysiology and Prediction of Gestational Diabetes Mellitus. Int J Mol Sci. 16**,** 13442-73.

Abraham, A. B., Bronstein, R., Reddy, A. S., Maletic-Savatic, M., Aguirre, A., Tsirka, S. E., 2014. Aberrant Neural Stem Cell Proliferation and Increased Adult Neurogenesis in Mice Lacking Chromatin Protein HMGB2. PLoS ONE. 8**,** e84838.

Adams, J., Faux, S. F., Nestor, P. G., Shenton, M., Marcy, B., Smith, S., McCarley, R. W., 1993. ERP abnormalities during semantic processing in schizophrenia. Schizophr Res. 10**,** 247-57.

Ade, K. K., Wan, Y., Chen, M., Gloss, B., Calakos, N., 2011. An Improved BAC Transgenic Fluorescent Reporter Line for Sensitive and Specific Identification of Striatonigral Medium Spiny Neurons. Front Syst Neurosci. 5**,** 32.

Adkins, B., 1999. T-cell function in newborn mice and humans. Immunol Today. 20**,** 330-5.

Aerts, L., Van Assche, F. A., 2001. Low taurine, gamma-aminobutyric acid and carnosine levels in plasma of diabetic pregnant rats: consequences for the offspring. J Perinat Med. 29**,** 81-4.

Aggarwal, B. B., 2003. Signalling pathways of the TNF superfamily: a double-edged sword. Nat Rev Immunol. 3**,** 745-756.

Aggarwal, B. B., Gupta, S. C., Kim, J. H., 2012. Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey. Blood. 119**,** 651-65.

Akassoglou, K., Probert, L., Kontogeorgos, G., Kollias, G., 1997. Astrocyte-specific but not neuron-specific transmembrane TNF triggers inflammation and degeneration in the central nervous system of transgenic mice. J Immunol. 158**,** 438-45.

Akin, D., Manier, D. H., Sanders-Bush, E., Shelton, R. C., 2005. Signal transduction abnormalities in melancholic depression. Int J Neuropsychopharmacol. 8**,** 5-16.

Akira, S., Takeda, K., 2004. Toll-like receptor signalling. Nat Rev Immunol. 4**,** 499-511.

Al-Damluji, S., 2004. The mechanism of action of antidepressants: a unitary hypothesis based on transport-p. Curr Drug Targets CNS Neurol Disord. 3**,** 201-16.

Alimohamad, H., Rajakumar, N., Seah, Y.-H., Rushlow, W., 2005. Antipsychotics alter the protein expression levels of β-catenin and GSK-3 in the rat medial prefrontal cortex and striatum. Biological Psychiatry. 57**,** 533-542.

Allansmith, M., McClellan, B. H., Butterworth, M., Maloney, J. R., 1968. The development of immunoglobulin levels in man. J Pediatr. 72**,** 276-90.

Allen, A. M., Graham, A., 2012. Mitochondrial function is involved in regulation of cholesterol efflux to apolipoprotein (apo)A-I from murine RAW 264.7 macrophages. Lipids Health Dis. 11**,** 169.

Alliot, F., Godin, I., Pessac, B., 1999. Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. Brain Res Dev Brain Res. 117**,** 145-52.

Almawi, W. Y., Beyhum, H. N., Rahme, A. A., Rieder, M. J., 1996. Regulation of cytokine and cytokine receptor expression by glucocorticoids. J Leukoc Biol. 60**,** 563-72.

Almoguera, B., Riveiro-Alvarez, R., Lopez-Castroman, J., Dorado, P., Lopez-Rodriguez, R., Fernandez-Navarro, P., . . . Ayuso, C., 2011. ATA homozigosity in the IL-10 gene promoter is a risk factor for schizophrenia in Spanish females: a case control study. BMC Med Genet. 12**,** 81.

Aloe, L., Properzi, F., Probert, L., Akassoglou, K., Kassiotis, G., Micera, A., Fiore, M., 1999. Learning abilities, NGF and BDNF brain levels in two lines of TNF-alpha transgenic mice, one characterized by neurological disorders, the other phenotypically normal. Brain Res. 840**,** 125-37.

Aluvihare, V. R., Kallikourdis, M., Betz, A. G., 2004. Regulatory T cells mediate maternal tolerance to the fetus. Nat Immunol. 5**,** 266-71.

Alvarez-Perez, J. C., Ernst, S., Demirci, C., Casinelli, G. P., Mellado-Gil, J. M. D., Rausell-Palamos, F., . . . Garcia-Ocaña, A., 2014. Hepatocyte Growth Factor/c-Met Signaling Is Required for β-Cell Regeneration. Diabetes. 63**,** 216-223.

Amano, Y., Lee, S. W., Allison, A. C., 1993. Inhibition by glucocorticoids of the formation of interleukin-1 alpha, interleukin-1 beta, and interleukin-6: mediation by decreased mRNA stability. Mol Pharmacol. 43**,** 176-82.

Anders, S., Kinney, D. K., 2015. Abnormal immune system development and function in schizophrenia helps reconcile diverse findings and suggests new treatment and prevention strategies. Brain Research. 1617**,** 93-112.

Anderson, P. D., Mehta, N. N., Wolfe, M. L., Hinkle, C. C., Pruscino, L., Comiskey, L. L., . . . Reilly, M. P., 2007. Innate Immunity Modulates Adipokines in Humans. The Journal of Clinical Endocrinology & Metabolism. 92**,** 2272-2279.

Andres, V., Pello, O. M., Silvestre-Roig, C., 2012. Macrophage proliferation and apoptosis in atherosclerosis. Curr Opin Lipidol. 23**,** 429-38.

Andrews, T., Zhang, P., Bhat, N. R., 1998. TNFalpha potentiates IFNgamma-induced cell death in oligodendrocyte progenitors. J Neurosci Res. 54**,** 574-83.

Anney, R., Klei, L., Pinto, D., Regan, R., Conroy, J., Magalhaes, T. R., . . . Hallmayer, J., 2010. A genome-wide scan for common alleles affecting risk for autism. Hum Mol Genet. 19**,** 4072-82.

Araki, K. Y., Sims, J. R., Bhide, P. G., 2007. Dopamine receptor mRNA and protein expression in the mouse corpus striatum and cerebral cortex during pre- and postnatal development. Brain Res. 1156**,** 31-45.

Arch, R. H., Gedrich, R. W., Thompson, C. B., 1998. Tumor necrosis factor receptor-associated factors (TRAFs)—a family of adapter proteins that regulates life and death. Genes & Development. 12**,** 2821-2830.

Arck, P. C., Hecher, K., 2013. Fetomaternal immune cross-talk and its consequences for maternal and offspring's health. Nat Med. 99**,** 548-556.

Arion, D., Unger, T., Lewis, D. A., Levitt, P., Mirnics, K., 2007. Molecular evidence for increased expression of genes related to immune and chaperone function in the prefrontal cortex in schizophrenia. Biol Psychiatry. 62**,** 711-21.

Arnsten, A. F., Wang, M. J., Paspalas, C. D., 2012. Neuromodulation of thought: flexibilities and vulnerabilities in prefrontal cortical network synapses. Neuron. 76**,** 223-39.

Arrode-Bruses, G., Bruses, J. L., 2012. Maternal immune activation by poly I:C induces expression of cytokines IL-1beta and IL-13, chemokine MCP-1 and colony stimulating factor VEGF in fetal mouse brain. J Neuroinflammation. 9**,** 83.

Arsenault, D., St-Amour, I., Cisbani, G., Rousseau, L. S., Cicchetti, F., 2014. The different effects of LPS and poly I:C prenatal immune challenges on the behavior, development and inflammatory responses in pregnant mice and their offspring. Brain Behav Immun. 38**,** 77-90.

Ashwell, K., 1991. The distribution of microglia and cell death in the fetal rat forebrain. Brain Res Dev Brain Res. 58**,** 1-12.

Ashwood, P., Corbett, B. A., Kantor, A., Schulman, H., Van de Water, J., Amaral, D. G., 2011a. In Search of Cellular Immunophenotypes in the Blood of Children with Autism. PLoS ONE. 6**,** e19299.

Ashwood, P., Enstrom, A., Krakowiak, P., Hertz-Picciotto, I., Hansen, R. L., Croen, L. A., . . . Van de Water, J., 2008. Decreased transforming growth factor beta1 in autism: a potential link between immune dysregulation and impairment in clinical behavioral outcomes. J Neuroimmunol. 204**,** 149-53.

Ashwood, P., Krakowiak, P., Hertz-Picciotto, I., Hansen, R., Pessah, I., Van de Water, J., 2011b. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain, Behavior, and Immunity. 25**,** 40-45.

Ashwood, P., Wakefield, A. J., 2006. Immune activation of peripheral blood and mucosal CD3+ lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms. J Neuroimmunol. 173.

Association, A. P., 2000. Diagnostic and Statistical Manual of Mental Disorders, Text Revision (DSM-IV-TR).

Atladóttir, H., Thorsen, P., Østergaard, L., Schendel, D., Lemcke, S., Abdallah, M., Parner, E., 2010. Maternal Infection Requiring Hospitalization During Pregnancy and Autism Spectrum Disorders. Journal of Autism and Developmental Disorders. 40**,** 1423-1430.

Atladottir, H. O., Pedersen, M. G., Thorsen, P., Mortensen, P. B., Deleuran, B., Eaton, W. W., Parner, E. T., 2009. Association of family history of autoimmune diseases and autism spectrum disorders. Pediatrics. 124**,** 687-94.

Avital, A., Goshen, I., Kamsler, A., Segal, M., Iverfeldt, K., Richter-Levin, G., Yirmiya, R., 2003. Impaired interleukin-1 signaling is associated with deficits in hippocampal memory processes and neural plasticity. Hippocampus. 13**,** 826-34.

Avitsur, R., Cohen, E., Yirmiya, R., 1997. Effects of interleukin-1 on sexual attractivity in a model of sickness behavior. Physiol Behav. 63**,** 25-30.

Avitsur, R., Yirmiya, R., 1999. Cytokines inhibit sexual behavior in female rats: I. Synergistic effects of tumor necrosis factor alpha and interleukin-1. Brain Behav Immun. 13**,** 14-32.

Ayala, J. E., Samuel, V. T., Morton, G. J., Obici, S., Croniger, C. M., Shulman, G. I., . . . McGuinness, O. P., 2010. Standard operating procedures for describing and performing metabolic tests of glucose homeostasis in mice. Dis Model Mech. 3**,** 525-34.

Aye, I. L. M. H., Lager, S., Ramirez, V. I., Gaccioli, F., Dudley, D. J., Jansson, T., Powell, T. L., 2014. Increasing Maternal Body Mass Index Is Associated with Systemic Inflammation in the Mother and the Activation of Distinct Placental Inflammatory Pathways. Biology of Reproduction. 90**,** 129, 1-9.

Ayroldi, E., Migliorati, G., Bruscoli, S., Marchetti, C., Zollo, O., Cannarile, L., . . . Riccardi, C., 2001. Modulation of T-cell activation by the glucocorticoid-induced leucine zipper factor via inhibition of nuclear factor kappaB. Blood. 98**,** 743-53.

Babon, J. J., Kershaw, N. J., Murphy, J. M., Varghese, L. N., Laktyushin, A., Young, S. N., . . . Nicola, N. A., 2012. Suppression of cytokine signaling by SOCS3: characterization of the mode of inhibition and the basis of its specificity. Immunity. 36**,** 239-50.

Babon, J. J., Varghese, L. N., Nicola, N. A., 2014. Inhibition of IL-6 family cytokines by SOCS3. Semin Immunol. 26**,** 13-9.

Babulas, V., Factor-Litvak, P., Goetz, R., Schaefer, C. A., Brown, A. S., 2006. Prenatal exposure to maternal genital and reproductive infections and adult schizophrenia. Am J Psychiatry. 163**,** 927-9.

Badger, A. M., Cook, M. N., Lark, M. W., Newman-Tarr, T. M., Swift, B. A., Nelson, A. H., . . . Kumar, S., 1998. SB 203580 inhibits p38 mitogen-activated protein kinase, nitric oxide production, and inducible nitric oxide synthase in bovine cartilage-derived chondrocytes. J Immunol. 161**,** 467-73.

Baerwald, K. D., Popko, B., 1998. Developing and mature oligodendrocytes respond differently to the immune cytokine interferon-gamma. J Neurosci Res. 52**,** 230-9.

Bahn, S., Chan, M. K., 2015. What Can We Learn About Depression from Gene Expression in Peripheral Tissues? Biological Psychiatry. 77**,** 207-209.

Bailey, M. T., Lubach, G. R., Coe, C. L., 2004. Prenatal stress alters bacterial colonization of the gut in infant monkeys. J Pediatr Gastroenterol Nutr. 38**,** 414-21.

Bakunina, N., Pariante, C. M., Zunszain, P. A., 2015. Immune mechanisms linked to depression via oxidative stress and neuroprogression. Immunology.

Balschun, D., Wetzel, W., Del Rey, A., Pitossi, F., Schneider, H., Zuschratter, W., Besedovsky, H. O., 2004. Interleukin-6: a cytokine to forget. Faseb j. 18**,** 1788-90.

Banks, W. A., 2005. Blood-brain barrier transport of cytokines: a mechanism for neuropathology. Curr Pharm Des. 11**,** 973-84.

Banks, W. A., Farr, S. A., La Scola, M. E., Morley, J. E., 2001. Intravenous human interleukin-1alpha impairs memory processing in mice: dependence on blood-brain barrier transport into posterior division of the septum. J Pharmacol Exp Ther. 299**,** 536-41.

Barnabe-Heider, F., Wasylnka, J. A., Fernandes, K. J., Porsche, C., Sendtner, M., Kaplan, D. R., Miller, F. D., 2005. Evidence that embryonic neurons regulate the onset of cortical gliogenesis via cardiotrophin-1. Neuron. 48**,** 253-65.

Barr, C. E., Mednick, S. A., Munk-Jorgensen, P., 1990. Exposure to influenza epidemics during gestation and adult schizophrenia. A 40-year study. Arch Gen Psychiatry. 47**,** 869-74.

Barreau, F., Ferrier, L., Fioramonti, J., Bueno, L., 2004. Neonatal maternal deprivation triggers long term alterations in colonic epithelial barrier and mucosal immunity in rats. Gut. 53**,** 501-6.

Barres, B. A., 2008. The mystery and magic of glia: a perspective on their roles in health and disease. Neuron. 60**,** 430-40.

Barres, B. A., Raff, M. C., 1994. Control of oligodendrocyte number in the developing rat optic nerve. Neuron. 12**,** 935-42.

Barres, B. A., Schmid, R., Sendnter, M., Raff, M. C., 1993. Multiple extracellular signals are required for long-term oligodendrocyte survival. Development. 118**,** 283-95.

Barry, H., Hardiman, O., Healy, D. G., Keogan, M., Moroney, J., Molnar, P. P., . . . Murphy, K. C., 2011. Anti-NMDA receptor encephalitis: an important differential diagnosis in psychosis. Br J Psychiatry. 199**,** 508-9.

Barton, G. M., Medzhitov, R., 2003. Toll-Like Receptor Signaling Pathways. Science. 300**,** 1524-1525.

Bastian, T. W., Santarriaga, S., Nguyen, T. A., Prohaska, J. R., Georgieff, M. K., Anderson, G. W., 2015. Fetal and neonatal iron deficiency but not copper deficiency increases vascular complexity in the developing rat brain. Nutr Neurosci. 18**,** 365-75.

Baudin, G., Godin, O., Lajnef, M., Aouizerate, B., Berna, F., Brunel, L., . . . Zinetti-Bertschy, A., 2016. Differential effects of childhood trauma and cannabis use disorders in patients suffering from schizophrenia. Schizophr Res.

Bauer, S., Kerr, B. J., Patterson, P. H., 2007. The neuropoietic cytokine family in development, plasticity, disease and injury. Nat Rev Neurosci. 8**,** 221-232.

Baumann, M. U., Deborde, S., Illsley, N. P., 2002. Placental glucose transfer and fetal growth. Endocrine. 19**,** 13-22.

Bazar, K. A., Yun, A. J., Lee, P. Y., 2005. "Starve a fever and feed a cold": feeding and anorexia may be adaptive behavioral modulators of autonomic and T helper balance. Med Hypotheses. 64**,** 1080-4.

Beattie, E. C., Stellwagen, D., Morishita, W., Bresnahan, J. C., Ha, B. K., Von Zastrow, M., . . . Malenka, R. C., 2002. Control of synaptic strength by glial TNFalpha. Science. 295**,** 2282-5.

Behrendt, G., Baer, K., Buffo, A., Curtis, M. A., Faull, R. L., Rees, M. I., . . . Dimou, L., 2013. Dynamic changes in myelin aberrations and oligodendrocyte generation in chronic amyloidosis in mice and men. Glia. 61**,** 273-86.

Bellinger, F. P., Madamba, S. G., Campbell, I. L., Siggins, G. R., 1995. Reduced long-term potentiation in the dentate gyrus of transgenic mice with cerebral overexpression of interleukin-6. Neurosci Lett. 198**,** 95-8.

Berda-Haddad, Y., Robert, S., Salers, P., Zekraoui, L., Farnarier, C., Dinarello, C. A., . . . Kaplanski, G., 2011. Sterile inflammation of endothelial cell-derived apoptotic bodies is mediated by interleukin-1α. Proceedings of the National Academy of Sciences. 108**,** 20684-20689.

Berk, M., Williams, L. J., Jacka, F. N., O'Neil, A., Pasco, J. A., Moylan, S., . . . Maes, M., 2013. So depression is an inflammatory disease, but where does the inflammation come from? BMC Med. 11**,** 200.

Besedovsky, H. O., Del Rey, A., Klusman, I., Furukawa, H., Monge Arditi, G., Kabiersch, A., 1991. Cytokines as modulators of the hypothalamus-pituitary-adrenal axis. The Journal of Steroid Biochemistry and Molecular Biology. 40**,** 613-618.

Bessis, A., Bernard, D., Triller, A., 2005. Tumor necrosis factor-alpha and neuronal development. Neuroscientist. 11**,** 277-81.

Beversdorf, D. Q., Manning, S. E., Hillier, A., Anderson, S. L., Nordgren, R. E., Walters, S. E., . . . Bauman, M. L., 2005. Timing of Prenatal Stressors and Autism. Journal of Autism and Developmental Disorders. 35**,** 471-478.

Bierut, L. J., Heath, A. C., Bucholz, K. K., Dinwiddie, S. H., Madden, P. A., Statham, D. J., . . . Martin, N. G., 1999. Major depressive disorder in a community-based twin sample: are there different genetic and environmental contributions for men and women? Arch Gen Psychiatry. 56**,** 557-63.

Bilbo, S. D., Tsang, V., 2010. Enduring consequences of maternal obesity for brain inflammation and behavior of offspring. Faseb j. 24**,** 2104-15.

Bilder, D. A., Bakian, A. V., Viskochil, J., Clark, E. A., Botts, E. L., Smith, K. R., . . . Coon, H., 2013. Maternal prenatal weight gain and autism spectrum disorders. Pediatrics. 132**,** e1276-83.

Blois, S. M., Joachim, R., Kandil, J., Margni, R., Tometten, M., Klapp, B. F., Arck, P. C., 2004. Depletion of CD8+ Cells Abolishes the Pregnancy Protective Effect of Progesterone Substitution with Dydrogesterone in Mice by Altering the Th1/Th2 Cytokine Profile. The Journal of Immunology. 172**,** 5893-5899.

Blomstrom, A., Gardner, R. M., Dalman, C., Yolken, R. H., Karlsson, H., 2015. Influence of maternal infections on neonatal acute phase proteins and their interaction in the development of non-affective psychosis. Transl Psychiatry. 5**,** e502.

Bloomfield, P. S., Selvaraj, S., Veronese, M., Rizzo, G., Bertoldo, A., Owen, D. R., . . . Howes, O. D., 2016. Microglial Activity in People at Ultra High Risk of Psychosis and in Schizophrenia: An [(11)C]PBR28 PET Brain Imaging Study. Am J Psychiatry. 173**,** 44-52.

Bluthe, R. M., Pawlowski, M., Suarez, S., Parnet, P., Pittman, Q., Kelley, K. W., Dantzer, R., 1994. Synergy between tumor necrosis factor alpha and interleukin-1 in the induction of sickness behavior in mice. Psychoneuroendocrinology. 19**,** 197-207.

Bocchio Chiavetto, L., Boin, F., Zanardini, R., Popoli, M., Michelato, A., Bignotti, S., . . . Gennarelli, M., 2002. Association between promoter polymorphic haplotypes of interleukin-10 gene and schizophrenia. Biol Psychiatry. 51**,** 480-4.

Bodey, B., 2002. Neuroendocrine influence on thymic haematopoiesis via the reticulo-epithelial cellular network. Expert Opin Ther Targets. 6**,** 57-72.

Bonaccorso, S., Puzella, A., Marino, V., Pasquini, M., Biondi, M., Artini, M., . . . Maes, M., 2001. Immunotherapy with interferon-alpha in patients affected by chronic hepatitis C induces an intercorrelated stimulation of the cytokine network and an increase in depressive and anxiety symptoms. Psychiatry Research. 105**,** 45-55.

Bonaguidi, M. A., McGuire, T., Hu, M., Kan, L., Samanta, J., Kessler, J. A., 2005. LIF and BMP signaling generate separate and discrete types of GFAP-expressing cells. Development. 132**,** 5503-14.

Borovikova, L. V., Ivanova, S., Zhang, M., Yang, H., Botchkina, G. I., Watkins, L. R., . . . Tracey, K. J., 2000. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature. 405**,** 458-62.

Borrell, J., Vela, J. M., Arevalo-Martin, A., Molina-Holgado, E., Guaza, C., 2002. Prenatal immune challenge disrupts sensorimotor gating in adult rats. Implications for the etiopathogenesis of schizophrenia. Neuropsychopharmacology. 26**,** 204-15.

Borrell, V., Marin, O., 2006. Meninges control tangential migration of hem-derived Cajal-Retzius cells via CXCL12/CXCR4 signaling. Nat Neurosci. 9, 1284-93.

Braida, D., Sacerdote, P., Panerai, A. E., Bianchi, M., Aloisi, A. M., Iosue, S., Sala, M., 2004. Cognitive function in young and adult IL (interleukin)-6 deficient mice. Behav Brain Res. 153, 423-9.

Breder, C. D., Dinarello, C. A., Saper, C. B., 1988. Interleukin-1 immunoreactive innervation of the human hypothalamus. Science. 240, 321-4.

Breder, C. D., Saper, C. B., 1996. Expression of inducible cyclooxygenase mRNA in the mouse brain after systemic administration of bacterial lipopolysaccharide. Brain Res. 713, 64-9.

Breder, C. D., Tsujimoto, M., Terano, Y., Scott, D. W., Saper, C. B., 1993. Distribution and characterization of tumor necrosis factor-alpha-like immunoreactivity in the murine central nervous system. J Comp Neurol. 337, 543-67.

Breier, G., Albrecht, U., Sterrer, S., Risau, W., 1992. Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation. Development. 114, 521-32.

Bresciani, G., da Cruz, I. B., Gonzalez-Gallego, J., 2015. Manganese superoxide dismutase and oxidative stress modulation. Adv Clin Chem. 68, 87-130.

Bronson, S. L., Bale, T. L., 2016. The Placenta as a Mediator of Stress Effects on Neurodevelopmental Reprogramming. Neuropsychopharmacology. 41, 207-18.

Brostjan, C., Anrather, J., Csizmadia, V., Natarajan, G., Winkler, H., 1997. Glucocorticoids inhibit E-selectin expression by targeting NF-kappaB and not ATF/c-Jun. J Immunol. 158, 3836-44.

Brown, A. S., 2002. Prenatal risk factors and schizophrenia. Expert Rev Neurother. 2, 53-60.

Brown, A. S., 2012. Epidemiologic studies of exposure to prenatal infection and risk of schizophrenia and autism. Developmental Neurobiology. 72, 1272-1276.

Brown, A. S., Begg, M. D., Gravenstein, S., Schaefer, C. A., Wyatt, R. J., Bresnahan, M., . . . Susser, E. S., 2004a. Serologic evidence of prenatal influenza in the etiology of schizophrenia. Arch Gen Psychiatry. 61, 774-80.

Brown, A. S., Cohen, P., Harkavy-Friedman, J., Babulas, V., Malaspina, D., Gorman, J. M., Susser, E. S., 2001. A.E. Bennett Research Award. Prenatal rubella, premorbid abnormalities, and adult schizophrenia. Biol Psychiatry. 49, 473-86.

Brown, A. S., Derkits, E. J., 2010. Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. Am J Psychiatry. 167.

Brown, A. S., Hooton, J., Schaefer, C. A., Zhang, H., Petkova, E., Babulas, V., . . . Susser, E. S., 2004b. Elevated maternal interleukin-8 levels and risk of schizophrenia in adult offspring. Am J Psychiatry. 161, 889-95.

Brown, A. S., Schaefer, C. A., Quesenberry, C. P., Jr., Liu, L., Babulas, V. P., Susser, E. S., 2005. Maternal exposure to toxoplasmosis and risk of schizophrenia in adult offspring. Am J Psychiatry. 162, 767-73.

Brown, A. S., van Os, J., Driessens, C., Hoek, H. W., Susser, E. S., 2000. Further evidence of relation between prenatal famine and major affective disorder. Am J Psychiatry. 157, 190-5.

Brown, J. A., Horvath, S., Garbett, K. A., Schmidt, M. J., Everheart, M., Gellert, L., . . . Mirnics, K., 2014. The role of cannabinoid 1 receptor expressing interneurons in behavior. Neurobiology of Disease. 63, 210-21.

Brown, J. A., Ramikie, T. S., Schmidt, M. J., Baldi, R., Garbett, K., Everheart, M. G., . . . Mirnics, K., 2015. Inhibition of parvalbumin-expressing interneurons results in complex behavioral changes. Mol Psychiatry.

Brucato, N., Guadalupe, T., Franke, B., Fisher, S. E., Francks, C., 2015. A schizophrenia-associated HLA locus affects thalamus volume and asymmetry. Brain, Behavior, and Immunity. 46, 311-318.

Brunton, P. J., Russell, J. A., 2010. Prenatal Social Stress in the Rat Programmes Neuroendocrine and Behavioural Responses to Stress in the Adult Offspring: Sex-Specific Effects. Journal of Neuroendocrinology. 22, 258-271.

Bryan, H. K., Olayanju, A., Goldring, C. E., Park, B. K., 2013. The Nrf2 cell defence pathway: Keap1-dependent and -independent mechanisms of regulation. Biochem Pharmacol. 85, 705-17.

Bugga, L., Gadient, R. A., Kwan, K., Stewart, C. L., Patterson, P. H., 1998. Analysis of neuronal and glial phenotypes in brains of mice deficient in leukemia inhibitory factor. J Neurobiol. 36, 509-24.

Buka, S. L., Cannon, T. D., Torrey, E. F., Yolken, R. H., 2008. Maternal exposure to herpes simplex virus and risk of psychosis among adult offspring. Biol Psychiatry. 63, 809-15.

Buka, S. L., Tsuang, M. T., Torrey, E. F., Klebanoff, M. A., Bernstein, D., Yolken, R. H., 2001a. Maternal infections and subsequent psychosis among offspring. Arch Gen Psychiatry. 58, 1032-7.

Buka, S. L., Tsuang, M. T., Torrey, E. F., Klebanoff, M. A., Wagner, R. L., Yolken, R. H., 2001b. Maternal cytokine levels during pregnancy and adult psychosis. Brain Behav Immun. 15, 411-20.

Burns, T. M., Clough, J. A., Klein, R. M., Wood, G. W., Berman, N. E., 1993. Developmental regulation of cytokine expression in the mouse brain. Growth Factors. 9, 253-8.

Cahill, M., Chant, D., Welham, J., McGrath, J., 2002. No significant association between prenatal exposure poliovirus epidemics and psychosis. Aust N Z J Psychiatry. 36, 373-5.

Campbell, D. B., D'Oronzio, R., Garbett, K., Ebert, P. J., Mirnics, K., Levitt, P., Persico, A. M., 2007. Disruption of cerebral cortex MET signaling in autism spectrum disorder. Annals of Neurology. 62, 243-50.

Campbell, D. B., Sutcliffe, J. S., Ebert, P. J., Militerni, R., Bravaccio, C., Trillo, S., . . . Levitt, P., 2006. A genetic variant that disrupts MET transcription is associated with autism. Proceedings of the National Academy of Sciences. 103, 16834-16839.

Campbell, I. L., Abraham, C. R., Masliah, E., Kemper, P., Inglis, J. D., Oldstone, M. B., Mucke, L., 1993. Neurologic disease induced in transgenic mice by cerebral overexpression of interleukin 6. Proc Natl Acad Sci U S A. 90, 10061-5.

Campbell, I. L., Hofer, M. J., Pagenstecher, A., 2010. Transgenic models for cytokine-induced neurological disease. Biochim Biophys Acta. 1802, 903-17.

Campbell, I. L., Stalder, A. K., Akwa, Y., Pagenstecher, A., Asensio, V. C., 1998. Transgenic Models to Study the Actions of Cytokines in the Central Nervous System. Neuroimmunomodulation. 5, 126-135.

Can, O. D., Ulupinar, E., Ozkay, U. D., Yegin, B., Ozturk, Y., 2012. The effect of simvastatin treatment on behavioral parameters, cognitive performance, and hippocampal morphology in rats fed a standard or a high-fat diet. Behav Pharmacol. 23, 582-92.

Cannell, J. J., 2008. Autism and vitamin D. Med Hypotheses. 70, 750-9.

Cannon, M., Cotter, D., Coffey, V. P., Sham, P. C., Takei, N., Larkin, C., . . . O'Callaghan, E., 1996. Prenatal exposure to the 1957 influenza epidemic and adult schizophrenia: a follow-up study. The British Journal of Psychiatry. 168**,** 368-371.

Cannon, M., Jones, P. B., Murray, R. M., 2002a. Obstetric Complications and Schizophrenia: Historical and Meta-Analytic Review. American Journal of Psychiatry. 159**,** 1080-1092.

Cannon, M., Jones, P. B., Murray, R. M., 2002b. Obstetric complications and schizophrenia: historical and meta-analytic review. Am J Psychiatry. 159**,** 1080-92.

Capuron, L., Gumnick, J. F., Musselman, D. L., Lawson, D. H., Reemsnyder, A., Nemeroff, C. B., Miller, A. H., 2002. Neurobehavioral effects of interferon-alpha in cancer patients: phenomenology and paroxetine responsiveness of symptom dimensions. Neuropsychopharmacology. 26**,** 643-52.

Carboni, E., Barros, V. G., Ibba, M., Silvagni, A., Mura, C., Antonelli, M. C., 2010. Prenatal restraint stress: an in vivo microdialysis study on catecholamine release in the rat prefrontal cortex. Neuroscience. 168**,** 156-66.

Carmeliet, P., 2003. Blood vessels and nerves: common signals, pathways and diseases. Nat Rev Genet. 4**,** 710-20.

Carpenter, L. L., Gawuga, C. E., Tyrka, A. R., Lee, J. K., Anderson, G. M., Price, L. H., 2010. Association between plasma IL-6 response to acute stress and early-life adversity in healthy adults. Neuropsychopharmacology. 35**,** 2617-23.

Carr, C. P., Martins, C. M., Stingel, A. M., Lemgruber, V. B., Juruena, M. F., 2013. The role of early life stress in adult psychiatric disorders: a systematic review according to childhood trauma subtypes. J Nerv Ment Dis. 201**,** 1007-20.

Carswell, E. A., Old, L. J., Kassel, R. L., Green, S., Fiore, N., Williamson, B., 1975. An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci U S A. 72**,** 3666-70.

Carvey, P. M., Chang, Q., Lipton, J. W., Ling, Z., 2003. Prenatal exposure to the bacteriotoxin lipopolysaccharide leads to long-term losses of dopamine neurons in offspring: a potential, new model of Parkinson's disease. Front Biosci. 8**,** s826-37.

Caspi, A., Moffitt, T. E., 2006. Gene-environment interactions in psychiatry: joining forces with neuroscience. Nat Rev Neurosci. 7**,** 583-590.

Castanon, N., Luheshi, G., Laye, S., 2015. Role of neuroinflammation in the emotional and cognitive alterations displayed by animal models of obesity. Front Neurosci. 9**,** 229.

Cebra, J. J., 1999. Influences of microbiota on intestinal immune system development. Am J Clin Nutr. 69**,** 1046s-1051s.

Cerf, M. E., Williams, K., van Rooyen, J., Esterhuyse, A. J., Muller, C. J., Louw, J., 2010. Gestational 30% and 40% fat diets increase brain GLUT2 and neuropeptide Y immunoreactivity in neonatal Wistar rats. Int J Dev Neurosci. 28**,** 625-30.

Chae, S., Sim, M., Lim, M., Na, J., Kim, D., 2015. Multivariate Analysis of Relationship between Childhood Trauma and Psychotic Symptoms in Patients with Schizophrenia. Psychiatry Investig. 12**,** 397-401.

Chalasani, S. H., Sabelko, K. A., Sunshine, M. J., Littman, D. R., Raper, J. A., 2003. A chemokine, SDF-1, reduces the effectiveness of multiple axonal repellents and is required for normal axon pathfinding. J Neurosci. 23**,** 1360-71.

Chandna, A. R., Kuhlmann, N., Bryce, C. A., Greba, Q., Campanucci, V. A., Howland, J. G., 2015. Chronic maternal hyperglycemia induced during mid-pregnancy in rats increases

RAGE expression, augments hippocampal excitability, and alters behavior of the offspring. Neuroscience. 303, 241-60.

Chandran, M., Phillips, S. A., Ciaraldi, T., Henry, R. R., 2003. Adiponectin: More Than Just Another Fat Cell Hormone? Diabetes Care. 26, 2442-2450.

Chang, M. Y., Park, C. H., Son, H., Lee, Y. S., Lee, S. H., 2004. Developmental stage-dependent self-regulation of embryonic cortical precursor cell survival and differentiation by leukemia inhibitory factor. Cell Death Differ. 11, 985-96.

Checchin, D., Sennlaub, F., Levavasseur, E., Leduc, M., Chemtob, S., 2006. Potential role of microglia in retinal blood vessel formation. Invest Ophthalmol Vis Sci. 47, 3595-602.

Chen, G., Goeddel, D. V., 2002. TNF-R1 signaling: a beautiful pathway. Science. 296, 1634-5.

Chen, L. W., Chien, C. Y., Yang, K. J., Kuo, S. F., Chen, C. H., Chien, R. N., 2015a. Helicobacter pylori Infection Increases Insulin Resistance and Metabolic Syndrome in Residents Younger than 50 Years Old: A Community-Based Study. PLoS One. 10, e0128671.

Chen, X., Wang, X., Hossain, S., O'Neill, F. A., Walsh, D., van den Oord, E., . . . Kendler, K. S., 2007. Interleukin 3 and schizophrenia: the impact of sex and family history. Mol Psychiatry. 12, 273-82.

Chen, Y., Molet, J., Gunn, B. G., Ressler, K., Baram, T. Z., 2015b. Diversity of Reporter Expression Patterns in Transgenic Mouse Lines Targeting Corticotropin-Releasing Hormone-Expressing Neurons. Endocrinology. 156, 4769-80.

Cheng, X., Folco, E. J., Shimizu, K., Libby, P., 2012. Adiponectin Induces Pro-inflammatory Programs in Human Macrophages and CD4+ T Cells. Journal of Biological Chemistry. 287, 36896-36904.

Chess, S., 1971. Autism in children with congenital rubella. J Autism Child Schizophr. 1, 33-47.

Chess, S., 1977. Follow-up report on autism in congenital rubella. J Autism Child Schizophr. 7, 69-81.

Chiang, C., Litingtung, Y., Lee, E., Young, K. E., Corden, J. L., Westphal, H., Beachy, P. A., 1996. Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function. Nature. 383, 407-13.

Chiang, C. S., Stalder, A., Samimi, A., Campbell, I. L., 1994. Reactive gliosis as a consequence of interleukin-6 expression in the brain: studies in transgenic mice. Dev Neurosci. 16, 212-21.

Chiang, J. J., Taylor, S. E., Bower, J. E., 2015. Early adversity, neural development, and inflammation. Developmental Psychobiology. 57, 887-907.

Chiang, S. S., Riedel, M., Schwarz, M., Mueller, N., 2013. Is T-helper type 2 shift schizophrenia-specific? Primary results from a comparison of related psychiatric disorders and healthy controls. Psychiatry Clin Neurosci. 67, 228-36.

Chourbaji, S., Urani, A., Inta, I., Sanchis-Segura, C., Brandwein, C., Zink, M., . . . Gass, P., 2006. IL-6 knockout mice exhibit resistance to stress-induced development of depression-like behaviors. Neurobiol Dis. 23, 587-94.

Christian, L. M., Porter, K., 2014. Longitudinal changes in serum proinflammatory markers across pregnancy and postpartum: effects of maternal body mass index. Cytokine. 70, 134-40.

Christoffels, V. M., Habets, P. E., Das, A. T., Clout, D. E., van Roon, M. A., Moorman, A. F., Lamers, W. H., 2000. A single regulatory module of the carbamoylphosphate synthetase I gene executes its hepatic program of expression. J Biol Chem. 275, 40020-7.

Cingolani, L. A., Thalhammer, A., Yu, L. M., Catalano, M., Ramos, T., Colicos, M. A., Goda, Y., 2008. Activity-dependent regulation of synaptic AMPA receptor composition and abundance by beta3 integrins. Neuron. 58**,** 749-62.

Clark, I. A., 2007. How TNF was recognized as a key mechanism of disease. Cytokine Growth Factor Rev. 18**,** 335-43.

Clarke, M. C., Tanskanen, A., Huttunen, M., Whittaker, J. C., Cannon, M., 2009. Evidence for an interaction between familial liability and prenatal exposure to infection in the causation of schizophrenia. Am J Psychiatry. 166**,** 1025-30.

Cleland, N., Lieblich, S., Schalling, M., Rahm, C., 2015. A 16-year-old girl with anti-NMDA-receptor encephalitis and family history of psychotic disorders. Acta Neuropsychiatr. 27**,** 375-9.

Coe, C. L., Kramer, M., Czeh, B., Gould, E., Reeves, A. J., Kirschbaum, C., Fuchs, E., 2003. Prenatal stress diminishes neurogenesis in the dentate gyrus of juvenile rhesus monkeys. Biol Psychiatry. 54**,** 1025-34.

Coe, C. L., Kramer, M., Kirschbaum, C., Netter, P., Fuchs, E., 2002. Prenatal stress diminishes the cytokine response of leukocytes to endotoxin stimulation in juvenile rhesus monkeys. J Clin Endocrinol Metab. 87**,** 675-81.

Coe, C. L., Lubach, G. R., 2000. Prenatal influences on neuroimmune set points in infancy. Ann N Y Acad Sci. 917**,** 468-77.

Coe, C. L., Lubach, G. R., 2003. Critical periods of special health relevance for psychoneuroimmunology. Brain, Behavior, and Immunity. 17**,** 3-12.

Coe, C. L., Lubach, G. R., 2005. Prenatal origins of individual variation in behavior and immunity. Neurosci Biobehav Rev. 29.

Coe, C. L., Lubach, G. R., CHAPTER 21 - Mother-infant Interactions and the Development of Immunity from Conception through Weaning A2 - Ader, Robert. Psychoneuroimmunology (Fourth Edition). Academic Press, Burlington, 2007, pp. 455-474.

Coe, C. L., Lubach, G. R., Izard, K. M., 1994. Progressive improvement in the transfer of maternal antibody across the order primates. American Journal of Primatology. 32**,** 51-55.

Coe, C. L., Lubach, G. R., Karaszewski, J. W., Ershler, W. B., 1996. Prenatal endocrine activation alters postnatal cellular immunity in infant monkeys. Brain, behavior, and immunity. 10**,** 221-234.

Coe, C. L., Lubach, G. R., Shirtcliff, E. A., 2007. Maternal stress during pregnancy predisposes for iron deficiency in infant monkeys impacting innate immunity. Pediatr Res. 61**,** 520-4.

Cohly, H. H., Panja, A., 2005. Immunological findings in autism. Int Rev Neurobiol. 71**,** 317-41.

Coiro, P., Padmashri, R., Suresh, A., Spartz, E., Pendyala, G., Chou, S., . . . Dunaevsky, A., 2015. Impaired synaptic development in a maternal immune activation mouse model of neurodevelopmental disorders. Brain, Behavior, and Immunity. 50**,** 249-258.

Colman, I., Ataullahjan, A., Naicker, K., Van Lieshout, R. J., 2012. Birth weight, stress, and symptoms of depression in adolescence: evidence of fetal programming in a national Canadian cohort. Can J Psychiatry. 57**,** 422-8.

Colotta, F., Re, F., Muzio, M., Bertini, R., Polentarutti, N., Sironi, M., . . . Mantovani, A., 1993. Interleukin-1 type II receptor: a decoy target for IL-1 that is regulated by IL-4. Science. 261**,** 472-5.

Comi, A. M., Zimmerman, A. W., Frye, V. H., Law, P. A., Peeden, J. N., 1999. Familial clustering of autoimmune disorders and evaluation of medical risk factors in autism. J Child Neurol. 14, 388-94.

Connor, C. M., Dincer, A., Straubhaar, J., Galler, J. R., Houston, I. B., Akbarian, S., 2012. Maternal immune activation alters behavior in adult offspring, with subtle changes in the cortical transcriptome and epigenome. Schizophr Res. 140, 175-84.

Consortium, S. P. G.-W. A. S. G., 2011. Genome-wide association study identifies five new schizophrenia loci. Nat Genet. 43, 969-76.

Consortium, T. N. a. P. A. S. o. t. P. G., 2015. Psychiatric genome-wide association study analyses implicate neuronal, immune and histone pathways. Nature neuroscience. 18, 199-209.

Coquet, J. M., Rausch, L., Borst, J., 2015. The importance of co-stimulation in the orchestration of T helper cell differentiation. Immunol Cell Biol. 93, 780-8.

Corbin, J. G., Kelly, D., Rath, E. M., Baerwald, K. D., Suzuki, K., Popko, B., 1996. Targeted CNS expression of interferon-gamma in transgenic mice leads to hypomyelination, reactive gliosis, and abnormal cerebellar development. Mol Cell Neurosci. 7, 354-70.

Corriveau, R. A., Huh, G. S., Shatz, C. J., 1998. Regulation of class I MHC gene expression in the developing and mature CNS by neural activity. Neuron. 21, 505-20.

Covic, T., Cumming, S. R., Pallant, J. F., Manolios, N., Emery, P., Conaghan, P. G., Tennant, A., 2012. Depression and anxiety in patients with rheumatoid arthritis: prevalence rates based on a comparison of the Depression, Anxiety and Stress Scale (DASS) and the hospital, Anxiety and Depression Scale (HADS). BMC Psychiatry. 12, 6.

Croker, B. A., Kiu, H., Pellegrini, M., Toe, J., Preston, S., Metcalf, D., . . . Roberts, A. W., 2012. IL-6 promotes acute and chronic inflammatory disease in the absence of SOCS3. Immunol Cell Biol. 90, 124-9.

Croker, B. A., Metcalf, D., Robb, L., Wei, W., Mifsud, S., DiRago, L., . . . Roberts, A. W., 2004. SOCS3 is a critical physiological negative regulator of G-CSF signaling and emergency granulopoiesis. Immunity. 20, 153-65.

Croonenberghs, J., Bosmans, E., Deboutte, D., Kenis, G., Maes, M., 2002. Activation of the inflammatory response system in autism. Neuropsychobiology. 45.

Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. The Lancet. 381, 1371-1379.

Cummings, C. M., Caporino, N. E., Kendall, P. C., 2014. Comorbidity of Anxiety and Depression in Children and Adolescents: 20 Years After. Psychological bulletin. 140, 816-845.

Curran, E. J., Watson, S. J., 1995. Dopamine receptor mRNA expression patterns by opioid peptide cells in the nucleus accumbens of the rat: a double in situ hybridization study. Journal of Comparative Neurology. 361, 57-76.

Cyster, J. G., Dang, E. V., Reboldi, A., Yi, T., 2014. 25-Hydroxycholesterols in innate and adaptive immunity. Nat Rev Immunol. 14, 731-43.

Czarny, P., Kwiatkowski, D., Kacperska, D., Kawczynska, D., Talarowska, M., Orzechowska, A., . . . Sliwinski, T., 2015. Elevated level of DNA damage and impaired repair of oxidative DNA damage in patients with recurrent depressive disorder. Med Sci Monit. 21, 412-8.

Czura, C. J., Rosas-Ballina, M., Tracey, K. J., CHAPTER 3 - Cholinergic Regulation of Inflammation A2 - Ader, Robert. Psychoneuroimmunology (Fourth Edition). Academic Press, Burlington, 2007, pp. 85-96.

D'Haenen H, A., Bossuyt, A., 1994. Dopamine D2 receptors in depression measured with single photon emission computed tomography. Biological Psychiatry. 35**,** 128-32.

Dada, T., Rosenzweig, J. M., Al Shammary, M., Firdaus, W., Al Rebh, S., Borbiev, T., . . . Burd, I., 2014. Mouse model of intrauterine inflammation: sex-specific differences in long-term neurologic and immune sequelae. Brain Behav Immun. 38**,** 142-50.

Dahl, J., Ormstad, H., Aass, H. C., Malt, U. F., Bendz, L. T., Sandvik, L., . . . Andreassen, O. A., 2014. The plasma levels of various cytokines are increased during ongoing depression and are reduced to normal levels after recovery. Psychoneuroendocrinology. 45**,** 77-86.

Danese, A., Caspi, A., Williams, B., Ambler, A., Sugden, K., Mika, J., . . . Arseneault, L., 2011. Biological embedding of stress through inflammation processes in childhood. Mol Psychiatry. 16**,** 244-6.

Danese, A., Pariante, C. M., Caspi, A., Taylor, A., Poulton, R., 2007. Childhood maltreatment predicts adult inflammation in a life-course study. Proceedings of the National Academy of Sciences. 104**,** 1319-1324.

Danesin, C., Houart, C., 2012. A Fox stops the Wnt: implications for forebrain development and diseases. Curr Opin Genet Dev. 22**,** 323-30.

Dantzer, R., 2006. Cytokine, sickness behavior, and depression. Neurol Clin. 24**,** 441-60.

Dantzer, R., CHAPTER 13 - Expression and Action of Cytokines in the Brain: Mechanisms and Pathophysiological Implications A2 - Ader, Robert. Psychoneuroimmunology (Fourth Edition). Academic Press, Burlington, 2007, pp. 271-280.

Dantzer, R., BluthÉ, R.-M., Castanon, N., Kelley, K. W., Konsman, J.-P., Laye, S., . . . Parnet, P., CHAPTER 14 - Cytokines, Sickness Behavior, and Depression A2 - Ader, Robert. Psychoneuroimmunology (Fourth Edition). Academic Press, Burlington, 2007, pp. 281-318.

Day, M., Wang, Z., Ding, J., An, X., Ingham, C. A., Shering, A. F., . . . Surmeier, D. J., 2006. Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models. Nat Neurosci. 9**,** 251-9.

Day, M., Wokosin, D., Plotkin, J. L., Tian, X., Surmeier, D. J., 2008. Differential excitability and modulation of striatal medium spiny neuron dendrites. J Neurosci. 28**,** 11603-14.

De Miranda, J., Yaddanapudi, K., Hornig, M., Villar, G., Serge, R., Lipkin, W. I., 2010. Induction of Toll-like receptor 3-mediated immunity during gestation inhibits cortical neurogenesis and causes behavioral disturbances. MBio. 1.

De Nardo, D., 2015. Toll-like receptors: Activation, signalling and transcriptional modulation. Cytokine. 74**,** 181-9.

De Souza, D., Fabri, L. J., Nash, A., Hilton, D. J., Nicola, N. A., Baca, M., 2002. SH2 domains from suppressor of cytokine signaling-3 and protein tyrosine phosphatase SHP-2 have similar binding specificities. Biochemistry. 41**,** 9229-36.

de Witte, L., Tomasik, J., Schwarz, E., Guest, P. C., Rahmoune, H., Kahn, R. S., Bahn, S., 2014. Cytokine alterations in first-episode schizophrenia patients before and after antipsychotic treatment. Schizophr Res. 154**,** 23-9.

Degos, B., Laforet, P., Jardel, C., Sedel, F., Jossay-Winter, M., Romero, N. B., . . . Tourbah, A., 2014. POLG mutations associated with remitting/relapsing neurological events. J Clin Neurosci. 21**,** 186-8.

Demirci, C., Ernst, S., Alvarez-Perez, J. C., Rosa, T., Valle, S., Shridhar, V., . . . García-Ocana, A., 2012. Loss of HGF/c-Met Signaling in Pancreatic β-Cells Leads to Incomplete Maternal β-Cell Adaptation and Gestational Diabetes Mellitus. Diabetes. 61**,** 1143-1152.

Demoulin, J.-B., Essaghir, A., 2014. PDGF receptor signaling networks in normal and cancer cells. Cytokine & Growth Factor Reviews. 25**,** 273-283.

Deng, M. Y., Lam, S., Meyer, U., Feldon, J., Li, Q., Wei, R., . . . McAlonan, G. M., 2011. Frontal-Subcortical Protein Expression following Prenatal Exposure to Maternal Inflammation. PLoS One. 6.

Derry, H. M., Padin, A. C., Kuo, J. L., Hughes, S., Kiecolt-Glaser, J. K., 2015. Sex Differences in Depression: Does Inflammation Play a Role? Curr Psychiatry Rep. 17**,** 78.

DeSisto, C. L., Kim, S. Y., Sharma, A. J., 2014. Prevalence estimates of gestational diabetes mellitus in the United States, Pregnancy Risk Assessment Monitoring System (PRAMS), 2007-2010. Prev Chronic Dis. 11**,** E104.

Deslauriers, J., Larouche, A., Sarret, P., Grignon, S., 2013. Combination of prenatal immune challenge and restraint stress affects prepulse inhibition and dopaminergic/GABAergic markers. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 45**,** 156-164.

Devereux, G., Seaton, A., Barker, R. N., 2001. In utero priming of allergen-specific helper T cells. Clin Exp Allergy. 31**,** 1686-95.

Deverman, B. E., Patterson, P. H., 2009. Cytokines and CNS development. Neuron. 64**,** 61-78.

Di Santo, E., Alonzi, T., Fattori, E., Poli, V., Ciliberto, G., Sironi, M., . . . Ghezzi, P., 1996. Overexpression of interleukin-6 in the central nervous system of transgenic mice increases central but not systemic proinflammatory cytokine production. Brain Research. 740**,** 239-244.

Dickens, C., McGowan, L., Clark-Carter, D., Creed, F., 2002. Depression in rheumatoid arthritis: a systematic review of the literature with meta-analysis. Psychosom Med. 64**,** 52-60.

Dinarello, C. A., 1987. The biology of interleukin 1 and comparison to tumor necrosis factor. Immunol Lett. 16**,** 227-31.

Dinarello, C. A., 2007. Mutations in cryopyrin: bypassing roadblocks in the caspase 1 inflammasome for interleukin-1beta secretion and disease activity. Arthritis Rheum. 56**,** 2817-22.

Dinarello, C. A., 2009. Immunological and Inflammatory Functions of the Interleukin-1 Family. Annual Review of Immunology. 27**,** 519-550.

Diniz, B. S., Teixeira, A. L., Talib, L., Gattaz, W. F., Forlenza, O. V., 2010. Interleukin-1beta serum levels is increased in antidepressant-free elderly depressed patients. Am J Geriatr Psychiatry. 18**,** 172-6.

Diz-Chaves, Y., Astiz, M., Bellini, M. J., Garcia-Segura, L. M., 2013. Prenatal stress increases the expression of proinflammatory cytokines and exacerbates the inflammatory response to LPS in the hippocampal formation of adult male mice. Brain Behav Immun. 28**,** 196-206.

Dobrian, A. D., Lieb, D. C., Cole, B. K., Taylor-Fishwick, D. A., Chakrabarti, S. K., Nadler, J. L., 2011. Functional and pathological roles of the 12- and 15-lipoxygenases. Progress in Lipid Research. 50**,** 115-131.

Dodds, L., Fell, D. B., Shea, S., Armson, B. A., Allen, A. C., Bryson, S., 2011. The role of prenatal, obstetric and neonatal factors in the development of autism. J Autism Dev Disord. 41**,** 891-902.

Doruk, M., Ugur, M., Oruc, A. S., Demirel, N., Yildiz, Y., 2014. Serum adiponectin in gestational diabetes and its relation to pregnancy outcome. J Obstet Gynaecol. 34, 471-5.

Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E. K., Lanctot, K. L., 2010. A meta-analysis of cytokines in major depression. Biol Psychiatry. 67, 446-57.

Drexhage, R. C., Knijff, E. M., Padmos, R. C., Heul-Nieuwenhuijzen, L., Beumer, W., Versnel, M. A., Drexhage, H. A., 2010. The mononuclear phagocyte system and its cytokine inflammatory networks in schizophrenia and bipolar disorder. Expert Rev Neurother. 10, 59-76.

Dripps, D. J., Brandhuber, B. J., Thompson, R. C., Eisenberg, S. P., 1991. Interleukin-1 (IL-1) receptor antagonist binds to the 80-kDa IL-1 receptor but does not initiate IL-1 signal transduction. J Biol Chem. 266, 10331-6.

Dunn, A. J., 1992. The role of interleukin-1 and tumor necrosis factor alpha in the neurochemical and neuroendocrine responses to endotoxin. Brain Res Bull. 29, 807-12.

Edvardsen, J., Torgersen, S., Roysamb, E., Lygren, S., Skre, I., Onstad, S., Oien, P. A., 2009. Unipolar depressive disorders have a common genotype. J Affect Disord. 117, 30-41.

Elahi, M. M., Cagampang, F. R., Mukhtar, D., Anthony, F. W., Ohri, S. K., Hanson, M. A., 2009. Long-term maternal high-fat feeding from weaning through pregnancy and lactation predisposes offspring to hypertension, raised plasma lipids and fatty liver in mice. Br J Nutr. 102, 514-9.

Elenkov, I. J., 2004. Glucocorticoids and the Th1/Th2 balance. Ann N Y Acad Sci. 1024, 138-46.

Elenkov, I. J., Papanicolaou, D. A., Wilder, R. L., Chrousos, G. P., 1996. Modulatory effects of glucocorticoids and catecholamines on human interleukin-12 and interleukin-10 production: clinical implications. Proc Assoc Am Physicians. 108, 374-81.

Elmer, B. M., McAllister, A. K., 2012. Major histocompatibility complex class I proteins in brain development and plasticity. Trends Neurosci. 35, 660-70.

Enayati, M., Solati, J., Hosseini, M. H., Shahi, H. R., Saki, G., Salari, A. A., 2012. Maternal infection during late pregnancy increases anxiety- and depression-like behaviors with increasing age in male offspring. Brain Res Bull. 87, 295-302.

Enquobahrie, D. A., Williams, M. A., Qiu, C., Meller, M., Sorensen, T. K., 2009. Global placental gene expression in gestational diabetes mellitus. Am J Obstet Gynecol. 200, 206.e1-13.

Entringer, S., Kumsta, R., Nelson, E. L., Hellhammer, D. H., Wadhwa, P. D., Wust, S., 2008. Influence of prenatal psychosocial stress on cytokine production in adult women. Dev Psychobiol. 50, 579-87.

Erdem, M., Celik, C., Cayci, T., Ozdemir, B., Kurt, Y. G., Akgul, E. O., . . . Uzun, O., 2011. Serum haptoglobin levels in patients with melancholic and nonmelancholic major depression. Prog Neuropsychopharmacol Biol Psychiatry. 35, 944-7.

Escobar, J., Teramo, K., Stefanovic, V., Andersson, S., Asensi, M. A., Arduini, A., . . . Vento, M., 2013. Amniotic fluid oxidative and nitrosative stress biomarkers correlate with fetal chronic hypoxia in diabetic pregnancies. Neonatology. 103, 193-8.

Escobar, M., Crouzin, N., Cavalier, M., Quentin, J., Roussel, J., Lante, F., . . . Vignes, M., 2011. Early, time-dependent disturbances of hippocampal synaptic transmission and plasticity after in utero immune challenge. Biol Psychiatry. 70, 992-9.

Fagundes, C. P., Glaser, R., Kiecolt-Glaser, J. K., 2013. Stressful early life experiences and immune dysregulation across the lifespan. Brain, Behavior, and Immunity. 27, 8-12.

Fantuzzi, G., 2008. Adiponectin and inflammation: consensus and controversy. J Allergy Clin Immunol. 121**,** 326-30.

Fasshauer, M., Blüher, M., Stumvoll, M., 2014. Adipokines in gestational diabetes. The Lancet Diabetes & Endocrinology. 2**,** 488-499.

Fatemi, S. H., Emamian, E. S., Kist, D., Sidwell, R. W., Nakajima, K., Akhter, P., . . . Bailey, K., 1999. Defective corticogenesis and reduction in Reelin immunoreactivity in cortex and hippocampus of prenatally infected neonatal mice. Mol Psychiatry. 4**,** 145-54.

Fatemi, S. H., Reutiman, T. J., Folsom, T. D., Huang, H., Oishi, K., Mori, S., . . . Juckel, G., 2008. Maternal infection leads to abnormal gene regulation and brain atrophy in mouse offspring: implications for genesis of neurodevelopmental disorders. Schizophr Res. 99**,** 56-70.

Fattori, E., Lazzaro, D., Musiani, P., Modesti, A., Alonzi, T., Ciliberto, G., 1995. IL-6 expression in neurons of transgenic mice causes reactive astrocytosis and increase in ramified microglial cells but no neuronal damage. Eur J Neurosci. 7**,** 2441-9.

Fergusson, D. M., Crane, J., Beasley, R., Horwood, L. J., 1997. Perinatal factors and atopic disease in childhood. Clin Exp Allergy. 27**,** 1394-401.

Fernandez-Estevez, M. A., Casarejos, M. J., Lopez Sendon, J., Garcia Caldentey, J., Ruiz, C., Gomez, A., . . . Mena, M. A., 2014. Trehalose reverses cell malfunction in fibroblasts from normal and Huntington's disease patients caused by proteosome inhibition. PLoS One. 9**,** e90202.

Fernandez-Pujals, A. M., Adams, M. J., Thomson, P., McKechanie, A. G., Blackwood, D. H., Smith, B. H., . . . McIntosh, A. M., 2015. Epidemiology and Heritability of Major Depressive Disorder, Stratified by Age of Onset, Sex, and Illness Course in Generation Scotland: Scottish Family Health Study (GS:SFHS). PLoS One. 10**,** e0142197.

Fila-Danilow, A., Kucia, K., Kowalczyk, M., Owczarek, A., Paul-Samojedny, M., Borkowska, P., . . . Kowalski, J., 2012. Association study of interleukin-4 polymorphisms with paranoid schizophrenia in the Polish population: a critical approach. Mol Biol Rep. 39**,** 7941-7.

Fillman, S. G., Cloonan, N., Catts, V. S., Miller, L. C., Wong, J., McCrossin, T., . . . Weickert, C. S., 2013. Increased inflammatory markers identified in the dorsolateral prefrontal cortex of individuals with schizophrenia. Mol Psychiatry. 18**,** 206-214.

Fiore, M., Alleva, E., Probert, L., Kollias, G., Angelucci, F., Aloe, L., 1998. Exploratory and displacement behavior in transgenic mice expressing high levels of brain TNF-alpha. Physiol Behav. 63**,** 571-6.

Fiore, M., Probert, L., Kollias, G., Akassoglou, K., Alleva, E., Aloe, L., 1996. Neurobehavioral alterations in developing transgenic mice expressing TNF-alpha in the brain. Brain Behav Immun. 10**,** 126-38.

First, M., Spitzer, R., Gibbon, M., Williams, J., 2002. Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Patient Edition (SCID-I/P). New York: New York State Psychiatric Institute.

Fischer, P., Lehmann, U., Sobota, R. M., Schmitz, J., Niemand, C., Linnemann, S., . . . Schaper, F., 2004. The role of the inhibitors of interleukin-6 signal transduction SHP2 and SOCS3 for desensitization of interleukin-6 signalling. Biochem J. 378**,** 449-60.

Fisher, H. L., Cohen-Woods, S., Hosang, G. M., Korszun, A., Owen, M., Craddock, N., . . . Uher, R., 2013. Interaction between specific forms of childhood maltreatment and the

serotonin transporter gene (5-HTT) in recurrent depressive disorder. J Affect Disord. 145, 136-41.

Fontaine, R. H., Cases, O., Lelievre, V., Mesples, B., Renauld, J. C., Loron, G., . . . Gressens, P., 2008. IL-9/IL-9 receptor signaling selectively protects cortical neurons against developmental apoptosis. Cell Death Differ. 15, 1542-52.

Forrest, C. M., Khalil, O. S., Pisar, M., Smith, R. A., Darlington, L. G., Stone, T. W., 2012. Prenatal activation of Toll-like receptors-3 by administration of the viral mimetic poly(I:C) changes synaptic proteins, N-methyl-D-aspartate receptors and neurogenesis markers in offspring. Mol Brain. 5, 22.

Fortier, M. E., Joober, R., Luheshi, G. N., Boksa, P., 2004. Maternal exposure to bacterial endotoxin during pregnancy enhances amphetamine-induced locomotion and startle responses in adult rat offspring. J Psychiatr Res. 38, 335-45.

Frank, M. G., Weber, M. D., Watkins, L. R., Maier, S. F., 2015. Stress sounds the alarmin: The role of the danger-associated molecular pattern HMGB1 in stress-induced neuroinflammatory priming. Brain Behav Immun. 48, 1-7.

French, L., Gray, C., Leonard, G., Perron, M., Pike, G. B., Richer, L., . . . Paus, T., 2015. Early Cannabis Use, Polygenic Risk Score for Schizophrenia and Brain Maturation in Adolescence. JAMA Psychiatry. 72, 1002-11.

Freyberg, Z., Ferrando, S. J., Javitch, J. A., 2010. Roles of the Akt/GSK-3 and Wnt signaling pathways in schizophrenia and antipsychotic drug action. American Journal of Psychiatry.

Frias, J. L., Frias, J. P., Frias, P. A., Martinez-Frias, M. L., 2007. Infrequently studied congenital anomalies as clues to the diagnosis of maternal diabetes mellitus. Am J Med Genet A. 143a, 2904-9.

Fride, E., Weinstock, M., 1988. Prenatal stress increases anxiety related behavior and alters cerebral lateralization of dopamine activity. Life Sci. 42, 1059-65.

Friis, C. M., Paasche Roland, M. C., Godang, K., Ueland, T., Tanbo, T., Bollerslev, J., Henriksen, T., 2013. Adiposity-related inflammation: Effects of pregnancy. Obesity. 21, E124-E130.

Frydecka, D., Misiak, B., Beszlej, J. A., Karabon, L., Pawlak-Adamska, E., Tomkiewicz, A., . . . Kiejna, A., 2013. Genetic variants in transforming growth factor-beta gene (TGFB1) affect susceptibility to schizophrenia. Mol Biol Rep. 40, 5607-14.

Fujigaki, H., Saito, K., Fujigaki, S., Takemura, M., Sudo, K., Ishiguro, H., Seishima, M., 2006. The signal transducer and activator of transcription 1alpha and interferon regulatory factor 1 are not essential for the induction of indoleamine 2,3-dioxygenase by lipopolysaccharide: involvement of p38 mitogen-activated protein kinase and nuclear factor-kappaB pathways, and synergistic effect of several proinflammatory cytokines. J Biochem. 139, 655-62.

Funa, K., Sasahara, M., 2014. The roles of PDGF in development and during neurogenesis in the normal and diseased nervous system. J Neuroimmune Pharmacol. 9, 168-81.

Gabellec, M. M., Griffais, R., Fillion, G., Haour, F., 1996. Interleukin-1 receptors type I and type II in the mouse brain: kinetics of mRNA expressions after peripheral administration of bacterial lipopolysaccharide. J Neuroimmunol. 66, 65-70.

Galli, R., Pagano, S. F., Gritti, A., Vescovi, A. L., 2000. Regulation of neuronal differentiation in human CNS stem cell progeny by leukemia inhibitory factor. Dev Neurosci. 22, 86-95.

Gallou-Kabani, C., Vige, A., Gross, M. S., Rabes, J. P., Boileau, C., Larue-Achagiotis, C., . . . Junien, C., 2007. C57BL/6J and A/J mice fed a high-fat diet delineate components of metabolic syndrome. Obesity (Silver Spring). 15**,** 1996-2005.

Gamez-Guadix, M., Orue, I., Smith, P. K., Calvete, E., 2013. Longitudinal and reciprocal relations of cyberbullying with depression, substance use, and problematic internet use among adolescents. J Adolesc Health. 53**,** 446-52.

Gangarossa, G., Espallergues, J., de Kerchove d'Exaerde, A., El Mestikawy, S., Gerfen, C. R., Herve, D., . . . Valjent, E., 2013a. Distribution and compartmental organization of GABAergic medium-sized spiny neurons in the mouse nucleus accumbens. Front Neural Circuits. 7**,** 22.

Gangarossa, G., Espallergues, J., Mailly, P., De Bundel, D., de Kerchove d'Exaerde, A., Herve, D., . . . Krieger, P., 2013b. Spatial distribution of D1R- and D2R-expressing medium-sized spiny neurons differs along the rostro-caudal axis of the mouse dorsal striatum. Front Neural Circuits. 7**,** 124.

Gangarossa, G., Longueville, S., De Bundel, D., Perroy, J., Herve, D., Girault, J. A., Valjent, E., 2012. Characterization of dopamine D1 and D2 receptor-expressing neurons in the mouse hippocampus. Hippocampus. 22**,** 2199-207.

Gao, X. L., Yang, H. X., Zhao, Y., 2008. Variations of tumor necrosis factor-alpha, leptin and adiponectin in mid-trimester of gestational diabetes mellitus. Chin Med J (Engl). 121**,** 701-5.

Garay, P. A., Hsiao, E. Y., Patterson, P. H., McAllister, A. K., 2013. Maternal immune activation causes age- and region-specific changes in brain cytokines in offspring throughout development. Brain Behav Immun. 31**,** 54-68.

Garbett, K., Ebert, P. J., Mitchell, A., Lintas, C., Manzi, B., Mirnics, K., Persico, A. M., 2008. Immune transcriptome alterations in the temporal cortex of subjects with autism. Neurobiol Dis. 30**,** 303-11.

Garbett, K. A., Horvath, S., Ebert, P. J., Schmidt, M. J., Lwin, K., Mitchell, A., . . . Mirnics, K., 2010. Novel animal models for studying complex brain disorders: BAC-driven miRNA-mediated in vivo silencing of gene expression. Mol Psychiatry. 15**,** 987-95.

Garbett, K. A., Hsiao, E. Y., Kalman, S., Patterson, P. H., Mirnics, K., 2012. Effects of maternal immune activation on gene expression patterns in the fetal brain. Transl Psychiatry. 2**,** e98.

Garbett, K. A., Vereczkei, A., Kalman, S., Brown, J. A., Taylor, W. D., Faludi, G., . . . Mirnics, K., 2015a. Coordinated messenger RNA/microRNA changes in fibroblasts of patients with major depression. Biol Psychiatry. 77**,** 256-65.

Garbett, K. A., Vereczkei, A., Kalman, S., Wang, L., Korade, Z., Shelton, R. C., Mirnics, K., 2015b. Fibroblasts from patients with major depressive disorder show distinct transcriptional response to metabolic stressors. Transl Psychiatry. 5**,** e523.

Gardener, H., Spiegelman, D., Buka, S. L., 2009. Prenatal risk factors for autism: comprehensive meta-analysis. Br J Psychiatry. 195**,** 7-14.

Gardner, R. M., Lee, B. K., Magnusson, C., Rai, D., Frisell, T., Karlsson, H., . . . Dalman, C., 2015. Maternal body mass index during early pregnancy, gestational weight gain, and risk of autism spectrum disorders: Results from a Swedish total population and discordant sibling study. Int J Epidemiol. 44**,** 870-83.

Garey, L., 2010. When cortical development goes wrong: schizophrenia as a neurodevelopmental disease of microcircuits. Journal of Anatomy. 217**,** 324-33.

Garey, L. J., Ong, W. Y., Patel, T. S., Kanani, M., Davis, A., Mortimer, A. M., . . . Hirsch, S. R., 1998. Reduced dendritic spine density on cerebral cortical pyramidal neurons in schizophrenia. J Neurol Neurosurg Psychiatry. 65**,** 446-53.

Gaspar, L., van de Werken, M., Johansson, A. S., Moriggi, E., Owe-Larsson, B., Kocks, J. W., . . . Brown, S. A., 2014. Human cellular differences in cAMP--CREB signaling correlate with light-dependent melatonin suppression and bipolar disorder. Eur J Neurosci. 40**,** 2206-15.

Gaspar, P., Bloch, B., Le Moine, C., 1995. D1 and D2 receptor gene expression in the rat frontal cortex: cellular localization in different classes of efferent neurons. European Journal of Neuroscience. 7**,** 1050-63.

Gasque, P., Neal, J. W., Singhrao, S. K., McGreal, E. P., Dean, Y. D., Van, B. J., Morgan, B. P., 2002. Roles of the complement system in human neurodegenerative disorders: pro-inflammatory and tissue remodeling activities. Mol Neurobiol. 25**,** 1-17.

Georgieff, M. K., 2006. The effect of maternal diabetes during pregnancy on the neurodevelopment of offspring. Minn Med. 89**,** 44-7.

Gerfen, C. R., Surmeier, D. J., 2011. Modulation of striatal projection systems by dopamine. Annu Rev Neurosci. 34**,** 441-66.

Gertler, T. S., Chan, C. S., Surmeier, D. J., 2008. Dichotomous anatomical properties of adult striatal medium spiny neurons. J Neurosci. 28**,** 10814-24.

Ghisletti, S., Huang, W., Ogawa, S., Pascual, G., Lin, M. E., Willson, T. M., . . . Glass, C. K., 2007. Parallel SUMOylation-dependent pathways mediate gene- and signal-specific transrepression by LXRs and PPARgamma. Mol Cell. 25**,** 57-70.

Gilvarry, C. M., Sham, P. C., Jones, P. B., Cannon, M., Wright, P., Lewis, S. W., . . . Murray, R. M., 1996. Family history of autoimmune diseases in psychosis. Schizophr Res. 19**,** 33-40.

Giordano, G. N., Ohlsson, H., Sundquist, K., Sundquist, J., Kendler, K. S., 2015. The association between cannabis abuse and subsequent schizophrenia: a Swedish national co-relative control study. Psychol Med. 45**,** 407-14.

Giovanoli, S., Engler, H., Engler, A., Richetto, J., Feldon, J., Riva, M. A., . . . Meyer, U., 2016. Preventive effects of minocycline in a neurodevelopmental two-hit model with relevance to schizophrenia. Transl Psychiatry. 6**,** e772-.

Giovanoli, S., Engler, H., Engler, A., Richetto, J., Voget, M., Willi, R., . . . Meyer, U., 2013. Stress in puberty unmasks latent neuropathological consequences of prenatal immune activation in mice. Science. 339**,** 1095-9.

Glantz, L. A., Lewis, D. A., 2000. DEcreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. Archives of General Psychiatry. 57**,** 65-73.

Glausier, J. R., Lewis, D. A., 2013. Dendritic spine pathology in schizophrenia. Neuroscience. 251**,** 90-107.

Goines, P. E., Croen, L. A., Braunschweig, D., Yoshida, C. K., Grether, J., Hansen, R., . . . Van de Water, J., 2011. Increased midgestational IFN-γ, IL-4 and IL-5 in women bearing a child with autism: A case-control study. Molecular Autism. 2**,** 1-11.

Golalipour, M. J., Kafshgiri, S. K., Ghafari, S., 2012. Gestational diabetes induced neuronal loss in CA1 and CA3 subfields of rat hippocampus in early postnatal life. Folia Morphol (Warsz). 71**,** 71-7.

Golan, H. M., Lev, V., Hallak, M., Sorokin, Y., Huleihel, M., 2005. Specific neurodevelopmental damage in mice offspring following maternal inflammation during pregnancy. Neuropharmacology. 48.

Golson, M. L., Misfeldt, A. A., Kopsombut, U. G., Petersen, C. P., Gannon, M., 2010. High Fat Diet Regulation of beta-Cell Proliferation and beta-Cell Mass. Open Endocrinol J. 4.

Gómez-Ambrosi, J., Becerril, S., Oroz, P., Zabalza, S., Rodríguez, A., Muruzábal, F. J., . . . Frühbeck, G., 2004. Reduced adipose tissue mass and hypoleptinemia in iNOS deficient mice: effect of LPS on plasma leptin and adiponectin concentrations. FEBS Letters. 577**,** 351-356.

Gong, S., Zheng, C., Doughty, M. L., Losos, K., Didkovsky, N., Schambra, U. B., . . . Heintz, N., 2003. A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. Nature. 425**,** 917-25.

Gottesman, II, Erlenmeyer-Kimling, L., 2001. Family and twin strategies as a head start in defining prodromes and endophenotypes for hypothetical early-interventions in schizophrenia. Schizophr Res. 51**,** 93-102.

Graff, L. A., Walker, J. R., Bernstein, C. N., 2009. Depression and anxiety in inflammatory bowel disease: a review of comorbidity and management. Inflamm Bowel Dis. 15**,** 1105-18.

Graham, A., Allen, A. M., 2015. Mitochondrial function and regulation of macrophage sterol metabolism and inflammatory responses. World J Cardiol. 7**,** 277-86.

Graham, D. L., Durai, H. H., Garden, J. D., Cohen, E. L., Echevarria, F. D., Stanwood, G. D., 2014. Loss of Dopamine D2 Receptors Increases Parvalbumin-Positive Interneurons in the Anterior Cingulate Cortex. ACS Chem Neurosci.

Grant, W. B., Soles, C. M., 2009. Epidemiologic evidence supporting the role of maternal vitamin D deficiency as a risk factor for the development of infantile autism. Dermatoendocrinol. 1**,** 223-8.

Grech, A., Takei, N., Murray, R. M., 1997. Maternal exposure to influenza and paranoid schizophrenia. Schizophr Res. 26**,** 121-5.

Green, M. J., Chia, T. Y., Cairns, M. J., Wu, J., Tooney, P. A., Scott, R. J., Carr, V. J., 2014. Catechol-O-methyltransferase (COMT) genotype moderates the effects of childhood trauma on cognition and symptoms in schizophrenia. J Psychiatr Res. 49**,** 43-50.

Grivennikov, S., Karin, M., 2010. Dangerous liaisons: STAT3 and NF-κB collaboration and crosstalk in cancer. Cytokine Growth Factor Rev. 21**,** 11-9.

Grossman, R. M., Krueger, J., Yourish, D., Granelli-Piperno, A., Murphy, D. P., May, L. T., . . . Gottlieb, A. B., 1989. Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. Proceedings of the National Academy of Sciences. 86**,** 6367-6371.

Guan, Z., Buckman, S. Y., Pentland, A. P., Templeton, D. J., Morrison, A. R., 1998. Induction of cyclooxygenase-2 by the activated MEKK1 --> SEK1/MKK4 --> p38 mitogen-activated protein kinase pathway. J Biol Chem. 273**,** 12901-8.

Gue, M., Bravard, A., Meunier, J., Veyrier, R., Gaillet, S., Recasens, M., Maurice, T., 2004. Sex differences in learning deficits induced by prenatal stress in juvenile rats. Behav Brain Res. 150**,** 149-57.

Guerini, F. R., Bolognesi, E., Chiappedi, M., De Silvestri, A., Ghezzo, A., Zanette, M., . . . Clerici, M., 2011. HLA polymorphisms in Italian children with autism spectrum disorders: results of a family based linkage study. J Neuroimmunol. 230**,** 135-42.

Guest, C. B., Gao, Y. A. N., O'Connor, J. C., Freund, G. G., CHAPTER 46 - Obesity and Immunity A2 - Ader, Robert. Psychoneuroimmunology (Fourth Edition). Academic Press, Burlington, 2007, pp. 993-1011.

Gui, J., Mustachio, L. M., Su, D. M., Craig, R. W., 2012. Thymus Size and Age-related Thymic Involution: Early Programming, Sexual Dimorphism, Progenitors and Stroma. Aging Dis. 3**,** 280-90.

Guma, M., Ronacher, L., Liu-Bryan, R., Takai, S., Karin, M., Corr, M., 2009. Caspase 1–independent activation of interleukin-1β in neutrophil-predominant inflammation. Arthritis & Rheumatism. 60**,** 3642-3650.

Guo, J. Y., Huo, H. R., Yang, Y. X., Li, C. H., Liu, H. B., Zhao, B. S., . . . Jiang, T. L., 2006. 2-methoxycinnamaldehyde reduces IL-1beta-induced prostaglandin production in rat cerebral endothelial cells. Biol Pharm Bull. 29**,** 2214-21.

Gupta, S., Ellis, S. E., Ashar, F. N., Moes, A., Bader, J. S., Zhan, J., . . . Arking, D. E., 2014. Transcriptome analysis reveals dysregulation of innate immune response genes and neuronal activity-dependent genes in autism. Nat Commun. 5.

Haddad, R., Guimiot, F., Six, E., Jourquin, F., Setterblad, N., Kahn, E., . . . Canque, B., 2006. Dynamics of thymus-colonizing cells during human development. Immunity. 24**,** 217-30.

Hagberg, B., Kyllerman, M., 1983. Epidemiology of mental retardation--a Swedish survey. Brain Dev. 5**,** 441-9.

Hallquist, N. A., McNeil, L. K., Lockwood, J. F., Sherman, A. R., 1992. Maternal-iron-deficiency effects on peritoneal macrophage and peritoneal natural-killer-cell cytotoxicity in rat pups. Am J Clin Nutr. 55**,** 741-6.

Hamlyn, J., Duhig, M., McGrath, J., Scott, J., 2013. Modifiable risk factors for schizophrenia and autism — Shared risk factors impacting on brain development. Neurobiology of Disease. 53**,** 3-9.

Hanisch, A., Silljé, H. H., Nigg, E. A., 2006. Timely anaphase onset requires a novel spindle and kinetochore complex comprising Ska1 and Ska2. The EMBO Journal. 25**,** 5504-5515.

Hansen, M. K., Daniels, S., Goehler, L. E., Gaykema, R. P., Maier, S. F., Watkins, L. R., 2000a. Subdiaphragmatic vagotomy does not block intraperitoneal lipopolysaccharide-induced fever. Auton Neurosci. 85**,** 83-7.

Hansen, M. K., Nguyen, K. T., Fleshner, M., Goehler, L. E., Gaykema, R. P., Maier, S. F., Watkins, L. R., 2000b. Effects of vagotomy on serum endotoxin, cytokines, and corticosterone after intraperitoneal lipopolysaccharide. Am J Physiol Regul Integr Comp Physiol. 278**,** R331-6.

Harden, L. M., Kent, S., Pittman, Q. J., Roth, J., 2015. Fever and sickness behavior: Friend or foe? Brain Behav Immun. 50**,** 322-33.

Haroon, E., Raison, C. L., Miller, A. H., 2012. Psychoneuroimmunology Meets Neuropsychopharmacology: Translational Implications of the Impact of Inflammation on Behavior. Neuropsychopharmacology. 37**,** 137-162.

Harris, L. K., Smith, S. D., Keogh, R. J., Jones, R. L., Baker, P. N., Knofler, M., . . . Aplin, J. D., 2010. Trophoblast- and vascular smooth muscle cell-derived MMP-12 mediates elastolysis during uterine spiral artery remodeling. Am J Pathol. 177**,** 2103-15.

Hart, B. L., 1988. Biological basis of the behavior of sick animals. Neurosci Biobehav Rev. 12**,** 123-37.

Hashimoto, R., Ohi, K., Yasuda, Y., Fukumoto, M., Yamamori, H., Takahashi, H., . . . Takeda, M., 2011. Variants of the RELA gene are associated with schizophrenia and their startle responses. Neuropsychopharmacology. 36**,** 1921-31.

Hashimoto, T., Arion, D., Unger, T., Maldonado-Aviles, J. G., Morris, H. M., Volk, D. W., . . . Lewis, D. A., 2008. Alterations in GABA-related transcriptome in the dorsolateral prefrontal cortex of subjects with schizophrenia. Mol Psychiatry. 13, 147-61.

Hashimoto, T., Zhang, X. M., Chen, B. Y., Yang, X. J., 2006. VEGF activates divergent intracellular signaling components to regulate retinal progenitor cell proliferation and neuronal differentiation. Development. 133, 2201-10.

Hashizume, M., Hayakawa, N., Suzuki, M., Mihara, M., 2009. IL-6/sIL-6R trans-signalling, but not TNF-alpha induced angiogenesis in a HUVEC and synovial cell co-culture system. Rheumatol Int. 29, 1449-54.

Hayashi, R., Wada, H., Ito, K., Adcock, I. M., 2004. Effects of glucocorticoids on gene transcription. Eur J Pharmacol. 500, 51-62.

Hayashi, T., Rizzuto, R., Hajnoczky, G., Su, T. P., 2009. MAM: more than just a housekeeper. Trends Cell Biol. 19, 81-8.

Hayashi, Y., Okamoto, Y., Takagaki, K., Okada, G., Toki, S., Inoue, T., . . . Yamawaki, S., 2015. Direct and indirect influences of childhood abuse on depression symptoms in patients with major depressive disorder. BMC Psychiatry. 15, 244.

Hayes, L. N., Severance, E. G., Leek, J. T., Gressitt, K. L., Rohleder, C., Coughlin, J. M., . . . Sawa, A., 2014. Inflammatory molecular signature associated with infectious agents in psychosis. Schizophr Bull. 40, 963-72.

Hazan, A. D., Smith, S. D., Jones, R. L., Whittle, W., Lye, S. J., Dunk, C. E., 2010. Vascular-leukocyte interactions: mechanisms of human decidual spiral artery remodeling in vitro. Am J Pathol. 177, 1017-30.

Hein, A. M., Zarcone, T. J., Parfitt, D. B., Matousek, S. B., Carbonari, D. M., Olschowka, J. A., O'Banion, M. K., 2012. Behavioral, structural and molecular changes following long-term hippocampal IL-1β overexpression in transgenic mice. J Neuroimmune Pharmacol. 7, 145-55.

Heinrich, P. C., Behrmann, I., Haan, S., Hermanns, H. M., Muller-Newen, G., Schaper, F., 2003. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J. 374, 1-20.

Heintz, N., 2004. Gene expression nervous system atlas (GENSAT). Nat Neurosci. 7, 483.

Hendrickx, A. G., Sawyer, R. H., Terrell, T. G., Osburn, B. I., Henrickson, R. V., Steffek, A. J., 1975. Teratogenic effects of triamcinolone on the skeletal and lymphoid systems in nonhuman primates. Fed Proc. 34, 1661-7.

Henry, C., Kabbaj, M., Simon, H., Le Moal, M., Maccari, S., 1994. Prenatal Stress Increases the Hypothalamo-Pituitary-Adrenal Axis Response in Young and Adult Rats. Journal of Neuroendocrinology. 6, 341-345.

Heuer, L., Braunschweig, D., Ashwood, P., Van de Water, J., Campbell, D. B., 2011. Association of a MET genetic variant with autism-associated maternal autoantibodies to fetal brain proteins and cytokine expression. Transl Psychiatry. 1, e48.

Heupel, K., Sargsyan, V., Plomp, J. J., Rickmann, M., Varoqueaux, F., Zhang, W., Krieglstein, K., 2008. Loss of transforming growth factor-beta 2 leads to impairment of central synapse function. Neural Dev. 3, 25.

Heyser, C. J., Masliah, E., Samimi, A., Campbell, I. L., Gold, L. H., 1997. Progressive decline in avoidance learning paralleled by inflammatory neurodegeneration in transgenic mice expressing interleukin 6 in the brain. Proc Natl Acad Sci U S A. 94, 1500-5.

Hirahara, K., Nakayama, T., 2016. CD4+ T-cell subsets in inflammatory diseases: beyond the Th1/Th2 paradigm. Int Immunol. 28, 163-71.

Hirano, T., Yasukawa, K., Harada, H., Taga, T., Watanabe, Y., Matsuda, T., . . . et al., 1986. Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. Nature. 324, 73-6.

Hodes, G. E., Pfau, M. L., Leboeuf, M., Golden, S. A., Christoffel, D. J., Bregman, D., . . . Russo, S. J., 2014. Individual differences in the peripheral immune system promote resilience versus susceptibility to social stress. Proc Natl Acad Sci U S A. 111, 16136-41.

Hollenbach, E., Neumann, M., Vieth, M., Roessner, A., Malfertheiner, P., Naumann, M., 2004. Inhibition of p38 MAP kinase- and RICK/NF-κB-signaling suppresses inflammatory bowel disease. The FASEB Journal.

Hooper, L. V., Gordon, J. I., 2001. Commensal host-bacterial relationships in the gut. Science. 292, 1115-8.

Hopwood, N., Maswanganyi, T., Harden, L. M., 2009. Comparison of anorexia, lethargy, and fever induced by bacterial and viral mimetics in rats. Canadian Journal of Physiology and Pharmacology. 87, 211-220.

Horvath, S., Mirnics, K., 2014. Immune system disturbances in schizophrenia. Biol Psychiatry. 75, 316-23.

Hovens, J. G., Giltay, E. J., Spinhoven, P., van Hemert, A. M., Penninx, B. W., 2015. Impact of childhood life events and childhood trauma on the onset and recurrence of depressive and anxiety disorders. J Clin Psychiatry. 76, 931-8.

Howren, M. B., Lamkin, D. M., Suls, J., 2009. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. Psychosom Med. 71, 171-86.

Hsiao, E. Y., McBride, S. W., Chow, J., Mazmanian, S. K., Patterson, P. H., 2012. Modeling an autism risk factor in mice leads to permanent immune dysregulation. Proceedings of the National Academy of Sciences of the United States of America. 109, 12776-12781.

Hsiao, E. Y., Patterson, P. H., 2011. Activation of the maternal immune system induces endocrine changes in the placenta via IL-6. Brain, Behavior, and Immunity. 25, 604-615.

Hsiao, E. Y., Patterson, P. H., 2012. Placental regulation of maternal-fetal interactions and brain development. Developmental Neurobiology. 72, 1317-1326.

Hu, V. W., Frank, B. C., Heine, S., Lee, N. H., Quackenbush, J., 2006. Gene expression profiling of lymphoblastoid cell lines from monozygotic twins discordant in severity of autism reveals differential regulation of neurologically relevant genes. BMC Genomics. 7, 1-18.

Hu, V. W., Nguyen, A., Kim, K. S., Steinberg, M. E., Sarachana, T., Scully, M. A., . . . Lee, N. H., 2009. Gene Expression Profiling of Lymphoblasts from Autistic and Nonaffected Sib Pairs: Altered Pathways in Neuronal Development and Steroid Biosynthesis. PLoS ONE. 4, e5775.

Huang, C. C., Liang, Y. C., Lee, C. C., Hsu, K. S., 2015. Cocaine Withdrawal Impairs mGluR5-Dependent Long-Term Depression in Nucleus Accumbens Shell Neurons of Both Direct and Indirect Pathways. Mol Neurobiol. 52, 1223-33.

Huh, G. S., Boulanger, L. M., Du, H., Riquelme, P. A., Brotz, T. M., Shatz, C. J., 2000. Functional Requirement for Class I MHC in CNS Development and Plasticity. Science. 290, 2155-2159.

Huppertz, B., 2008. The anatomy of the normal placenta. J Clin Pathol. 61, 1296-302.

Huttunen, M. O., Niskanen, P., 1978. PRenatal loss of father and psychiatric disorders. Archives of General Psychiatry. 35, 429-431.

Ieraci, A., Forni, P. E., Ponzetto, C., 2002. Viable hypomorphic signaling mutant of the Met receptor reveals a role for hepatocyte growth factor in postnatal cerebellar development. Proc Natl Acad Sci U S A. 99, 15200-5.

Insel, T., Cuthbert, B., Garvey, M., Heinssen, R., Pine, D. S., Quinn, K., . . . Wang, P., 2010. Research domain criteria (RDoC): toward a new classification framework for research on mental disorders. Am J Psychiatry. 167, 748-51.

Ishibashi, T., Kimura, H., Shikama, Y., Uchida, T., Kariyone, S., Hirano, T., . . . Akiyama, Y., 1989. Interleukin-6 is a potent thrombopoietic factor in vivo in mice. Blood. 74, 1241-1244.

Jacobs, M. M., Fogg, R. L., Emeson, R. B., Stanwood, G. D., 2009. ADAR1 and ADAR2 expression and editing activity during forebrain development. Dev Neurosci. 31, 223-37.

Jaffee, S. R., Price, T. S., 2007. Gene-environment correlations: a review of the evidence and implications for prevention of mental illness. Mol Psychiatry. 12, 432-42.

Janeway, C. A., Jr., Medzhitov, R., 2002. Innate immune recognition. Annu Rev Immunol. 20, 197-216.

Jankowsky, J. L., Derrick, B. E., Patterson, P. H., 2000. Cytokine responses to LTP induction in the rat hippocampus: a comparison of in vitro and in vivo techniques. Learn Mem. 7, 400-12.

Jansen, R., Penninx, B. W. J. H., Madar, V., Xia, K., Milaneschi, Y., Hottenga, J. J., . . . Sullivan, P. F., 2016. Gene expression in major depressive disorder. Mol Psychiatry. 21, 339-347.

Jansson, N., Rosario, F. J., Gaccioli, F., Lager, S., Jones, H. N., Roos, S., . . . Powell, T. L., 2013. Activation of Placental mTOR Signaling and Amino Acid Transporters in Obese Women Giving Birth to Large Babies. The Journal of Clinical Endocrinology & Metabolism. 98, 105-113.

Jansson, T., Powell, T. L., 2007. Role of the placenta in fetal programming: underlying mechanisms and potential interventional approaches. Clin Sci (Lond). 113, 1-13.

Jansson, T., Wennergren, M., Powell, T. L., 1999. Placental glucose transport and GLUT 1 expression in insulin-dependent diabetes. American Journal of Obstetrics and Gynecology. 180, 163-168.

Jawerbaum, A., Gonzalez, E., 2006. Diabetic pregnancies: the challenge of developing in a pro-inflammatory environment. Current medicinal chemistry. 13, 2127-2138.

Jia, H., Morris, C. D., Williams, R. M., Loring, J. F., Thomas, E. A., 2015. HDAC inhibition imparts beneficial transgenerational effects in Huntington's disease mice via altered DNA and histone methylation. Proc Natl Acad Sci U S A. 112, E56-64.

Jia, N., Yang, K., Sun, Q., Cai, Q., Li, H., Cheng, D., . . . Zhu, Z., 2010. Prenatal stress causes dendritic atrophy of pyramidal neurons in hippocampal CA3 region by glutamate in offspring rats. Dev Neurobiol. 70, 114-25.

Jia, P., Kao, C. F., Kuo, P. H., Zhao, Z., 2011. A comprehensive network and pathway analysis of candidate genes in major depressive disorder. BMC Syst Biol. 5 Suppl 3, S12.

Jiang, B., Kumar, S. D., Loh, W. T., Manikandan, J., Ling, E. A., Tay, S. S., Dheen, S. T., 2008. Global gene expression analysis of cranial neural tubes in embryos of diabetic mice. J Neurosci Res. 86, 3481-93.

Jin, K., Zhu, Y., Sun, Y., Mao, X. O., Xie, L., Greenberg, D. A., 2002. Vascular endothelial growth factor (VEGF) stimulates neurogenesis in vitro and in vivo. Proc Natl Acad Sci U S A. 99, 11946-50.

Jin, K. L., Mao, X. O., Greenberg, D. A., 2000. Vascular endothelial growth factor: direct neuroprotective effect in in vitro ischemia. Proc Natl Acad Sci U S A. 97**,** 10242-7.

Jing, Y. H., Song, Y. F., Yao, Y. M., Yin, J., Wang, D. G., Gao, L. P., 2014. Retardation of fetal dendritic development induced by gestational hyperglycemia is associated with brain insulin/IGF-I signals. Int J Dev Neurosci. 37**,** 15-20.

Joachim, R., Zenclussen, A. C., Polgar, B., Douglas, A. J., Fest, S., Knackstedt, M., . . . Arck, P. C., 2003. The progesterone derivative dydrogesterone abrogates murine stress-triggered abortion by inducing a Th2 biased local immune response. Steroids. 68**,** 931-40.

Johe, K. K., Hazel, T. G., Muller, T., Dugich-Djordjevic, M. M., McKay, R. D., 1996. Single factors direct the differentiation of stem cells from the fetal and adult central nervous system. Genes Dev. 10**,** 3129-40.

Johnson, M. H., Jones, E. J. H., Gliga, T., 2015. Brain adaptation and alternative developmental trajectories. Development and Psychopathology. 27**,** 425-442.

Johnson, R. W., von Borell, E., 1994. Lipopolysaccharide-induced sickness behavior in pigs is inhibited by pretreatment with indomethacin. J Anim Sci. 72**,** 309-14.

Johnson, V. J., Yucesoy, B., Luster, M. I., 2005. Prevention of IL-1 signaling attenuates airway hyperresponsiveness and inflammation in a murine model of toluene diisocyanate-induced asthma. J Allergy Clin Immunol. 116**,** 851-8.

Jones, H. N., Woollett, L. A., Barbour, N., Prasad, P. D., Powell, T. L., Jansson, T., 2009. High-fat diet before and during pregnancy causes marked up-regulation of placental nutrient transport and fetal overgrowth in C57/BL6 mice. Faseb j. 23**,** 271-8.

Jones, P. B., Rantakallio, P., Hartikainen, A. L., Isohanni, M., Sipila, P., 1998. Schizophrenia as a long-term outcome of pregnancy, delivery, and perinatal complications: a 28-year follow-up of the 1966 north Finland general population birth cohort. Am J Psychiatry. 155**,** 355-64.

Joosten, L. A. B., Netea, M. G., Fantuzzi, G., Koenders, M. I., Helsen, M. M. A., Sparrer, H., . . . van den Berg, W. B., 2009. Inflammatory arthritis in caspase 1 gene–deficient mice: Contribution of proteinase 3 to caspase 1–independent production of bioactive interleukin-1β. Arthritis & Rheumatism. 60**,** 3651-3662.

Kagiwada, K., Chida, D., Sakatani, T., Asano, M., Nambu, A., Kakuta, S., Iwakura, Y., 2004. Interleukin (IL)-6, but not IL-1, induction in the brain downstream of cyclooxygenase-2 is essential for the induction of febrile response against peripheral IL-1alpha. Endocrinology. 145**,** 5044-8.

Kaiser, S., Sachser, N., 2005. The effects of prenatal social stress on behaviour: mechanisms and function. Neuroscience & Biobehavioral Reviews. 29**,** 283-294.

Kalman, S., Garbett, K. A., Vereczkei, A., Shelton, R. C., Korade, Z., Mirnics, K., 2014. Metabolic stress-induced microRNA and mRNA expression profiles of human fibroblasts. Exp Cell Res. 320**,** 343-53.

Kaneko, M., Stellwagen, D., Malenka, R. C., Stryker, M. P., 2008. Tumor Necrosis Factor-α Mediates One Component of Competitive, Experience-Dependent Plasticity in Developing Visual Cortex. Neuron. 58**,** 673-680.

Kang, H. J., Adams, D. H., Simen, A., Simen, B. B., Rajkowska, G., Stockmeier, C. A., . . . Duman, R. S., 2007. Gene expression profiling in postmortem prefrontal cortex of major depressive disorder. J Neurosci. 27**,** 13329-40.

Kang, S. S., Kurti, A., Fair, D. A., Fryer, J. D., 2014. Dietary intervention rescues maternal obesity induced behavior deficits and neuroinflammation in offspring. J Neuroinflammation. 11**,** 156.

Kao, C. F., Jia, P., Zhao, Z., Kuo, P. H., 2012. Enriched pathways for major depressive disorder identified from a genome-wide association study. Int J Neuropsychopharmacol. 15**,** 1401-11.

Katsuki, H., Nakai, S., Hirai, Y., Akaji, K., Kiso, Y., Satoh, M., 1990. Interleukin-1 beta inhibits long-term potentiation in the CA3 region of mouse hippocampal slices. Eur J Pharmacol. 181**,** 323-6.

Kautzky-Willer, A., Pacini, G., Tura, A., Bieglmayer, C., Schneider, B., Ludvik, B., . . . Waldhäusl, W., 2001. Increased plasma leptin in gestational diabetes. Diabetologia. 44**,** 164-172.

Kawai, M., Minabe, Y., Takagai, S., Ogai, M., Matsumoto, H., Mori, N., Takei, N., 2004. Poor maternal care and high maternal body mass index in pregnancy as a risk factor for schizophrenia in offspring. Acta Psychiatr Scand. 110**,** 257-63.

Kayser, M. S., Titulaer, M. J., Gresa-Arribas, N., Dalmau, J., 2013. Frequency and characteristics of isolated psychiatric episodes in anti-N-methyl-d-aspartate receptor encephalitis. JAMA Neurol. 70**,** 1133-9.

Keller, M. C., Simonson, M. A., Ripke, S., Neale, B. M., Gejman, P. V., Howrigan, D. P., . . . Sullivan, P. F., 2012. Runs of homozygosity implicate autozygosity as a schizophrenia risk factor. PLoS Genet. 8**,** e1002656.

Kendall, M. D., Johnson, H. R., Singh, J., 1980. The weight of the human thymus gland at necropsy. J Anat. 131**,** 483-97.

Kendell, R. E., Kemp, I. W., 1989. Maternal influenza in the etiology of schizophrenia. Arch Gen Psychiatry. 46**,** 878-82.

Kendler, K. S., Prescott, C. A., 1999. A population-based twin study of lifetime major depression in men and women. Archives of General Psychiatry. 56**,** 39-44.

Kent, A., Scorrer, T., Pollard, A. J., Snape, M. D., Clarke, P., Few, K., . . . Heath, P. T., 2016. Lymphocyte subpopulations in premature infants: an observational study. Arch Dis Child Fetal Neonatal Ed.

Kent, S., Bluthe, R. M., Dantzer, R., Hardwick, A. J., Kelley, K. W., Rothwell, N. J., Vannice, J. L., 1992. Different receptor mechanisms mediate the pyrogenic and behavioral effects of interleukin 1. Proc Natl Acad Sci U S A. 89**,** 9117-20.

Kerns, C. M., Newschaffer, C. J., Berkowitz, S. J., 2015. Traumatic Childhood Events and Autism Spectrum Disorder. J Autism Dev Disord. 45**,** 3475-86.

Kershaw, N. J., Murphy, J. M., Liau, N. P., Varghese, L. N., Laktyushin, A., Whitlock, E. L., . . . Babon, J. J., 2013. SOCS3 binds specific receptor-JAK complexes to control cytokine signaling by direct kinase inhibition. Nat Struct Mol Biol. 20**,** 469-76.

Kessler, R. C., Aguilar-Gaxiola, S., Alonso, J., Chatterji, S., Lee, S., Ormel, J., . . . Wang, P. S., 2009. The global burden of mental disorders: An update from the WHO World Mental Health (WMH) Surveys. Epidemiol Psichiatr Soc. 18**,** 23-33.

Khalil, O. S., Forrest, C. M., Pisar, M., Smith, R. A., Darlington, L. G., Stone, T. W., 2013. Prenatal activation of maternal TLR3 receptors by viral-mimetic poly(I:C) modifies GluN2B expression in embryos and sonic hedgehog in offspring in the absence of kynurenine pathway activation. Immunopharmacol Immunotoxicol. 35**,** 581-93.

Khan, D., Fernando, P., Cicvaric, A., Berger, A., Pollak, A., Monje, F. J., Pollak, D. D., 2014. Long-term effects of maternal immune activation on depression-like behavior in the mouse. Transl Psychiatry. 4**,** e363.

Kidd, P., 2003. Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. Altern Med Rev. 8**,** 223-46.

Kiecolt-Glaser, J. K., Derry, H. M., Fagundes, C. P., 2015. Inflammation: Depression Fans the Flames and Feasts on the Heat. American Journal of Psychiatry. 172**,** 1075-1091.

Kiecolt-Glaser, J. K., Gouin, J. P., Weng, N. P., Malarkey, W. B., Beversdorf, D. Q., Glaser, R., 2011. Childhood adversity heightens the impact of later-life caregiving stress on telomere length and inflammation. Psychosom Med. 73**,** 16-22.

Kievit, P., Howard, J. K., Badman, M. K., Balthasar, N., Coppari, R., Mori, H., . . . Flier, J. S., 2006. Enhanced leptin sensitivity and improved glucose homeostasis in mice lacking suppressor of cytokine signaling-3 in POMC-expressing cells. Cell Metab. 4**,** 123-32.

Kim, S. U., de Vellis, J., 2005. Microglia in health and disease. J Neurosci Res. 81**,** 302-13.

Kim, Y. S., Leventhal, B. L., 2015. Genetic Epidemiology and Insights into Interactive Genetic and Environmental Effects in Autism Spectrum Disorders. Biol Psychiatry. 77**,** 66-74.

Kimura, M., Toth, L. A., Agostini, H., Cady, A. B., Majde, J. A., Krueger, J. M., 1994. Comparison of acute phase responses induced in rabbits by lipopolysaccharide and double-stranded RNA. Am J Physiol. 267**,** R1596-605.

Kinalski, M., Telejko, B., Kuzmicki, M., Kretowski, A., Kinalska, I., 2005. Tumor necrosis factor alpha system and plasma adiponectin concentration in women with gestational diabetes. Horm Metab Res. 37**,** 450-4.

Kinney, D. K., Hintz, K., Shearer, E. M., Barch, D. H., Riffin, C., Whitley, K., Butler, R., 2010. A unifying hypothesis of schizophrenia: Abnormal immune system development may help explain roles of prenatal hazards, post-pubertal onset, stress, genes, climate, infections, and brain dysfunction. Medical Hypotheses. 74**,** 555-563.

Kinney, D. K., Miller, A. M., Crowley, D. J., Huang, E., Gerber, E., 2008. Autism prevalence following prenatal exposure to hurricanes and tropical storms in Louisiana. J Autism Dev Disord. 38**,** 481-8.

Kishimoto, T., 1985. Factors affecting B-cell growth and differentiation. Annu Rev Immunol. 3**,** 133-57.

Kishimoto, T., 1989. The biology of interleukin-6. Blood. 74**,** 1-10.

Kishimoto, T., Akira, S., Narazaki, M., Taga, T., 1995. Interleukin-6 family of cytokines and gp130. Blood. 86**,** 1243-54.

Klengel, T., Binder, E. B., 2015. Epigenetics of Stress-Related Psychiatric Disorders and Gene x Environment Interactions. Neuron. 86**,** 1343-57.

Kneeland, R. E., Fatemi, S. H., 2013. Viral infection, inflammation and schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry. 42**,** 35-48.

Knud Larsen, J., Bendsen, B. B., Foldager, L., Munk-Jorgensen, P., 2010. Prematurity and low birth weight as risk factors for the development of affective disorder, especially depression and schizophrenia: a register study. Acta Neuropsychiatr. 22**,** 284-91.

Koblar, S. A., Turnley, A. M., Classon, B. J., Reid, K. L., Ware, C. B., Cheema, S. S., . . . Bartlett, P. F., 1998. Neural precursor differentiation into astrocytes requires signaling through the leukemia inhibitory factor receptor. Proc Natl Acad Sci U S A. 95**,** 3178-81.

Kochanowski, B. A., Sherman, A. R., 1985. Decreased antibody formation in iron-deficient rat pups--effect of iron repletion. Am J Clin Nutr. 41**,** 278-84.

Koenig, J. I., Elmer, G. I., Shepard, P. D., Lee, P. R., Mayo, C., Joy, B., . . . Brady, D. L., 2005. Prenatal exposure to a repeated variable stress paradigm elicits behavioral and neuroendocrinological changes in the adult offspring: potential relevance to schizophrenia. Behav Brain Res. 156**,** 251-61.

Koido, K., Eller, T., Kingo, K., Koks, S., Traks, T., Shlik, J., . . . Maron, E., 2010. Interleukin 10 family gene polymorphisms are not associated with major depressive disorder and panic disorder phenotypes. J Psychiatr Res. 44**,** 275-7.

Konsman, J. P., Parnet, P., Dantzer, R., 2002. Cytokine-induced sickness behaviour: mechanisms and implications. Trends Neurosci. 25**,** 154-9.

Korn, T., Bettelli, E., Oukka, M., Kuchroo, V. K., 2009. IL-17 and Th17 Cells. Annu Rev Immunol. 27**,** 485-517.

Kozak, M. J., Cuthbert, B. N., 2016. The NIMH Research Domain Criteria Initiative: Background, Issues, and Pragmatics. Psychophysiology. 53**,** 286-97.

Kramina, S., Kevere, L., Bezborodovs, N., Purvina, S., Rozentals, G., Strautmanis, J., Viksna, Z., 2015. Acute psychosis due to non-paraneoplastic anti-NMDA-receptor encephalitis in a teenage girl: Case report. Psych J. 4**,** 226-30.

Krause, D., Weidinger, E., Dippel, C., Riedel, M., Schwarz, M. J., Muller, N., Myint, A. M., 2013. Impact of different antipsychotics on cytokines and tryptophan metabolites in stimulated cultures from patients with schizophrenia. Psychiatr Danub. 25**,** 389-97.

Krause, D. L., Riedel, M., Muller, N., Weidinger, E., Schwarz, M. J., Myint, A. M., 2012. Effects of antidepressants and cyclooxygenase-2 inhibitor on cytokines and kynurenines in stimulated in vitro blood culture from depressed patients. Inflammopharmacology. 20**,** 169-76.

Kreitzer, A. C., Malenka, R. C., 2007. Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. Nature. 445**,** 643-7.

Kreitzer, A. C., Malenka, R. C., 2008. Striatal plasticity and basal ganglia circuit function. Neuron. 60**,** 543-54.

Krishnadas, R., Mallon, V., McInnes, I., Cavanagh, J., 2011. P01-380 - Correlates of depression and quality of life in patients with inflammatory arthritides. European Psychiatry. 26, Supplement 1**,** 383.

Krishnan, R., Cella, D., Leonardi, C., Papp, K., Gottlieb, A. B., Dunn, M., . . . Jahreis, A., 2007. Effects of etanercept therapy on fatigue and symptoms of depression in subjects treated for moderate to severe plaque psoriasis for up to 96 weeks. Br J Dermatol. 157**,** 1275-7.

Kudinova, A. Y., Deak, T., Hueston, C. M., McGeary, J. E., Knopik, V. S., Palmer, R. H., Gibb, B. E., 2016. Cross-species evidence for the role of interleukin-33 in depression risk. J Abnorm Psychol. 125**,** 482-94.

Kumari, D., Bhattacharya, A., Nadel, J., Moulton, K., Zeak, N. M., Glicksman, A., . . . Usdin, K., 2014. Identification of fragile X syndrome specific molecular markers in human fibroblasts: a useful model to test the efficacy of therapeutic drugs. Hum Mutat. 35**,** 1485-94.

Kunugi, H., Nanko, S., Takei, N., Saito, K., Hayashi, N., Kazamatsuri, H., 1995. Schizophrenia following in utero exposure to the 1957 influenza epidemics in Japan. Am J Psychiatry. 152**,** 450-2.

Kurian, S. M., Le-Niculescu, H., Patel, S. D., Bertram, D., Davis, J., Dike, C., . . . Niculescu, A. B., 2011. Identification of blood biomarkers for psychosis using convergent functional genomics. Mol Psychiatry. 16**,** 37-58.

La Rocca, C., Carbone, F., Longobardi, S., Matarese, G., 2014. The immunology of pregnancy: regulatory T cells control maternal immune tolerance toward the fetus. Immunol Lett. 162, 41-8.

LaFerla, F. M., Sugarman, M. C., Lane, T. E., Leissring, M. A., 2000. Regional hypomyelination and dysplasia in transgenic mice with astrocyte-directed expression of interferon-gamma. J Mol Neurosci. 15, 45-59.

Lalovic, A., Klempan, T., Sequeira, A., Luheshi, G., Turecki, G., 2010. Altered expression of lipid metabolism and immune response genes in the frontal cortex of suicide completers. J Affect Disord. 120, 24-31.

Langridge, A. T., Glasson, E. J., Nassar, N., Jacoby, P., Pennell, C., Hagan, R., . . . Stanley, F. J., 2013. Maternal conditions and perinatal characteristics associated with autism spectrum disorder and intellectual disability. PLoS One. 8, e50963.

Las, G., Shirihai, O. S., 2014. Miro1: new wheels for transferring mitochondria. Embo j. 33, 939-41.

Lawlor, K. E., Vince, J. E., 2014. Ambiguities in NLRP3 inflammasome regulation: is there a role for mitochondria? Biochim Biophys Acta. 1840, 1433-40.

Lazarini, F., Tham, T. N., Casanova, P., Arenzana-Seisdedos, F., Dubois-Dalcq, M., 2003. Role of the alpha-chemokine stromal cell-derived factor (SDF-1) in the developing and mature central nervous system. Glia. 42, 139-48.

Lazarus, M., Saper, C. B., CHAPTER 15 - The Differential Role of Prostaglandin E2 Receptors in the CNS Response to Systemic Immune Challenge A2 - Ader, Robert. Psychoneuroimmunology (Fourth Edition). Academic Press, Burlington, 2007, pp. 319-336.

Le Moine, C., Gaspar, P., 1998. Subpopulations of cortical GABAergic interneurons differ by their expression of D1 and D2 dopamine receptor subtypes. Brain Research. Molecular Brain Research. 58, 231-6.

Leboyer, M., Oliveira, J., Tamouza, R., Groc, L., 2016. Is it time for immunopsychiatry in psychotic disorders? Psychopharmacology (Berl). 233, 1651-60.

Lee, B. K., Magnusson, C., Gardner, R. M., Blomstrom, A., Newschaffer, C. J., Burstyn, I., . . . Dalman, C., 2015. Maternal hospitalization with infection during pregnancy and risk of autism spectrum disorders. Brain Behav Immun. 44, 100-5.

Lee, L. C., Zachary, A. A., Leffell, M. S., Newschaffer, C. J., Matteson, K. J., Tyler, J. D., Zimmerman, A. W., 2006. HLA-DR4 in families with autism. Pediatr Neurol. 35, 303-7.

Lee, R. H., Mills, E. A., Schwartz, N., Bell, M. R., Deeg, K. E., Ruthazer, E. S., . . . Aizenman, C. D., 2010a. Neurodevelopmental effects of chronic exposure to elevated levels of pro-inflammatory cytokines in a developing visual system. Neural Dev. 5, 2.

Lee, S., Hjerling-Leffler, J., Zagha, E., Fishell, G., Rudy, B., 2010b. The largest group of superficial neocortical GABAergic interneurons expresses ionotropic serotonin receptors. J Neurosci. 30, 16796-808.

Lee, S. H., Ripke, S., Neale, B. M., Faraone, S. V., Purcell, S. M., Perlis, R. H., . . . Wray, N. R., 2013. Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. Nat Genet. 45, 984-94.

Lehmann, J., Pryce, C. R., Feldon, J., 2000. Lack of effect of an early stressful life event on sensorimotor gating in adult rats. Schizophr Res. 41, 365-71.

Lemaire, V., Koehl, M., Le Moal, M., Abrous, D. N., 2000. Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. Proc Natl Acad Sci U S A. 97, 11032-7.

LeMay, L. G., Vander, A. J., Kluger, M. J., 1990. The effects of psychological stress on plasma interleukin-6 activity in rats. Physiology & Behavior. 47, 957-961.

Lencz, T., Lambert, C., DeRosse, P., Burdick, K. E., Morgan, T. V., Kane, J. M., . . . Malhotra, A. K., 2007. Runs of homozygosity reveal highly penetrant recessive loci in schizophrenia. Proc Natl Acad Sci U S A. 104, 19942-7.

Lenczowski, M. J., Bluthe, R. M., Roth, J., Rees, G. S., Rushforth, D. A., van Dam, A. M., . . . Luheshi, G. N., 1999. Central administration of rat IL-6 induces HPA activation and fever but not sickness behavior in rats. Am J Physiol. 276, R652-8.

Levy, D. E., Darnell, J. E., Jr., 2002. Stats: transcriptional control and biological impact. Nat Rev Mol Cell Biol. 3, 651-62.

Li, H. P., Chen, X., Li, M. Q., 2013. Gestational diabetes induces chronic hypoxia stress and excessive inflammatory response in murine placenta. Int J Clin Exp Pathol. 6, 650-9.

Li, J., Vestergaard, M., Obel, C., Christensen, J., Precht, D. H., Lu, M., Olsen, J., 2009. A nationwide study on the risk of autism after prenatal stress exposure to maternal bereavement. Pediatrics. 123, 1102-7.

Li, S., Goorha, S., Ballou, L. R., Blatteis, C. M., 2003a. Intracerebroventricular interleukin-6, macrophage inflammatory protein-1 beta and IL-18: pyrogenic and PGE(2)-mediated? Brain Res. 992, 76-84.

Li, W., Knowlton, D., Woodward, W. R., Habecker, B. A., 2003b. Regulation of noradrenergic function by inflammatory cytokines and depolarization. J Neurochem. 86, 774-83.

Lian, Q., Dheen, S. T., Liao, D., Tay, S. S., 2004. Enhanced inflammatory response in neural tubes of embryos derived from diabetic mice exposed to a teratogen. J Neurosci Res. 75, 554-64.

Liang, C., DeCourcy, K., Prater, M. R., 2010. High-saturated-fat diet induces gestational diabetes and placental vasculopathy in C57BL/6 mice. Metabolism. 59, 943-50.

Liang, C., Oest, M. E., Prater, M. R., 2009. Intrauterine exposure to high saturated fat diet elevates risk of adult-onset chronic diseases in C57BL/6 mice. Birth Defects Res B Dev Reprod Toxicol. 86, 377-84.

Liao, D. M., Ng, Y. K., Tay, S. S., Ling, E. A., Dheen, S. T., 2004. Altered gene expression with abnormal patterning of the telencephalon in embryos of diabetic Albino Swiss mice. Diabetologia. 47, 523-31.

Libbey, J. E., Sweeten, T. L., McMahon, W. M., Fujinami, R. S., 2005. Autistic disorder and viral infections. J Neurovirol. 11, 1-10.

Lichtenstein, P., Carlstrom, E., Rastam, M., Gillberg, C., Anckarsater, H., 2010. The genetics of autism spectrum disorders and related neuropsychiatric disorders in childhood. Am J Psychiatry. 167, 1357-63.

Liden, J., Rafter, I., Truss, M., Gustafsson, J. A., Okret, S., 2000. Glucocorticoid effects on NF-kappaB binding in the transcription of the ICAM-1 gene. Biochem Biophys Res Commun. 273, 1008-14.

Light, G., Greenwood, T. A., Swerdlow, N. R., Calkins, M. E., Freedman, R., Green, M. F., . . . Braff, D. L., 2014. Comparison of the heritability of schizophrenia and endophenotypes in the COGS-1 family study. Schizophr Bull. 40, 1404-11.

Limosin, F., Rouillon, F., Payan, C., Cohen, J. M., Strub, N., 2003. Prenatal exposure to influenza as a risk factor for adult schizophrenia. Acta Psychiatr Scand. 107**,** 331-5.

Lin, Y. L., Wang, S., 2014. Prenatal lipopolysaccharide exposure increases depression-like behaviors and reduces hippocampal neurogenesis in adult rats. Behav Brain Res. 259**,** 24-34.

Lisi, L., Camardese, G., Treglia, M., Tringali, G., Carrozza, C., Janiri, L., . . . Navarra, P., 2013. Monocytes from depressed patients display an altered pattern of response to endotoxin challenge. PLoS One. 8**,** e52585.

Liu, D., Ellis, H., 2016. The Mystery of the Thymus Gland. Clin Anat.

Liu, F., Xia, Y., Parker, A. S., Verma, I. M., 2012. IKK Biology. Immunol Rev. 246**,** 239-53.

Liu, J., Shen, Y., Li, M., Shi, Q., Zhang, A., Miao, F., . . . Zhang, J., 2013. The expression pattern of classical MHC class I molecules in the development of mouse central nervous system. Neurochem Res. 38**,** 290-9.

Liu, S. Y., Sanchez, D. J., Cheng, G., 2011a. New developments in the induction and antiviral effectors of type I interferon. Curr Opin Immunol. 23**,** 57-64.

Liu, Y., Wang, L., Kikuiri, T., Akiyama, K., Chen, C., Xu, X., . . . Shi, S., 2011b. Mesenchymal stem cell-based tissue regeneration is governed by recipient T lymphocytes via IFN-[gamma] and TNF-[alpha]. Nat Med. 17**,** 1594-1601.

Liuzzi, J. P., Lichten, L. A., Rivera, S., Blanchard, R. K., Aydemir, T. B., Knutson, M. D., . . . Cousins, R. J., 2005. Interleukin-6 regulates the zinc transporter Zip14 in liver and contributes to the hypozincemia of the acute-phase response. Proceedings of the National Academy of Sciences of the United States of America. 102**,** 6843-6848.

Lo Fermo, S., Barone, R., Patti, F., Laisa, P., Cavallaro, T. L., Nicoletti, A., Zappia, M., 2010. Outcome of psychiatric symptoms presenting at onset of multiple sclerosis: a retrospective study. Mult Scler. 16**,** 742-8.

Loibichler, C., Pichler, J., Gerstmayr, M., Bohle, B., Kisst, H., Urbanek, R., Szepfalusi, Z., 2002. Materno-fetal passage of nutritive and inhalant allergens across placentas of term and pre-term deliveries perfused in vitro. Clin Exp Allergy. 32**,** 1546-51.

López-Soldado, I., Herrera, E., 2003. Different diabetogenic response to moderate doses of streptozotocin in pregnant rats, and its long-term consequences in the offspring. Exp Diabesity Res. 4**,** 107-18.

Louis, J. C., Magal, E., Takayama, S., Varon, S., 1993. CNTF protection of oligodendrocytes against natural and tumor necrosis factor-induced death. Science. 259**,** 689-92.

Lu, J., Wu, Y., Sousa, N., Almeida, O. F., 2005. SMAD pathway mediation of BDNF and TGF beta 2 regulation of proliferation and differentiation of hippocampal granule neurons. Development. 132**,** 3231-42.

Lu, J., Zhang, Y. H., Chou, T. C., Gaus, S. E., Elmquist, J. K., Shiromani, P., Saper, C. B., 2001. Contrasting effects of ibotenate lesions of the paraventricular nucleus and subparaventricular zone on sleep-wake cycle and temperature regulation. J Neurosci. 21**,** 4864-74.

Lu, S., Peng, H., Wang, L., Vasish, S., Zhang, Y., Gao, W., . . . Li, L., 2013. Elevated specific peripheral cytokines found in major depressive disorder patients with childhood trauma exposure: a cytokine antibody array analysis. Compr Psychiatry. 54**,** 953-61.

Lu, X. Y., Ghasemzadeh, M. B., Kalivas, P. W., 1998. Expression of D1 receptor, D2 receptor, substance P and enkephalin messenger RNAs in the neurons projecting from the nucleus accumbens. Neuroscience. 82**,** 767-80.

Lubach, G. R., Coe, C. L., 2006. Preconception maternal iron status is a risk factor for iron deficiency in infant rhesus monkeys (Macaca mulatta). J Nutr. 136, 2345-9.

Lukic, I., Mitic, M., Djordjevic, J., Tatalovic, N., Bozovic, N., Soldatovic, I., . . . Adzic, M., 2014. Lymphocyte levels of redox-sensitive transcription factors and antioxidative enzymes as indicators of pro-oxidative state in depressive patients. Neuropsychobiology. 70, 1-9.

Luo, Z., Volkow, N. D., Heintz, N., Pan, Y., Du, C., 2011. Acute cocaine induces fast activation of D1 receptor and progressive deactivation of D2 receptor striatal neurons: in vivo optical microprobe [Ca2+]i imaging. J Neurosci. 31, 13180-90.

Ma, D., Salyakina, D., Jaworski, J. M., Konidari, I., Whitehead, P. L., Andersen, A. N., . . . Pericak-Vance, M. A., 2009. A genome-wide association study of autism reveals a common novel risk locus at 5p14.1. Ann Hum Genet. 73, 263-73.

Machón, R. A., Mednick, S. A., Huttunen, M. O., 1997. ADult major affective disorder after prenatal exposure to an influenza epidemic. Archives of General Psychiatry. 54, 322-328.

Mackall, C. L., Gress, R. E., 1997. Thymic aging and T-cell regeneration. Immunol Rev. 160, 91-102.

MacSweeney, D., Timms, P., Johnson, A., 1978. Thryo-endocrine pathology, obstetric morbidity and schizophrenia: survey of a hundred families with a schizophrenic proband. Psychol Med. 8, 151-5.

Madden, C. J., Morrison, S. F., 2003. Excitatory amino acid receptor activation in the raphe pallidus area mediates prostaglandin-evoked thermogenesis. Neuroscience. 122, 5-15.

Madden, C. J., Morrison, S. F., 2004. Excitatory amino acid receptors in the dorsomedial hypothalamus mediate prostaglandin-evoked thermogenesis in brown adipose tissue. Am J Physiol Regul Integr Comp Physiol. 286, R320-5.

Maes, M., 1995. Evidence for an immune response in major depression: a review and hypothesis. Prog Neuropsychopharmacol Biol Psychiatry. 19, 11-38.

Maes, M., Bosmans, E., De Jongh, R., Kenis, G., Vandoolaeghe, E., Neels, H., 1997. Increased serum IL-6 and IL-1 receptor antagonist concentrations in major depression and treatment resistant depression. Cytokine. 9, 853-8.

Maes, M., Meltzer, H. Y., Bosmans, E., Bergmans, R., Vandoolaeghe, E., Ranjan, R., Desnyder, R., 1995. Increased plasma concentrations of interleukin-6, soluble interleukin-6, soluble interleukin-2 and transferrin receptor in major depression. J Affect Disord. 34, 301-9.

Maes, M., Scharpe, S., Meltzer, H. Y., Bosmans, E., Suy, E., Calabrese, J., Cosyns, P., 1993. Relationships between interleukin-6 activity, acute phase proteins, and function of the hypothalamic-pituitary-adrenal axis in severe depression. Psychiatry Res. 49, 11-27.

Maes, M., Yirmyia, R., Noraberg, J., Brene, S., Hibbeln, J., Perini, G., . . . Maj, M., 2009. The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. Metab Brain Dis. 24, 27-53.

Maftei, O., Whitrow, M. J., Davies, M. J., Giles, L. C., Owens, J. A., Moore, V. M., 2015. Maternal body size prior to pregnancy, gestational diabetes and weight gain: associations with insulin resistance in children at 9-10 years. Diabet Med. 32, 174-80.

Maier, S. F., Goehler, L. E., Fleshner, M., Watkins, L. R., 1998. The role of the vagus nerve in cytokine-to-brain communication. Ann N Y Acad Sci. 840, 289-300.

Major Depressive Disorder Working Group of the Psychiatric, G. C., 2013. A mega-analysis of genome-wide association studies for major depressive disorder. Mol Psychiatry. 18, 497-511.

Makhseed, M., Raghupathy, R., Azizieh, F., Omu, A., Al-Shamali, E., Ashkanani, L., 2001. Th1 and Th2 cytokine profiles in recurrent aborters with successful pregnancy and with subsequent abortions. Hum Reprod. 16**,** 2219-26.

Makinodan, M., Tatsumi, K., Manabe, T., Yamauchi, T., Makinodan, E., Matsuyoshi, H., . . . Wanaka, A., 2008. Maternal immune activation in mice delays myelination and axonal development in the hippocampus of the offspring. J Neurosci Res. 86**,** 2190-200.

Malkova, N. V., Yu, C. Z., Hsiao, E. Y., Moore, M. J., Patterson, P. H., 2012. Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism. Brain, Behavior, and Immunity. 26**,** 607-616.

Mandal, M., Donnelly, R., Elkabes, S., Zhang, P., Davini, D., David, B. T., Ponzio, N. M., 2013. Maternal immune stimulation during pregnancy shapes the immunological phenotype of offspring. Brain, Behavior, and Immunity. 33**,** 33-45.

Manier, D. H., Shelton, R. C., Ellis, T. C., Peterson, C. S., Eiring, A., Sulser, F., 2000. Human fibroblasts as a relevant model to study signal transduction in affective disorders. J Affect Disord. 61**,** 51-8.

Manuvakhova, M. S., Johnson, G. G., White, M. C., Ananthan, S., Sosa, M., Maddox, C., . . . Grimaldi, M., 2011. Identification of novel small molecule activators of nuclear factor-kappaB with neuroprotective action via high-throughput screening. J Neurosci Res. 89**,** 58-72.

Marin-Teva, J. L., Dusart, I., Colin, C., Gervais, A., van Rooijen, N., Mallat, M., 2004. Microglia promote the death of developing Purkinje cells. Neuron. 41**,** 535-47.

Marin, O., 2013. Cellular and molecular mechanisms controlling the migration of neocortical interneurons. European Journal of Neuroscience. 38**,** 2019-29.

Markham, J. A., Koenig, J. I., 2011. Prenatal stress: Role in psychotic and depressive diseases. Psychopharmacology (Berl). 214**,** 89-106.

Markham, J. A., Taylor, A. R., Taylor, S. B., Bell, D. B., Koenig, J. I., 2010. Characterization of the cognitive impairments induced by prenatal exposure to stress in the rat. Front Behav Neurosci. 4**,** 173.

Martin, K. E., Grivell, R. M., Yelland, L. N., Dodd, J. M., 2015. The influence of maternal BMI and gestational diabetes on pregnancy outcome. Diabetes Res Clin Pract. 108**,** 508-13.

Martin, P., Palmer, G., Vigne, S., Lamacchia, C., Rodriguez, E., Talabot-Ayer, D., . . . Gabay, C., 2013. Mouse neutrophils express the decoy type 2 interleukin-1 receptor (IL-1R2) constitutively and in acute inflammatory conditions. J Leukoc Biol. 94**,** 791-802.

Martino, J., Sebert, S., Segura, M. T., García-Valdés, L., Florido, J., Padilla, M. C., . . . Campoy, C., 2016. Maternal Body Weight and Gestational Diabetes Differentially Influence Placental and Pregnancy Outcomes. The Journal of Clinical Endocrinology & Metabolism. 101**,** 59-68.

Mathiasen, R., Hansen, B. M., Forman, J. L., Kessing, L. V., Greisen, G., 2011. The risk of psychiatric disorders in individuals born prematurely in Denmark from 1974 to 1996. Acta Paediatr. 100**,** 691-9.

Matousek, S. B., Hein, A. M., Shaftel, S. S., Olschowka, J. A., Kyrkanides, S., O'Banion, M. K., 2010. Cyclooxygenase-1 mediates prostaglandin E(2) elevation and contextual memory impairment in a model of sustained hippocampal interleukin-1beta expression. J Neurochem. 114**,** 247-58.

Matson, B. C., Caron, K. M., 2014. Uterine natural killer cells as modulators of the maternal-fetal vasculature. Int J Dev Biol. 58**,** 199-204.

Matsuzaki, H., Tamatani, M., Yamaguchi, A., Namikawa, K., Kiyama, H., Vitek, M. P., . . . Tohyama, M., 2001. Vascular endothelial growth factor rescues hippocampal neurons from glutamate-induced toxicity: signal transduction cascades. Faseb j. 15**,** 1218-20.

McCarthy, M. J., Wei, H., Marnoy, Z., Darvish, R. M., McPhie, D. L., Cohen, B. M., Welsh, D. K., 2013. Genetic and clinical factors predict lithium's effects on PER2 gene expression rhythms in cells from bipolar disorder patients. Transl Psychiatry. 3**,** e318.

McGrath, J., Eyles, D., Mowry, B., Yolken, R., Buka, S., 2003. Low maternal vitamin D as a risk factor for schizophrenia: a pilot study using banked sera. Schizophr Res. 63**,** 73-8.

McGrath, J. E., Tschan, F., The complex nature of time. Temporal matters in social psychology: Examining the role of time in the lives of groups and individuals. American Psychological Association, Washington, DC, US, 2004, pp. 13-25.

McGrath, J. J., Eyles, D. W., Pedersen, C. B., Anderson, C., Ko, P., Burne, T. H., . . . Mortensen, P. B., 2010. Neonatal vitamin D status and risk of schizophrenia: a population-based case-control study. Arch Gen Psychiatry. 67**,** 889-94.

McGue, M., Gottesman, II, 1991. The genetic epidemiology of schizophrenia and the design of linkage studies. Eur Arch Psychiatry Clin Neurosci. 240**,** 174-81.

McGuffin, P., Katz, R., Watkins, S., Rutherford, J., 1996. A hospital-based twin register of the heritability of DSM-IV unipolar depression. Arch Gen Psychiatry. 53**,** 129-36.

McKinsey, T. A., Zhang, C. L., Olson, E. N., 2002. MEF2: a calcium-dependent regulator of cell division, differentiation and death. Trends in Biochemical Sciences. 27**,** 40-47.

McMahan, C. J., Slack, J. L., Mosley, B., Cosman, D., Lupton, S. D., Brunton, L. L., . . . et al., 1991. A novel IL-1 receptor, cloned from B cells by mammalian expression, is expressed in many cell types. Embo j. 10**,** 2821-32.

McOmish, C. E., Burrows, E. L., Hannan, A. J., 2014. Identifying novel interventional strategies for psychiatric disorders: integrating genomics, 'enviromics' and gene-environment interactions in valid preclinical models. Br J Pharmacol. 171**,** 4719-28.

Medawar, P. B., Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. Symp Soc Exp Biol, Vol. 7, 1953, pp. 38.

Mednick, S. A., Machon, R. A., Huttunen, M. O., Bonett, D., 1988. Adult schizophrenia following prenatal exposure to an influenza epidemic. Archives of General Psychiatry. 45**,** 189-192.

Mellado-Gil, J., Rosa, T. C., Demirci, C., Gonzalez-Pertusa, J. A., Velazquez-Garcia, S., Ernst, S., . . . Garcia-Ocaña, A., 2011. Disruption of Hepatocyte Growth Factor/c-Met Signaling Enhances Pancreatic β-Cell Death and Accelerates the Onset of Diabetes. Diabetes. 60**,** 525-536.

Melo, A. M., Benatti, R. O., Ignacio-Souza, L. M., Okino, C., Torsoni, A. S., Milanski, M., . . . Torsoni, M. A., 2014. Hypothalamic endoplasmic reticulum stress and insulin resistance in offspring of mice dams fed high-fat diet during pregnancy and lactation. Metabolism. 63**,** 682-92.

Meredith, R. M., 2015. Sensitive and critical periods during neurotypical and aberrant neurodevelopment: a framework for neurodevelopmental disorders. Neurosci Biobehav Rev. 50**,** 180-8.

Merlot, E., Couret, D., Otten, W., 2008. Prenatal stress, fetal imprinting and immunity. Brain, Behavior, and Immunity. 22**,** 42-51.

Metin, C., Vallee, R. B., Rakic, P., Bhide, P. G., 2008. Modes and mishaps of neuronal migration in the mammalian brain. Journal of Neuroscience. 28**,** 11746-52.

Meyer, H. M., Jr., 1969. The control of rubella and other virus infections in the prevention of mental retardation. Ment Retard. 7**,** 17-8.

Meyer, U., 2014. Prenatal Poly(I:C) Exposure and Other Developmental Immune Activation Models in Rodent Systems. Biological Psychiatry. 75**,** 307-315.

Meyer, U., Engler, A., Weber, L., Schedlowski, M., Feldon, J., 2008. Preliminary evidence for a modulation of fetal dopaminergic development by maternal immune activation during pregnancy. Neuroscience. 154**,** 701-9.

Meyer, U., Feldon, J., 2012. To poly(I:C) or not to poly(I:C): advancing preclinical schizophrenia research through the use of prenatal immune activation models. Neuropharmacology. 62**,** 1308-21.

Meyer, U., Feldon, J., Schedlowski, M., Yee, B. K., 2006. Immunological stress at the maternal-foetal interface: a link between neurodevelopment and adult psychopathology. Brain Behav Immun. 20**,** 378-88.

Meyer, U., Yee, B. K., Feldon, J., 2007. The neurodevelopmental impact of prenatal infections at different times of pregnancy: the earlier the worse? Neuroscientist. 13.

Michaelson, M. D., Mehler, M. F., Xu, H., Gross, R. E., Kessler, J. A., 1996. Interleukin-7 is trophic for embryonic neurons and is expressed in developing brain. Dev Biol. 179**,** 251-63.

Michel, M., Schmidt, M. J., Mirnics, K., 2012. Immune system gene dysregulation in autism and schizophrenia. Developmental Neurobiology. 72**,** 1277-1287.

Millan, M. J., Andrieux, A., Bartzokis, G., Cadenhead, K., Dazzan, P., Fusar-Poli, P., . . . Weinberger, D., 2016. Altering the course of schizophrenia: progress and perspectives. Nat Rev Drug Discov. advance online publication.

Miller, B. J., Buckley, P., Seabolt, W., Mellor, A., Kirkpatrick, B., 2011. Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects. Biol Psychiatry. 70**,** 663-71.

Miller, G. E., Chen, E., Sze, J., Marin, T., Arevalo, J. M., Doll, R., . . . Cole, S. W., 2008. A functional genomic fingerprint of chronic stress in humans: blunted glucocorticoid and increased NF-kappaB signaling. Biol Psychiatry. 64**,** 266-72.

Mino, Y., Oshima, I., Okagami, K., 2000a. Mood disorders and influenza epidemics in Japan. Psychiatry and Clinical Neurosciences. 54**,** 59-65.

Mino, Y., Oshima, I., Tsuda, T., Okagami, K., 2000b. No relationship between schizophrenic birth and influenza epidemics in Japan. J Psychiatr Res. 34**,** 133-8.

Miranda-Filho Dde, B., Martelli, C. M., Ximenes, R. A., Araujo, T. V., Rocha, M. A., Ramos, R. C., . . . Rodrigues, L. C., 2016. Initial Description of the Presumed Congenital Zika Syndrome. Am J Public Health. 106**,** 598-600.

Mirnics, K., Middleton, F. A., Marquez, A., Lewis, D. A., Levitt, P., 2000. Molecular Characterization of Schizophrenia Viewed by Microarray Analysis of Gene Expression in Prefrontal Cortex. Neuron. 28**,** 53-67.

Missault, S., Van den Eynde, K., Vanden Berghe, W., Fransen, E., Weeren, A., Timmermans, J. P., . . . Dedeurwaerdere, S., 2014. The risk for behavioural deficits is determined by the maternal immune response to prenatal immune challenge in a neurodevelopmental model. Brain Behav Immun. 42**,** 138-46.

Moffett, A., Loke, C., 2006. Immunology of placentation in eutherian mammals. Nat Rev Immunol. 6**,** 584-94.

Moieni, M., Irwin, M. R., Jevtic, I., Olmstead, R., Breen, E. C., Eisenberger, N. I., 2015. Sex Differences in Depressive and Socioemotional Responses to an Inflammatory Challenge: Implications for Sex Differences in Depression. Neuropsychopharmacology. 40**,** 1709-1716.

Money, K. M., Stanwood, G. D., 2013. Developmental origins of brain disorders: roles for dopamine. Frontiers in Cellular Neuroscience. 7.

Monje, F. J., Cabatic, M., Divisch, I., Kim, E. J., Herkner, K. R., Binder, B. R., Pollak, D. D., 2011. Constant darkness induces IL-6-dependent depression-like behavior through the NF-kappaB signaling pathway. J Neurosci. 31**,** 9075-83.

Moraga, I., Spangler, J., Mendoza, J. L., Garcia, K. C., 2014. Multifarious determinants of cytokine receptor signaling specificity. Adv Immunol. 121**,** 1-39.

Moreno, J. L., Kurita, M., Holloway, T., Lopez, J., Cadagan, R., Martinez-Sobrido, L., . . . Gonzalez-Maeso, J., 2011. Maternal influenza viral infection causes schizophrenia-like alterations of 5-HT(2)A and mGlu(2) receptors in the adult offspring. J Neurosci. 31**,** 1863-72.

Morgan, M. J., Liu, Z. G., 2010. Reactive oxygen species in TNFalpha-induced signaling and cell death. Mol Cells. 30**,** 1-12.

Morgan, V., Castle, D., Page, A., Fazio, S., Gurrin, L., Burton, P., . . . Jablensky, A., 1997. Influenza epidemics and incidence of schizophrenia, affective disorders and mental retardation in Western Australia: no evidence of a major effect. Schizophr Res. 26**,** 25-39.

Mori, H., Hanada, R., Hanada, T., Aki, D., Mashima, R., Nishinakamura, H., . . . Yoshimura, A., 2004. Socs3 deficiency in the brain elevates leptin sensitivity and confers resistance to diet-induced obesity. Nat Med. 10**,** 739-43.

Morrison, S. F., 2004. Central pathways controlling brown adipose tissue thermogenesis. News Physiol Sci. 19**,** 67-74.

Morrow, J. D., Opp, M. R., 2005. Sleep-wake behavior and responses of interleukin-6-deficient mice to sleep deprivation. Brain Behav Immun. 19**,** 28-39.

Mortensen, P. B., Norgaard-Pedersen, B., Waltoft, B. L., Sorensen, T. L., Hougaard, D., Torrey, E. F., Yolken, R. H., 2007. Toxoplasma gondii as a risk factor for early-onset schizophrenia: analysis of filter paper blood samples obtained at birth. Biol Psychiatry. 61**,** 688-93.

Mosmann, T. R., Sad, S., 1996. The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol Today. 17**,** 138-46.

Moss, B. G., Chugani, D. C., 2014. Increased risk of very low birth weight, rapid postnatal growth, and autism in underweight and obese mothers. Am J Health Promot. 28**,** 181-8.

Mostafa, G. A., Shehab, A. A., 2010. The link of C4B null allele to autism and to a family history of autoimmunity in Egyptian autistic children. J Neuroimmunol. 223**,** 115-9.

Mueller, B. R., Bale, T. L., 2008. Sex-specific programming of offspring emotionality after stress early in pregnancy. J Neurosci. 28**,** 9055-65.

Mukherjee, S., Guha, S., Ikeda, M., Iwata, N., Malhotra, A. K., Pe'er, I., . . . Lencz, T., 2014. Excess of homozygosity in the major histocompatibility complex in schizophrenia. Hum Mol Genet. 23**,** 6088-95.

Muller, N., 2014. Immunology of major depression. Neuroimmunomodulation. 21**,** 123-30.

Muller, N., Schwarz, M. J., 2006. Neuroimmune-endocrine crosstalk in schizophrenia and mood disorders. Expert Rev Neurother. 6**,** 1017-38.

Muly, E. C., 3rd, Szigeti, K., Goldman-Rakic, P. S., 1998. D1 receptor in interneurons of macaque prefrontal cortex: distribution and subcellular localization. Journal of Neuroscience. 18**,** 10553-65.

Murray, C. A., Lynch, M. A., 1998. Evidence that increased hippocampal expression of the cytokine interleukin-1 beta is a common trigger for age- and stress-induced impairments in long-term potentiation. J Neurosci. 18**,** 2974-81.

Murray, C. J., Vos, T., Lozano, R., Naghavi, M., Flaxman, A. D., Michaud, C., . . . Memish, Z. A., 2012. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 380**,** 2197-223.

Musselman, D. L., Lawson, D. H., Gumnick, J. F., Manatunga, A. K., Penna, S., Goodkin, R. S., . . . Miller, A. H., 2001. Paroxetine for the prevention of depression induced by high-dose interferon alfa. N Engl J Med. 344**,** 961-6.

Naik, U. S., Gangadharan, C., Abbagani, K., Nagalla, B., Dasari, N., Manna, S. K., 2011. A study of nuclear transcription factor-kappa B in childhood autism. PLoS One. 6**,** e19488.

Nakashima, K., Wiese, S., Yanagisawa, M., Arakawa, H., Kimura, N., Hisatsune, T., . . . Taga, T., 1999. Developmental requirement of gp130 signaling in neuronal survival and astrocyte differentiation. J Neurosci. 19**,** 5429-34.

Narazaki, M., Yasukawa, K., Saito, T., Ohsugi, Y., Fukui, H., Koishihara, Y., . . . Kishimoto, T., 1993. Soluble forms of the interleukin-6 signal-transducing receptor component gp130 in human serum possessing a potential to inhibit signals through membrane-anchored gp130. Blood. 82**,** 1120-1126.

Nauli, S. M., Williams, J. M., Gerthoffer, W. T., Pearce, W. J., 2005. Chronic hypoxia modulates relations among calcium, myosin light chain phosphorylation, and force differently in fetal and adult ovine basilar arteries. J Appl Physiol (1985). 99**,** 120-7.

Neeley, E. W., Berger, R., Koenig, J. I., Leonard, S., 2011. Prenatal stress differentially alters brain-derived neurotrophic factor expression and signaling across rat strains. Neuroscience. 187**,** 24-35.

Nelson, A. B., Hang, G. B., Grueter, B. A., Pascoli, V., Luscher, C., Malenka, R. C., Kreitzer, A. C., 2012. A comparison of striatal-dependent behaviors in wild-type and hemizygous Drd1a and Drd2 BAC transgenic mice. J Neurosci. 32**,** 9119-23.

Nemeth, E., Rivera, S., Gabayan, V., Keller, C., Taudorf, S., Pedersen, B. K., Ganz, T., 2004. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin Invest. 113**,** 1271-6.

Nergiz, S., Altinkaya, O. S., Kucuk, M., Yuksel, H., Sezer, S. D., Kurt Omurlu, I., Odabasi, A. R., 2014. Circulating galanin and IL-6 concentrations in gestational diabetes mellitus. Gynecol Endocrinol. 30**,** 236-40.

Ness, G. C., 2015. Physiological feedback regulation of cholesterol biosynthesis: Role of translational control of hepatic HMG-CoA reductase and possible involvement of oxylanosterols. Biochim Biophys Acta. 1851**,** 667-73.

Neubauer, H., Cumano, A., Muller, M., Wu, H., Huffstadt, U., Pfeffer, K., 1998. Jak2 deficiency defines an essential developmental checkpoint in definitive hematopoiesis. Cell. 93**,** 397-409.

Nicodemus, K. K., Marenco, S., Batten, A. J., Vakkalanka, R., Egan, M. F., Straub, R. E., Weinberger, D. R., 2008. Serious obstetric complications interact with hypoxia-

regulated/vascular-expression genes to influence schizophrenia risk. Mol Psychiatry. 13**,** 873-7.

Niemand, C., Nimmesgern, A., Haan, S., Fischer, P., Schaper, F., Rossaint, R., . . . Müller-Newen, G., 2003. Activation of STAT3 by IL-6 and IL-10 in Primary Human Macrophages Is Differentially Modulated by Suppressor of Cytokine Signaling 3. The Journal of Immunology. 170**,** 3263-3272.

Nilsson, I., Shibuya, M., Wennstrom, S., 2004. Differential activation of vascular genes by hypoxia in primary endothelial cells. Exp Cell Res. 299**,** 476-85.

Nishikawa, T., Edelstein, D., Du, X. L., Yamagishi, S., Matsumura, T., Kaneda, Y., . . . Brownlee, M., 2000. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature. 404**,** 787-90.

Niwa, M., Kamiya, A., Murai, R., Kubo, K., Gruber, A. J., Tomita, K., . . . Nabeshima, T., 2010. Knockdown of DISC1 by in utero gene transfer disturbs postnatal dopaminergic maturation in the frontal cortex and leads to adult behavioral deficits. Neuron. 65**,** 480-9.

O'Callaghan, E., Sham, P., Takei, N., Glover, G., Murray, R. M., 1991. Schizophrenia after prenatal exposure to 1957 A2 influenza epidemic. Lancet. 337**,** 1248-50.

O'Callaghan, E., Sham, P. C., Takei, N., Murray, G., Glover, G., Hare, E. H., Murray, R. M., 1994. The relationship of schizophrenic births to 16 infectious diseases. Br J Psychiatry. 165**,** 353-6.

O'Donovan, A., Cohen, B. E., Seal, K. H., Bertenthal, D., Margaretten, M., Nishimi, K., Neylan, T. C., 2015. Elevated risk for autoimmune disorders in iraq and afghanistan veterans with posttraumatic stress disorder. Biol Psychiatry. 77**,** 365-74.

Odell, D., Maciulis, A., Cutler, A., Warren, L., McMahon, W. M., Coon, H., . . . Torres, A., 2005. Confirmation of the association of the C4B null allelle in autism. Hum Immunol. 66**,** 140-5.

Ohtsuka, N., Badurek, S., Busslinger, M., Benes, F. M., Minichiello, L., Rudolph, U., 2013. GABAergic neurons regulate lateral ventricular development via transcription factor Pax5. Genesis. 51**,** 234-45.

Oitzl, M. S., van Oers, H., Schobitz, B., de Kloet, E. R., 1993. Interleukin-1 beta, but not interleukin-6, impairs spatial navigation learning. Brain Res. 613**,** 160-3.

Okada, K., Hashimoto, K., Iwata, Y., Nakamura, K., Tsujii, M., Tsuchiya, K. J., . . . Mori, N., 2007. Decreased serum levels of transforming growth factor-β1 in patients with autism. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 31**,** 187-190.

Okada, M., Kitahara, M., Kishimoto, S., Matsuda, T., Hirano, T., Kishimoto, T., 1988. IL-6/BSF-2 functions as a killer helper factor in the in vitro induction of cytotoxic T cells. The Journal of Immunology. 141**,** 1543-9.

Okunishi, K., Dohi, M., Nakagome, K., Tanaka, R., Mizuno, S., Matsumoto, K., . . . Yamamoto, K., 2005. A novel role of hepatocyte growth factor as an immune regulator through suppressing dendritic cell function. J Immunol. 175**,** 4745-53.

Olson, C. M., Strawderman, M. S., Dennison, B. A., 2009. Maternal weight gain during pregnancy and child weight at age 3 years. Matern Child Health J. 13**,** 839-46.

Onore, C. E., Schwartzer, J. J., Careaga, M., Berman, R. F., Ashwood, P., 2014. Maternal immune activation leads to activated inflammatory macrophages in offspring. Brain Behav Immun. 38**,** 220-6.

Orlando, S., Matteucci, C., Fadlon, E. J., Buurman, W. A., Bardella, M. T., Colotta, F., . . . Mantovani, A., 1997. TNF-alpha, unlike other pro- and anti-inflammatory cytokines,

induces rapid release of the IL-1 type II decoy receptor in human myelomonocytic cells. J Immunol. 158, 3861-8.

Ornoy, A., Ratzon, N., Greenbaum, C., Peretz, E., Soriano, D., Dulitzky, M., 1998. Neurobehaviour of school age children born to diabetic mothers. Arch Dis Child Fetal Neonatal Ed. 79, F94-9.

Ornoy, A., Ratzon, N., Greenbaum, C., Wolf, A., Dulitzky, M., 2001. School-age children born to diabetic mothers and to mothers with gestational diabetes exhibit a high rate of inattention and fine and gross motor impairment. J Pediatr Endocrinol Metab. 14 Suppl 1, 681-9.

Ornoy, A., Reece, E. A., Pavlinkova, G., Kappen, C., Miller, R. K., 2015. Effect of maternal diabetes on the embryo, fetus, and children: congenital anomalies, genetic and epigenetic changes and developmental outcomes. Birth Defects Res C Embryo Today. 105, 53-72.

Ortega-Senovilla, H., Schaefer-Graf, U., Meitzner, K., Abou-Dakn, M., Graf, K., Kintscher, U., Herrera, E., 2011. Gestational Diabetes Mellitus Causes Changes in the Concentrations of Adipocyte Fatty Acid–Binding Protein and Other Adipocytokines in Cord Blood. Diabetes Care. 34, 2061-2066.

Osada-Oka, M., Ikeda, T., Imaoka, S., Akiba, S., Sato, T., 2008. VEGF-enhanced proliferation under hypoxia by an autocrine mechanism in human vascular smooth muscle cells. J Atheroscler Thromb. 15, 26-33.

Ozgen, H., Kahya, N., de Jonge, J. C., Smith, G. S., Harauz, G., Hoekstra, D., Baron, W., 2014. Regulation of cell proliferation by nucleocytoplasmic dynamics of postnatal and embryonic exon-II-containing MBP isoforms. Biochim Biophys Acta. 1843, 517-30.

Pace, T. W., Mletzko, T. C., Alagbe, O., Musselman, D. L., Nemeroff, C. B., Miller, A. H., Heim, C. M., 2006. Increased stress-induced inflammatory responses in male patients with major depression and increased early life stress. Am J Psychiatry. 163, 1630-3.

Pace, T. W., Wingenfeld, K., Schmidt, I., Meinlschmidt, G., Hellhammer, D. H., Heim, C. M., 2012. Increased peripheral NF-kappaB pathway activity in women with childhood abuse-related posttraumatic stress disorder. Brain Behav Immun. 26, 13-7.

Pagano, S. F., Impagnatiello, F., Girelli, M., Cova, L., Grioni, E., Onofri, M., . . . Parati, E. A., 2000. Isolation and characterization of neural stem cells from the adult human olfactory bulb. Stem Cells. 18, 295-300.

Pala, H. G., Ozalp, Y., Yener, A. S., Gerceklioglu, G., Uysal, S., Onvural, A., 2015. Adiponectin levels in gestational diabetes mellitus and in pregnant women without glucose intolerance. Adv Clin Exp Med. 24, 85-92.

Paliogianni, F., Boumpas, D. T., 1995. Glucocorticoids regulate calcineurin-dependent trans-activating pathways for interleukin-2 gene transcription in human T lymphocytes. Transplantation. 59, 1333-9.

Palmen, S. J., van Engeland, H., Hof, P. R., Schmitz, C., 2004. Neuropathological findings in autism. Brain. 127, 2572-83.

Palomo, J., Dietrich, D., Martin, P., Palmer, G., Gabay, C., 2015. The interleukin (IL)-1 cytokine family--Balance between agonists and antagonists in inflammatory diseases. Cytokine. 76, 25-37.

Pan, W., Stone, K. P., Hsuchou, H., Manda, V. K., Zhang, Y., Kastin, A. J., 2011. Cytokine signaling modulates blood-brain barrier function. Curr Pharm Des. 17, 3729-40.

Pang, D., Syed, S., Fine, P., Jones, P. B., 2009. No association between prenatal viral infection and depression in later life--a long-term cohort study of 6152 subjects. Can J Psychiatry. 54**,** 565-70.

Papadogiannakis, N., Johnsen, S., Olding, L., Suppressor cell activity in human cord blood. The immunology of the fetus. CRC Press Boca Raton, FL, 1990, pp. 215-227.

Parganas, E., Wang, D., Stravopodis, D., Topham, D. J., Marine, J. C., Teglund, S., . . . Ihle, J. N., 1998. Jak2 is essential for signaling through a variety of cytokine receptors. Cell. 93**,** 385-95.

Park, K., Volkow, N. D., Pan, Y., Du, C., 2013a. Chronic cocaine dampens dopamine signaling during cocaine intoxication and unbalances D1 over D2 receptor signaling. J Neurosci. 33**,** 15827-36.

Park, S., Kim, M. Y., Baik, S. H., Woo, J. T., Kwon, Y. J., Daily, J. W., . . . Kim, S. H., 2013b. Gestational diabetes is associated with high energy and saturated fat intakes and with low plasma visfatin and adiponectin levels independent of prepregnancy BMI. Eur J Clin Nutr. 67**,** 196-201.

Paschen, W., Gissel, C., Linden, T., Althausen, S., Doutheil, J., 1998. Activation of gadd153 expression through transient cerebral ischemia: evidence that ischemia causes endoplasmic reticulum dysfunction. Molecular Brain Research. 60**,** 115-122.

Pasek, R. C., Gannon, M., 2013. Advancements and challenges in generating accurate animal models of gestational diabetes mellitus. American Journal of Physiology - Endocrinology and Metabolism. 305**,** E1327-E1338.

Patterson, P. H., 2009. Immune involvement in schizophrenia and autism: Etiology, pathology and animal models. Behavioural Brain Research. 204**,** 313-321.

Patterson, P. H., 2011. Maternal infection and immune involvement in autism. Trends in Molecular Medicine. 17**,** 389-394.

Paul-Samojedny, M., Kowalczyk, M., Suchanek, R., Owczarek, A., Fila-Danilow, A., Szczygiel, A., Kowalski, J., 2010. Functional polymorphism in the interleukin-6 and interleukin-10 genes in patients with paranoid schizophrenia--a case-control study. J Mol Neurosci. 42**,** 112-9.

Paul, W. E., Seder, R. A., 1994. Lymphocyte responses and cytokines. Cell. 76**,** 241-51.

Pavlov, V. A., Wang, H., Czura, C. J., Friedman, S. G., Tracey, K. J., 2003. The cholinergic anti-inflammatory pathway: a missing link in neuroimmunomodulation. Mol Med. 9**,** 125-34.

Pearce, W. J., Butler, S. M., Abrassart, J. M., Williams, J. M., 2011. Fetal cerebral oxygenation: the homeostatic role of vascular adaptations to hypoxic stress. Adv Exp Med Biol. 701**,** 225-32.

Pearce, W. J., Williams, J. M., White, C. R., Lincoln, T. M., 2009. Effects of chronic hypoxia on soluble guanylate cyclase activity in fetal and adult ovine cerebral arteries. J Appl Physiol (1985). 107**,** 192-9.

Pekkanen, J., Xu, B., Jarvelin, M. R., 2001. Gestational age and occurrence of atopy at age 31--a prospective birth cohort study in Finland. Clin Exp Allergy. 31**,** 95-102.

Pelton, R. W., Saxena, B., Jones, M., Moses, H. L., Gold, L. I., 1991. Immunohistochemical localization of TGF beta 1, TGF beta 2, and TGF beta 3 in the mouse embryo: expression patterns suggest multiple roles during embryonic development. J Cell Biol. 115**,** 1091-105.

Penn, A. A., Riquelme, P. A., Feller, M. B., Shatz, C. J., 1998. Competition in retinogeniculate patterning driven by spontaneous activity. Science. 279**,** 2108-12.

Perkins, J. M., Dunn, J. P., Jagasia, S. M., 2007. Perspectives in Gestational Diabetes Mellitus: A Review of Screening, Diagnosis, and Treatment. Clinical Diabetes. 25**,** 57-62.

Perregaux, D. G., Dean, D., Cronan, M., Connelly, P., Gabel, C. A., 1995. Inhibition of interleukin-1 beta production by SKF86002: evidence of two sites of in vitro activity and of a time and system dependence. Mol Pharmacol. 48**,** 433-42.

Pineda, E., Shin, D., You, S. J., Auvin, S., Sankar, R., Mazarati, A., 2013. Maternal immune activation promotes hippocampal kindling epileptogenesis in mice. Ann Neurol. 74**,** 11-9.

Pinto, L., Gotz, M., 2007. Radial glial cell heterogeneity--the source of diverse progeny in the CNS. Prog Neurobiol. 83**,** 2-23.

Pitman, M., Emery, B., Binder, M., Wang, S., Butzkueven, H., Kilpatrick, T. J., 2004. LIF receptor signaling modulates neural stem cell renewal. Mol Cell Neurosci. 27**,** 255-66.

Pitossi, F., del Rey, A., Kabiersch, A., Besedovsky, H., 1997. Induction of cytokine transcripts in the central nervous system and pituitary following peripheral administration of endotoxin to mice. J Neurosci Res. 48**,** 287-98.

Plagemann, A., Harder, T., Lindner, R., Melchior, K., Rake, A., Rittel, F., . . . Dorner, G., 1998a. Alterations of hypothalamic catecholamines in the newborn offspring of gestational diabetic mother rats. Brain Res Dev Brain Res. 109**,** 201-9.

Plagemann, A., Harder, T., Rake, A., Melchior, K., Rittel, F., Rohde, W., Dorner, G., 1998b. Hypothalamic insulin and neuropeptide Y in the offspring of gestational diabetic mother rats. Neuroreport. 9**,** 4069-73.

Plata-Salaman, C. R., Sonti, G., Borkoski, J. P., Wilson, C. D., French-Mullen, J. M. b., 1996. Anorexia induced by chronic central administration of cytokines at estimated pathophysiological concentrations. Physiol Behav. 60**,** 867-75.

Poli, V., Balena, R., Fattori, E., Markatos, A., Yamamoto, M., Tanaka, H., . . . Costantini, F., 1994. Interleukin-6 deficient mice are protected from bone loss caused by estrogen depletion. Embo j. 13**,** 1189-96.

Powell, E. M., Campbell, D. B., Stanwood, G. D., Davis, C., Noebels, J. L., Levitt, P., 2003. Genetic disruption of cortical interneuron development causes region- and GABA cell type-specific deficits, epilepsy, and behavioral dysfunction. Journal of Neuroscience. 23**,** 622-31.

Powell, E. M., Mars, W. M., Levitt, P., 2001. Hepatocyte growth factor/scatter factor is a motogen for interneurons migrating from the ventral to dorsal telencephalon. Neuron. 30**,** 79-89.

Pratt, L., Ni, L., Ponzio, N. M., Jonakait, G. M., 2013. Maternal inflammation promotes fetal microglial activation and increased cholinergic expression in the fetal basal forebrain: role of interleukin-6. Pediatr Res. 74**,** 393-401.

Probert, L., Akassoglou, K., Pasparakis, M., Kontogeorgos, G., Kollias, G., 1995. Spontaneous inflammatory demyelinating disease in transgenic mice showing central nervous system-specific expression of tumor necrosis factor alpha. Proc Natl Acad Sci U S A. 92**,** 11294-8.

Quak, J., Doornbos, B., Roest, A. M., Duivis, H. E., Vogelzangs, N., Nolen, W. A., . . . de Jonge, P., 2014. Does tryptophan degradation along the kynurenine pathway mediate the association between pro-inflammatory immune activity and depressive symptoms? Psychoneuroendocrinology. 45**,** 202-10.

Raber, J., O'Shea, R. D., Bloom, F. E., Campbell, I. L., 1997. Modulation of hypothalamic-pituitary-adrenal function by transgenic expression of interleukin-6 in the CNS of mice. J Neurosci. 17, 9473-80.

Rai, D., Golding, J., Magnusson, C., Steer, C., Lewis, G., Dalman, C., 2012. Prenatal and early life exposure to stressful life events and risk of autism spectrum disorders: population-based studies in Sweden and England. PLoS One. 7, e38893.

Raison, C. L., Borisov, A. S., Majer, M., Drake, D. F., Pagnoni, G., Woolwine, B. J., . . . Miller, A. H., 2009. Activation of central nervous system inflammatory pathways by interferon-alpha: relationship to monoamines and depression. Biol Psychiatry. 65, 296-303.

Raison, C. L., Capuron, L., Miller, A. H., 2006. Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol. 27, 24-31.

Rajan, P., McKay, R. D., 1998. Multiple routes to astrocytic differentiation in the CNS. J Neurosci. 18, 3620-9.

Rajkowska, G., Mahajan, G., Maciag, D., Sathyanesan, M., Iyo, A. H., Moulana, M., . . . Newton, S. S., 2015. Oligodendrocyte morphometry and expression of myelin - Related mRNA in ventral prefrontal white matter in major depressive disorder. J Psychiatr Res. 65, 53-62.

Rapoport, J. L., Giedd, J. N., Gogtay, N., 2012. Neurodevelopmental model of schizophrenia: update 2012. Molecular Psychiatry. 17, 1228-1238.

Ratzon, N., Greenbaum, C., Dulitzky, M., Ornoy, A., 2000. Comparison of the motor development of school-age children born to mothers with and without diabetes mellitus. Phys Occup Ther Pediatr. 20, 43-57.

Ray, A., Prefontaine, K. E., 1994. Physical association and functional antagonism between the p65 subunit of transcription factor NF-kappa B and the glucocorticoid receptor. Proc Natl Acad Sci U S A. 91, 752-6.

Rees, S., Harding, R., Walker, D., 2008. An adverse intrauterine environment: implications for injury and altered development of the brain. Int J Dev Neurosci. 26, 3-11.

Reichenberg, A., Gorman, J. M., Dieterich, D. T., 2005. Interferon-induced depression and cognitive impairment in hepatitis C virus patients: a 72 week prospective study. Aids. 19 Suppl 3, S174-8.

Reus, G. Z., Nacif, M. P., Abelaira, H. M., Tomaz, D. B., dos Santos, M. A., Carlessi, A. S., . . . Quevedo, J., 2015. Ketamine ameliorates depressive-like behaviors and immune alterations in adult rats following maternal deprivation. Neurosci Lett. 584, 83-7.

Reyes-Vazquez, C., Prieto-Gomez, B., Dafny, N., 1994. Alpha-interferon suppresses food intake and neuronal activity of the lateral hypothalamus. Neuropharmacology. 33, 1545-52.

Reynolds, L. C., Inder, T. E., Neil, J. J., Pineda, R. G., Rogers, C. E., 2014. Maternal obesity and increased risk for autism and developmental delay among very preterm infants. J Perinatol. 34, 688-92.

Ricci, S., Businaro, R., Ippoliti, F., Lo Vasco, V. R., Massoni, F., Onofri, E., . . . Archer, T., 2013. Altered Cytokine and BDNF Levels in Autism Spectrum Disorder. Neurotoxicity Research. 24, 491-501.

Richardson, H. N., Zorrilla, E. P., Mandyam, C. D., Rivier, C. L., 2006. Exposure to repetitive versus varied stress during prenatal development generates two distinct anxiogenic and neuroendocrine profiles in adulthood. Endocrinology. 147, 2506-17.

Rivera, H. M., Christiansen, K. J., Sullivan, E. L., 2015. The role of maternal obesity in the risk of neuropsychiatric disorders. Front Neurosci. 9, 194.

Rivest, S., 2003. Molecular insights on the cerebral innate immune system. Brain Behav Immun. 17**,** 13-9.

Rizzo, T. A., Dooley, S. L., Metzger, B. E., Cho, N. H., Ogata, E. S., Silverman, B. L., 1995. Prenatal and perinatal influences on long-term psychomotor development in offspring of diabetic mothers. Am J Obstet Gynecol. 173**,** 1753-8.

Roberts, A. L., Koenen, K. C., Lyall, K., Robinson, E. B., Weisskopf, M. G., 2015. Association of autistic traits in adulthood with childhood abuse, interpersonal victimization, and posttraumatic stress. Child Abuse Negl. 45**,** 135-42.

Roberts, A. W., Robb, L., Rakar, S., Hartley, L., Cluse, L., Nicola, N. A., . . . Alexander, W. S., 2001. Placental defects and embryonic lethality in mice lacking suppressor of cytokine signaling 3. Proc Natl Acad Sci U S A. 98**,** 9324-9.

Rodig, S. J., Meraz, M. A., White, J. M., Lampe, P. A., Riley, J. K., Arthur, C. D., . . . Schreiber, R. D., 1998. Disruption of the Jak1 gene demonstrates obligatory and nonredundant roles of the Jaks in cytokine-induced biologic responses. Cell. 93**,** 373-83.

Romero, E., Ali, C., Molina-Holgado, E., Castellano, B., Guaza, C., Borrell, J., 2006. Neurobehavioral and Immunological Consequences of Prenatal Immune Activation in Rats. Influence of Antipsychotics. Neuropsychopharmacology. 32**,** 1791-1804.

Rong, C., Cui, X., Chen, J., Qian, Y., Jia, R., Hu, Y., 2015. DNA methylation profiles in placenta and its association with gestational diabetes mellitus. Exp Clin Endocrinol Diabetes. 123**,** 282-8.

Ross, E. J., Graham, D. L., Money, K. M., Stanwood, G. D., 2015. Developmental consequences of fetal exposure to drugs: what we know and what we still must learn. Neuropsychopharmacology. 40**,** 61-87.

Roth, T. L., Sweatt, J. D., 2011. Annual Research Review: Epigenetic mechanisms and environmental shaping of the brain during sensitive periods of development. J Child Psychol Psychiatry. 52**,** 398-408.

Rothermundt, M., Arolt, V., Fenker, J., Gutbrodt, H., Peters, M., Kirchner, H., 2001. Different immune patterns in melancholic and non-melancholic major depression. Eur Arch Psychiatry Clin Neurosci. 251**,** 90-7.

Roussa, E., Wiehle, M., Dunker, N., Becker-Katins, S., Oehlke, O., Krieglstein, K., 2006. Transforming growth factor beta is required for differentiation of mouse mesencephalic progenitors into dopaminergic neurons in vitro and in vivo: ectopic induction in dorsal mesencephalon. Stem Cells. 24**,** 2120-9.

Rubenstein, J. L. R., 2011. Development of the Cerebral Cortex: Implications for Neurodevelopmental Disorders. J Child Psychol Psychiatry. 52**,** 339-55.

Sadler, T. W., Liu, E. T., Augustine, K. A., 1995. Antisense targeting of engrailed-1 causes abnormal axis formation in mouse embryos. Teratology. 51**,** 292-9.

Saetre, P., Emilsson, L., Axelsson, E., Kreuger, J., Lindholm, E., Jazin, E., 2007. Inflammation-related genes up-regulated in schizophrenia brains. BMC Psychiatry. 7**,** 46.

Salbaum, J. M., Kappen, C., 2012. Responses of the embryonic epigenome to maternal diabetes. Birth Defects Res A Clin Mol Teratol. 94**,** 770-81.

Salihagić-Kadić, A., Medić, M., Jugović, D., Kos, M., Latin, V., Kušan Jukić, M., Arbeille, P., 2006. Fetal cerebrovascular response to chronic hypoxia—implications for the prevention of brain damage. The Journal of Maternal-Fetal & Neonatal Medicine. 19**,** 387-396.

Sandin, S., Lichtenstein, P., Kuja-Halkola, R., Larsson, H., Hultman, C. M., Reichenberg, A., 2014. The familial risk of autism. Jama. 311**,** 1770-7.

Santana, N., Mengod, G., Artigas, F., 2009. Quantitative analysis of the expression of dopamine D1 and D2 receptors in pyramidal and GABAergic neurons of the rat prefrontal cortex. Cereb Cortex. 19**,** 849-60.

Sasaki, A., de Vega, W. C., St-Cyr, S., Pan, P., McGowan, P. O., 2013. Perinatal high fat diet alters glucocorticoid signaling and anxiety behavior in adulthood. Neuroscience. 240**,** 1-12.

Sasayama, D., Wakabayashi, C., Hori, H., Teraishi, T., Hattori, K., Ota, M., . . . Kunugi, H., 2011. Association of plasma IL-6 and soluble IL-6 receptor levels with the Asp358Ala polymorphism of the IL-6 receptor gene in schizophrenic patients. J Psychiatr Res. 45**,** 1439-44.

Savitz, J., Drevets, W. C., Wurfel, B. E., Ford, B. N., Bellgowan, P. S., Victor, T. A., . . . Dantzer, R., 2015. Reduction of kynurenic acid to quinolinic acid ratio in both the depressed and remitted phases of major depressive disorder. Brain Behav Immun. 46**,** 55-9.

Schaefer, C. A., Brown, A. S., Wyatt, R. J., Kline, J., Begg, M. D., Bresnahan, M. A., Susser, E. S., 2000. Maternal prepregnant body mass and risk of schizophrenia in adult offspring. Schizophr Bull. 26**,** 275-86.

Schmidt-Kastner, R., van Os, J., Esquivel, G., Steinbusch, H. W., Rutten, B. P., 2012. An environmental analysis of genes associated with schizophrenia: hypoxia and vascular factors as interacting elements in the neurodevelopmental model. Mol Psychiatry. 17**,** 1194-205.

Schmidt-Kastner, R., van Os, J., H, W. M. S., Schmitz, C., 2006. Gene regulation by hypoxia and the neurodevelopmental origin of schizophrenia. Schizophr Res. 84**,** 253-71.

Schmidt, M. J., Horvath, S., Ebert, P., Norris, J. L., Seeley, E. H., Brown, J., . . . Mirnics, K., 2014. Modulation of behavioral networks by selective interneuronal inactivation. Mol Psychiatry. 19**,** 580-7.

Schmidt, M. J., Mirnics, K., 2012. Modeling interneuron dysfunction in schizophrenia. Dev Neurosci. 34**,** 152-8.

Schmitz, J., Weissenbach, M., Haan, S., Heinrich, P. C., Schaper, F., 2000. SOCS3 Exerts Its Inhibitory Function on Interleukin-6 Signal Transduction through the SHP2 Recruitment Site of gp130. Journal of Biological Chemistry. 275**,** 12848-12856.

Schneider, C. A., Rasband, W. S., Eliceiri, K. W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. 9**,** 671-5.

Schneider, H., Pitossi, F., Balschun, D., Wagner, A., del Rey, A., Besedovsky, H. O., 1998. A neuromodulatory role of interleukin-1beta in the hippocampus. Proc Natl Acad Sci U S A. 95**,** 7778-83.

Schoneveld, O. J. L. M., Cidlowski, J. A., CHAPTER 1 - Glucocorticoids and Immunity: Mechanisms of Regulation A2 - Ader, Robert. Psychoneuroimmunology (Fourth Edition). Academic Press, Burlington, 2007, pp. 45-61.

Schreiber, W., Pollmacher, T., Fassbender, K., Gudewill, S., Vedder, H., Wiedemann, K., . . . Holsboer, F., 1993. Endotoxin- and corticotropin-releasing hormone-induced release of ACTH and cortisol. A comparative study in men. Neuroendocrinology. 58**,** 123-8.

Schreuder, H., Tardif, C., Trump-Kallmeyer, S., Soffientini, A., Sarubbi, E., Akeson, A., . . . Barrett, R. W., 1997. A new cytokine-receptor binding mode revealed by the crystal structure of the IL-1 receptor with an antagonist. Nature. 386**,** 194-200.

Schubert, D., Martens, G. J. M., Kolk, S. M., 2015. Molecular underpinnings of prefrontal cortex development in rodents provide insights into the etiology of neurodevelopmental disorders. Mol Psychiatry. 20**,** 795-809.

Schwarz, M. J., Chiang, S., Muller, N., Ackenheil, M., 2001. T-helper-1 and T-helper-2 responses in psychiatric disorders. Brain Behav Immun. 15**,** 340-70.

Schwarz, M. J., Kronig, H., Riedel, M., Dehning, S., Douhet, A., Spellmann, I., . . . Muller, N., 2006. IL-2 and IL-4 polymorphisms as candidate genes in schizophrenia. Eur Arch Psychiatry Clin Neurosci. 256**,** 72-6.

Schwarz, Q., Gu, C., Fujisawa, H., Sabelko, K., Gertsenstein, M., Nagy, A., . . . Ruhrberg, C., 2004. Vascular endothelial growth factor controls neuronal migration and cooperates with Sema3A to pattern distinct compartments of the facial nerve. Genes Dev. 18**,** 2822-34.

Scott, D. K., Stromstedt, P. E., Wang, J. C., Granner, D. K., 1998. Further characterization of the glucocorticoid response unit in the phosphoenolpyruvate carboxykinase gene. The role of the glucocorticoid receptor-binding sites. Mol Endocrinol. 12**,** 482-91.

Seiki, K., Sakabe, K., 1997. Sex hormones and the thymus in relation to thymocyte proliferation and maturation. Archives of histology and cytology. 60**,** 29-38.

Sekar, A., Bialas, A. R., de Rivera, H., Davis, A., Hammond, T. R., Kamitaki, N., . . . McCarroll, S. A., 2016. Schizophrenia risk from complex variation of complement component 4. Nature. 530**,** 177-83.

Sellmann, C., Villarin Pildain, L., Schmitt, A., Leonardi-Essmann, F., Durrenberger, P. F., Spanagel, R., . . . Matthaus, F., 2014. Gene expression in superior temporal cortex of schizophrenia patients. Eur Arch Psychiatry Clin Neurosci. 264**,** 297-309.

Selten, J.-P., Frissen, A., Lensvelt-Mulders, G., Morgan, V. A., 2010. Schizophrenia and 1957 Pandemic of Influenza: Meta-analysis. Schizophrenia Bulletin. 36**,** 219-228.

Selye, H., Marion, D., 1955. On the direct thymolytic effect of hexestrol. Acta Anat (Basel). 23**,** 180-4.

Seong, H. J., Carter, A. G., 2012. D1 receptor modulation of action potential firing in a subpopulation of layer 5 pyramidal neurons in the prefrontal cortex. J Neurosci. 32**,** 10516-21.

Setiawan, E., Wilson, A. A., Mizrahi, R., Rusjan, P. M., Miler, L., Rajkowska, G., . . . Meyer, J. H., 2015. Role of translocator protein density, a marker of neuroinflammation, in the brain during major depressive episodes. JAMA Psychiatry. 72**,** 268-75.

Severance, E. G., Alaedini, A., Yang, S., Halling, M., Gressitt, K. L., Stallings, C. R., . . . Yolken, R. H., 2012. Gastrointestinal inflammation and associated immune activation in schizophrenia. Schizophr Res. 138**,** 48-53.

Shaftel, S. S., Carlson, T. J., Olschowka, J. A., Kyrkanides, S., Matousek, S. B., O'Banion, M. K., 2007a. Chronic interleukin-1beta expression in mouse brain leads to leukocyte infiltration and neutrophil-independent blood brain barrier permeability without overt neurodegeneration. J Neurosci. 27**,** 9301-9.

Shaftel, S. S., Kyrkanides, S., Olschowka, J. A., Miller, J. N., Johnson, R. E., O'Banion, M. K., 2007b. Sustained hippocampal IL-1 beta overexpression mediates chronic neuroinflammation and ameliorates Alzheimer plaque pathology. J Clin Invest. 117**,** 1595-604.

Sham, P. C., O'Callaghan, E., Takei, N., Murray, G. K., Hare, E. H., Murray, R. M., 1992. Schizophrenia following pre-natal exposure to influenza epidemics between 1939 and 1960. Br J Psychiatry. 160, 461-6.

Sharpe, L. J., Brown, A. J., 2013. Controlling Cholesterol Synthesis beyond 3-Hydroxy-3-methylglutaryl-CoA Reductase (HMGCR). Journal of Biological Chemistry. 288, 18707-18715.

Shatz, C. J., 2009. MHC class I: an unexpected role in neuronal plasticity. Neuron. 64, 40-5.

Shatz, C. J., Stryker, M. P., 1988. Prenatal tetrodotoxin infusion blocks segregation of retinogeniculate afferents. Science. 242, 87-9.

Shavit, Y., Wolf, G., Goshen, I., Livshits, D., Yirmiya, R., 2005. Interleukin-1 antagonizes morphine analgesia and underlies morphine tolerance. Pain. 115, 50-9.

Shelton, R. C., Claiborne, J., Sidoryk-Wegrzynowicz, M., Reddy, R., Aschner, M., Lewis, D. A., Mirnics, K., 2011. Altered expression of genes involved in inflammation and apoptosis in frontal cortex in major depression. Mol Psychiatry. 16, 751-62.

Shelton, R. C., Liang, S., Liang, P., Chakrabarti, A., Manier, D. H., Sulser, F., 2004. Differential expression of pentraxin 3 in fibroblasts from patients with major depression. Neuropsychopharmacology. 29, 126-32.

Shelton, R. C., Mainer, D. H., Sulser, F., 1996. cAMP-dependent protein kinase activity in major depression. Am J Psychiatry. 153, 1037-42.

Shen, W., Flajolet, M., Greengard, P., Surmeier, D. J., 2008. Dichotomous dopaminergic control of striatal synaptic plasticity. Science. 321, 848-51.

Shi, J., Levinson, D. F., Duan, J., Sanders, A. R., Zheng, Y., Pe'er, I., . . . Gejman, P. V., 2009. Common variants on chromosome 6p22.1 are associated with schizophrenia. Nature. 460, 753-7.

Shi, L., Fatemi, S. H., Sidwell, R. W., Patterson, P. H., 2003. Maternal Influenza Infection Causes Marked Behavioral and Pharmacological Changes in the Offspring. The Journal of Neuroscience. 23, 297-302.

Shi, L., Tu, N., Patterson, P. H., 2005. Maternal influenza infection is likely to alter fetal brain development indirectly: the virus is not detected in the fetus. International Journal of Developmental Neuroscience. 23, 299-305.

Shibata, N., Glass, C. K., 2010. Macrophages, oxysterols and atherosclerosis. Circ J. 74, 2045-51.

Shibata, T., Yamagata, H., Uchida, S., Otsuki, K., Hobara, T., Higuchi, F., . . . Watanabe, Y., 2013. The alteration of hypoxia inducible factor-1 (HIF-1) and its target genes in mood disorder patients. Prog Neuropsychopharmacol Biol Psychiatry. 43, 222-9.

Shimazaki, T., Shingo, T., Weiss, S., 2001. The ciliary neurotrophic factor/leukemia inhibitory factor/gp130 receptor complex operates in the maintenance of mammalian forebrain neural stem cells. J Neurosci. 21, 7642-53.

Shuen, J. A., Chen, M., Gloss, B., Calakos, N., 2008. Drd1a-tdTomato BAC transgenic mice for simultaneous visualization of medium spiny neurons in the direct and indirect pathways of the basal ganglia. J Neurosci. 28, 2681-5.

Sibley, W. A., Bamford, C. R., Clark, K., 1985. Clinical viral infections and multiple sclerosis. Lancet. 1, 1313-5.

Silvagni, A., Barros, V. G., Mura, C., Antonelli, M. C., Carboni, E., 2008. Prenatal restraint stress differentially modifies basal and stimulated dopamine and noradrenaline release in

the nucleus accumbens shell: an 'in vivo' microdialysis study in adolescent and young adult rats. Eur J Neurosci. 28**,** 744-58.

Silverman, B. L., Rizzo, T. A., Cho, N. H., Metzger, B. E., 1998. Long-term effects of the intrauterine environment. The Northwestern University Diabetes in Pregnancy Center. Diabetes Care. 21 Suppl 2**,** B142-9.

Sim, R. B., Tsiftsoglou, S. A., 2004. Proteases of the complement system. Biochem Soc Trans. 32**,** 21-7.

Simanek, A. M., Meier, H. C. S., 2015. Association Between Prenatal Exposure to Maternal Infection and Offspring Mood Disorders: A Review of the Literature. Current Problems in Pediatric and Adolescent Health Care. 45**,** 325-364.

Singh, G. K., Kenney, M. K., Ghandour, R. M., Kogan, M. D., Lu, M. C., 2013. Mental Health Outcomes in US Children and Adolescents Born Prematurely or with Low Birthweight. Depress Res Treat. 2013**,** 570743.

Singh, V. K., 1996. Plasma increase of interleukin-12 and interferon-gamma. Pathological significance in autism. Journal of Neuroimmunology. 66**,** 143-145.

Slavich, G. M., Irwin, M. R., 2014. From stress to inflammation and major depressive disorder: a social signal transduction theory of depression. Psychol Bull. 140**,** 774-815.

Slopen, N., Lewis, T. T., Gruenewald, T. L., Mujahid, M. S., Ryff, C. D., Albert, M. A., Williams, D. R., 2010. Early life adversity and inflammation in African Americans and whites in the midlife in the United States survey. Psychosom Med. 72**,** 694-701.

Smith, B. N., Dou, P., Barber, W. D., Dudek, F. E., 1998. Vagally evoked synaptic currents in the immature rat nucleus tractus solitarii in an intact in vitro preparation. J Physiol. 512 (Pt 1)**,** 149-62.

Smith, G. S., Paez, P. M., Spreuer, V., Campagnoni, C. W., Boggs, J. M., Campagnoni, A. T., Harauz, G., 2011. Classical 18.5-and 21.5-kDa isoforms of myelin basic protein inhibit calcium influx into oligodendroglial cells, in contrast to golli isoforms. J Neurosci Res. 89**,** 467-80.

Smith, R. J., Lobo, M. K., Spencer, S., Kalivas, P. W., 2013. Cocaine-induced adaptations in D1 and D2 accumbens projection neurons (a dichotomy not necessarily synonymous with direct and indirect pathways). Curr Opin Neurobiol. 23**,** 546-52.

Smith, S. E., Li, J., Garbett, K., Mirnics, K., Patterson, P. H., 2007. Maternal immune activation alters fetal brain development through interleukin-6. J Neurosci. 27.

Smolders, S., Smolders, S. M., Swinnen, N., Gartner, A., Rigo, J. M., Legendre, P., Brone, B., 2015. Maternal immune activation evoked by polyinosinic:polycytidylic acid does not evoke microglial cell activation in the embryo. Front Cell Neurosci. 9**,** 301.

Smoller, J. W., Finn, C. T., 2003. Family, twin, and adoption studies of bipolar disorder. Am J Med Genet C Semin Med Genet. 123c**,** 48-58.

Sondell, M., Lundborg, G., Kanje, M., 1999. Vascular endothelial growth factor has neurotrophic activity and stimulates axonal outgrowth, enhancing cell survival and Schwann cell proliferation in the peripheral nervous system. J Neurosci. 19**,** 5731-40.

Sondell, M., Sundler, F., Kanje, M., 2000. Vascular endothelial growth factor is a neurotrophic factor which stimulates axonal outgrowth through the flk-1 receptor. Eur J Neurosci. 12**,** 4243-54.

Song, G. G., Kim, J. H., Lee, Y. H., 2013. Genome-wide pathway analysis in major depressive disorder. J Mol Neurosci. 51**,** 428-36.

Song, X., Fan, X., Li, X., Zhang, W., Gao, J., Zhao, J., . . . Lv, L., 2014. Changes in pro-inflammatory cytokines and body weight during 6-month risperidone treatment in drug naive, first-episode schizophrenia. Psychopharmacology (Berl). 231**,** 319-25.

Song, X., Li, W., Yang, Y., Zhao, J., Jiang, C., Li, W., Lv, L., 2011. The nuclear factor-kappaB inhibitor pyrrolidine dithiocarbamate reduces polyinosinic-polycytidilic acid-induced immune response in pregnant rats and the behavioral defects of their adult offspring. Behav Brain Funct. 7**,** 50.

Song, X. Q., Lv, L. X., Li, W. Q., Hao, Y. H., Zhao, J. P., 2009. The interaction of nuclear factor-kappa B and cytokines is associated with schizophrenia. Biol Psychiatry. 65**,** 481-8.

Sorensen, H. J., Mortensen, E. L., Reinisch, J. M., Mednick, S. A., 2009. Association between prenatal exposure to bacterial infection and risk of schizophrenia. Schizophr Bull. 35**,** 631-7.

Soumiya, H., Fukumitsu, H., Furukawa, S., 2011a. Prenatal immune challenge compromises development of upper-layer but not deeper-layer neurons of the mouse cerebral cortex. J Neurosci Res. 89**,** 1342-50.

Soumiya, H., Fukumitsu, H., Furukawa, S., 2011b. Prenatal immune challenge compromises the normal course of neurogenesis during development of the mouse cerebral cortex. J Neurosci Res. 89**,** 1575-85.

Sousa, I., Clark, T. G., Toma, C., Kobayashi, K., Choma, M., Holt, R., . . . Monaco, A. P., 2009. MET and autism susceptibility: family and case-control studies. Eur J Hum Genet. 17**,** 749-58.

Spassky, N., Goujet-Zalc, C., Parmantier, E., Olivier, C., Martinez, S., Ivanova, A., . . . Thomas, J. L., 1998. Multiple restricted origin of oligodendrocytes. J Neurosci. 18**,** 8331-43.

Spauwen, J., Krabbendam, L., Lieb, R., Wittchen, H. U., van Os, J., 2004. Early maternal stress and health behaviours and offspring expression of psychosis in adolescence. Acta Psychiatr Scand. 110**,** 356-64.

Spellman, P. T., Sherlock, G., Zhang, M. Q., Iyer, V. R., Anders, K., Eisen, M. B., . . . Futcher, B., 1998. Comprehensive Identification of Cell Cycle–regulated Genes of the Yeast Saccharomyces cerevisiae by Microarray Hybridization. Molecular Biology of the Cell. 9**,** 3273-3297.

Spencer, G. E., Klumperman, J., Syed, N. I., 1998. Neurotransmitters and neurodevelopment. Role of dopamine in neurite outgrowth, target selection and specific synapse formation. Perspectives on Developmental Neurobiology. 5**,** 451-67.

Spinazzola, A., Zeviani, M., 2009. Disorders from perturbations of nuclear-mitochondrial intergenomic cross-talk. J Intern Med. 265**,** 174-92.

Sretavan, D. W., Shatz, C. J., Stryker, M. P., 1988. Modification of retinal ganglion cell axon morphology by prenatal infusion of tetrodotoxin. Nature. 336**,** 468-71.

St Clair, D., Xu, M., Wang, P., Yu, Y., Fang, Y., Zhang, F., . . . He, L., 2005. Rates of adult schizophrenia following prenatal exposure to the Chinese famine of 1959-1961. Jama. 294**,** 557-62.

Stanwood, G. D., Leitch, D. B., Savchenko, V., Wu, J., Fitsanakis, V. A., Anderson, D. J., . . . McLaughlin, B., 2009. Manganese exposure is cytotoxic and alters dopaminergic and GABAergic neurons within the basal ganglia. J Neurochem. 110**,** 378-89.

Stanwood, G. D., Parlaman, J. P., Levitt, P., 2005. Anatomical abnormalities in dopaminoceptive regions of the cerebral cortex of dopamine D1 receptor mutant mice. J Comp Neurol. 487, 270-82.

Stanwood, G. D., Washington, R. A., Levitt, P., 2001. Identification of a Sensitive Period of Prenatal Cocaine Exposure that Alters the Development of the Anterior Cingulate Cortex. Cerebral Cortex. 11, 430-440.

Steffensen, S. C., Campbell, I. L., Henriksen, S. J., 1994. Site-specific hippocampal pathophysiology due to cerebral overexpression of interleukin-6 in transgenic mice. Brain Res. 652, 149-53.

Steiner, J., Walter, M., Glanz, W., Sarnyai, Z., Bernstein, H. G., Vielhaber, S., . . . Stoecker, W., 2013. Increased prevalence of diverse N-methyl-D-aspartate glutamate receptor antibodies in patients with an initial diagnosis of schizophrenia: specific relevance of IgG NR1a antibodies for distinction from N-methyl-D-aspartate glutamate receptor encephalitis. JAMA Psychiatry. 70, 271-8.

Stellwagen, D., Malenka, R. C., 2006. Synaptic scaling mediated by glial TNF-alpha. Nature. 440, 1054-9.

Stevens, B., Allen, N. J., Vazquez, L. E., Howell, G. R., Christopherson, K. S., Nouri, N., . . . Barres, B. A., 2007. The classical complement cascade mediates CNS synapse elimination. Cell. 131, 1164-78.

Stewart, F. M., Freeman, D. J., Ramsay, J. E., Greer, I. A., Caslake, M., Ferrell, W. R., 2007. Longitudinal Assessment of Maternal Endothelial Function and Markers of Inflammation and Placental Function throughout Pregnancy in Lean and Obese Mothers. The Journal of Clinical Endocrinology & Metabolism. 92, 969-975.

Stice, E., Yokum, S., Burger, K. S., Epstein, L. H., Small, D. M., 2011. Youth at risk for obesity show greater activation of striatal and somatosensory regions to food. J Neurosci. 31, 4360-6.

Stitt, J. T., Bernheim, H. A., 1985. Differences in endogenous pyrogen fevers induced by iv and icv routes in rabbits. J Appl Physiol (1985). 59, 342-7.

Stubbs, E. G., Crawford, M. L., Burger, D. R., Vandenbark, A. A., 1977. Depressed lymphocyte responsiveness in autistic children. Journal of autism and childhood schizophrenia. 7, 49-55.

Stumm, R. K., Zhou, C., Ara, T., Lazarini, F., Dubois-Dalcq, M., Nagasawa, T., . . . Schulz, S., 2003. CXCR4 regulates interneuron migration in the developing neocortex. J Neurosci. 23, 5123-30.

Su, S., Miller, A. H., Snieder, H., Bremner, J. D., Ritchie, J., Maisano, C., . . . Vaccarino, V., 2009. Common genetic contributions to depressive symptoms and inflammatory markers in middle-aged men: the Twins Heart Study. Psychosom Med. 71, 152-8.

Sukoff Rizzo, S. J., Neal, S. J., Hughes, Z. A., Beyna, M., Rosenzweig-Lipson, S., Moss, S. J., Brandon, N. J., 2012. Evidence for sustained elevation of IL-6 in the CNS as a key contributor of depressive-like phenotypes. Transl Psychiatry. 2, e199.

Sullivan, P. F., 2005. The genetics of schizophrenia. PLoS Med. 2, e212.

Sullivan, P. F., Kendler, K. S., Neale, M. C., 2003. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry. 60, 1187-92.

Sullivan, P. F., Lin, D., Tzeng, J. Y., van den Oord, E., Perkins, D., Stroup, T. S., . . . Close, S. L., 2008. Genomewide association for schizophrenia in the CATIE study: results of stage 1. Mol Psychiatry. 13, 570-84.

Sullivan, P. F., Neale, M. C., Kendler, K. S., 2000. Genetic Epidemiology of Major Depression: Review and Meta-Analysis. American Journal of Psychiatry. 157, 1552-1562.

Susser, E., Lin, S. P., Brown, A. S., Lumey, L. H., Erlenmeyer-Kimling, L., 1994. No relation between risk of schizophrenia and prenatal exposure to influenza in Holland. Am J Psychiatry. 151, 922-4.

Susser, E. S., Lin, S. P., 1992. Schizophrenia after prenatal exposure to the Dutch Hunger Winter of 1944-1945. Arch Gen Psychiatry. 49, 983-8.

Sutton, C., Brereton, C., Keogh, B., Mills, K. H., Lavelle, E. C., 2006. A crucial role for interleukin (IL)-1 in the induction of IL-17-producing T cells that mediate autoimmune encephalomyelitis. J Exp Med. 203, 1685-91.

Suvisaari, J., Haukka, J., Tanskanen, A., Hovi, T., Lonnqvist, J., 1999. Association between prenatal exposure to poliovirus infection and adult schizophrenia. Am J Psychiatry. 156, 1100-2.

Swarbrick, M. M., Havel, P. J., 2008. Physiological, pharmacological, and nutritional regulation of circulating adiponectin concentrations in humans. Metab Syndr Relat Disord. 6.

Sweeten, T. L., Posey, D. J., McDougle, C. J., 2003. High Blood Monocyte Counts and Neopterin Levels in Children With Autistic Disorder. American Journal of Psychiatry. 160, 1691-1693.

Sweeten, T. L., Posey, D. J., McDougle, C. J., 2004. Brief report: autistic disorder in three children with cytomegalovirus infection. J Autism Dev Disord. 34, 583-6.

Szczesny, E., Basta-Kaim, A., Slusarczyk, J., Trojan, E., Glombik, K., Regulska, M., . . . Lason, W., 2014. The impact of prenatal stress on insulin-like growth factor-1 and pro-inflammatory cytokine expression in the brains of adult male rats: the possible role of suppressors of cytokine signaling proteins. J Neuroimmunol. 276, 37-46.

Szelényi, J., 2001. Cytokines and the central nervous system. Brain Research Bulletin. 54, 329-338.

Szymanska, M., Budziszewska, B., Jaworska-Feil, L., Basta-Kaim, A., Kubera, M., Leskiewicz, M., . . . Lason, W., 2009. The effect of antidepressant drugs on the HPA axis activity, glucocorticoid receptor level and FKBP51 concentration in prenatally stressed rats. Psychoneuroendocrinology. 34, 822-32.

Tahara, Y., Ido, A., Yamamoto, S., Miyata, Y., Uto, H., Hori, T., . . . Tsubouchi, H., 2003. Hepatocyte growth factor facilitates colonic mucosal repair in experimental ulcerative colitis in rats. J Pharmacol Exp Ther. 307, 146-51.

Tak, P. P., Firestein, G. S., 2001. NF-κB: a key role in inflammatory diseases. J Clin Invest. 107, 7-11.

Takao, T., Tracey, D. E., Mitchell, W. M., De Souza, E. B., 1990. Interleukin-1 receptors in mouse brain: characterization and neuronal localization. Endocrinology. 127, 3070-8.

Takebayashi, H., Ikenaka, K., 2015. Oligodendrocyte generation during mouse development. Glia. 63, 1350-6.

Takei, N., Murray, R. M., Sham, P., O'Callaghan, E., 1995. Schizophrenia risk for women from in utero exposure to influenza. Am J Psychiatry. 152, 150-1.

Takei, N., O'Callaghan, E., Sham, P. C., Glover, G., Murray, R. M., 1993. Does prenatal influenza divert susceptible females from later affective psychosis to schizophrenia? Acta Psychiatr Scand. 88, 328-36.

Takei, N., Sham, P., O'Callaghan, E., Murray, G. K., Glover, G., Murray, R. M., 1994. Prenatal exposure to influenza and the development of schizophrenia: is the effect confined to females? Am J Psychiatry. 151**,** 117-9.

Tanaka, T., Narazaki, M., Kishimoto, T., 2014. IL-6 in Inflammation, Immunity, and Disease. Cold Spring Harbor Perspectives in Biology. 6.

Tancredi, V., D'Arcangelo, G., Grassi, F., Tarroni, P., Palmieri, G., Santoni, A., Eusebi, F., 1992. Tumor necrosis factor alters synaptic transmission in rat hippocampal slices. Neurosci Lett. 146**,** 176-8.

Tang, X., Qin, Q., Xie, X., He, P., 2015. Protective effect of sRAGE on fetal development in pregnant rats with gestational diabetes mellitus. Cell Biochem Biophys. 71**,** 549-56.

Taniguchi, H., 2014. Genetic dissection of GABAergic neural circuits in mouse neocortex. Front Cell Neurosci. 8**,** 8.

Tartter, M., Hammen, C., Bower, J. E., Brennan, P. A., Cole, S., 2015. Effects of chronic interpersonal stress exposure on depressive symptoms are moderated by genetic variation at IL6 and IL1beta in youth. Brain Behav Immun. 46**,** 104-11.

Tatsumi, M., Sasaki, T., Sakai, T., Kamijima, K., Fukuda, R., Kunugi, H., . . . Nanko, S., 1997. Genes for interleukin-2 receptor beta chain, interleukin-1 beta, and schizophrenia: no evidence for the association or linkage. Am J Med Genet. 74**,** 338-41.

Taylor, S. E., Lehman, B. J., Kiefe, C. I., Seeman, T. E., 2006. Relationship of early life stress and psychological functioning to adult C-reactive protein in the coronary artery risk development in young adults study. Biol Psychiatry. 60**,** 819-24.

Tchelingerian, J. L., Monge, M., Le Saux, F., Zalc, B., Jacque, C., 1995. Differential oligodendroglial expression of the tumor necrosis factor receptors in vivo and in vitro. J Neurochem. 65**,** 2377-80.

Teixeira, C. M., Masachs, N., Muhaisen, A., Bosch, C., Perez-Martinez, J., Howell, B., Soriano, E., 2014. Transient Downregulation of Dab1 Protein Levels during Development Leads to Behavioral and Structural Deficits: Relevance for Psychiatric Disorders. Neuropsychopharmacology. 39**,** 556-568.

Thibault, D., Loustalot, F., Fortin, G. M., Bourque, M. J., Trudeau, L. E., 2013. Evaluation of D1 and D2 dopamine receptor segregation in the developing striatum using BAC transgenic mice. PLoS One. 8**,** e67219.

Thompson, B. L., Stanwood, G. D., Levitt, P., 2010. Specificity of prenatal cocaine exposure effects on cortical interneurons is independent from dopamine D1 receptor co-localization. J Chem Neuroanat. 39**,** 228-34.

Tian, X., Kai, L., Hockberger, P. E., Wokosin, D. L., Surmeier, D. J., 2010. MEF-2 regulates activity-dependent spine loss in striatopallidal medium spiny neurons. Mol Cell Neurosci. 44**,** 94-108.

Tick, B., Bolton, P., Happe, F., Rutter, M., Rijsdijk, F., 2016. Heritability of autism spectrum disorders: a meta-analysis of twin studies. J Child Psychol Psychiatry. 57**,** 585-95.

Toda, H., Inoue, T., Tsunoda, T., Nakai, Y., Tanichi, M., Tanaka, T., . . . Kusumi, I., 2016. Affective temperaments play an important role in the relationship between childhood abuse and depressive symptoms in major depressive disorder. Psychiatry Res. 236**,** 142-7.

Toma, K., Kumamoto, T., Hanashima, C., 2014. The Timing of Upper-Layer Neurogenesis Is Conferred by Sequential Derepression and Negative Feedback from Deep-Layer Neurons. The Journal of Neuroscience. 34**,** 13259-13276.

Torres-Espinola, F. J., Berglund, S. K., Garcia-Valdes, L. M., Segura, M. T., Jerez, A., Campos, D., . . . Campoy, C., 2015. Maternal Obesity, Overweight and Gestational Diabetes Affect the Offspring Neurodevelopment at 6 and 18 Months of Age--A Follow Up from the PREOBE Cohort. PLoS One. 10**,** e0133010.

Torres, A. R., Sweeten, T. L., Cutler, A., Bedke, B. J., Fillmore, M., Stubbs, E. G., Odell, D., 2006. The association and linkage of the HLA-A2 class I allele with autism. Hum Immunol. 67**,** 346-51.

Torrey, E. F., 1988. Stalking the schizovirus. Schizophr Bull. 14**,** 223-9.

Tourjman, V., Kouassi, E., Koue, M. E., Rocchetti, M., Fortin-Fournier, S., Fusar-Poli, P., Potvin, S., 2013. Antipsychotics' effects on blood levels of cytokines in schizophrenia: a meta-analysis. Schizophr Res. 151**,** 43-7.

Tozuka, Y., Kumon, M., Wada, E., Onodera, M., Mochizuki, H., Wada, K., 2010. Maternal obesity impairs hippocampal BDNF production and spatial learning performance in young mouse offspring. Neurochem Int. 57**,** 235-47.

Tozuka, Y., Wada, E., Wada, K., 2009. Diet-induced obesity in female mice leads to peroxidized lipid accumulations and impairment of hippocampal neurogenesis during the early life of their offspring. Faseb j. 23**,** 1920-34.

Traherne, J. A., 2008. Human MHC architecture and evolution: implications for disease association studies. Int J Immunogenet. 35**,** 179-92.

Traks, T., Koido, K., Balotsev, R., Eller, T., Koks, S., Maron, E., . . . Vasar, V., 2015. Polymorphisms of IKBKE gene are associated with major depressive disorder and panic disorder. Brain Behav. 5**,** e00314.

Traversari, C., Russo, V., 2012. Control of the immune system by oxysterols and cancer development. Curr Opin Pharmacol. 12**,** 729-35.

Trinchieri, G., 1995. Natural killer cells wear different hats: effector cells of innate resistance and regulatory cells of adaptive immunity and of hematopoiesis. Semin Immunol. 7**,** 83-8.

Tsuang, M. T., Stone, W. S., Faraone, S. V., 2001. Genes, environment and schizophrenia. The British Journal of Psychiatry. 178**,** s18-s24.

Tsuchihashi, H., Yamamoto, H., Maeda, K., Ugi, S., Mori, T., Shimizu, T., . . . Tani, T., 2006. Circulating concentrations of adiponectin, an endogenous lipopolysaccharide neutralizing protein, decrease in rats with polymicrobial sepsis. J Surg Res. 134.

Turnley, A. M., Miller, J. F., Bartlett, P. F., 1991a. Regulation of MHC molecules on MBP positive oligodendrocytes in mice by IFN-gamma and TNF-alpha. Neurosci Lett. 123**,** 45-8.

Turnley, A. M., Morahan, G., Okano, H., Bernard, O., Mikoshiba, K., Allison, J., . . . Miller, J. F., 1991b. Dysmyelination in transgenic mice resulting from expression of class I histocompatibility molecules in oligodendrocytes. Nature. 353**,** 566-9.

Turrigiano, G. G., Nelson, S. B., 2004. Homeostatic plasticity in the developing nervous system. Nat Rev Neurosci. 5**,** 97-107.

Tyring, S., Gottlieb, A., Papp, K., Gordon, K., Leonardi, C., Wang, A., . . . Krishnan, R., 2006. Etanercept and clinical outcomes, fatigue, and depression in psoriasis: double-blind placebo-controlled randomised phase III trial. Lancet. 367**,** 29-35.

Ullian, E. M., Sapperstein, S. K., Christopherson, K. S., Barres, B. A., 2001. Control of synapse number by glia. Science. 291**,** 657-61.

Valiente, M., Marin, O., 2010. Neuronal migration mechanisms in development and disease. Current Opinion in Neurobiology. 20**,** 68-78.

Valjent, E., Bertran-Gonzalez, J., Herve, D., Fisone, G., Girault, J. A., 2009. Looking BAC at striatal signaling: cell-specific analysis in new transgenic mice. Trends Neurosci. 32**,** 538-47.

Vallieres, L., Campbell, I. L., Gage, F. H., Sawchenko, P. E., 2002. Reduced hippocampal neurogenesis in adult transgenic mice with chronic astrocytic production of interleukin-6. J Neurosci. 22**,** 486-92.

Van Heek, M., Compton, D. S., France, C. F., Tedesco, R. P., Fawzi, A. B., Graziano, M. P., . . . Davis, H. R., Jr., 1997. Diet-induced obese mice develop peripheral, but not central, resistance to leptin. J Clin Invest. 99**,** 385-90.

van Leeuwen, J. E., Samelson, L. E., 1999. T cell antigen-receptor signal transduction. Curr Opin Immunol. 11**,** 242-8.

Van Lieshout, R. J., Robinson, M., Boyle, M. H., 2013. Maternal pre-pregnancy body mass index and internalizing and externalizing problems in offspring. Can J Psychiatry. 58**,** 151-9.

Van Lieshout, R. J., Voruganti, L. P., 2008. Diabetes mellitus during pregnancy and increased risk of schizophrenia in offspring: a review of the evidence and putative mechanisms. J Psychiatry Neurosci. 33**,** 395-404.

van Os, J., Selten, J. P., 1998. Prenatal exposure to maternal stress and subsequent schizophrenia. The May 1940 invasion of The Netherlands. The British Journal of Psychiatry. 172**,** 324-326.

van Zuiden, M., Kavelaars, A., Amarouchi, K., Maas, M., Vermetten, E., Geuze, E., Heijnen, C. J., 2012. IL-1beta reactivity and the development of severe fatigue after military deployment: a longitudinal study. J Neuroinflammation. 9**,** 205.

Vanbesien-Mailliot, C. C., Wolowczuk, I., Mairesse, J., Viltart, O., Delacre, M., Khalife, J., . . . Maccari, S., 2007. Prenatal stress has pro-inflammatory consequences on the immune system in adult rats. Psychoneuroendocrinology. 32**,** 114-24.

Vincent, A., Bien, C. G., 2008. Anti-NMDA-receptor encephalitis: a cause of psychiatric, seizure, and movement disorders in young adults. Lancet Neurol. 7**,** 1074-5.

Vitkovic, L., Bockaert, J., Jacque, C., 2000. "Inflammatory" cytokines: neuromodulators in normal brain? J Neurochem. 74**,** 457-71.

Vitoratos, N., Salamalekis, E., Kassanos, D., Loghis, C., Panayotopoulos, N., Kouskouni, E., Creatsas, G., 2001. Maternal Plasma Leptin Levels and Their Relationship to Insulin and Glucose in Gestational-Onset Diabetes. Gynecologic and Obstetric Investigation. 51**,** 17-21.

Vogt, M. C., Paeger, L., Hess, S., Steculorum, S. M., Awazawa, M., Hampel, B., . . . Bruning, J. C., 2014. Neonatal insulin action impairs hypothalamic neurocircuit formation in response to maternal high-fat feeding. Cell. 156**,** 495-509.

Voineagu, I., Wang, X., Johnston, P., Lowe, J. K., Tian, Y., Horvath, S., . . . Geschwind, D. H., 2011. Transcriptomic analysis of autistic brain reveals convergent molecular pathology. Nature. 474**,** 380-384.

Voineskos, A. N., Felsky, D., Kovacevic, N., Tiwari, A. K., Zai, C., Chakravarty, M. M., . . . Kennedy, J. L., 2013. Oligodendrocyte genes, white matter tract integrity, and cognition in schizophrenia. Cereb Cortex. 23**,** 2044-57.

Voorhees, J. L., Tarr, A. J., Wohleb, E. S., Godbout, J. P., Mo, X., Sheridan, J. F., . . . Marsh, C. B., 2013. Prolonged restraint stress increases IL-6, reduces IL-10, and causes persistent depressive-like behavior that is reversed by recombinant IL-10. PLoS One. 8**,** e58488.

Voronov, E., Shouval, D. S., Krelin, Y., Cagnano, E., Benharroch, D., Iwakura, Y., . . . Apte, R. N., 2003. IL-1 is required for tumor invasiveness and angiogenesis. Proc Natl Acad Sci U S A. 100**,** 2645-50.

Vos, T., Flaxman, A. D., Naghavi, M., Lozano, R., Michaud, C., Ezzati, M., . . . Memish, Z. A., 2012. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 380**,** 2163-96.

Vuillermot, S., Weber, L., Feldon, J., Meyer, U., 2010. A Longitudinal Examination of the Neurodevelopmental Impact of Prenatal Immune Activation in Mice Reveals Primary Defects in Dopaminergic Development Relevant to Schizophrenia. The Journal of Neuroscience. 30**,** 1270-1287.

Walder, D. J., Laplante, D. P., Sousa-Pires, A., Veru, F., Brunet, A., King, S., 2014. Prenatal maternal stress predicts autism traits in 6(1/2) year-old children: Project Ice Storm. Psychiatry Res. 219**,** 353-60.

Walshe, J., Mason, I., 2003. Unique and combinatorial functions of Fgf3 and Fgf8 during zebrafish forebrain development. Development. 130**,** 4337-49.

Walters, J. T. R., Rujescu, D., Franke, B., Giegling, I., Vásquez, A. A., Hargreaves, A., . . . Owen, M. J., 2013. The Role of the Major Histocompatibility Complex Region in Cognition and Brain Structure: A Schizophrenia GWAS Follow-Up. American Journal of Psychiatry. 170**,** 877-885.

Wang, F., Flanagan, J., Su, N., Wang, L. C., Bui, S., Nielson, A., . . . Luo, Y., 2012. RNAscope: a novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues. J Mol Diagn. 14**,** 22-9.

Wang, H., Yu, M., Ochani, M., Amella, C. A., Tanovic, M., Susarla, S., . . . Tracey, K. J., 2003. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. Nature. 421**,** 384-8.

Wang, Q., Yang, C., Gelernter, J., Zhao, H., 2015. Pervasive pleiotropy between psychiatric disorders and immune disorders revealed by integrative analysis of multiple GWAS. Human Genetics. 134**,** 1195-1209.

Ward, A. J., 1990. A comparison and analysis of the presence of family problems during pregnancy of mothers of "autistic" children and mothers of normal children. Child Psychiatry and Human Development. 20**,** 279-288.

Warren, R. P., Burger, R. A., Odell, D., Torres, A. R., Warren, W. L., 1994. Decreased plasma concentrations of the C4B complement protein in autism. Arch Pediatr Adolesc Med. 148**,** 180-3.

Warren, R. P., Foster, A. N. N., Margaretten, N. C., 1987. Reduced Natural Killer Cell Activity in Autism. Journal of the American Academy of Child & Adolescent Psychiatry. 26**,** 333-335.

Warren, R. P., Singh, V. K., Cole, P., Odell, J. D., Pingree, C. B., Warren, W. L., White, E., 1991. Increased frequency of the null allele at the complement C4b locus in autism. Clin Exp Immunol. 83**,** 438-40.

Watanabe, Y., Nunokawa, A., Shibuya, M., Kaneko, N., Nawa, H., Someya, T., 2008. Association study of interleukin 2 (IL2) and IL4 with schizophrenia in a Japanese population. Eur Arch Psychiatry Clin Neurosci. 258**,** 422-7.

Watson, C. G., Kucala, T., Tilleskjor, C., Jacobs, L., 1984. Schizophrenic birth seasonality in relation to the incidence of infectious diseases and temperature extremes. Arch Gen Psychiatry. 41**,** 85-90.

Watson, J. B., Mednick, S. A., Huttunen, M., Wang, X., 1999. Prenatal teratogens and the development of adult mental illness. Dev Psychopathol. 11**,** 457-66.

Wegelius, A., Tuulio-Henriksson, A., Pankakoski, M., Haukka, J., Lehto, U., Paunio, T., . . . Suvisaari, J., 2011. An association between high birth weight and schizophrenia in a Finnish schizophrenia family study sample. Psychiatry Res. 190**,** 181-6.

Wei, H., Zou, H., Sheikh, A. M., Malik, M., Dobkin, C., Brown, W. T., Li, X., 2011. IL-6 is increased in the cerebellum of autistic brain and alters neural cell adhesion, migration and synaptic formation. Journal of Neuroinflammation. 8**,** 1-10.

Weiss, L. A., Arking, D. E., Daly, M. J., Chakravarti, A., 2009. A genome-wide linkage and association scan reveals novel loci for autism. Nature. 461**,** 802-8.

Wermter, A. K., Laucht, M., Schimmelmann, B. G., Banaschewski, T., Sonuga-Barke, E. J., Rietschel, M., Becker, K., 2010. From nature versus nurture, via nature and nurture, to gene x environment interaction in mental disorders. Eur Child Adolesc Psychiatry. 19**,** 199-210.

Whiteman, V. E., Salemi, J. L., Mejia De Grubb, M. C., Ashley Cain, M., Mogos, M. F., Zoorob, R. J., Salihu, H. M., 2015. Additive effects of Pre-pregnancy body mass index and gestational diabetes on health outcomes and costs. Obesity (Silver Spring). 23**,** 2299-308.

Wilk, S., Scheibenbogen, C., Bauer, S., Jenke, A., Rother, M., Guerreiro, M., . . . Skurk, C., 2011. Adiponectin is a negative regulator of antigen-activated T cells. European Journal of Immunology. 41**,** 2323-2332.

Willi, R., Harmeier, A., Giovanoli, S., Meyer, U., 2013. Altered GSK3β signaling in an infection-based mouse model of developmental neuropsychiatric disease. Neuropharmacology. 73**,** 56-65.

Williams, J. M., Pearce, W. J., 2006. Age-dependent modulation of endothelium-dependent vasodilatation by chronic hypoxia in ovine cranial arteries. J Appl Physiol (1985). 100**,** 225-32.

Winter, C., Djodari-Irani, A., Sohr, R., Morgenstern, R., Feldon, J., Juckel, G., Meyer, U., 2009. Prenatal immune activation leads to multiple changes in basal neurotransmitter levels in the adult brain: implications for brain disorders of neurodevelopmental origin such as schizophrenia. Int J Neuropsychopharmacol. 12**,** 513-24.

Workman, A. D., Charvet, C. J., Clancy, B., Darlington, R. B., Finlay, B. L., 2013. Modeling Transformations of Neurodevelopmental Sequences across Mammalian Species. Journal of Neuroscience. 33**,** 7368-83.

Wray, N. R., Gottesman, II, 2012. Using summary data from the danish national registers to estimate heritabilities for schizophrenia, bipolar disorder, and major depressive disorder. Front Genet. 3**,** 118.

Wright, P., Sham, P. C., Gilvarry, C. M., Jones, P. B., Cannon, M., Sharma, T., Murray, R. M., 1996. Autoimmune diseases in the pedigrees of schizophrenic and control subjects. Schizophrenia Research. 20**,** 261-267.

Wu, Y., Wang, F., Fu, M., Wang, C., Quon, M. J., Yang, P., 2015. Cellular Stress, Excessive Apoptosis, and the Effect of Metformin in a Mouse Model of Type 2 Diabetic Embryopathy. Diabetes. 64**,** 2526-36.

Wynn, T. A., 2003. IL-13 effector functions. Annu Rev Immunol. 21**,** 425-56.

Xiang, A. H., Wang, X., Martinez, M. P., Walthall, J. C., Curry, E. S., Page, K., . . . Getahun, D., 2015. Association of maternal diabetes with autism in offspring. Jama. 313**,** 1425-34.

Xu, G., Jing, J., Bowers, K., Liu, B., Bao, W., 2014. Maternal diabetes and the risk of autism spectrum disorders in the offspring: a systematic review and meta-analysis. J Autism Dev Disord. 44**,** 766-75.

Yaka, R., Salomon, S., Matzner, H., Weinstock, M., 2007. Effect of varied gestational stress on acquisition of spatial memory, hippocampal LTP and synaptic proteins in juvenile male rats. Behav Brain Res. 179**,** 126-32.

Yamada, K., Hattori, E., Iwayama, Y., Toyota, T., Iwata, Y., Suzuki, K., . . . Yoshikawa, T., 2015. Population-dependent contribution of the major histocompatibility complex region to schizophrenia susceptibility. Schizophr Res. 168**,** 444-9.

Yamada, K., Iida, R., Miyamoto, Y., Saito, K., Sekikawa, K., Seishima, M., Nabeshima, T., 2000. Neurobehavioral alterations in mice with a targeted deletion of the tumor necrosis factor-alpha gene: implications for emotional behavior. J Neuroimmunol. 111**,** 131-8.

Yamashita, Y., Fujimoto, C., Nakajima, E., Isagai, T., Matsuishi, T., 2003. Possible association between congenital cytomegalovirus infection and autistic disorder. J Autism Dev Disord. 33**,** 455-9.

Yanai, H., Ban, T., Wang, Z., Choi, M. K., Kawamura, T., Negishi, H., . . . Taniguchi, T., 2009. HMGB proteins function as universal sentinels for nucleic-acid-mediated innate immune responses. Nature. 462**,** 99-103.

Yee, N., Schwarting, R. K., Fuchs, E., Wohr, M., 2012. Increased affective ultrasonic communication during fear learning in adult male rats exposed to maternal immune activation. J Psychiatr Res. 46**,** 1199-205.

Yirmiya, R., Pollak, Y., Morag, M., Reichenberg, A., Barak, O., Avitsur, R., . . . Pollmacher, T., 2000. Illness, cytokines, and depression. Ann N Y Acad Sci. 917**,** 478-87.

Yirmiya, R., Winocur, G., Goshen, I., 2002. Brain interleukin-1 is involved in spatial memory and passive avoidance conditioning. Neurobiol Learn Mem. 78**,** 379-89.

Yokota, T., Oritani, K., Takahashi, I., Ishikawa, J., Matsuyama, A., Ouchi, N., . . . Matsuzawa, Y., 2000. Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. Blood. 96**,** 1723-1732.

Yue, W. H., Wang, H. F., Sun, L. D., Tang, F. L., Liu, Z. H., Zhang, H. X., . . . Zhang, D., 2011. Genome-wide association study identifies a susceptibility locus for schizophrenia in Han Chinese at 11p11.2. Nat Genet. 43**,** 1228-31.

Zalcman, S., Murray, L., Dyck, D. G., Greenberg, A. H., Nance, D. M., 1998. Interleukin-2 and -6 induce behavioral-activating effects in mice. Brain Res. 811**,** 111-21.

Zarubin, T., Han, J., 2005. Activation and signaling of the p38 MAP kinase pathway. Cell Res. 15**,** 11-18.

Zerbo, O., Qian, Y., Yoshida, C., Grether, J. K., Van de Water, J., Croen, L. A., 2015. Maternal Infection During Pregnancy and Autism Spectrum Disorders. J Autism Dev Disord. 45**,** 4015-25.

Zetterstrom, R., 2004. Iron deficiency and iron deficiency anaemia during infancy and childhood. Acta Paediatr. 93**,** 436-9.

Zhang, A., Yu, H., He, Y., Shen, Y., Zhang, Y., Liu, J., . . . Zhang, J., 2015. Developmental expression and localization of MHC class I molecules in the human central nervous system. Exp Brain Res. 233**,** 2733-43.

Zhang, A. J. X., To, K. K. W., Li, C., Lau, C. C. Y., Poon, V. K. M., Chan, C. C. S., . . . Yuen, K.-Y., 2013. Leptin Mediates the Pathogenesis of Severe 2009 Pandemic Influenza A(H1N1) Infection Associated With Cytokine Dysregulation in Mice With Diet-Induced Obesity. Journal of Infectious Diseases. 207**,** 1270-1280.

Zhang, Y., Taveggia, C., Melendez-Vasquez, C., Einheber, S., Raine, C. S., Salzer, J. L., . . . John, G. R., 2006. Interleukin-11 potentiates oligodendrocyte survival and maturation, and myelin formation. J Neurosci. 26**,** 12174-85.

Zhang, Y., Zhang, Y., Gu, W., He, L., Sun, B., Th1/Th2 Cell's Function in Immune System. In: B. Sun, (Ed.), T Helper Cell Differentiation and Their Function. Springer Netherlands, Dordrecht, 2014, pp. 45-65.

Zhang, Y. H., Lu, J., Elmquist, J. K., Saper, C. B., 2003. Specific roles of cyclooxygenase-1 and cyclooxygenase-2 in lipopolysaccharide-induced fever and Fos expression in rat brain. J Comp Neurol. 463**,** 3-12.

Zhang, Z., van Praag, H., 2015. Maternal immune activation differentially impacts mature and adult-born hippocampal neurons in male mice. Brain Behav Immun. 45**,** 60-70.

Zhang, Z. W., Burke, M. W., Calakos, N., Beaulieu, J. M., Vaucher, E., 2010. Confocal Analysis of Cholinergic and Dopaminergic Inputs onto Pyramidal Cells in the Prefrontal Cortex of Rodents. Front Neuroanat. 4**,** 21.

Zhou, X., Michal, J. J., Zhang, L., Ding, B., Lunney, J. K., Liu, B., Jiang, Z., 2013. Interferon induced IFIT family genes in host antiviral defense. Int J Biol Sci. 9**,** 200-8.

Zimmerman, A. W., Connors, S. L., 2010. Maternal Influences on Fetal Neurodevelopment: Clinical and Research Aspects. Springer Science & Business Media.

Zorrilla, E. P., Luborsky, L., McKay, J. R., Rosenthal, R., Houldin, A., Tax, A., . . . Schmidt, K., 2001. The relationship of depression and stressors to immunological assays: a meta-analytic review. Brain Behav Immun. 15**,** 199-226.

Zorumski, C. F., Paul, S. M., Izumi, Y., Covey, D. F., Mennerick, S., 2013. Neurosteroids, stress and depression: potential therapeutic opportunities. Neurosci Biobehav Rev. 37**,** 109-22.