# The Effect of Gestational Immune Activation on Estrogen Receptor Alpha Levels in the Medial Preoptic Area and Bed Nucleus of the Stria Terminalis of the Maternal Rat Brain

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# Contents

## ABSTRACT

Maternal Immune Activation (MIA) has been widely used as a model for the development of neurological disorders in offspring in large part due to the effect of neuroinflammation. Despite the multitude of research that explores direct effects on offspring, relatively little is known about the effects on the maternal brain.

Studies have demonstrated that the frequency and quality of maternal care with which a mother provides her offspring can significantly affect the behavioral and neurological development of her offspring. Maternal behavior is primarily regulated by the action of estrogen with its nuclear receptor, ER-α, in key brain regions like the media preoptic area (MPOA) and bed nucleus of the stria terminalis (BNST). Furthermore, environmental enrichment (EE) has been shown to be neuroprotective following exposure to a stressor and is used as a model to counteract the deleterious effect of neuroinflammation. The question is then posed whether MIA-induced neuroinflammation has the capacity to cause a reduction in ER-α levels in the MPOA and BNST which could impact the quality of maternal care provided to offspring; moreover, does EE protect against this purported damage.

Nulliparous female Sprague-Dawley rats housed in varying levels of environmental complexity were mated, and on gestational day 11, dams were administered an intraperitoneal injection of 100 µg/kg LPS or equivalent volume of saline. At postpartum day 21 dams were sacrificed, brains were collected and were prepared for immunohistochemical labeling of ER-α positive cells in the MPOA and BNST.

MIA caused a significant decrease in ER-α in the BNST (but not the MPOA), a loss which was prevented in dams exposed to EE. These results suggest that MIA may negatively impact the maternal brain which can potentially result in altered levels of maternal care, ultimately affecting the development of offspring.

*L'activation immunitaire maternelle (AIM) a été largement utilisée comme modèle pour mimer des troubles se développement neurologique chez la progéniture, et ceci, à cause de l'effet de la neuroinflammation. Malgré toutes les études qui explorent les effets directs de celle-ci sur la progéniture, on en connait relativement peu sur les effets de l'AIM sur le cerveau maternel.*

*Des études ont démontré que la fréquence et la qualité des soins maternels peuvent affecter de façon significative le développement comportemental et neurologique de sa progéniture. Le comportement maternel est principalement régulé par l'action des oestrogènes à leur récepteur nucléaire, soit le ER-α, et ce, dans des régions cérébrales comme l'aire préoptique médiane (MPOA) et le noyau du lit de la strie terminale (BNST). De plus, il a été démontré que l'enrichissement environnemental (EE) serait neuroprotecteur suite à un stress, et sert de modèle pour opposer les effets cérébraux de la neuroinflammation. La question qui demande d'être posée est donc de savoir si la neuroinflammation induite par l'AIM est capable de réduire les niveaux de ER-α dans la MPOA et le BNST, ce qui pourrait avoir un impact sur la qualité des soins maternels apportés à la progéniture; de plus, nous voulons savoir si l'exposition à EE protègerait contre l'endommagement potential du cerveau.*

*Des rates de souche Sprague-Dawley, nullipares ont été hébergées dans des environnements à divers niveaux de complexité. Elles ont été accouplées etlors du 11ième jour gestationnel, elles ont reçu du LPS (100 µg / kg) ou un volume équivalent de solution saline par*

*injection péritonéale. Au jour 21 postnatal, les mères ont été sacrifiées, les cerveaux prélevés et préparés pour le marquage immunohistochimique des ER-α.*

*Nos résultats démontrent que l'AIM a provoqué une réduction significative du nombre de ER-α dans le BNST (mais pas dans la MPOA), une perte qui a été contrée dans le cerveau des mères exposées à EE. Ces résultats suggèrent que l'AIM peut avoir un impact négatif sur le cerveau maternel, ce qui peut éventuellement entraîner une modification à la qualité de soins maternels, ce qui peut, en fin de compte, affecter le neurodéveloppement de la progéniture.*

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## ABBREVIATIONS

ACC: animal care control
BNST: bed nucleus of the stria terminalis
CORT: corticosterone
DBS: deep brain stimulation
EE: environmental enrichment
ER-α/β: estrogen receptor alpha/beta
FDA: Food and Drug Administration
IFN: interferon
IL: interleukin
LPS: lipopolysaccharide
MIA: maternal immune activation
MPOA: medial preoptic area
SE: social enrichment
TLR: toll-like receptor
TNF-α: tumor necrosis factor alpha

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**Figure 8. Representative pictographs of the BNST:** ER-α immunopositive cells visualized at a 400x in the BNST of dams housed in ACC, SE and EE conditions when treated with saline (a, b, c) and LPS (d, e, f), respectively.

**Figure 9. Mean ER-α levels in the MPOA:** ER-α levels represented by mean ± SEM in the MPOA of dams housed in ACC, SE and EE conditions. A Treatment by Housing interaction was detected ($F_{2,35}$=4.07; $p<0.05$); simple effects did not detect any specific pairwise Treatment differences at each Housing condition.

**Figure 10. Mean ER-α levels in the BNST:** ER-α levels represented by mean ± SEM in the BNST of dams housed in ACC, SE and EE conditions. A significant interaction between Treatment and Housing was detected ($F_{2,35}$=3.93; $p<0.05$). A difference between saline and LPS treated dams in the number of ER-α positive cells was only seen in the BNST in ACC housed dams ($F_{1,35}$=16.50;$p < 0.05$).

## 1. BACKGROUND

As time moves on, research continues to show the vast amount of factors that exist which affect our health and well-being. From early childhood experiences, to socio-economic status, exposure to environmental factors and genetics, it seems that every one of our experiences plays some part in shaping how we respond to, adapt and recover from stressors. Plenty of research has looked at the transgenerational effect of these types of exposures and experiences during the vulnerable period of pregnancy, as maternal exposure to certain stressors during this time can have a direct impact on developing offspring. Particular interest has been placed on maternal immune activation (MIA), where a female is exposed to either a viral or bacterial infection at a particular time during pregnancy in order to observe how the offspring are affected. However, little research has explored the effects on the mother during this delicate time, in particular what effects immune activation can have on brain plasticity during pregnancy and the postpartum period and how this could impact behavior. Thus, this study aims to shed light on this missing piece of the puzzle in order to provide a better understanding of how the maternal brain may be impacted by gestational immune activation to hopefully serve as a foundation for future behavioral studies.

### *1.1 Immune Activation*

When exposed to a foreign pathogen, the body is capable of initiating a cascade of responses as part of either the innate or adaptive immune system. The innate immune system, as the name suggests, is an inherent form of protection our bodies employ upon invasion of a pathogen. It is the first line of defense and is responsible for distinguishing the body's cells from foreign invaders, generalizing and attacking anything considered "non-self". This type of immunity involves various lines of defense against pathogens, including the use of physical and chemical barriers such as the skin and mucous membranes, the recruitment of immune cells such

as neutrophils and macrophages to infection sites and the activation of the adaptive immune system.

The innate immune system's cellular defence involves the recognition of pathogens by dendritic cells, neutrophils and macrophages. Pattern recognition receptors (PRR's) such as Toll-like receptors (TLR's) on these immune cells are able to recognize certain structures present on the surface of pathogens called pathogen-associated molecular patterns (PAMP's) (Blasius & Beutler, 2010; Kawai & Akira, 2010; Takeuchi & Akira, 2010). The innate immune system thereby can distinguish self from non-self via these patterns, albeit in a non-specific manner, enough so to identify that a bacterial, viral or parasitic breach has occurred.

When TLR's and other PAMP's on immune cells recognize a foreign pathogen, an immune response is quickly initiated. This involves the activation of the complement system which triggers a cascade of cell signalling to fight off the pathogen (Blasius & Beutler, 2010; Kumar, Kawai & Akira, 2009). Cellular signalling by TLR's on dendritic cells and macrophages in particular can trigger the transcription and production of type 1 interferons and other cytokines (Kumar et al. 2009). These TLR's are particularly capable of recognizing bacterial infections, seen by TLR's high affinity for LPS, a component of the cell wall of gram-negative bacteria (Hoshino et al. 1999; Poltorak et al. 1998).

Whereas the innate immune system recognizes PAMPs on "non-self" pathogens in a generalized manner, the adaptive immune system recognizes antigens on pathogens that allow a unique response to be produced to help combat a specific invader. In adaptive immunity, B and T lymphocytes produce either a humoral (antibody) or cell mediated response, respectively, to target a specific invader. Though slow to initiate during the first exposure to a pathogen, gene rearrangement occurs in B and T cells which is passed down to future cell lines allowing for a

stored library of responses that can be quickly triggered upon future exposure to the same pathogen (Iwasaki & Medzhitov, 2010; Hoebe et al. 2004).

The innate immune system works in tandem with the adaptive immune system, in that dendritic cells and macrophages of the innate immune system present pathogenic antigens on their cell membrane after engulfment; these antigens are combined with the immune cell's major histocompatibility complex (MHC) so that they can then be presented to and "read" by T cells, priming them and triggering an adaptive immune response (Iwasaki & Medzhitov, 2010). Once cytotoxic T cells are activated, they travel through the body and trigger lysis in cells displaying these antigen/MHC complexes. Some of these T cells remain after an infection and become memory cells so that a quick response can be initiated upon future infection by the same pathogen. B cells also recognize antigens from pathogens, however, unlike T cells which must be presented with an antigen/MHC complex by an antigen-presenting cell, B cells can recognize antigens in their natural state on the pathogens. Specific antibody receptors are produced on the B cells – once these receptors identify the presence of the pathogen, B cells can agglutinate the invaders, initiate cell lysis and inflammation, and facilitate phagocytosis by macrophages. Similar to T cells, a small percentage of B cells remain after infection to mount a quick response to specific pathogens upon future infection.

### *1.2 Cytokines*

Depending on the type and extent of infection, an acute, systemic inflammatory response may be produced. Both cell-signalling during the innate immune response and B and T cells in the adaptive response can activate pro-inflammatory cytokine production. While cytokines can refer to a wide range of compounds, those that are often referred to when discussing their involvement with the inflammatory response are tumor necrosis factor alpha (TNF-α),

interleukins (IL's) and interferons (IFN's) (Abram et al. 2000). These cytokines are essentially signalling compounds that act as part of the cascade to regulate the release and recruitment of mediators and immune cells to protect against a pathogen and are involved in the inflammatory response to infection.

**Tumor Necrosis Factor alpha (TNF-α)** is largely produced by macrophages, although it can be secreted by a number of other immune cells (Flynn et al. 1995; Pfeffer et al. 1993). Macrophages quickly respond to an infection to initiate their phagocytic abilities, and upon doing so, they release TNF-α which is one of the first compounds produced to regulate the inflammatory response cascade (Maini et al. 1995; Feldman et al. 1994). Like other cytokines, TNF-α increases vascular permeability and improves the ability of neutrophils to be recruited to a site of infection, which is one of the hallmarks of inflammation (Janeway et al. 2001). Interestingly, macrophages themselves are stimulated by TNF-α through their TLR's to further release more TNF-α, thereby upregulating the release of this inflammatory marker (Parameswaran & Patial, 2010). A sustained inflammatory response can be produced when TNF-α works in conjunction with interferon-γ (IFN-γ), as they act together to trigger the transcription of inflammatory marker genes and increase sensitivity of macrophages to TNF-α, thereby perpetuating the production and maintenance of the inflammatory response (Yarilina et al. 2008).

**Interferons (IFNs)** are produced by immune cells to amplify the antiviral abilities of nearby cells to mitigate the replication and spread of a viral infection (Isaacs-Alick et al. 1957). They aid in the recruitment of macrophages and natural killer cells of the innate immune system and can facilitate the expression of antigen/MHC complexes for targeted destruction by T cells of the adaptive immune system (Parkin &Cohen, 2001). Similar to TNF-α, IFN's further stimulate the release of cytokines by immune cells (McNab et al. 2015; Ivashkiv & Donlin,

2014). Interferons are grouped into type 1, type 2 and type 3; IFN-γ is the sole interferon in type 2 and is largely responsible for the inflammatory response through recruiting macrophages and amplifying their activity (McNab et al. 2015; Mao et al. 2013; Mishra et al. 2013; Medzhitov, 2008). The release of both type 1 and 2 interferons can be stimulated by other cytokines or in the presence of bacterial or viral markers (viral RNA for example); their anti-viral properties aid in recognizing and targeting pathogens as well as promote inflammation through recruitment and activation of macrophages (Schneider et al. 2014; Iwasaki, 2012; Trinchier, 2010). TLR's on macrophages and dendritic cells are widely responsible for the recognition of pathogenic PAMPs that ultimately lead to the transcription and production of interferons and up-regulate pro-inflammatory cytokine production (Fang et al. 2012; Kawai & Akira, 2009; Apostolou & Thanos, 2008).

**Interleukins (ILs)** are produced by immune cells such as T lymphocytes and macrophages. Some, like IL-1 and IL-2, help activate B and T cells to quickly respond to an infection, while others such as IL-6 along with IL-1 are involved in the inflammatory response. Other interleukins can upregulate the production of T cells as well as antibodies by B cells (Park et al. 2005; Mizel, 1989)

Interleukins can be distinguished as having either anti-inflammatory or pro-inflammatory roles in the immune response. Anti-inflammatory interleukins (IL-4, 9, 10 and 13 for example) typically exert their effect by inhibiting cytokine production in helper T cells and macrophages and regulate the secretion of INF-γ and TNF-α, the two potent pro-inflammatory cytokines previously discussed (Godfrained et al. 1998; Paul, 1991).

IL-1 and IL-6 are interleukins that are widely recognized as the pro-inflammatory variant (Di Paolo & Shayakhmenov 2016). IL-1α/β stimulates recruitment of neutrophils and promotes

inflammation in response to pathogenic invasion, whereas IL-6 can have both anti-inflammatory and pro-inflammatory properties (Scheller et al. 2011). The action of IL-6 is dependent on how it is recognized in circulation. Classic signalling involves IL-6 binding to IL-6 receptors directly on cells which is associated with IL-6's anti-inflammatory effects, while trans-signalling sees IL-6 binding to a soluble form of its receptor with the complex then acting on cells, producing pro-inflammatory effects (Scheller et al. 2011). As few cells actually have IL-6 receptors, the trans-signalling method allows for a vastly wider range of cells to be activated by IL-6, meaning that the pro-inflammatory properties of this cytokine are more widely employed (Scheller et al. 2011).

These pro-inflammatory cytokines, particularly IL-1, IL-12, IL-18, IFN-γ and TNF-α, are typically produced by macrophages, mast cells and by B and T cells (Cavaillon, 2001; Isaacs-Alick et al. 1957). As part of the inflammatory response these cytokines produce, vascular permeability is increased and immune cells such as neutrophils and macrophages are recruited to the site of infection (Wilkins & Gale, 2010). Signalling by cytokines can also trigger the release of more cytokines through gene transcription thereby amplifying the inflammatory response; oxidative stress can result from extensive inflammation (Khansari et al. 2009), which in itself contributes to further cytokine production, creating a positive feedback/up-regulation of inflammatory markers and a potentially chronic state of inflammation (Chokkalingam et al. 2013; David et al.2007; Carpenter et al. 2002; Vlahopoulos et al. 1999).

### *1.3 Immune Activation in the CNS*

Once this systemic immune response is produced, circulating cytokines can communicate this to the brain, ultimately leading to immune activation within the central nervous system. Nerve pathways are the quickest route of transmitting this message to the brain, although this can

also occur through humoral and endocrine pathways (Konsman et al. 2002). The gut-brain axis has also been shown to be a supporting route, with the gut microbiome acting as an effective messenger signalling the initiation of an immune response to the brain.

Cytokines are also capable of passing through the blood brain barrier to act directly on brain areas like the hypothalamus, which regulates the peripheral immune response through neuroendocrine pathways (Dantzer et al. 2008; Otmishi et al. 2008). The HPA-axis plays a role in the immune response acting as a regulating factor. Upon stimulation by cytokines, the adrenal glands produce cortisol and other glucocorticoids that are released into circulation. These act to counter the immune response; in particular, glucocorticoids can inhibit T–cell activity and prevent their interleukin-stimulated proliferation (Patterson et al. 2013), as well as inhibit the immune response triggered by cytokines by decreasing vasodilation and vascular permeability (Perretti & Ahluwalia, 2000). As in the periphery, cytokines like TNF- α and IL-1β are capable of stimulating their own transcription and that of the other in the CNS, consequently magnifying their effect (Skelly et al. 2013). Cytokines are also centrally produced by mast cells, astrocytes and inflammatory-phenotypic microglia (Kohman & Rhodes, 2013; Ransohoff & Brown, 2012; Skaper et al. 2012). Activation of the immune response in the CNS can lead to observable behavioral changes known as sickness behaviors in animals, which include changes in body temperature, social withdrawal, fatigue, and decreased motor activity (Kohman & Rhodes, 2013).

The induction of pro-inflammatory cytokines in the CNS can lead to neuroinflammation, which can have significant consequences on brain chemistry and development. Microglia that display an inflammatory phenotype contribute to amplifying this immune response by further releasing pro-inflammatory cytokines and increasing the size of their cell body (Kettenmann et

al. 2011). The combination of centrally-produced pro-inflammatory cytokines and inflammatory microglia can lead to neuroinflammation, which impairs proper brain development by decreasing neurogenesis, neuron survival, dendritic volume, and leads to fewer synaptic processes as seen in animal studies observing the impact of immune activation via LPS exposure on adult neurogenesis (Valero et al. 2014; Bastos et al. 2008; Ekdahl et al. 2003). The dangers of neuroinflammation caused by an immune response vary depending on which brain areas are damaged by inflammation - a host of neurological disorders can develop in offspring when exposed *in utero* to immune stress, and mothers are vulnerable to changes to their brain chemistry and behavior (Lombardo et al. 2017; Weir et al. 2015; Hsiao et al. 2012; Meyer & Feldon, 2012).

Neuroinflammation should not be considered to be the same as the inflammatory response that occurs in the periphery – rather, neuroinflammation is unique in the sense that it is a result of microglial activation in response to infection. As reviewed by Streit, Mrak and Griffin (2004), inflammatory microglial activation is central to the immune response in the CNS. These activated microglia have the capacity to produce cytokines centrally, exacerbating the inflammatory response by potentially damaging healthy neurons and oligodendrocytes despite the purpose of cytokines being to mitigate the spread of infection (Streit & Kincaid-Colton, 1995). In addition, the activation of microglia can contribute to oxidative stress by releasing oxygen and nitrate free radicals, adding to the cumulative inflammatory damage to the brain (Tanaka et al. 2006; Sugino et al. 1987).

Acute neuroinflammation can result from microglial activation without a damaged or compromised blood-brain barrier (BBB). In the presence of minor insults to the brain, such as the inflammatory response caused by cytokines crossing the BBB, rapid microglial activation

can occur with the potential result of neuroinflammation even in the absence of BBB breakdown or leukocytic infiltration, which in itself could cause more significant damage (Sroga et al. 2003; Streit et al. 1998; Ito et al. 1997; Kreutzberg et al. 1996). These studies reflect the benefits of microglial activation and the inflammatory response following severe injury and leukocytic infiltration as they restrict the spread of infection; however, in the case of a minor insult, the situation does not warrant such a strong response. In these scenarios the negative effects of neuroinflammation outweigh the benefits as the brain may ultimately suffer from an unnecessary, exaggerated immune response. This should be contrasted with chronic neuroinflammation, in which a vicious cycle of cytokine release and microglial activation can cause a persistent state of neuroinflammation which is believed to play a role in major neurodegenerative diseases such as Parkinson's, Alzheimer's and Huntington's diseases (Akiyama et al. 2000).

### *1.4 Animal Models of Maternal Immune Activation*

MIA has been used extensively as a model for the development of neurodevelopmental disorders in offspring such as depression, autism and schizophrenia. Gestational MIA can impair or interrupt fetal brain development via humoral and neural mechanisms by which the immune response is communicated from mother to fetus (Oskvig et al. 2012; Zaretsky et al. 2004). Although the exact mechanisms underlying such disruptions are not well understood, it is postulated that the crossing of cytokines through the fetal blood brain barrier may be one such mechanism by which increased brain size, dysregulated genetic expression and changes in dendritic structure can occur (Lombardo et al. 2017; Weir et al. 2015; Le Belle et al. 2014; Saunders et al. 2012). This disruption can contribute to the development of the aforementioned disorders depending on the timing of the MIA.

There are a number of models by which MIA can be induced in animals. These mechanisms typically involve either live microorganisms to cause illness, or immunogenic compounds that, while not live, are capable of triggering an immune response in the host. There are advantages and disadvantages to both types of mechanisms.

*i) Live Microorganisms*

Experimental exposure to live organisms such as the influenza virus produces illness mimicking what would occur naturally and over a similar timeframe; the resultant immune activation is more representative of the typical course of an illness (Ronovsky et al. 2012). This also allows for experimental exposure to specific pathogens such as the human influenza virus or cytomegalovirus in order to observe pathogen-specific immune responses (Barry et al. 2006; Shi et al. 2003). However, models using live microorganisms typically produce longer periods of illness which removes some experimental control for researchers wanting to isolate the immune activation to a specific time point (Harvey & Boksa, 2012).

*ii) Immunogenic Compounds*

Immunogenic compounds are the counterpart to using live microorganisms in that they are typically easier to use without requiring specialized equipment and produce shorter periods of illness, usually between 1-2 days, allowing for tighter control over the timing of immune activation (Ronovsky et al. 2012; Cunningham et al.2007; Meyer et al.2005). However, while they do trigger an acute immune response, they cannot perfectly simulate the normal course of an infection as would be produced when using live microorganisms.

*iii) Turpentine*

Turpentine is an immunogenic compound administered intramuscularly to trigger an immune response. The nature of turpentine allows it to remain in the tissue near the site of

injection, creating a localized response that can be studied (Wusteman et al. 1990). It has been shown to be an activator of surrounding immune cells stimulating the release of cytokines such as TNF-α and IL-1 and leads to higher levels of circulating IL-6 (Luheshi et al. 1997). Neuroinflammation can result from turpentine-induced cytokine release into circulation which can interfere with the development of hippocampal interneurons in offspring leading to schizophrenia-like behaviors in adulthood (Aguilar-Valles et al. 2012; Aguilar-Valles & Luheshi, 2011; Heckers & Konradi, 2002; Heckers, 2001).

*iv) Polyinosinic:Polycytidylic Acid (Poly(I:C))*

Poly(I:C) is a double stranded RNA chain similar to that found in viral replication; as such, this immunogen causes an immune response similar to that of a viral infection. Poly(I:C) is shown to stimulate significant release of IFN-1 as part of the antiviral response and primarily acts on TLR-3 on immune cells to trigger the cascade leading to this cytokine's production (Van den Boorn et al. 2012; Alexopoulou et al. 2001). Immune activation by poly(I:C) also leads to the production of IL-6 and IL-12 - IL-6 being a pro-inflammatory cytokine as previously discussed, whereas IL-12 aids in the growth and differentiation of T cells and stimulates the production of IFN-γ and TNF-α, the other main pro-inflammatory cytokines which amplify the inflammatory response (Guidotti & Chisari, 2001; Stark et al. 1998). Prenatal exposure to poly(I:C) has been effectively used in animals to model a number of neurodevelopmental disorders in offspring, including schizophrenia, autism and depression (Hsiao et al. 2012; Meyer & Feldon, 2012; Markham & Koenig, 2011). In addition, the neuroinflammatory properties of poly(I:C) are well demonstrated when administered in adulthood and are implicated in the development of depression behaviors in adult rats (Gibney et al. 2013).

*v) Lipopolysaccharide (LPS)*

LPS is a bacterial endotoxin found on the outer membrane of Gram-negative bacteria which can produce a strong, acute immune response. LPS is capable of binding to TLR-4, which in turn causes the release of pro-inflammatory cytokines both systemically and centrally (Konsman et al. 2002).

The mechanism by which LPS can induce neuroinflammation involves its demonstrated ability to trigger pro-inflammatory cytokine production by activating microglia (Quan et al. 1994). This, in combination with oxidative stress caused by said activation, can damage neurons as is seen through reduced function of hippocampal neurons in rats that are treated with LPS (Lee et al. 2003). Furthermore, Nizri et al. (2006) explored the anti-inflammatory properties of acetylcholinesterase in immune cells in the CNS, demonstrating that ACH inhibitors could reduce the production of cytokines in the brain. These anti-inflammatory neurotransmitter systems are suspected of being impaired in the presence of neuroinflammation caused by LPS, further exacerbating the inflammatory response and subsequent potential damage to the brain (Tyagi et al. 2008; Kawashima & Fuji, 2003; Anisman et al. 1993).

Multiple studies use LPS as an immune activator due to its intense yet short lived effect; sickness behavior expression, levels of circulating cytokines and genetic transcription of inflammatory agents all reach their peak intensity within 2-4 hours following intraperitoneal injection with LPS (Murray et al. 2012). A single exposure to LPS in adult mice for example can have long term effects due to neuroinflammation such as memory deficits and decreased hippocampal neurogenesis and dendritic volume (Valero et al. 2014). This effect is further exacerbated when LPS exposure occurs in subjects already suffering from neurodegenerative diseases like Alzheimer's disease (Perry et al. 2007).

Similar to poly(I:C) and other immunogenic compounds, LPS has been used extensively to model autism and schizophrenia in offspring of dams having undergone MIA during pregnancy. LPS administered to dams during gestation showed effects on the dysregulation of genes in offspring, in that genes that were penetrant for autism were downregulated, indicating that immune activation in combination with genetic susceptibility is a risk factor for the development of this disorder (Lombardo et al. 2017). The cytokines produced from exposure to LPS, in particular IL-6, are implicated in the disruption of proper gene expression ultimately leading to autism typical behaviors in offspring exposed to an immune challenge *in utero* (Smith et al. 2007). Prenatal exposure can also lead to stem cell dysfunction and increases in brain size as a result of neuroinflammation (Le Belle et al. 2014).

Sex and age differences exist in the duration and intensity of the immune response to LPS exposure. Adult mice appear more vulnerable to the immune challenge as they show greater levels of plasma cytokines and more intense sickness behavior when compared to pubertal mice, with male mice exhibiting a higher degree of sickness behavior than female mice (Cai et al. 2016). Adult female mice also display a greater level of circulating corticosterone following LPS exposure compared to male mice (Girard-Joyal et al. 2015). Puberty is a markedly vulnerable period in its own regard, as female mice exposed to LPS during puberty display reduced sexual receptivity and sensitivity to estrogen in adulthood (Ismail et al. 2011). Pubertal female mice are also more susceptible to the development of anxiety and depressive behaviors following LPS exposure compared to adults (Ismail et al. 2013; Olesen et al. 2011).

### *1.5 Maternal Behavior*

From an evolutionary standpoint, maternal behavior is believed to have developed out of the need for a mother to ensure the survival of her young, especially in the case of humans and

species that have relatively few offspring (Kinsley & Armory-Meyer, 2011). There is particular importance placed on the quality of maternal care provided to offspring in the time following parturition; both the mother and offspring continue to develop during this time, and improper or maladaptive care has implications on both the cognitive development of the child and the mother's mental health as seen in postpartum depression studies (Pooblan et al. 2007). However, human studies are fairly limited in their scope due to the ethical issues surrounding interventional studies with pregnant women and newborns. As a result, animal models have been developed and used rather effectively to demonstrate how changes in maternal care can affect both the mother and offspring, and while there is the limitation that animal models cannot fully replicate the social context in which humans experience life, there are advantages that allow for tight control of experimental interventions with results that can potentially be extrapolated to human behavior (Pires et al. 2013). In addition to a greater degree of experimental control, the shorter life cycle of many animal models allows for efficient study of this life stage, along with greater freedom to apply experimental interventions that are not ethically suitable for human studies. There is the added benefit that animal models of maternal behavior are very easily observed and quantified; these behaviors are typically expressed in rodents as acts of licking and grooming pups, nursing pups, nest building and pup retrieval (Numan & Insel, 2003). Moreover, various protocols exist to evaluate the expression of maternal behavior, be it the duration and frequency of multiple behaviors, the expression of all behaviors, or the frequency of a specific behavior like licking and grooming (Lonstein & Stern, 1999; Felicio et al. 1991; Myers et al. 1989). These models and protocols ultimately provide some understanding of how alterations in the development of the maternal brain can be translated into changes in behavior.

### *1.6 Neural Circuitry of Maternal Behavior*

Studying the neural circuitry of maternal behaviour using rats as an animal model can provide some insight into how this behavior is regulated in the mammalian brain given that these particular brain areas evolved from genetically older regions of the brain, meaning that what is learned using animal models is likely accurately applicable to what could be expected in humans (Numan, 1988). Plenty of work describing the neural circuitry underlying the pathways of maternal behavior has been conducted by Numan and colleagues (1985; 1977; 1974), along with other contributors as reviewed in detail by Numan (1988).

The medial preoptic area of the hypothalamus (MPOA) had been suspected to be the origin point for the transmission of signals necessary to engage in maternal behavior as seen in lesion studies where damage to the MPOA resulted in decreased expressions of maternal behavior (Gray & Brooks, 1984 Jacobson et al., 1980; Numan et al., 1977; Numan, 1974). There remained the possibility that lesions were damaging other crucial neurons whose axons simply passed through the MPOA, however, it was later confirmed that the MPOA was the origin of these signals from studies using N-methyl-DL-aspartic acid, or NMDA, which targeted and destroyed neuron cell bodies only. Leaving passing axons unharmed, the targeted destruction of MPOA neuron cell bodies resulted in a near absence of maternal behavior, specifically the complete absence of pup retrieval and poorer nest building both of size and quality, as well as fewer instances of nursing (Gray & Brooks, 1984; Jacobson et al., 1980). This supports previous findings that this brain region was crucial for this behavioral regulation (Hastings, Winn & Dunnett, 1985).

With the initiation of maternal behavior originating in the MPOA, neural circuits have been described using lesion techniques to better understand the pathway and brain areas involved in this behavior. Two main pathways of interest have been described. The first is a direct

pathway from the MPOA to the ventral tegmental area (VTA), and the other being through projections from the MPOA, to the lateral preoptic area (LPOA) then to the VTA (Chiba & Murata, 1985; Swanson et al., 1984; Phillipson, 1979; Conrad & Pfaff, 1976; Swanson, 1976). Despite projections from the MPOA to multiple other brain areas, it is these connections to the VTA that have been found to be necessary to control maternal behavior; in female rats with lesions to lateral projections from the MPOA, nearly half displayed a total absence of all maternal behaviors, with the rest showing a complete absence of pup retrieval and poor nest building (Numan and Callahan, 1980). The VTA has projections to the striatum (Swanson, 1982; Beckstead et al., 1979), suggesting that this may be a way for maternal behavior to be translated into motor responses (Heimer et al., 1982; Nauta et al., 1978). While the direct MPOA-VTA route has some involvement in regulating maternal behavior, the initiation and control of these behaviors are severely disrupted only when the more indirect MPOA-LPOA-VTA circuit is damaged (Numan et al. 1985).

Sensory information can also contribute to the initiation of maternal behavior in postpartum mothers. Interestingly, studies using virgin female rats show that the odors from pups act as an inhibitory factor preventing the initiation of maternal behavior (Fleming et al. 1979). However, virgin rats who had both the primary olfactory epithelium and vomeronasal organ removed began displaying maternal behaviors with pups (Mayer & Rosenblatt, 1977; Fleming & Rosenblatt, 1974). The primary olfactory epithelium projects to the MPOA while the vomeronasal organ projects downstream from the VTA. The projections from the primary olfactory epithelium are integrated with signals from the VTA at the olfactory tubercle, which can account for sensorimotor signals whereby maternal behaviors are triggered in response to odors (Haber et al., 1985; Young et al., 1984; Scott et al., 1980; Newman & Winans, 1980). The

vomeronasal nerve on the other hand ultimately leads to the bed nucleus of the stria terminalis (BNST), which has projections to the amygdala which then passes to the MPOA (Simerly & Swanson, 1986; Raisman, 1972). These neurons in the BNST-VTA pathway are shown to regulate rewarding or anxious states in response to stimuli as a way of controlling behaviors (Jennings et al. 2013), suggesting the potential to encourage or inhibit maternal behavior in response to sensory stimuli. Taken together, this research offers one explanation as to how olfactory inputs may be inhibited by hormonal activation in the MPOA towards the end of gestation which trigger sensorimotor pathways allowing the mother to perform these maternal behaviors in response to their surroundings and stimulation from pups.

### *1.7 Estrogen (17-β Estradiol)*

The three most common types of estrogen naturally circulating in females are estrone ($E_1$), estradiol ($E_2$) and estriol ($E_3$), with 17-β estradiol being the most common estrogen. Throughout the lifespan the levels of these estrogens fluctuate, each peaking at different times. During most of the reproductive life of a female, estradiol is most prevalent. In pregnancy, estriol reaches the highest serum levels, while menopausal females have the highest levels of estrone (De Hertogh et al. 1975).

While estrogens can be produced in the brain, skin, and fat cells, they are primarily synthesized in the ovaries, as well as in the placenta during pregnancy; in the ovaries, estrogens are synthesized from enzymatic actions by aromatase and 17β-hydroxysteroid dehydrogenase in granulosa and theca cells, and in the mitochondria of cells throughout the body (Nelson & Bulun, 2001; Ryan, 1982; Canick & Ryan, 1978). During the estrus phase of the cycle in female rodents, estrogen increases sexual receptivity (Christensen et al. 2011), triggers lordosis behavior and is implicated in the commencement of ovulation by stimulating the secretion of luteinizing

hormone (Handa et al. 2012). Estradiol production can occur in the brain independently of production in peripheral sex organs, mainly by the conversion of androgen precursors by aromatase, and is the leading factor establishing sex differences in the perinatal brain (Konkle & McCarthy, 2011). In particular, it is shown that high aromatase activity in the hypothalamus is correlated with higher estradiol concentrations in this area.

### *1.8 Estrogen Receptor Alpha and Beta (ERα, ERβ)*

Estrogen action is mediated by estrogen receptors located inside the cell. Estrogen receptors exist as G-protein coupled membrane-bound receptors or as nuclear receptors, with estrogen exerting its effects through binding as a ligand. There are two types of nuclear estrogen receptors, estrogen receptor-alpha (ER-α) and estrogen receptor-beta (ER-β). While a small proportion of ER-α and ER-β exist as free-floating proteins in the cytoplasm, the vast majority are located in the nucleus and estrogen's steroidal nature allows it to move into the cell to access these receptors easily (Htun et al. 1999).

The primary location of ER-α is in hypothalamic neurons of the brain as well as in endometrial cells (Yaghmaie et al. 2005), while ER-β is found more so in the heart, brain and ovaries (Babiker et al. 2002). While all three major estrogens can bind to these nuclear receptors, estradiol is capable of binding to both ER-α and ER-β equally (Zhu et al. 2006). When estrogen binds to either ER-α or ER-β, the receptor can dimerize upon entering the nucleus and attaches to the estrogen response elements (ERE) on DNA, activating transcription of target genes (Smith & O'Malley, 2004). Upon binding to its receptor, the complex can activate transcription of certain genes. As a result, the scale of estrogen activity is controlled by the number of receptors present in the cells.

### *1.9 ER-α and ER-β in Maternal Behavior*

In the CNS, estradiol primes the maternal brain throughout gestation to initiate maternal behavior upon parturition and plays a role in the immune response through its anti-inflammatory properties (Maggioli et al. 2016; Mann & Bridges, 2001). Furthermore, estrogen signalling can play a role in emotional control, as impaired signalling has been shown to reduce the excitation threshold of neurons in the amygdala leading to the development of anxiety-like behaviors in female mice (Krezel et al. 2001).

During gestation, the maternal brain undergoes restructuring and adaptations to maintain a healthy pregnancy and prepares to initiate maternal care following parturition (Brunton & Russell, 2008). Studies observing the structural and functional changes have observed alterations in neurogenesis and cytokine profiles throughout gestation. Interestingly, recent work by Eid et al. (2019) has demonstrated a significant decrease in hippocampal neurogenesis and cell proliferation throughout pregnancy and the post-partum period in pregnant rats when compared to nulliparous controls, which later began increasing to greater levels than controls 1-month post partum. This was in conjunction with decreased microglial size and increased microglial density in the hippocampus, as well as lower circulating levels of IL-4, IL-10 and IFN-γ (Eid et al. 2019), adding onto their lab's previous work showing that increased estradiol levels during pregnancy can reduce cell proliferation in the dentate gyrus (Galea et al. 2006). Other structural alterations include changes in brain plasticity and reductions in gray matter volume in regions related to social cognition (Hoekzema et al. 2016; Galea et al. 2000) and increased dendritic growth in the medial prefrontal cortex (Leuner & Gould, 2010). Pregnancy also leads to long-lasting changes to brain structure that persist later in life; these include increased neuroplasticity, improved cognition and longer cell life (Galea et al. 2018; Barha et al. 2016; Barha et al. 2015; Colucci et al. 2006). In addition, there appears to be significant alterations to the maternal brain's

diffusivity. A gradual decrease in cellular taurine throughout gestation causes an efflux of intracellular fluid as it leaves the cell, leading to glial cell shrinking which increases the extracellular space (Zhou et al. 2013; Pasantes-Morales & Schousboe, 1997; Law, 1994); this increased space allows for more extracellular fluid which facilitates diffusion of molecules (Fan et al. 2013; Chan et al. 2015; Pasco et al. 2006; Oatridge et al. 2002). Ultimately these structural changes are necessary to lead to the behavioral changes that are initiated upon giving birth to improve the maternal bond with offspring and ensure their survival (Moya et al. 2014; Kinsley & Lambert, 2006).

Animal models help us understand the factors affecting the onset and intensity of maternal behavior, which could have a significant impact on offspring health due to the quality of care they receive (Champagne et al 2003). Many of these behavioral changes are initiated in response to the actions of hormones like estradiol, progesterone and oxytocin. During gestation, the increasing levels of estrogen, progesterone and prolactin prime the maternal brain in preparation to quickly initiate maternal behavior upon parturition, triggered by the sudden withdrawal of progesterone (Mann & Bridges, 2001). Studies have shown that maternal behavior is triggered by increased levels of estrogen in the MPOA (Fahrbach & Pfaff, 1986; Numan et al., 1977), as well as increasing concentrations of estrogen receptors in the MPOA during gestation (Rainbow et al., 1982; Pfaff & Keiner, 1973). The sharp increase in central estrogen levels towards the end of gestation plays a large part in the initiation of maternal behaviors; studies where rats have had their pregnancy terminated at later times initiate maternal care with substitute pups sooner than rats with earlier terminated pregnancies (Mackinnon & Stern, 1977). With this considered, estrogen appears to stimulate the expression of behaviors while progesterone has inhibitory properties.

Estrogen mediates maternal behavior through binding with its receptors. Specifically, higher levels of ER-α mRNA in the MPOA are associated with a greater expression of one subtype of maternal behavior observed in rats, that is, higher rates of licking and grooming of pups (Champagne et al. 2003). In late pregnancy, increased levels of ER-α are seen in the MPOA, with up to 45% of neurons expressing this receptor (Lonstein et al. 2000). Higher quality of maternal care has in fact been correlated with increased levels of ER-α mRNA in the MPOA, which is also associated with decreased anxiety behavior post-partum (Champagne et al. 2003). The MPOA expresses greater amounts of estrogen receptors and fewer progesterone receptors in late pregnancy, matching a similar pattern to the fluctuating levels of these hormones throughout gestation, thereby optimizing the excitatory and inhibitory effect of these hormones in the promotion of maternal care (Mann & Babb, 2005; Giordano et al. 1990). Similarly, the BNST contains ER-immunoreactive cells contributing to the onset and maintenance of maternal care. Animal studies show that females displaying higher levels of amicability towards new pups and higher rates of licking and grooming had greater ER-α protein levels in the BNST (Wu et al. 2011). This same correlation is seen when observing estrogen-sensitive oxytocin receptor levels in the BNST (Champagne et al 2001). A greater number of ER-immunoreactive cells are seen in the BNST of pregnant mice compared to virgin females; this high density of estrogen receptors in key brain areas precedes the initiation of maternal behavior, at which point there is a sharp increase in estrogen in the BNST upon seeing pups for the first time. (Ehret & Buckenmaier, 1994).

### *1.10 The Role of Immune Activation on Estrogen and ER-α Activity*

In the brain, estrogen and ER-α serve a neuroprotective function through their anti-inflammatory and transcriptional properties. Estrogen helps prevent pathogenic inflammatory damage in the brain by reducing permeability of the endothelium in the blood brain barrier, namely by estrogen enhancing annexin-1 transcription (Maggioli et al. 2016). Cranial administration of LPS has been shown to cause long term microglial activation in the brain, and estrogen can block the inflammatory effects of LPS while ER-α deficient mice are unable to do so (Marriott et al. 2007). Human studies have shown that exposure to LPS results in a nine-fold increase in ER-α expression in female blood monocyte-derived macrophages with a 26-fold increase seen in males, as well as an increase in receptor activation (Campesi et al. 2017). In fact, the cytokines that are commonly released as part of the immune response, such as TNF-α and IL-6, also stimulate aromatase, the enzyme responsible for estrogen synthesis, as this cytokine-induced synthesis is seen in breast cancer tumors which rely on estrogen to continue growing (Purohit et al. 2002). Both estradiol and cytokines have the capacity to phosphorylate ER-α which increases binding of ligands and coactivators, essentially regulating ER-α activity and DNA binding (Kassi & Moutsatsou, 2010). Taken together, it's possible that the rise in estrogen synthesis as well as increased estrogen circulation to brain areas involved in maternal behavior may play a role in attenuating the severity of an immune response.

### *1.11 Environmental Enrichment*

Environmental enrichment (EE) is a means of providing a social and physical environment capable of providing stimulation that usually translates into an increase in brain synaptogenesis and changes to the dendritic processes. In animal studies, the concept aims to replicate a sensory rich environment that animals would find in the wild. This could include, but is not limited to, offering opportunities to forage for food, items for physical activity, unique

treats of food items that offer different taste and olfactory stimulation, offering novel play items and can include structures that encourage exploration (CCAC, 2017). This can often include a social component where animals are housed in groups and are free to interact with one another. Essentially, it offers a chance for animals to be reared in an environment that would provide the stimulation mimicking what they would experience in the wild, and sometimes more so.

Plenty of research has investigated the role EE plays in improving cognitive capacity, as well as a preventative and restorative mechanism following exposure to a stressor. In animal research, EE is often employed by using specific housing conditions that typically involve combinations of certain components such as a multi-level cage, novel toys or objects, activity wheels and group housing to offer physical, cognitive and social stimulation (Jurgens & Johnson, 2012; Rosenzweig & Bennett, 1996). EE must be distinguished from social enrichment (SE) or communal nesting (CN), which typically only offer the social component in the form of group housing without the physical activities or novel stimuli. For decades, EE has been incorporated into studies for its ability to restore neurological function after exposure to a stressor, as well as promote neurogenesis and neural plasticity (Greenough et al. 1986; Green et al. 1983; Diamond et al. 1976). The benefits and consequences of rearing in an enriched environment have been more thoroughly explored since these concepts were developed.

A fair share of research has focused on the benefits of rearing offspring in EE at the time of exposure to a stressor, with results demonstrating that EE is effective in preventing or restoring the many potential neurological deficits that could be caused by stressor exposure, as previously discussed. When MIA activation was used as a stressor, EE was observed to have regulated the stress response of male offspring by attenuating corticosterone (CORT) levels produced in the brain, as well as reduced instances of observed social anxiety or agitation

(Connors et al. 2014). It is believed that EE reflects a similarly complex environment that rats would typically live in when in the wild, thereby priming offspring to respond to certain stressors such as maternal separation as would be seen when dams leave the nest to forage for food (Connors et al. 2015). How EE affects the dams in the case of MIA is not very well understood - apart from spending less time on nests, rates of licking/grooming and pup retrieval have been unchanged when dams are exposed to EE during gestation and while raising pups, both with and without the presence of an LPS immune challenge (Connors et al. 2014; 2015).

Adult male Long Evans and Sprague Dawley rats that were housed in EE showed higher baseline CORT levels and quicker return to baseline levels following stressor application, as well as an overall higher resiliency to stress (Konkle et al. 2010; Francis et al. 2002). In one of the earliest studies to investigate the protective role of EE with a viral infection in the host, male Balb/c mice housed in EE for four months that were later exposed to a peripheral influenza infection were shown to have reduced severity of neuroinflammation in the hippocampus as well as lower prevalence of cognitive deficits (Jurgens & Johnson, 2012). Pro-inflammatory cytokines IL-1β and TNF-α were effectively reduced in the hippocampus, and effects were also seen in the ventral tegmental area of rats housed in an enriched environment where central IL-6 levels were significantly reduced following exposure to an LPS immune challenge (Kentner et al. 2008). EE has also been shown to reduce microglial inflammation and circulating levels of pro-inflammatory cytokines following inflammation due to exposure to human A oligomers as well as minor traumatic brain injuries (Xu et al. 2016; Briones et al. 2013). It has been suggested that it is specifically the voluntary exercise component of EE that is primarily responsible for the redistribution of immune markers (rather than a flat decrease in production) and ultimately improved immune function (Patten et al. 2013; Gleeson & Bishop, 2005).

Rearing in an enriched environment from an early age has further demonstrated that memory and learning behaviors can be enhanced in adulthood (Bruel-Jungerman et al. 2005; Leggio et al. 2005) and can reduce the expression of anxiety behaviors and regulate stress reactivity (Schloesser et al. 2010; Fox et al. 2006). These benefits to stress reactivity, decreased anxiety behaviors and improved cognitive function appear to be seen when animals are exposed to an enriched environment at various times in their lifespan (Connors et al. 2015; Ravenelle et al. 2013; Baldini et al. 2013; Girbovan & Plamondon, 2013). The improvements to cognitive function and mental health among animals is in conjunction with studies that have observed physical changes to the brain, including increased brain weight and cortical thickness, neurogenesis and synaptic formation and strength (Alwis & Rajan, 2014; Sale et al. 2014). The social component of EE also appears to contribute to increased social stability between housed animals, which typically show decreased circulating CORT (Kentner 2015; Branchi et al. 2013).

While initially these results seem compelling, in reality the benefits and consequences of EE vary significantly from study to study. In a thorough review of multiple studies incorporating EE, Simpson & Kelly (2011) outlined the need to standardize an EE housing protocol, as variations in age of EE onset, duration of EE exposure, cage structure, types of objects and activities and number of housed animals all result in different reported consequences in animal mental health, cognitive ability and immune function. Studies that use EE have shown to either decrease (Hendriksen et al. 2010; Schloesser et al. 2010; Hellemans et al. 2005), increase (Brenes et al. 2009; Francis et al. 2002) or show no effect on expression of anxiety behaviors (Hoffman et al. 2009). Similarly, the regulation of circulating CORT in response to a stressor varies greatly from one study to another, where some report decreases (Laviola et al. 2004; Belz et al. 2003), increases (Moncek et al. 2004; Dronjak et al. 2004; Marashi et al. 2003; Francis et

al. 2002) or no change (Morley-Fletcher et al. 2003; Schrijver et al. 2002) after housing in an enriched environment. Early or later-life initiation of EE exposure can also result in different brain regions being affected for better or worse (Mychasiuk et al. 2014), and as expected different EE protocols are shown to affect different brain regions depending on the type and amount of stimuli provided (Lambert et al. 2005). To further complicate matters, there is no concrete method of measuring how each animal responds to the different stimuli and how much they intrinsically enjoy it or not (Kentner et al. 2008). Social hierarchies formed in group housing can result in some animals having a satisfying experience while others do not, which in itself has been shown to result in greater immunocompetence in the dominant animal (Wrona et al. 2005). Despite the numerous studies boasting the anxiolytic effects of rearing in an enriched environment, it is not uncommon to find those who report increases in anxiety behaviors in animals exposed to EE (Cymerblit-Sabba et al. 2013; Rosenfeld & Weller, 2012; Workman et al. 2011).

Moreover, the sex and strain of animals adds another variable, as different strains react differently to the physical and social components of EE, whereby preventative effects observed in one strain can have drastically opposite effects in another. Wistar rats for example tend to be more anxious than Long Evans rats and do not benefit from EE in comparison (Simpson & Kelly, 2011). McQuaid et al. (2013) report the strain difference seen when using balb/cByJ mice, which they describe as being non-aggressive yet more anxious. The communal nesting component of the enriched environment led to competition between male mice and social defeat in the less dominant animals, ultimately resulting in increased CRH and cytokine production in the brain, despite other strains of mice benefiting from the effect of EE (Kentner 2015; Jurgens & Johnson, 2012). This social defeat experienced as a result of EE can increase both central and

peripheral mRNA levels of pro-inflammatory cytokines TNF-α and IL-6 (Audet et al. 2011; Bartolomucci et al. 2003), which can unsurprisingly have harmful effects in the form of neuroinflammation.

With this in mind, it can be difficult to predict the effect of EE without a standardized protocol in place. Here, we report the impact of environmental enrichment conditions as described in the methods and its effect on female Sprague Dawley rats during gestation and following parturition.

## 2. RATIONALE

Much research surrounding gestational immune stress has focused on the direct impact of *in utero* exposure on offspring health. In fact, gestational administration of LPS is used to model a variety of neurodevelopmental disorders like anxiety, autism and schizophrenia as it triggers a similar immune response as would a typical infection during gestation which has the capacity to impede proper neural development through neuroinflammation, as reviewed by Harvey & Boska (2012). However, little is known about how MIA alters the mother's brain and behavior and how this may negatively impact the maternal care she provides, consequently affecting the neurodevelopment of the offspring. This can in turn lead to life-long dysfunctions in offspring; for example, animal work has established a non-genetic mechanism by which offspring who received improper maternal care carried over those same maladaptive traits when providing maternal care to their future offspring (Champagne et al. 2003). This association has also been shown in human studies in which mothers who provided self reports of having received poor maternal care provided lower quality care to their own offspring (Neuwald et al. 2014). Furthermore, human studies show that decreased maternal sensitivity has also been associated with greater negative affect and temperament in infants (Jonas et al. 2015), higher rates of

obesity in female offspring (Wendland et al. 2014), and a greater risk of developing neurodevelopmental disorders like ADHD (Neuwald et al. 2014). Thus, this study will further our understanding of how the maternal brain changes following infection during pregnancy.

## 3. RESEARCH QUESTIONS

Question1: Does LPS exposure affect the number of estrogen receptor-alpha in brain areas responsible for maternal behavior?

Question 2: Can environmental enrichment counteract any effects of immune stress on the number of estrogen receptors in these brain areas?

## 4. OBJECTIVES

To observe the effect of maternal immune activation during gestation on estrogen receptor levels (specifically ER-α) in the medial preoptic area of the hypothalamus and bed nucleus of the stria terminalis through immunohistochemical analyses. This can be intersected with housing conditions of pregnant dams to determine whether social stimulation or rewarding activities impact vulnerability to an immune challenge.

## 5. METHODOLOGY

All brains used in this study were acquired from a collaboration with the Kentner laboratory (Massachusetts College of Pharmacy and Health Sciences) as part of a larger study (Connors et al. 2014); the original work focused on environmental enrichment as a protective factor for offspring having been exposed to maternal immune activation *in utero*.

### *5.1 Animals*

Male and female Sprague-Dawley rats were obtained from Charles River. Rats were pair-housed separately by sex in standard conditions (regular cage with toys, Nestlets and tube)

conditions. They were housed at 20ºC on a 12-hour light/dark cycle with *ad libitum* access to food and water. Rats were given one week to acclimatize, after which the estrous cycle of females were monitored to determine optimal time for breeding. Once they began the proestrous phase, rats were housed in 2:1 ratio of females to males to optimize chance of copulation. Spermatozoa present in vaginal lavage samples later confirmed breeding, and pregnancy (gestational day 1) was confirmed by continuous weight gain over the following days, as well as cytology confirming the beginning of diestrus. Body weight was chronicled weekly, except at the time of treatment - see measures of sickness behaviour below.

### *5.2 Housing Conditions*

Pregnant dams were pair housed in either Animal Care Control (ACC; n=16), Social Enrichment (SE; n=14) or Environmental Enrichment (EE; n=14) conditions. ACC housing included a standard cage, tube, chew bone and Nestlets ©; SE housing included a large, single-level cage with tube, chew bone, Nestlets © and offered more opportunity for social engagement; EE housing included a large, multi-level cage, toys that were changed twice weekly to maintain novelty, chew bone, Nestlets ©, as well as ramps (Critter Nation, Muncie IN). See Connors et al. (2015; 2014) for additional details.

### *5.3 Experimental Groups and Treatments*

On gestational day 11 the dams were exposed to either an LPS immune challenge or saline control. Gestational day 11 was selected based on research that has demonstrated the link between maternal immune activation and a greater risk for offspring to develop symptoms associate with neurodevelopmental disorders such as autism and schizophrenia when exposed to an immune challenge at this stage of fetal development. For example, studies have show that maternal immune activation between gestational day 9-12 is related to increased brain size (Le

Belle et al. 2014), deficits in Purkinje cells within the cerebellum (Shi et al. 2009), reduced Reelin production (Meyer et al. 2006) and behavioral changes including decreases sociability, range of vocalizations and increased expression of stereotypical behaviors (Malkova et al. 2012). These physiological and behavioral changes are consistent with autism spectrum disorder and schizophrenia.

ACC dams were separated from their cage mate into separate cages in order to monitor sickness behavior (and eventually mark litters to the appropriate dam). SE dams were separated by a custom-made stainless-steel divider placed in the large home cage (Connors et al. 2015; Thermo-Craft Engineering, Lynn, MA) to maintain olfactory and auditory contact. EE dams were separated by a divider placed in the home cage that allowed for visual, tactile, olfactory and auditory contact, which are necessary factors of EE (Connors et al. 2014).

Each dam received an intraperitoneal injection of either 100 µg/kg LPS (Escherichia coli, serotype 026:B6) in saline, or a similar volume of 0.9% saline vehicle. Dams were scored on piloerection, ptosis and lethargy, after which a total score was composed averaging the severity of each behavior to provide an overall score for sickness behavior. Available water and food were weighed immediately before injection and at 4 and 20 hours post-treatment to determine anorectic effects of the treatment. Additionally, body weights were chronicalled for dams to monitor whether the treatment appaeared to threaten the pregnancy.

### *5.4 Parturition and Maternal Behavior*

At parturition, number of pups and the distribution of males and females was noted. Litters were culled to 10 pups each and an even ratio of female to male pups was maintained. Ten one-minute observations took place at 6:00, 12:00 and 20:30 from postnatal day 3-6 to monitor the frequency of maternal behavior in the home cage. Observers recorded the frequency

of pup retrieval, licking and grooming, and the amount of time spent on the nest; these are reported in Connors et al (2014). At parturition day 22, the offspring were weaned and housed in separate cages; further behavioral tests and specimen collection took place for the Connors et al. (2014) study.

### *5.5 Brain Collection*

Brains of dams were collected along with those of the offspring on postpartum day 21. The adult female rats were given a 40-80/5-10 mg/kg IP injection of a Ketamine/Xylazine mixture to anesthetize them, at which point they were intracardially perfused with saline, followed by fixation with 4% paraformaldehyde. Brains were collected and stored for at least 48 hours in 20% sucrose solution until the time of sectioning. Brains were sectioned at 40 µm using a Leica 1850 cryostat and sections stored in an antifreeze solution at -20$^{o}$C until the time of immunohistochemistry.

### *5.6 Immunohistochemistry for ER-α*

Standard immunohistochemical procedures were used as are typically done in our laboratory. Briefly, sections were washed in phosphate buffered saline (PBS) (3 x 5 min) followed by washes in PBS-t (0.4% Triton X-100) (3 x 5 min). The sections were then incubated in 5% dimethyl sulfoxide (DMSO) for 10 minutes, followed by three more 5 minute washes in 0.4% PBS-t. This was followed by a 20-minute wash in 0.06% $H_2O_2$ in PBS after which sections were washed again in PBS-t (3 x 5 min). Sections were then incubated for 30 minutes in 10% normal goat serum (NGS; Vector Labs, S1000) at room temperature. Afterwards, sections were incubated in primary rabbit ER-α antibody (Millipore 06-935; 1:1000 in 0.4% PBS-t) for 1 hour at room temperature and then moved to a 4$^{o}$C cold room to incubate over 24 hours. Following primary antibody incubation, sections were washed in 0.4% PBS-t and then incubated for 2 hours

at room temperature with biotinylated goat anti-rabbit secondary antibody (Vector Labs, BA1000; 1:1000 in 0.4% PBS-t and 10% NGS). Afterwards, sections were incubated for 1 hour in ABC reagent (Vector Labs, PK6100) at room temperature. Sections were then washed in PBS (3 x 5 min) and developed with the chromagen 3.3'-diaminobenzidine (DAB) in 0.1M PBS and 35% $H_2O_2$ for 2-3 minutes, with the reaction stopped by adding distilled $H_2O$.

Using the Paxinos and Watson (2007) brain atlas, sections were arranged from anterior to posterior and mounted on glass slides. After drying and adhering, the slides were placed in increasing concentration of ethanol (50%, 70%, 95%) for 2 minutes each, followed by two 5-minute rinses in xylene. Slides were then coverslipped with Permount.

### *5.7 Microscopy and Cell Counting*

Using the Paxinos and Watson (2007) brain atlas, sections containing the MPOA and BNST were identified along with their bregma value. Vizualizing the sections was done using a Zeiss Axio Imager microscope at 10x magnification. Sections previously identified as containing the MPOA or BNST were visualized and the brain area of interest was defined with reference to the brain atlas and centered in the microscope's field of view. By centering the brain area, images captured at 10x, 20x and 40x magnification ensured that cell counts could be performed for receptors within to the nucleus of the brain area as to avoid counting those from other brain areas that may overlap near the edge of the area of interest. Images were captured at these three magnifications for both the left and right side of sections containing the MPOA or BNST (Figure 1a-e).

### *5.8 Cell Counting*

Cell counts were performed for images captured at 40x magnification for both left and right sides of the brain section. Using Image J software, a grid was placed over each image and a

standard area of 520 x 345 pixels (180,000 square pixels) was defined near the center of each image in which immunopositive cells would be identified. Only full receptors contained within this defined grid space were counted – those that touched or crossed the border of this defined area were omitted. Data were entered into an Excel spreadsheet for subsequent statistical analysis. Only sections ranging from bregma value +0.72 to -0.96 were used for the BNST, and from +0.48 to -0.96 for the MPOA to remain consistent in the areas being counted (Figure 2).

### *5.9 Statistical Analysis*

The average number of immunopositive cells within each brain area was calculated so that the mPOA and BNST could be analyzed seperately. A Levene's test for homogeneity of variances was conducted, and upon confirmation of homogeneity, a two-way factorial ANOVA was conducted using Treatment (2 levels) and Housing (3 levels) as factors. When a significant interaction was observed, simple effects analyses were conducted to further elucidate where the differences lie. A *p-value* of $< 0.05$ was considered statistically significant. These methods were similarily employed to analysize differences in dam body weights, food and water intake, and expressions of maternal behaviors in the post-partum period.

## 6. RESULTS

### *6.1 Body Weight, Sickness Behaviour and Maternal Behavior*

Data pertaining to pregnancy and sickness behaviour are presented here to showcase the effectiveness of the treatment with respect to inducing acute sickness behaviour but also to show an assessment of the treatment on measures related to pregnancy. Sickness behaviour data were originally reported in Connors et al. (2014) where a significant effect of treatment was observed such that LPS-treated dams displayed greater expressions of sickness behaviors than saline-treated dams at 105 ($p = 0.0001$), 120 ($p = 0.011$) and 210 ($p = 0.004$) minutes following

treatment. No significant difference between treatments was observed in body weights of dams at baseline. The expected increase in body weight across days was seen during pregnancy with a reduction at parturition and a weight gain resuming thereafter (Figure 3). We also found no effect of treatment on the relative offspring survival rates.

Following treatment on gestational day 11, water intake of dams showed a significant increase from 4 hours to 20 hours post treatment. A significant interaction between time and treatment on water intake was observed ($p = 0.013$); simple effects analyses showed that water intake was similarly low between the groups at 4h post treatment ($p < 0.05$) whereas saline treated animals drank significantly more than LPS treated animals at 20h post treatment ($F_{1,35} = 9.09$; $p = 0.005$) (Figure 4a-b). A similar pattern was seen with respect to food intake where we found a significant interaction between time and treatment ($F_{1,35} = 11.93$; $p = 0.001$). Simple effects analyses showed that the difference in food intake between the two treatment groups was only evident at 20h post injection ($F_{1,35} = 18.49$; $p = 0.0001$) (Figure 5a-b).

Observations of maternal behaviors reported by Connors et al. (2014) were assessed over the four-day observation period. For observed expressions of both licking and grooming and of pup nursing, no significant interaction or main effects were revealed (Figures 6a-b). When observing expressions of nest building, a significant main effect of housing was revealed where animals in the SE condition displayed higher rates of nest building compared to animals in the ACC or EE conditions ($F_{2,31} = 3.57$; $p = 0.04$). There was also a significant interaction between Treatment by Housing ($p = 0.034$); simple effects analysis revealed that LPS-treated animals housed in the SE condition displayed greater expressions of nest building compared to other housing and treatment groups ($F_{1,31} = 8.018$; $p = 0.008$) (Figure 6c). Furthermore, analysis of expressed behaviors of pup retrieval showed a main effect of housing, such that animals housed

in SE conditions displayed greater expressions of pup retrieval compared to animals in ACC or EE conditions ($F_{2,31} = 7.969$; $p < 0.002$) (Figure 6d).

### *6.2 MPOA*

Please refer to Figures 7 and 8 for pictographs of the MPOA and BNST for visual representation of cell counts for each treatment and housing combination. We found a significant interaction between treatment and housing on the number of ER-α cells ($F_{2,35} = 4.073$; $p = 0.025$). Simple effects analyses confirmed that that there was no significant difference between saline and LPS ($F_{1,35} = 2.58$; $p = 0.116$) in the animal care control condition, nor between saline and LPS treated dams in the social enrichment condition ($F_{1, 35} = 2.661$; $p = 0.112$). In the environmental enrichment condition, while LPS treated dams showed fewer estrogen alpha receptors than saline treated dams, this difference only approached significance, with means of 10.25 ±1.73 and 15.04 ± 2.22, respectively ($F_{1, 35} = 3.748$; $p = 0.060$) (Figure 9). However, data were re-analyzed with the removal of the SE housing condition to compare ACC and EE dams only. This analysis revealed a main effect of treatment such that LPS-treated dams displayed significantly reduced ER-α levels compared to saline-treated dams in both ACC and EE conditions ($F_{1,21} = 6.817$; $p = 0.016$).

### *6.3 BNST*

Contrary to observed results in the MPOA, ER-α levels in the BNST were significantly affected in a manner consistent with our hypothesis. We again show a significant interaction between Treatment and Housing on the number of ER-α cells ($F_{2,35} = 3.93$; $p = 0.028$). Simple effects analyses revealed that in ACC conditions lacking any kind of enrichment, LPS-treated dams displayed significantly lower ER-α levels compared to saline-treated dams with means of 6.04 ±1.79 and 15.96 ± 1.58, respectively ($F_{1, 35} = 16.49$; $p < 0.001$) (Figure 10). Both social

enrichment and environmental enrichment appeared to have a protective effect in mitigating ER-α loss such that no significant effect of treatment was seen in SE ($F_{1.35} = 1.59$ $p = 0.214$) or EE ($F_{1.35} = 0.56$; $p = 0.458$ conditions. These results suggest that exposure to social or environmental enrichment prevent the loss of ER-α as a result of an immune challenge.

## 7. DISCUSSION

The effect of MIA in combination with EE housing are consistent with our hypothesis within the BNST: an LPS immune challenge significantly decreased ER-α protein levels in this brain region, with exposure to EE having a protective effect such that dams housed in this condition displayed levels of immunopositive cells similar to saline-treated controls. However, this effect was not seen in the MPOA – in fact, LPS did not produce the expected significant loss of ER-α in control conditions, and EE did not have a significant effect on ER-α levels; if anything, there was a tendency for LPS to reduce the ER-α counts in the EE housing condition, though this did not reach significance. It is possible that since there wasn't a significant reduction in ER-α caused by LPS in the MPOA there was not enough of a deficit for EE to protect against. However, the fact that LPS-treated animals housed in EE conditions approached significantly lower ER-α levels than their saline-treated counterparts suggests that EE may not have had any beneficial effect in this brain area; this will be further explored in the following paragraphs.

### *7.1 Body Weight and Sickness Behavior*

Body weight and sickness behavior data were collected to determine whether a significant immune response took place, which also did not threaten the pregnancy. A consistent rise in body weight throughout gestation, followed by a small drop at parturition, is what would be expected during a normal pregnancy. Body weights continued to increase during the post-partum period indicating dams were still growing during this time – it's expected that the body

weight of older animals would have plateaued in this period but because these were young animals (weighing on average 253g pre-conception), their weight continued to increase following parturition. Considering that a drop in body weight was not observed in the time shortly after administration of the immune challenge, it can be said that the pregnancy was not threatened.

Food and water intake were also measured to complement observations of expressed sickness behaviors to determine that a strong enough immune response took place. Typically, what would be seen is that food and water intake would be significantly reduced 4 hours and 20 hours post-treatment in animals exposed to the immune challenge compared to those treated with the saline control – a quantifiable measure of the anorectic effects of the immune challenge. Differences in food and water intake between LPS and saline-treated animals were observed by Connors et al. (2014) only at 20 hours post-treatment. This may be a result of the timing of the immune challenge. As the animals were housed on a 12 hour light/dark cycle (0700h-1900h light) with lights on at 7h and the treatment was administered at 1200h, it's possible the animals were still sleeping, as they are nocturnal animals, before becoming more active nearing 1900h. If true, this would explain why relatively no effect of LPS administration was observed in food and water intake at 4 hours post-treatment. Using a reverse light/dark cycle and administering the treatment during the animal's dark hours may have yielded different results at the 4 hour mark.

### *7.2 Immune Challenge and MPOA*

Initially, results seem to conflict with our hypothesis – LPS did not cause a significant reduction in ER-α levels in the MPOA in the ACC housing condition, and EE did not have a protective or restorative effect against the effects of the immune challenge. However, despite only approaching significance, it is important to note that LPS-treated animals in the ACC

condition displayed nearly 40% lower ER-α levels than saline-treated controls in the same housing condition. It is possible that results from animals in the SE condition may be skewing results the overall factorial analysis, considering a moderate increase in ER-α levels in LPS-treated dams in the SE housing condition. We conducted further analyses that omitted SE data and found a significant decrease in ER-α levels in LPS-treated dams in both the ACC and EE conditions, which is what we would have expected to see in the ACC group. With this secondary analysis in mind, it could be said that the immune challenge did cause a significant decrease in ER-α levels in the MPOA, an effect that was not protected against by exposure to an enriched environment. As observations of sickness behaviors by Connors et al. (2014) confirmed an acute immune response, the variation in observed results between the brain areas must rest in differences between the MPOA and BNST themselves.

Should the results of the initial factorial analysis be considered, there are some potential explanations for why there was not a significant (albeit nearly significant) decrease in ER-α levels in the MPOA. One possible reason for the lack of an LPS-induced reduction in ER-α in the MPOA could be that of the anti-inflammatory properties of estrogen. As discussed, during the course of gestation the maternal brain undergoes not only structural changes but cellular changes as well, such that there is a significant rise in the expression of ER-α among cells in the MPOA, as well as a redirection of estrogen that is concentrated within the MPOA throughout pregnancy in preparation for parturition (Brunton & Russell, 2008; Mann & Bridges, 2001; Lonstein et al. 2000; Fahrbach & Pfaff, 1986; Rainbow et al., 1982; Numan et al., 1977; Pfaff & Keiner, 1973). This sharp increase in both estrogen as well as receptor expression does not appear to be documented in the BNST, at least not at the same magnitude as in the MPOA (McHenry et al. 2015; Mann & Babb, 2005; Giordano et al. 1990). It is possible that the

concentrated estrogen in the MPOA may be producing a localized anti-inflammatory effect which counteracts the central immune response, an effect we do not see in the BNST.

There are a number of mechanisms by which a high concentration of estrogen in the MPOA could counteract this response. One well studied avenue is estrogen's ability to promote a Th2 (T-helper 2) immune response over a Th1 response. Despite the Th1 response being typical of bacterial or viral infection where the Th2 response is initiated with parasitic invasion, the Th2 response is produced when there is a sustained increase in the levels of estrogen, and this response is in fact seen more often during pregnancy due to the increase in circulating estrogen (Pernis, 2007; Al-Shammri et al. 2004; O'Garra & Arai, 2000; Whitacre et al. 1999; Buyon, 1998; Krishnan et al. 1996; Mosmann & Coffman, 1989). The Th1 response involves the release of pro-inflammatory cytokines IFN-γ, IL-2 and TNF-α, whereas the Th2 response produces interleukins, in particular the anti-inflammatory variants IL-4, IL-10 and IL-13, which act to inhibit pro-inflammatory cytokine production (Perkel, 2001; Godfrained et al. 1998; Paul, 1991). This is in addition to the fact that anti-inflammatory cytokines of the Th2 response further promote this method of immunity while simultaneously blocking the Th1 response and the subsequent release of pro-inflammatory cytokines (Perkel 2001; Abbas et al. 1996). Estrogen's ability to regulate the production of pro and anti-inflammatory cytokines is thereby one method of controlling the central immune response.

Another mechanism is estrogen's ability to reduce microglial inflammation (Su et al. 2008; Liu et al. 2005; Maggi et al. 2004). Studies by Vegeto et al. (2001; 2008) have demonstrated that estrogen can have a significant effect on reducing microglial activation and inflammation in the presence of LPS exposure. Inducing encephalitis in mice and thereby causing microglial activation to simulate multiple sclerosis has supported other findings that

suggest that estrogen activity within microglia, specifically upon binding to ER-α rather than ER-β (Merchanthaler et al. 2004; Mitra et al. 2003), minimizes microglial inflammation (Vegeto et al. 2008; Polanczyk et al. 2005; Garidou et al. 2004; Vegeto et al. 2003; Dubal et al. 2001). This has also demonstrated estrogen's ability to promote central production of anti-inflammatory cytokines while inhibiting pro-inflammatory cytokine production (Subramanian et al. 2003; Ito et al. 2001; Bebo et a. 2001; Matejuk et al. 2001). This protective effect of estrogen with ER-α is also supported with the use of ER-α agonists and ligands such as PPT (pyrazole triol) as it reflects similar results in preventing inflammation and cell death (Vegeto et al. 2008; Brown et al. 2008; Morales et al. 2006; Miller et al. 2005; Elloso et al. 2005; D'Astous et al 2004). This reduction in microglial activation has been shown to prevent damage to the brain, with the magnitude of microglial inhibition being correlated with the degree of neuronal protection (Aharoni et al. 2005; Heppner et al. 2005). A contributing mechanism to microglial control involves p65, a transcription factor that initiates production of inflammatory agents. While this factor is found free-floating in the cytoplasm of microglia and macrophages, it has been demonstrated that immune activation by LPS triggers p65's movement into the nucleus to transcribe genes necessary for an inflammatory response, an event that it effectively inhibited by treatment with estrogen prior to and during infection. This is achieved by restricting the movement of p65 into the nucleus (Vegeto et al. 2008; Granucci et al. 2003).

Estrogen and ER-α have been shown to play a vital role in mitigating the inflammatory response in the brain due to the anti-inflammatory properties of estrogen action via its receptor. By promoting the expression of anti-inflammatory cytokines while simultaneously inhibiting pro-inflammatory variants, as well as regulating microglial activation such that the magnitude of inflammation is effectively controlled, high concentrations of estrogen and its receptor could

very well produce enough of a protective effect to avoid significant cell damage in the brain. Considering the rise in estrogen and ER-α seen in the MPOA during pregnancy which may not be present in the BNST, it is possible that increased hormone and receptor expression produced a localized protective effect that prevented significant damage in the MPOA and not in the BNST, such that LPS did not cause a significant loss of ER-α in this brain region. There also remains the possibility that an effect of LPS in the MPOA was transient, such that the restructuring of the maternal brain during pregnancy obfuscated any significant impact on the distribution of ER-immunopositive cells. Again, marketed decreases in neurogenesis, cell proliferation and dendritic volume and density have been observed in multiple brain areas throughout gestation (Eid et al. 2019; Galea et al. 2006) that actually return to and exceed baseline activity at one-month post-partum (Galea, 2018; Barha et al. 2016). The fact that brain collection occurred at post partum day 21 for this study may suggest that any earlier effect on the MPOA would have been missed, as cells within the MPOA may have already returned to baseline levels of neurogenic activity such that it compensated for the loss in ER-immunopositive cells.

### *7.3 Environmental Enrichment and BNST*

Given that LPS did not produce a significant loss of ER-α in the MPOA, at least at the time point we explored, we cannot conclude that there was any restorative effect from EE. Looking at the MPOA, the fact that LPS-treated dams housed in EE had a tendency toward lower levels of ER-α when compared to their saline-treated counterparts would suggest that EE did not have a clear role in protecting the MPOA – however, EE played a strong role in preventing ER-α loss in the BNST. There is the potential that EE could have acted as a stressor, enough so that it not only offered no protection against ER-α loss, but that such a stressor could have added to the inflammatory response and actually contribute to the deficit in immunopositive cells in the

MPOA. This is purely speculation however, as research regarding circulating CORT levels at baseline and following exposure to stress show varying positive and negative results across brain areas, which also seems to depend highly on the specific components of the EE model as well as the species and strain of animal used. With this in mind, our observed results could then be due to differences between brain regions with the BNST supposedly responding better to the effect of EE.

Another possible explanation for the difference between the MPOA and BNST could be that the BNST is stimulated more as a result of the rewarding activities provided in the enriched environment, which then by some mechanisms attenuates the damage that would be caused by LPS-induced damage to the brain. As previously discussed, the BNST has pathways associated with the VTA, which together help control both anxious and rewarding states to dictate future behaviors (Jennings et al. 2013). In fact, the BNST has been described in many studies as a theoretical extension of the amygdala due to its role in receiving information related to emotions, stress and learning to integrate into pathways responsible for anxiolytic effects as well as feelings of motivation and reward (Luyton et al. 2016; Jennings et al. 2013; Davis et al. 2010; Jalabert et al. 2009; Harris & Ashton-Jones, 2007; Burrow et al. 2005; Walker et al. 2003). This reward circuitry is thereby regulated and is plastic based on the incoming excitation from either rewarding or stressful stimuli (Dumont et al, 2005). Given that the environmental enrichment housing condition was the only condition that involved a complex housing structure, novel play items and physical activities, this may have stimulated reward pathways that the BNST is involved with, motivating future behavior to continue with these activities. The pathways that the BNST shares with the hippocampus, limbic cortical areas, medial prefrontal cortex and VTA have all been described as playing a role in responding to stress as well as directing reward-

seeking behaviors, and respond to stimuli based on the motivation to obtain some form of intrinsic enjoyment (Grace et al. 2007; Herman & Mueller, 2006; Vertes, 2006; Goldstein & Volkow, 2002; Dong et al. 2001; McDonald et al. 1999). Furthermore, the anteroventral and anterolateral areas of the BNST communicate with the VTA to guide rewarding behaviors (Jalabert et al. 2009; Dumont et al. 2005).

With this in mind, the persistent act of stimulating these pathways by taking part in rewarding physical activities may have ultimately improved cell genesis and plasticity in this brain region, which may provide an explanation for how the expected loss of ER-α was counteracted. Studies using deep brain stimulation (DBS) have demonstrated the restorative effects of stimulating certain brain areas, although typically in the context of Parkinson's Disease or Major Depressive Disorder, in potentially offering neuroprotection though improving plasticity and neurogenesis (Herrington et al. 2015). Animal studies have shown that stimulation of subthalamic regions ultimately induced plasticity and synapse formation in a relatively short amount of time within the networks that were activated (Caroni et al. 2014). However, stimulation may not be restricted to one area as activation of a particular brain region can in turn activate networks that ultimately shape brain plasticity and provide protective and restorative effects within multiple brain areas involved in the activated pathways. This is seen in studies that stimulate brain areas like the nucleus accumbens or substantia nigra which in turn activate dopaminergic pathways, with the presence of dopamine being the trigger that increases synaptic plasticity within the whole activated network (Creed et al. 2015; Herrington et al. 2015; Yamawaki et al. 2012). The neuroprotective effects of brain stimulation are further seen in improved cell survival in Parkinson's and lesion models (Temel et al. 2006; Maesawa et al. 2004). The proposed mechanisms by which survival is improved are through reducing toxic

glutamate production from hyperactive brain areas when the dopamine pathways are not activated, as well as by increasing production of brain-derived neurotrophic factor (BDNF) and glia-derived neurotrophic factor (GDNF) which promote growth and maintenance of brain cells (Ho et al 2014; Spieles-Engemann et al. 2011; Wallace et al. 2007). These studies would suggest that the physical components present in EE conditions, and not the social component seen in SE conditions, act as rewarding stimuli that activate BNST pathways and promote neurogenesis and synaptic plasticity in this brain region which minimize the detrimental effects of neuroinflammation following an immune challenge.

### *7.4 ER-α and Maternal Behavior*

Despite there being reductions in ER-α levels within both brain areas in the ACC housing condition following LPS-treatment, there were no reductions in expressed maternal behaviors. In fact, there were actually increased expressions of some behaviors in LPS-treated dams in the SE condition. The lack of reduction in expressed maternal behaviors could suggest that other brain areas are able to compensate for any deficits within the MPOA or BNST. Work in our lab is currently investigating the role of microglial activation within these brain areas following LPS treatment, as well as how ER-α levels in the hippocampus may play a role in secondary maternal behaviors such as spatial reasoning and how that may be involved in maternal behaviors proper (for example, being able to remember where the nest is after foraging). In this sense it is possible that deficits caused by an immune response within these brain areas alone are not enough to translate into significant reductions in expressed maternal behaviors. There is also the potential that the timing of observed maternal behaviors affected the obtained results. Connors et al. (2014) conducted observations at 0600h, 1200h and 2030h, meaning measurements were taken at the tail ends of the animal's dark cycle, and in the middle of their light cycle when they would

typically be sleeping. Once again, if a reverse light/dark cycle was to be employed so observations could take place during the animal's active hours, it's possible different results may have been observed. The skew in rates of maternal behaviors in SE-housed animals could therefore simply be a results of timing of the observations.

Another potential explanation for differences in maternal behaviors (or lack there-of) could be due to similarities between ACC and SE housing conditions. The main differences between these conditions were structural, in that SE housing involved a larger cage and separation via a steel divider during observation periods – dams were still pair-housed in both conditions. It may be advantageous to have animals housed individually in an ACC to act as a social control, eliminating any chance for social engagement. The fact that animals were pair housed in the ACC condition could suggest that the social effects of pair-housing affected, or rather prevented, any reduced expressions of maternal behaviors, suggesting a floor effect from the pair housing.

Despite no differences in maternal behaviors being observed in this study, the long-term reduction in ER-α levels seen in the MPOA and BNST at three weeks post-partum may impact future behaviors. Considering these relatively long-lasting decreases in ER-α levels, it would be interesting to investigate how future pregnancies, and subsequently future immune challenges, further affect ER-α and whether this could eventually translate to altered maternal behaviors. The cumulative loss of ER-α in these brain areas may reach a threshold after which other brain areas may not be able to compensate as previously speculated.

## 8. SIGNIFICANCE OF THIS WORK - AN INTERDISCIPLINARY PERSPECTIVE

Animal research has been and continues to be extremely valuable in expanding our understanding of the impact our environment has on our health. Some discretion must be taken

when generalizing results to humans, especially in drug trials when results can vary between mice, rats, rabbits, monkeys and humans (Bracken, 2008). Pharmacological treatment responses have sometimes only been in accordance with human responses around 50% of the time (Perel et al 2007); the disparity has often been attributed to the difficulty in modelling complex human diseases in animals (Akhtar, 2015). This being said, animal work that draws conclusions from brain and behavior studies have been widely generalized to humans and our understanding of human health and development, as depicted by the numerous studies referenced herein. Studies that observe effects on phylogenetically older parts of the brain (such as the MPOA and BNST) are more confidently applied to our understanding of the human brain, as these brain regions existed among common ancestors with evolutional diversity occurring later in time. Therefore, studies like this provide an opportunity to expand on knowledge of human health and allow us to pose questions on current state of affairs based on relevant findings. With a relative lack of research that involves pregnant women due to ethical or safety concerns, animals research can help provide some perspective into issues that women may face in today's healthcare system. Thus, the impact and the significance of our work can be lightly extended to humans and speaks to grave concerns surrounding women's health and healthcare. We have approached these within an interdisciplinary context in the following sections.

### *8.1 Social Determinants of Health*

Social determinants of health are an appropriate starting point when discussing the interdisciplinary nature of this type of research. Income and social status, education, and access to health services are just some examples by which men and women experience health and illness differently. For example, women with little access to formal education can show less knowledge about health and illness which can result in fewer presentations to health services due

to potentially un-recognized health problems (Vlassoff 2007). Studies looking at both developing and developed countries have noticed a pattern whereby women often show more time being spent in what is characterized as “reproductive work”, which not to be confused with pregnancy, refers to activities such as volunteer or unpaid labor that aims to enhance culture or human attitudes. This results in lower income earned for women participating in these activities and a reflected decrease in sense of autonomy and control of one’s life resulting from inability to gain health care coverage from employers or to pay for uninsured health services (Rathegeber & Vlassof, 1993). In regard to access to health services, with a focus on mental health, women have also reported higher rates of depression and anxiety and express greater illness burden than men, such that mental illness is more disabling to women (Hantsoo & Epperson, 2017; McLean et al. 2011; Rickwood & Braithwaite, 1994). This is further complicated by the fact that women tend to view their illness more negatively than men, further contributing to illness burden (Lernmark et al. 1999; Greca et al. 1995).

Social determinants help to put these health differences we experience into perspective, but an interesting point of view comes from how these factors are considered in the practice of medicine, as well as how patients play a larger role in directing their care in the age of patient-centered medicine and the issues surrounding these practices. An important topic of discussion is the quality of sought medical care during pregnancy. Issues surrounding drug trials in pregnancy to determine safe medications, to social influences of appropriate prenatal medical care and ethical dilemmas faced by physicians all influence the quality of care that a pregnant woman will receive during pregnancy, and to that effect, the impact and severity of any experienced illness or infection, as related to the work reported herein.

### *8.2 Health Research During Pregnancy*

Pregnancy is a period unique in its vulnerability such that women have a modified immune response that can often result in more severe symptoms and hospitalizations from infections such as influenza (Dawood et al. 2018; Regan et al. 2017). However, in a review by Mor & Cardena (2010), the point is made that illness during pregnancy cannot be considered under an umbrella, as though the change in immune response to one pathogen is the same for all pathogens – in fact, different pathogens interact with the mother, fetus and placental barrier in different ways, ultimately requiring different approaches to provide appropriate treatment (Laibl & Sheffield, 2005; Goodnight & Soper, 2005). This has in fact been a barrier to adequate medical care during pregnancy, not so much because pregnant women are given limited access to prenatal care, but rather that simple treatments by general practitioners or family medicine for common illnesses like streptoccocus, influenza, or upper respiratory tract infections suddenly become more complicated during pregnancy. This is a result of both how the pregnant body responds to infection and the restrictions in medications and treatments available during pregnancy due to lack of testing during this period or their administration being not advised during pregnancy. It is therefore more appropriate to see pregnancy as a time of modified immune function with altered responses to pathogens, rather that considering it as immune suppression as a whole (Mor & Cardena, 2010).

Much of the knowledge that exists regarding the safety of medications for pregnant women comes from animal research. Since 1979 and up until June 30$^{th}$ 2015, in the USA, the FDA had issued a labelling system for prescription medications using a five letter system that was intended to categorize medications based on outcomes in animal studies as well as human studies, if any existed, in an effort to simplify the identification of safe medications and those to avoid during pregnancy. However, this rule seemed to oversimplify the risks and benefits such

that it made it difficult for a consumer to conceptualize the difference, for example, between group B medications where animal studies show that offspring do not exhibit problems when the pregnant animals had received the drug, and group C medications, where some offspring had demonstrate negative effects but the medication could still sometimes benefit mother and offspring more than harm them. An over-reliance on animal studies was seen, which cannot guarantee the exact same outcomes in humans. Note that the FDA testing guidelines require studies to demonstrate consistent effects in more than one species of animal; this could help support an assumption of similar effects in humans (FDA, 2014). As a result of critiques of the ambiguous nature of these labelling categories, the FDA replaced the lettering system with what they term as the Pregnancy and Lactation Labelling Rule (PLLR), a more narrative based format that aims to help pregnant women make more informed decisions regarding the medications they take, rather than leaving it solely at their physician's discretion. Rather than simply stating whether benefit or harm came to offspring in animal studies, it also mandates the inclusion of the pregnancy exposure registry, which must now be included to show what the exact results were of medications that were tested on pregnant women.

### *8.3 Standardized Medical Guidelines*

Despite the revised system, decisions regarding medical treatment for pregnant women still remain rather subject to interpretation. The safety of medications prescribed for chronic diseases, like Metformin or insulin for diabetes, Ventolin for asthma, and lithium for bipolar disorder, all have potential dangers when taken during pregnancy despite being the standard treatment for these conditions. Medications prescribed for acute illness are in the same situation – the safety of antibiotics that are commonly prescribed for bacterial infections like urinary tract infections or strep throat is rather unclear during pregnancy. Some antibiotics like penicillin and

amoxicillin are safe after 15 weeks of pregnancy, while others like tetracycline or doxycycline can cause birth defects and have been associated with an increased risk of miscarriages (Muanda et al. 2017). This poses the risk that pregnant women may forgo treatment if they are allergic to a particular family of antibiotics, or avoid medications altogether knowing that certain drugs may cause birth defects. One might suggest over the counter medications for symptom management, though they do not treat the root cause of the illness. To further complicate matters, over the counter medications and supplements are not as closely regulated by the FDA, and they do not carry the category markings that indicate levels of safety for consumption during pregnancy.

Although pregnant women are typically closely followed by obstetricians or OB/GYNs, it is not uncommon for urgent care to be sought for acute illnesses. This then puts into question how medical care is standardized for pregnant women suffering from acute illnesses when guidelines are not clearly stated and treatment could potentially vary from physician to physician. Exaggerated immune responses during pregnancy combined with this model of limited pharmacological treatment for pregnant women could result in women suffering from longer lasting or highly symptomatic illnesses, which as discussed in this paper and countless others, could pose significant risk to proper fetal development.

### *8.4 Social Media and Patient-Centered Medicine*

Subjectivity on appropriate medical care for pregnant women is of course not limited to physicians; in the age of patient-centered medicine, patients are playing a larger role than ever in decisions made regarding their health care. The wealth of information accessible to the layman empowers us to make decisions based on our health-related goals and values, and by what we feel is best for us. However, the information available over the internet or through social media is not always helpful nor is it always safe.

The impact of falsified and manipulated research linking vaccines to autism years ago still remains today, and unfortunately the beliefs in the anti-vaccination movement are as strong as ever. One can see the division of beliefs when looking at the issue of receiving vaccination for influenza (the flu shot) during pregnancy. Numerous human and animal studies have observed the risks of influenza in compromising healthy pregnancies, in that exposure to this viral illness has shown to decrease fetal growth rate and increase the risk for premature birth, miscarriage and stillbirth which has been widely attributed to the inflammatory response and altered nutrient and cytokine transfer through the placenta upon infection (Littauer et al. 2017; Nunes & Madhi, 2015; Martin et al. 2013; Uchide et al. 2012; McNeil et al. 2011). The risks to the fetus are numerous, as described in this paper; exposure to maternal immune activation *in utero* can cause a number of deficits in the developing offspring. With reference to influenza specifically, maternal exposure to the virus and subsequent immune activation at certain times during gestation are linked with an increased risk of developmental delays and neurodevelopmental disorders like autism and schizophrenia in offspring. The mechanisms by which these are believed to occur include alterations in cytokine profiles, modified gene expression, dendritic morphology and increased brain size (Lombardo et al. 2017; Weir et al. 2015; Le Belle et al. 2014; Zerbo et al. 2013; Short et al 2013; Garbett et al. 2012; Atladottir et al. 2012; Canetta & Brown, 2012; Brown 2011; Patterson, 2011; Shi et al. 2009; Smith et al. 2007; Limosin et al. 2003). The annual influenza vaccine has been a proven method of reducing the incidence as well as severity of influenza among the population, offering a safe a minimally invasive way of offering protection for pregnant women (Molgaard-Nielsen et al. 2019; Donzelli, 2019; Esser et al. 2017; Tapia et al. 2016; Madhi et al. 2014; Xia et al. 2014; Temma et al. 2009; Zaman et al. 2008; Munoz et al. 2005; Black et al. 2004). Despite the fairly consistent advocacy by both

scientific literature and health care professionals in practice, a rift remains between professional encouragement and public condemnation of seeking the flu shot during pregnancy. Social media provides a platform for the sharing of many opinions, and pregnant women seeking information regarding the safety or efficacy of the vaccine may be exposed to harmful views and ideas. Dangers can arise when pregnant women take as fact the negative views regarding vaccination during pregnancy. Considering the mere exposure effect in psychology, ideas may seem more agreeable solely for the fact that is unconsciously familiar to us. This could lead women to believe false information online after being bombarded by it, even unknowingly so (Bornstein & D'Agostino, 1992). As a harmful example, we cite a recent study by Donahue et al. (2017), which attributed a potential increase in miscarriage as a result of obtaining two consecutive annual influenza vaccines during pregnancy. This article was posted on the CDC website and was weaponized by social media anti-vaccination activists as undeniable proof that vaccinations are harmful to pregnant women. It is important to note that these activists ignored the fact that the report specified that an association was only observed in women who received one particular strain of the vaccine (not applicable to all influenza vaccinations) and that a causal relationship between influenza vaccination and miscarriage could not be established. Furthermore, also ignored was the fact that the CDC continued to advocate for the administration of the vaccine due to its undeniable wealth of health benefits that far outweighed the slight increase in risk of miscarriage in that particular strain of the vaccine. The issue becomes problematic when women forego beneficial, preventative treatments based on the views they are exposed to in the media, putting them at an increased risk of infection with subsequent potential dangers to both mother and child.

### *8.5 Ethical Treatment of Acute Illness During Pregnancy*

How is it then that physicians respond to expecting mothers who refuse recommended treatments when it appears to put the fetus at risk? Are there policies in place to empower women to make decisions regarding their health and well-being both in clinical situations and in the workplace? In medical practice physicians are guided by the three pillars of ethics: autonomy, beneficence/non-maleficence and justice. The American College of Obstetricians and Gynecologists has developed guidelines based on ethical treatment of both woman and fetus. Though administration of the influenza vaccine is a rather simple medical intervention and these guidelines are likely more widely referred to in cases requiring more complex treatments, the principles remain the same. A misconception in medicine is that the physician's responsibility lies with protecting the developing child. Though the physician must consider all prospects of benefitting or harming the fetus, the supposition that the fetus is the focal point of concern treats women as merely a vehicle for the fetus, when in reality fetal intervention impacts the woman's body as well, and therefore cannot be performed without expressed consent (ACOG, 2011; 2009; Annas, 1986). The patient and fetus cannot be considered as separate patients, otherwise resultant ethical dilemmas arise when priority is put on the developing child at the expense of the mother (Minkoff et al. 2014; McCullough & Chervenak, 2008; Harris & Paltrow, 2008). To this effect, women play a much larger role in decisions regarding their health care during pregnancy as we've moved away from the paternalistic view of medicine. Playing a larger role in the decision-making process can in fact help women choose treatments that align with their values and goals – benefits to the women often lead to benefits to the fetus, which may ultimately have positive effects in the long term when at the moment it may appear counterintuitive to a physician (WHO, 2015). In more severe cases that go far beyond seeking vaccination during pregnancy, there is the possibility of court ordered medical treatment by forced compliance, the

implementation of which is extremely controversial as it completely undermines the autonomy and decision-making capabilities of the patient (Adams et al. 2003; Glass, 2001). The possibility of forced treatment may also cause some women to avoid seeking prenatal care, an important problem in itself considering the potential harm this can have on the fetus (Paltrow & Flavin 2013).

Some may question the possibility of instituting mandatory flu vaccination in pregnant women (bar exclusion for medical reasons) as the benefits far outweigh the risks to the developing child. Afterall, some schools require mandatory vaccinations for students to attend – since 1982, Ontario and New Brunswick have been the only provinces to make routine vaccinations mandatory in school aged children (Walkinshaw, 2011), and according to the "Immunization of School's Pupil's Act", exemptions for medical, philosophical or religious reasons are harder to obtain with parents being required to attend a vaccine safety education session prior to acceptance of exemption. Could mandatory flu vaccinations be on the horizon, and would this be an ethical practice? In reality we are not likely to see the institution of a mandatory flu vaccination policy any time in the near future. The distinguishing parameters surrounding prenatal vaccination to vaccination in school aged children is apparent with an understanding of Tort law (Wright, 1985; Fleming, 1967). Tort law is the process by which civil disputes can render someone amenable to compensation or damages based on emotional or physical distress, breach of privacy, negligence and similar matters, much like the civil version of criminal law. Vaccination is mandated in schools because being negligent in the adherence to vaccination schedules can put other children at risk of harm. When considering autonomy, a person who is of sound mind has every right to either accept a dangerous treatment or refuse a beneficial treatment that can ultimately lead to harm, if they are the only ones affected by the

decision. In this light, the decision for a pregnant woman to forego vaccination for influenza is considered to be only affecting her – again, referring to previous discussions, the fetus is not considered a separate patient, and thus the woman is fully within her rights to refuse such a treatment. For better or for worse, the decision remains with the patient, and as the patient begins to play a more significant role in her health care, she may be in the position to dictate the factors that can help or harm her or her developing child.

It is important to recognize the practical applications of research like ours which investigates an issue from a different perspective – in this case, it is research that focuses on the maternal experience of illness and how this could potentially affect her health and that of her offspring. It is our hope that research which looks through the lens of maternal health be further developed by future studies. Afterall, research into maternal health is not limited to a wet lab or a clinic. Rather, it is necessary to use lab-based discoveries in understanding the mechanisms underlying certain aspects of maternal health and to apply them to further our understanding of issues in current practices regarding maternal health, and likewise to consider current policies and lived experiences of women to guide lab-based research to ultimately improve women's health on a larger scale.

## 9. LIMITATIONS

As an extension of the study by Connors et al. (2014), experimental control was limited in some regards, but we were nonetheless able to achieve some rather interesting results. One experimental manipulation that would have been beneficial and could be worth exploring in future studies is the collection of brains at different times post-partum. It was briefly mentioned that the possibility exists that the MPOA may have begun returning to or exceeding baseline levels of neurogenesis by post-partum day 21, an effect that could have diluted any significant

effect of the immune challenge. Comparing brains collected within a few days following parturition to those at different time points thereafter would be useful to determine the acute changes to these brain areas and whether they naturally return to baseline levels as we may have witnessed. It would also be beneficial to compare pregnant dams to a nulliparous group of control dams who were exposed to similar environmental conditions and immune challenge. This would help determine actual baseline levels of ER-α following parturition without needing to rely on speculation and would more accurately demonstrate the changes the maternal brain undergoes during gestation. Closer observation and documentation of how much dams interact with components of the enriched environments could be useful to gauge whether they obtain some form of enjoyment from the stimulating activities and social interaction or whether they appear stressed in such situations - alternatively, tests of stress reactivity could be useful to assess the effects of EE in female dams. As an additional measure of control, having equal numbers of dams in treatment group could strengthen the significance of our results. Finally, it is important to once again emphasize that these results were obtained under specific EE conditions that were described in the methods section. Considering some of the various effects EE has been reported to have on the brain, some of these observed effects may not be generalizable to all species or strains of animals.

## 10. CONCLUSION

Although we obtained seemingly conflicting results regarding the efficacy of LPS to induce neuroinflammation and subsequently reduce ER-α levels across brain areas, as well as the potential for EE to protect against this deficit, this study has nonetheless shed some light on the vulnerability of the maternal brain during pregnancy. Our findings suggest that brain areas may differ in terms of their susceptibility to neuroinflammatory damage due to the plastic nature of

the maternal brain as well as the changes in hormone levels throughout pregnancy. The perpetual rise in estrogen and expression of ER-α specifically in the MPOA appeared to have provided a localized anti-inflammatory response that in itself protected against the effects of neuroinflammation, maintaining relatively normal levels of ER-α with no significant decrease. In contrast, the BNST does not seem to benefit from such a pronounced rise in estrogen and ER-α, thereby succumbing to prolonged significant damage and loss of ER-α as a result of the immune challenge. However, the stimulating activities that are fundamental to an enriched environment may have affected the BNST more so than the MPOA based on the role of the BNST in motivating rewarding behaviors. This may have in itself led to greater synaptic plasticity, neurogenesis and longer cell life in the BNST, as seen in normalized levels of ER-α when housed in EE conditions during an immune challenge. Taken together, it would appear that both brain areas have vulnerabilities and protective factors based on their function and reorganization during gestation. While the maternal brain is still not immune to the affects of neuroinflammation, it is promising to see that avenues such as EE can offer some form of protection. It is important to again note that these results were obtained under very specific EE conditions that were previously outlined. As discussed, research related to EE can vary significantly based on the types of stimulation offered as well as housing structure, strain of animal and number of animals housed together. With this model of MIA during pregnancy using Sprague-Dawley rats, results suggest that MIA can affect levels of ER-α in at least one key brain area involved in maternal behavior which could ultimately affect the quality and quantity of maternal care offered to offspring, which in itself can have long lasting effects on offspring development. If future studies were to consistently show similar results with different models of EE, as well as in in different species and strains of animals, these results could be more strongly

generalized to humans. This would reinforce the concept that getting sick during pregnancy not only affects the offspring directly *in utero*, but could also just as significantly affect the maternal brain in areas that could compromise the frequency and quality of maternal care provided to newborns, with the potential to affect the mother-child bond which can contribute to altering offspring neurodevelopment throughout the lifespan.

# 11. FIGURES

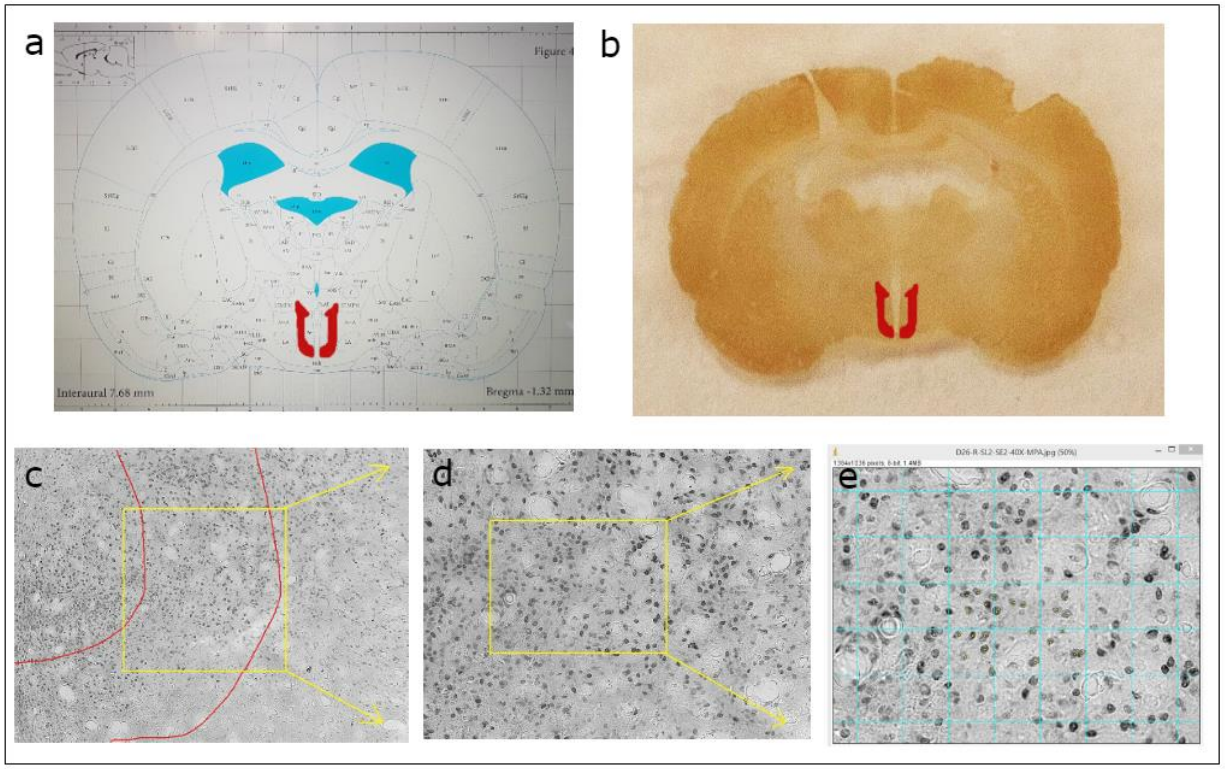



**Figure 1: Identification and microscopy of brain areas.** Brain areas were identified using the Paxinos and Watson Brain Atlas (2007) (a) and associated with corresponding brain sections to determine bregma values and location of brain areas of interest (b). Brain areas were then identified under the microscope and photos were taken at 10x, 20x and 40x objectives (c-d).

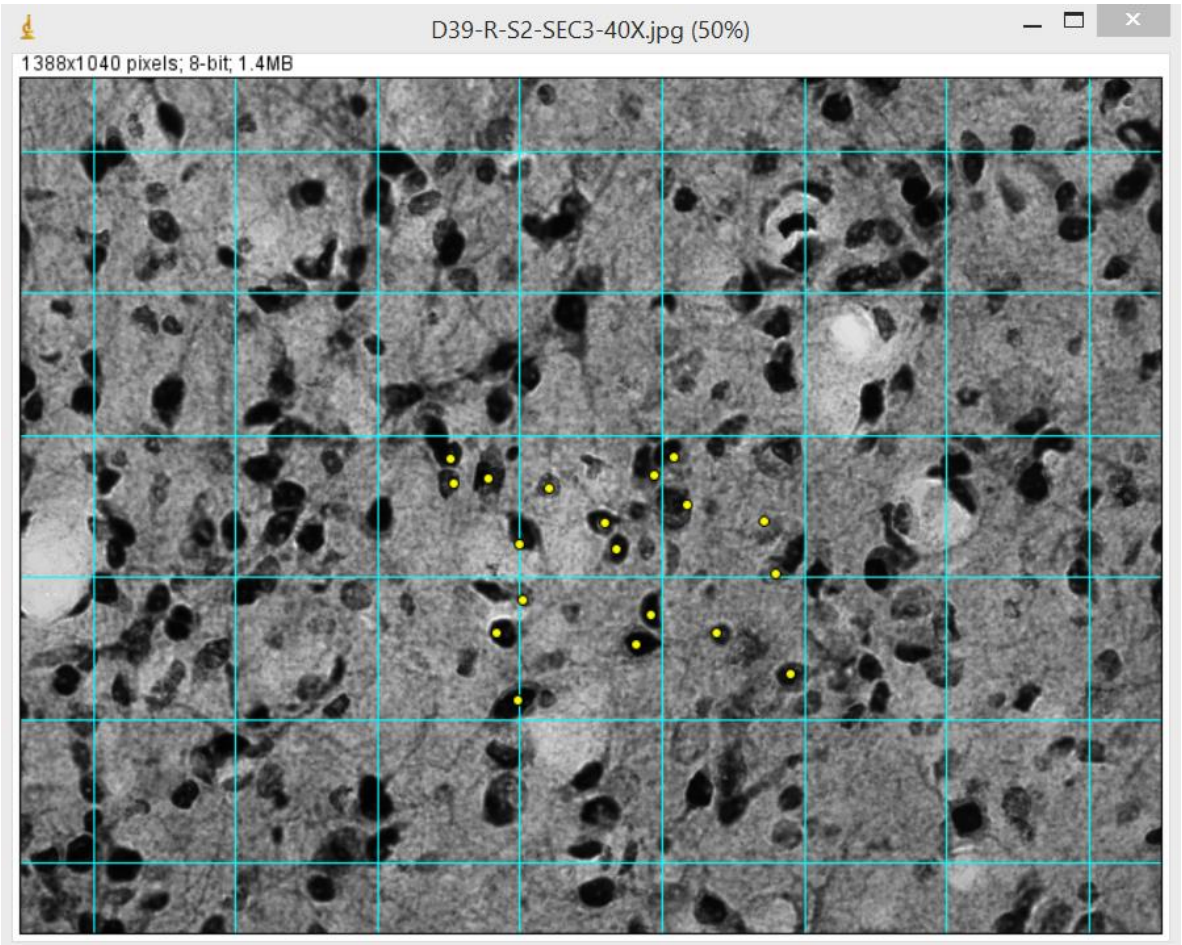



**Figure 2. Example of Cell Counting at 400x.** The same sized grid was laid over all images taken with the 40x objective; receptors were counted only if the complete receptor fell within the central 2x3 area without crossing the outer border.

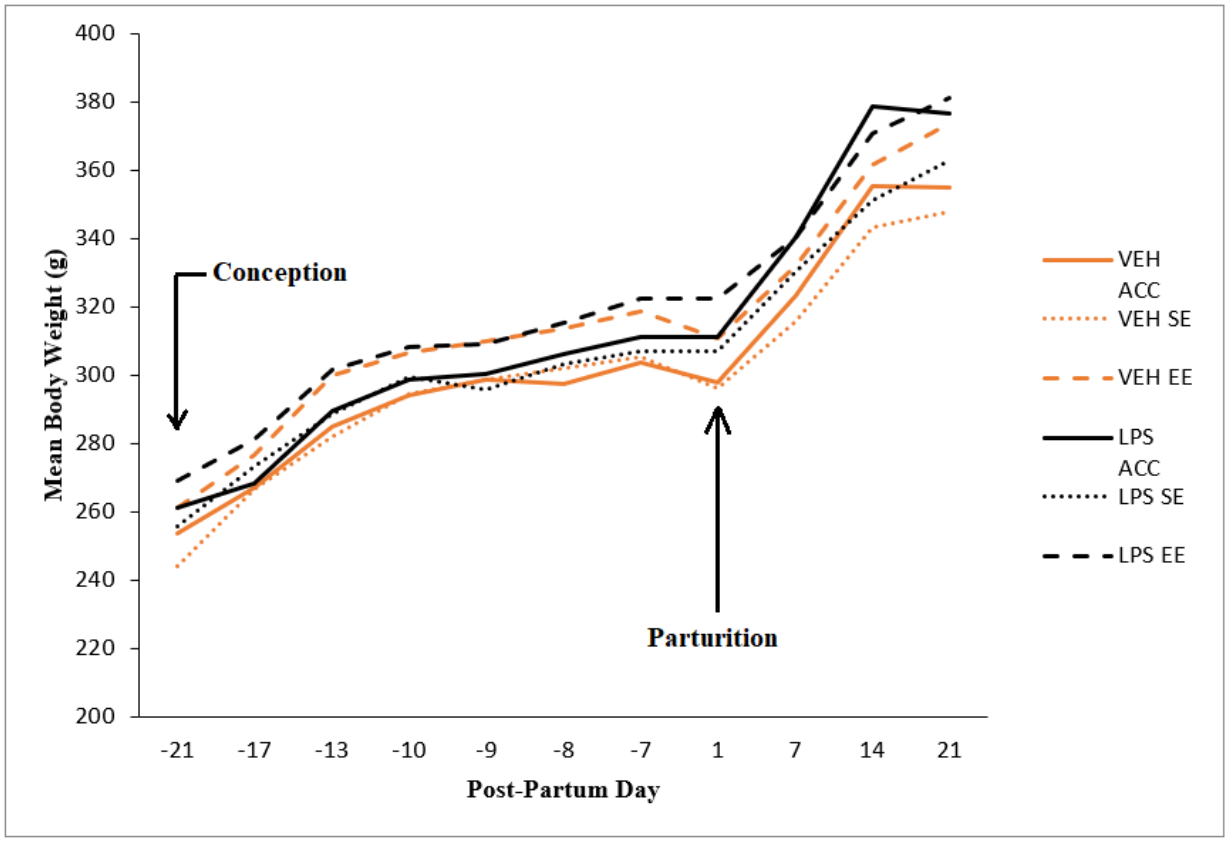
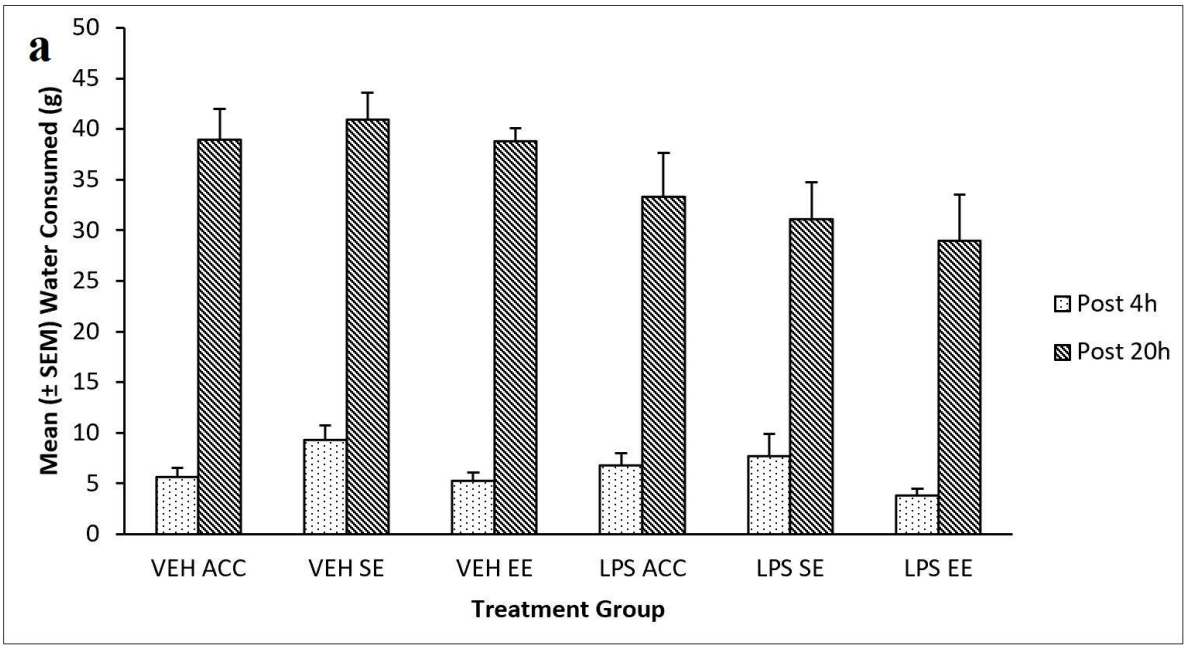



**Figure 3. Mean body weights during gestation:** Dams were weighed at varying time points from conception (21 days prior to parturition) up to 21 days post-partum. Comparisons were made between LPS and saline-treated dams across all housing conditions. We observe an effect of time but no seemingly important difference in weight gain due to treatment and housing.



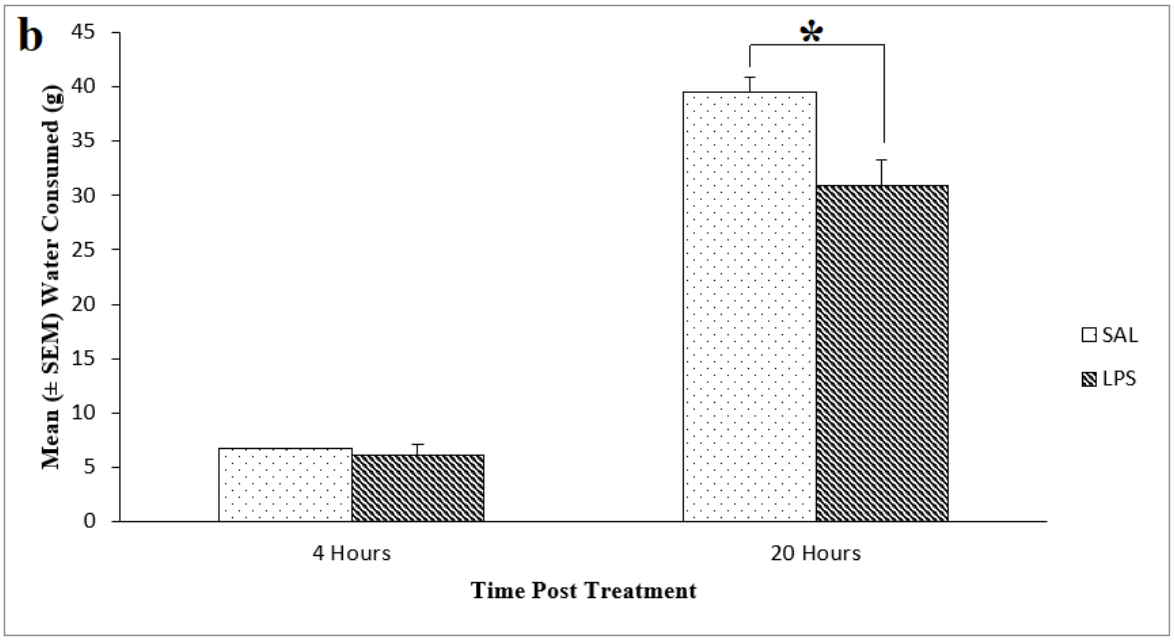




**Figure 4. Mean water intake following treatment:** a.Values represent mean ± SEM grams of water consumption in dams at 4 hours and 20 hours post-treatment across all treatment and housing groups. We found a significant Time by Treatment interaction ($F_{1,35}$=6.84; $p<0.05$). b.Values represent mean ± SEM grams of water consumption, with a focus on the comparison made between LPS and saline-treated dams 4 hours and 20 hours post-treatment. A significant Treatment difference was only seen at 20h post treatment ($F_{1,35}$=9.09; $p<0.05$).

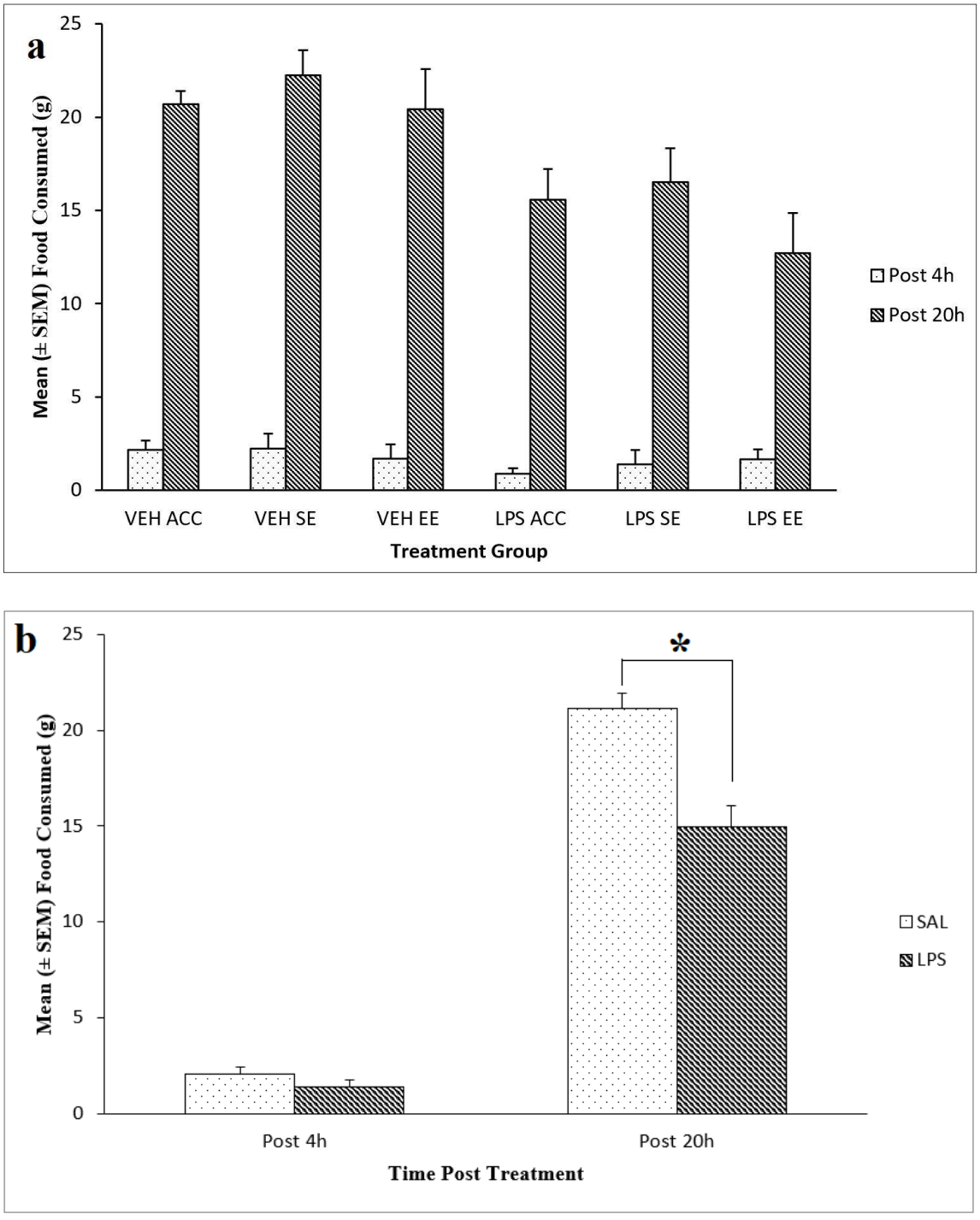



**Figure 5. Mean food intake following treatment:** a. Values represent mean ± SEM grams of food consumption in dams at 4 hours and 20 hours post-treatment across all treatment and housing groups. We found a significant interaction between Time and Treatment ($F_{1,35}$=11.93; $p<0.05$). b. Values represent mean ± SEM grams of food consumption, with a focus on the comparison made between LPS and saline-treated dams 4 hours and 20 hours post-treatment. Significant different in Treatment was only seen at 20h post treatment ($F_{1,35}$=18.49; $p<0.05$).

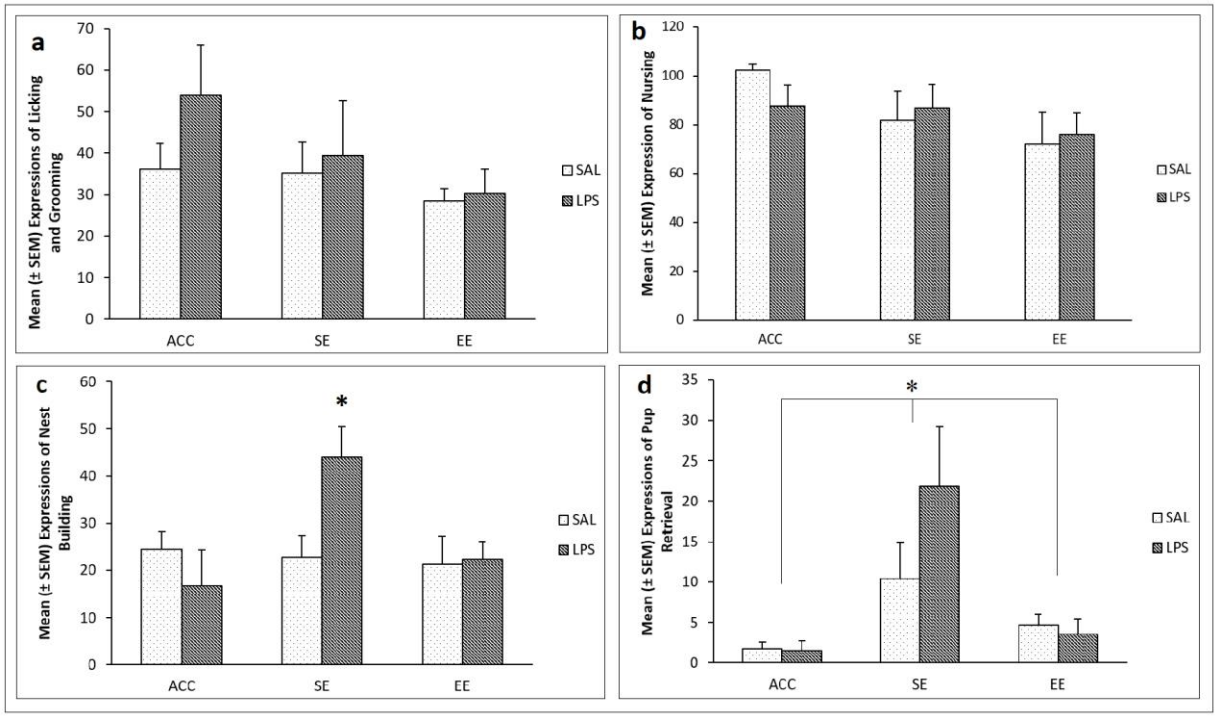



**Figure 6. Mean expression of maternal behaviors:** a. Values represent mean ± SEM total instances of observed licking and grooming over post-partum day 2-6 across all treatment and housing groups. No significant differences were observed. b. Values represent mean ± SEM total instances of nursing over post-partum day 2-6 across all treatment and housing groups. No significant differences were observed. c. Values represent mean ± SEM total instances of observed nest building over post-partum day 2-6 across all treatment and housing groups. A significant interaction of Housing by Treatment was observed ($F_{1,31}$=8.02; p<0.008). d. Values represent mean ± SEM total instances of observed pup retrieval over post-partum day 2-6 across all treatment and housing groups. A significant effect of Housing was observed ($F_{2,31}$=7.97; p<0.002). (Connors et al. 2014)

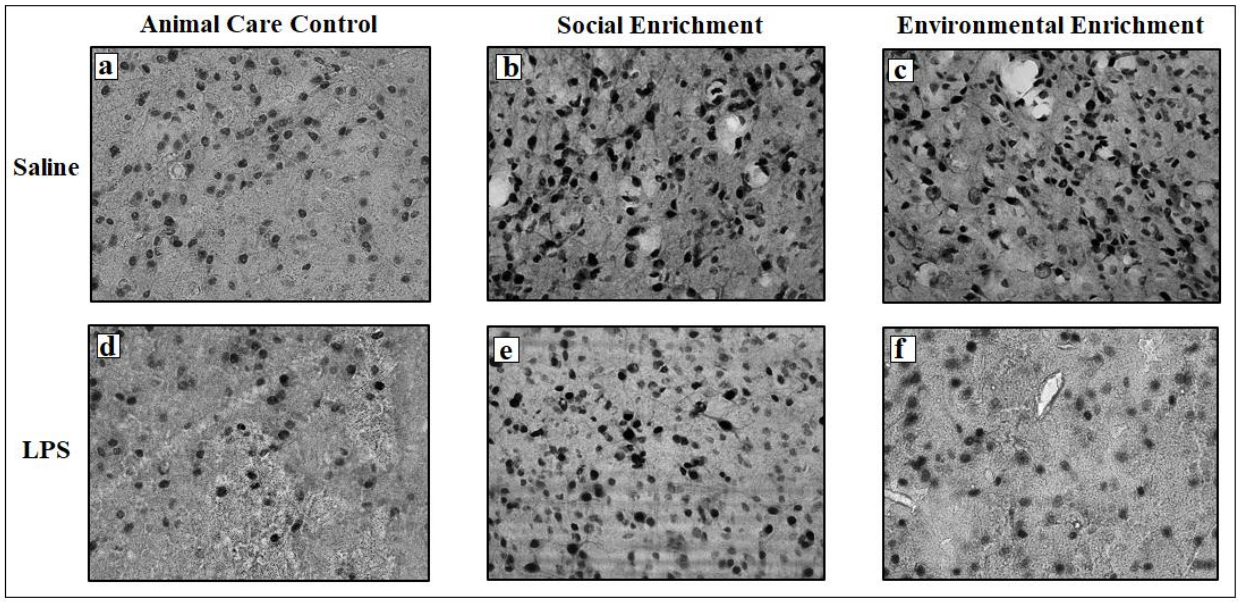



**Figure 7. Representative pictographs of the MPOA:** ER-α immunopositive cells visualized at a 400x in the MPOA of dams housed in ACC, SE and EE conditions when treated with saline (a, b, c) and LPS (d, e, f), respectively.

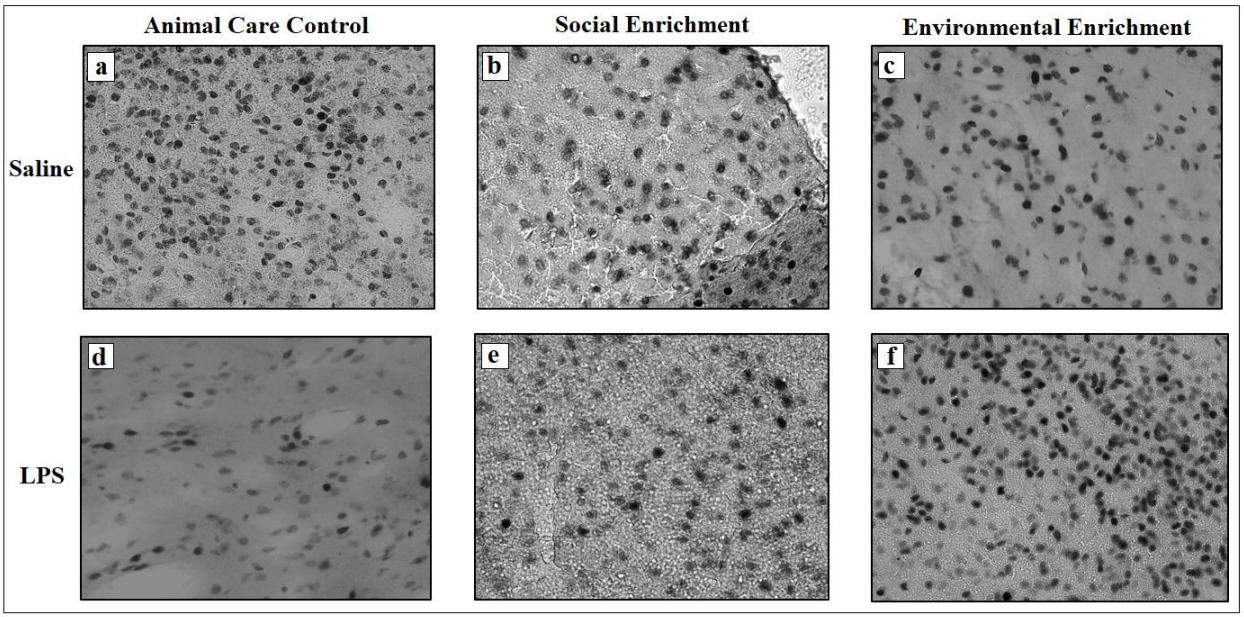



**Figure 8. Representative pictographs of the BNST:** ER-α immunopositive cells visualized at a 400x in the BNST of dams housed in ACC, SE and EE conditions when treated with saline (a, b, c) and LPS (d, e, f), respectively.

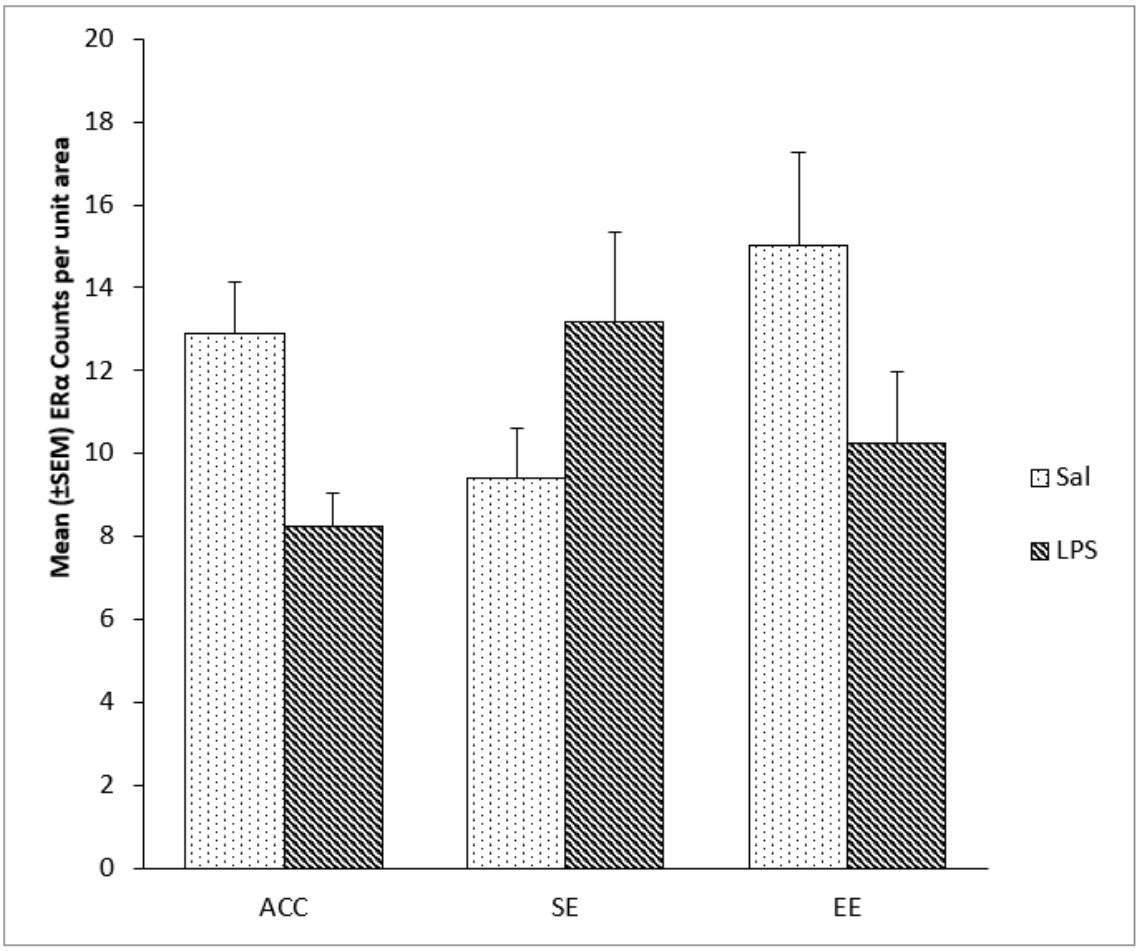



**Figure 9. Mean ER-α levels in the MPOA:** ER-α levels represented by mean ± SEM in the MPOA of dams housed in ACC, SE and EE conditions. A Treatment by Housing interaction was detected ($F_{2,35}$=4.07; $p<0.05$); simple effects did not detect any specific pairwise Treatment differences at each Housing condition.

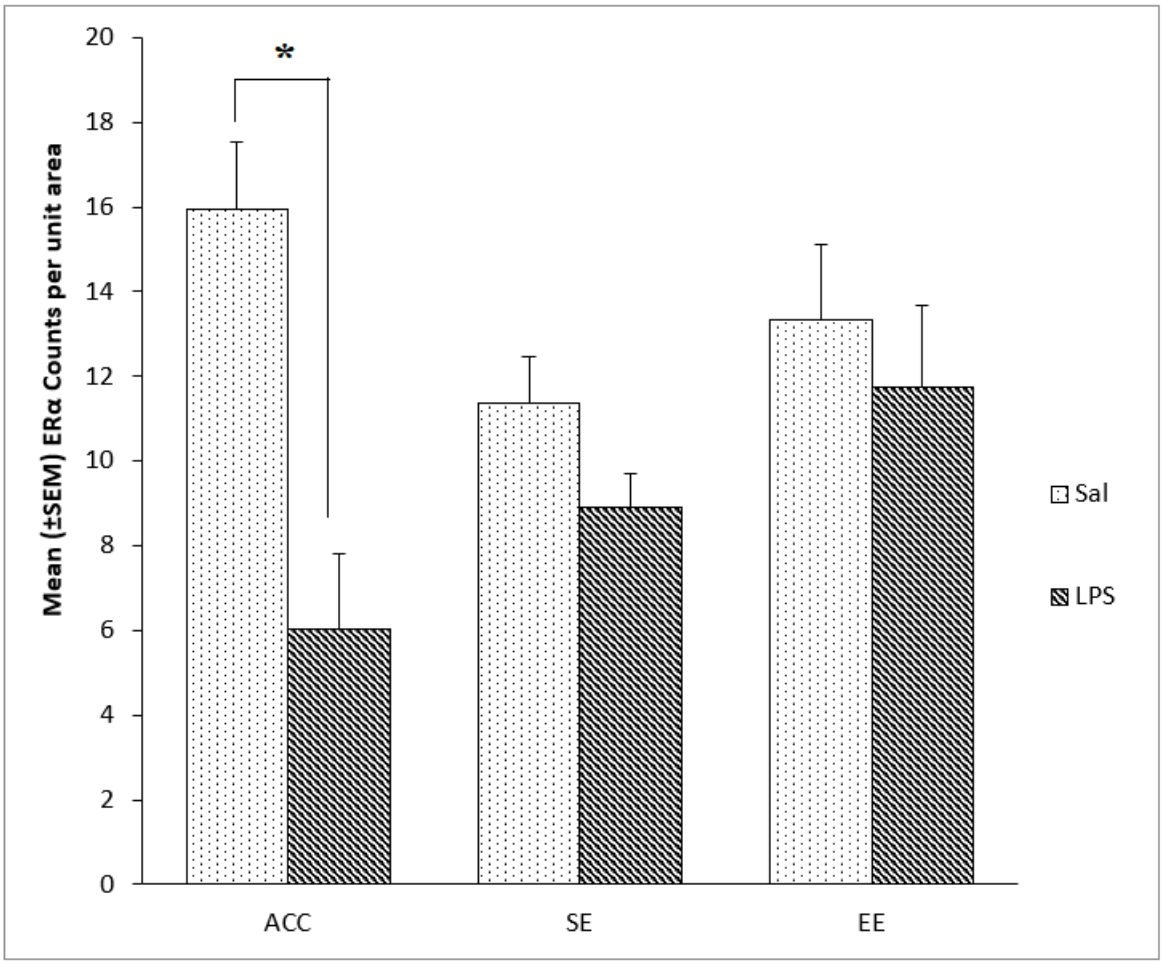



**Figure 10. Mean ER-α levels in the BNST:** ER-α levels represented by mean ± SEM in the BNST of dams housed in ACC, SE and EE conditions. A significant interaction between Treatment and Housing was detected ($F_{2,35}$=3.93; $p<0.05$). A difference between saline and LPS treated dams in the number of ER-α positive cells was only seen in the BNST in ACC housed dams ($F_{1,35}$=16.50;$p < 0.05$).

## 12. REFERENCES


Abbas, A. K., Murphy, K. M., & Sher, A. (1996). Functional diversity of helper T lymphocytes. *Nature*, *383*(6603), 787–793. https://doi.org/10.1038/383787a0

Abram, M., Vu ković, D., Wraber, B., & Dorić, M. (2000). Plasma cytokine response in mice with bacterial infection. *Mediators of Inflammation*, *9*(5), 229–234. https://doi.org/10.1080/09629350020025746

ACOG Committee on Ethics. (2009). ACOG Committee Opinion No. 439: Informed consent. *Obstetrics and Gynecology*, *114*(2 Pt 1), 401–408. https://doi.org/10.1097/AOG.0b013e3181b48f7f

Adams, S. F., Mahowald, M. B., & Gallagher, J. (2003). Refusal of treatment during pregnancy. *Clinics in Perinatology*, *30*(1), 127–140, vii–viii.

Aguilar-Valles, A., Jung, S., Poole, S., Flores, C., & Luheshi, G. N. (2012). Leptin and interleukin-6 alter the function of mesolimbic dopamine neurons in a rodent model of prenatal inflammation. *Psychoneuroendocrinology*, *37*(7), 956–969. https://doi.org/10.1016/j.psyneuen.2011.11.003

Aguilar-Valles, A., & Luheshi, G. N. (2011). Alterations in cognitive function and behavioral response to amphetamine induced by prenatal inflammation are dependent on the stage of pregnancy. *Psychoneuroendocrinology*, *36*(5), 634–648. https://doi.org/10.1016/j.psyneuen.2010.09.006

Aharoni, R., Arnon, R., & Eilam, R. (2005). Neurogenesis and neuroprotection induced by peripheral immunomodulatory treatment of experimental autoimmune encephalomyelitis. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *25*(36), 8217–8228. https://doi.org/10.1523/JNEUROSCI.1859-05.2005

AKHTAR, A. (2015). The Flaws and Human Harms of Animal Experimentation. *Cambridge Quarterly of Healthcare Ethics*, *24*(4), 407–419. https://doi.org/10.1017/S0963180115000079

Akiyama, H., Barger, S., Barnum, S., Bradt, B., Bauer, J., Cole, G. M., Cooper, N. R., Eikelenboom, P., Emmerling, M., Fiebich, B. L., Finch, C. E., Frautschy, S., Griffin, W. S. T., Hampel, H., Hull, M., Landreth, G., Lue, L., Mrak, R., Mackenzie, I. R., … Wyss–Coray, T. (2000). Inflammation and Alzheimer's disease. *Neurobiology of Aging*, *21*(3), 383–421. https://doi.org/10.1016/S0197-4580(00)00124-X

Alexopoulou, L., Holt, A. C., Medzhitov, R., & Flavell, R. A. (2001). Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature*, *413*(6857), 732–738. https://doi.org/10.1038/35099560

Al-Shammri, S., Rawoot, P., Azizieh, F., AbuQoora, A., Hanna, M., Saminathan, T. R., & Raghupathy, R. (2004). Th1/Th2 cytokine patterns and clinical profiles during and after pregnancy in women with multiple sclerosis. *Journal of the Neurological Sciences*, *222*(1–2), 21–27. https://doi.org/10.1016/j.jns.2004.03.027

Alwis, D. S., & Rajan, R. (2014). Environmental enrichment and the sensory brain: The role of enrichment in remediating brain injury. *Frontiers in Systems Neuroscience*, *8*, 156. https://doi.org/10.3389/fnsys.2014.00156

American College of Obstetricians and Gynecologists. Committee on Ethics, & American Academy of Pediatrics.Committee on Bioethics. (2011). Committee opinion no. 501: Maternal-fetal intervention and fetal care centers. *Obstetrics and Gynecology*, *118*(2 Pt 1), 405–410. https://doi.org/10.1097/AOG.0b013e31822c99af

Anisman, H., Zalcman, S., & Zacharko, R. M. (1993). The impact of stressors on immune and central neurotransmitter activity: Bidirectional communication. *Reviews in the Neurosciences*, *4*(2), 147–180.

Annas, G. J. (1986). Pregnant women as fetal containers. *The Hastings Center Report*, *16*(6), 13–14.

Apostolou, E., & Thanos, D. (2008). Virus Infection Induces NF-kappaB-dependent interchromosomal associations mediating monoallelic IFN-beta gene expression. *Cell*, *134*(1), 85–96. https://doi.org/10.1016/j.cell.2008.05.052

Atladottir, H. O., Henriksen, T. B., Schendel, D. E., & Parner, E. T. (2012). Autism after infection, febrile episodes, and antibiotic use during pregnancy: An exploratory study. *Pediatrics*, *130*(6), e1447-1454. https://doi.org/10.1542/peds.2012-1107

Audet, M.-C., Jacobson-Pick, S., Wann, B. P., & Anisman, H. (2011). Social defeat promotes specific cytokine variations within the prefrontal cortex upon subsequent aggressive or endotoxin challenges. *Brain, Behavior, & Immunity*, *25*(6), 1197–1205. https://doi.org/10.1016/j.bbi.2011.03.010

Babiker, F. A., De Windt, L. J., van Eickels, M., Grohe, C., Meyer, R., & Doevendans, P. A. (2002). Estrogenic hormone action in the heart: Regulatory network and function. [Review] [119 refs]. *Cardiovascular Research*, *53*(3), 709–719.

Baldini, S., Restani, L., Baroncelli, L., Coltelli, M., Franco, R., Cenni, M. C., Maffei, L., & Berardi, N. (2013). Enriched Early Life Experiences Reduce Adult Anxiety-Like Behavior in Rats: A Role for Insulin-Like Growth Factor 1. *Journal of Neuroscience*, *33*(28), 11715–11723. https://doi.org/10.1523/JNEUROSCI.3541-12.2013

Barha, C. K., Hanna, C. W., Salvante, K. G., Wilson, S. L., Robinson, W. P., Altman, R. M., & Nepomnaschy, P. A. (2016). Number of Children and Telomere Length in Women: A Prospective, Longitudinal Evaluation. *PLOS ONE*, *11*(1), e0146424. https://doi.org/10.1371/journal.pone.0146424

Barha, C. K., Lieblich, S. E., Chow, C., & Galea, L. A. M. (2015). Multiparity-induced enhancement of hippocampal neurogenesis and spatial memory depends on ovarian hormone status in middle

age. *Neurobiology of Aging*, *36*(8), 2391–2405. https://doi.org/10.1016/j.neurobiolaging.2015.04.007

Baroncelli, L., Braschi, C., Spolidoro, M., Begenisic, T., Sale, A., & Maffei, L. (2010). Nurturing brain plasticity: Impact of environmental enrichment. *Cell Death and Differentiation*, *17*(7), 1092–1103. https://doi.org/10.1038/cdd.2009.193

Barry, P. A., Lockridge, K. M., Salamat, S., Tinling, S. P., Yue, Y., Zhou, S. S., Gospe, S. M., Britt, W. J., & Tarantal, A. F. (2006). Nonhuman primate models of intrauterine cytomegalovirus infection. *ILAR Journal*, *47*(1), 49–64. https://doi.org/10.1093/ilar.47.1.49

Bartolomucci, A., Palanza, P., Sacerdote, P., Panerai, A. E., Sgoifo, A., Dantzer, R., & Parmigiani, S. (2005). Social factors and individual vulnerability to chronic stress exposure. [Review] [103 refs]. *Neuroscience & Biobehavioral Reviews*, *29*(1), 67–81.

Bastos, G. N., Moriya, T., Inui, F., Katura, T., & Nakahata, N. (2008). Involvement of cyclooxygenase-2 in lipopolysaccharide-induced impairment of the newborn cell survival in the adult mouse dentate gyrus. *Neuroscience*, *155*(2), 454–462. https://doi.org/10.1016/j.neuroscience.2008.06.020

Bayne, K. (2005). Potential for unintended consequences of environmental enrichment for laboratory animals and research results. [Review] [116 refs]. *Ilar Journal*, *46*(2), 129–139.

Beato, M. (1989). Gene regulation by steroid hormones. [Review] [122 refs]. *Cell*, *56*(3), 335–344.

Bebo, B. F., Fyfe-Johnson, A., Adlard, K., Beam, A. G., Vandenbark, A. A., & Offner, H. (2001). Low-dose estrogen therapy ameliorates experimental autoimmune encephalomyelitis in two different inbred mouse strains. *Journal of Immunology (Baltimore, Md.: 1950)*, *166*(3), 2080–2089. https://doi.org/10.4049/jimmunol.166.3.2080

Beckstead, R. M., Domesick, V. B., & Nauta, W. J. (1979). Efferent connections of the substantia nigra and ventral tegmental area in the rat. *Brain Research*, *175*(2), 191–217. https://doi.org/10.1016/0006-8993(79)91001-1

Belz, E. E., Kennell, J. S., Czambel, R. K., Rubin, R. T., & Rhodes, M. E. (2003). Environmental enrichment lowers stress-responsive hormones in singly housed male and female rats. *Pharmacology, Biochemistry & Behavior*, *76*(3–4), 481–486.

Beutler, B., Greenwald, D., Hulmes, J. D., Chang, M., Pan, Y. C., Mathison, J., Ulevitch, R., & Cerami, A. (1985). Identity of tumour necrosis factor and the macrophage-secreted factor cachectin. *Nature*, *316*(6028), 552–554. https://doi.org/10.1038/316552a0

Black, S. B., Shinefield, H. R., France, E. K., Fireman, B. H., Platt, S. T., Shay, D., & Vaccine Safety Datalink Workgroup. (2004). Effectiveness of influenza vaccine during pregnancy in preventing hospitalizations and outpatient visits for respiratory illness in pregnant women and their infants. *American Journal of Perinatology*, *21*(6), 333–339. https://doi.org/10.1055/s-2004-831888

Blasius, A. L., & Beutler, B. (2010). Intracellular toll-like receptors. *Immunity*, *32*(3), 305–315. https://doi.org/10.1016/j.immuni.2010.03.012

Bornstein, R. F., & D'Agostino, P. R. (1992). Stimulus recognition and the mere exposure effect. *Journal of Personality and Social Psychology*, *63*(4), 545–552. https://doi.org/10.1037/0022-3514.63.4.545

Bracken, M. B. (2009). Why animal studies are often poor predictors of human reactions to exposure. *Journal of the Royal Society of Medicine*, *102*(3), 120–122. https://doi.org/10.1258/jrsm.2008.08k033

Branchi, I., Santarelli, S., D'Andrea, I., & Alleva, E. (2013). Not all stressors are equal: Early social enrichment favors resilience to social but not physical stress in male mice. *Hormones and Behavior*, *63*(3), 503–509. https://doi.org/10.1016/j.yhbeh.2013.01.003

Brenes, J. C., Padilla, M., & Fornaguera, J. (2009). A detailed analysis of open-field habituation and behavioral and neurochemical antidepressant-like effects in postweaning enriched rats. *Behavioural Brain Research*, *197*(1), 125–137. https://doi.org/10.1016/j.bbr.2008.08.014

Brenes, J. C., Rodriguez, O., & Fornaguera, J. (2008). Differential effect of environment enrichment and social isolation on depressive-like behavior, spontaneous activity and serotonin and norepinephrine concentration in prefrontal cortex and ventral striatum. *Pharmacology, Biochemistry & Behavior*, *89*(1), 85–93.

Briones, T. L., Woods, J., & Rogozinska, M. (2013). Decreased neuroinflammation and increased brain energy homeostasis following environmental enrichment after mild traumatic brain injury is associated with improvement in cognitive function. *Acta Neuropathologica Communications*, *1*, 57. https://doi.org/10.1186/2051-5960-1-57

Brown, A. S. (2011). Exposure to prenatal infection and risk of schizophrenia. *Frontiers in Psychiatry Frontiers Research Foundation*, *1*, 63. https://doi.org/10.3389/fpsyt.2011.00063

Brown, C. M., Choi, E., Xu, Q., Vitek, M. P., & Colton, C. A. (2008). The APOE4 genotype alters the response of microglia and macrophages to 17beta-estradiol. *Neurobiology of Aging*, *29*(12), 1783–1794. https://doi.org/10.1016/j.neurobiolaging.2007.04.018

Brown, K. J., & Grunberg, N. E. (1995). Effects of housing on male and female rats: Crowding stresses male but calm females. *Physiology & Behavior*, *58*(6), 1085–1089.

Bruel-Jungerman, E., Laroche, S., & Rampon, C. (2005). New neurons in the dentate gyrus are involved in the expression of enhanced long-term memory following environmental enrichment. *Journal of Neuroscience*, *21*(2), 513–521.

Brunton, P. J., & Russell, J. A. (2008). The expectant brain: Adapting for motherhood. [Review] [182 refs]. *Nature Reviews Neuroscience*, *9*(1), 11–25. https://doi.org/10.1038/nrn2280

Burow, A., Day, H. E. W., & Campeau, S. (2005). A detailed characterization of loud noise stress: Intensity analysis of hypothalamo-pituitary-adrenocortical axis and brain activation. *Brain Research*, *1062*(1–2), 63–73. https://doi.org/10.1016/j.brainres.2005.09.031

Buyon, J. P. (1998). The effects of pregnancy on autoimmune diseases. *Journal of Leukocyte Biology*, *63*(3), 281–287. https://doi.org/10.1002/jlb.63.3.281

Cai, K. C., van Mil, S., Murray, E., Mallet, J.-F., Matar, C., & Ismail, N. (2016). Age and sex differences in immune response following LPS treatment in mice. *Brain, Behavior, and Immunity*, *58*, 327–337. https://doi.org/10.1016/j.bbi.2016.08.002

Campesi, I., Marino, M., Montella, A., Pais, S., & Franconi, F. (2017). Sex Differences in Estrogen Receptor alpha and beta Levels and Activation Status in LPS-Stimulated Human Macrophages. *Journal of Cellular Physiology*, *232*(2), 340–345. https://doi.org/10.1002/jcp.25425

Canetta, S. E., & Brown, A. S. (2012). Prenatal infection, maternal immune activation, and risk for schizophrenia. *Translational Neuroscience*, *3*(4), 320–327. https://doi.org/10.2478/s13380-012-0045-6

Canick, J. A., & Ryan, K. J. (1978a). Properties of the aromatase system associated with the mitochondrial fraction of human placenta. *Steroids*, *32*(4), 499–509.

Canick, J. A., & Ryan, K. J. (1978b). Properties of the aromatase system associated with the mitochondrial fraction of human placenta. *Steroids*, *32*(4), 499–509.

Caroni, P., Chowdhury, A., & Lahr, M. (2014). Synapse rearrangements upon learning: From divergent–sparse connectivity to dedicated sub-circuits. *Trends in Neurosciences*, *37*(10), 604–614. https://doi.org/10.1016/j.tins.2014.08.011

Carpenter, L. R., Moy, J. N., & Roebuck, K. A. (2002). Respiratory syncytial virus and TNF alpha induction of chemokine gene expression involves differential activation of Rel A and NF-kappa B1. *BMC Infectious Diseases*, *2*, 5.

Cavaillon, J. M. (2001). Pro- versus anti-inflammatory cytokines: Myth or reality. *Cellular and Molecular Biology (Noisy-Le-Grand, France)*, *47*(4), 695–702.

*CCAC - Canadian Council on Animal Care: Environmental Enrichment*. (n.d.). Retrieved September 18, 2019, from https://www.ccac.ca/en/training/modules/animals-housed-in-vivaria-stream/environmental-enrichment.html

Champagne, F. A., Weaver, I. C. G., Diorio, J., Sharma, S., & Meaney, M. J. (2003). Natural variations in maternal care are associated with estrogen receptor alpha expression and estrogen sensitivity in the medial preoptic area. *Endocrinology*, *144*(11), 4720–4724. https://doi.org/10.1210/en.2003-0564

Champagne, F., Diorio, J., Sharma, S., & Meaney, M. J. (2001). Naturally occurring variations in maternal behavior in the rat are associated with differences in estrogen-inducible central oxytocin receptors. *Proceedings of the National Academy of Sciences*, *98*(22), 12736–12741. https://doi.org/10.1073/pnas.221224598

Chan, R. W., Ho, L. C., Zhou, I. Y., Gao, P. P., Chan, K. C., & Wu, E. X. (2015). Structural and Functional Brain Remodeling during Pregnancy with Diffusion Tensor MRI and Resting-State Functional MRI. *PLoS ONE [Electronic Resource]*, *10*(12), e0144328. https://doi.org/10.1371/journal.pone.0144328

Chapillon, P., Patin, V., Roy, V., Vincent, A., & Caston, J. (2002). Effects of pre- and postnatal stimulation on developmental, emotional, and cognitive aspects in rodents: A review. *Developmental Psychobiology*, *41*(4), 373–387. https://doi.org/10.1002/dev.10066

Charles A Janeway, J., Travers, P., Walport, M., & Shlomchik, M. J. (2001). *The complement system and innate immunity*. https://www.ncbi.nlm.nih.gov/books/NBK27100/

Chiba, T., & Murata, Y. (1985). Afferent and efferent connections of the medial preoptic area in the rat: A WGA-HRP study. *Brain Research Bulletin*, *14*(3), 261–272. https://doi.org/10.1016/0361-9230(85)90091-7

Chokkalingam, V., Tel, J., Wimmers, F., Liu, X., Semenov, S., Thiele, J., Figdor, C. G., & Huck, W. T. S. (2013). Probing cellular heterogeneity in cytokine-secreting immune cells using droplet-based microfluidics. *Lab on a Chip*, *13*(24), 4740–4744. https://doi.org/10.1039/c3lc50945a

Christensen, A., Dewing, P., & Micevych, P. (2011). Membrane-initiated estradiol signaling induces spinogenesis required for female sexual receptivity. *Journal of Neuroscience*, *31*(48), 17583–17589. https://doi.org/10.1523/JNEUROSCI.3030-11.2011

Colucci, M., Cammarata, S., Assini, A., Croce, R., Clerici, F., Novello, C., Mazzella, L., Dagnino, N., Mariani, C., & Tanganelli, P. (2006). The number of pregnancies is a risk factor for Alzheimer's disease. *European Journal of Neurology*, *13*(12), 1374–1377. https://doi.org/10.1111/j.1468-1331.2006.01520.x

Connors, E. J., Migliore, M. M., Pillsbury, S. L., Shaik, A. N., & Kentner, A. C. (2015). Environmental enrichment models a naturalistic form of maternal separation and shapes the anxiety response patterns of offspring. *Psychoneuroendocrinology*, *1*, 153–167. https://doi.org/10.1016/j.psyneuen.2014.10.021

Connors, E. J., Shaik, A. N., Migliore, M. M., & Kentner, A. C. (2014a). Environmental enrichment mitigates the sex-specific effects of gestational inflammation on social engagement and the hypothalamic pituitary adrenal axis-feedback system. *Brain, Behavior, & Immunity*, 178–190. https://doi.org/10.1016/j.bbi.2014.06.020

Connors, E. J., Shaik, A. N., Migliore, M. M., & Kentner, A. C. (2014b). Environmental enrichment mitigates the sex-specific effects of gestational inflammation on social engagement and the hypothalamic pituitary adrenal axis-feedback system. *Brain, Behavior, & Immunity*, *1*, 178–190. https://doi.org/10.1016/j.bbi.2014.06.020

Conrad, L. C. A., & Pfaff, D. W. (1976). Efferents from medial basal forebrain and hypothalamus in the rat. II. An autoradiographic study of the anterior hypothalamus. *Journal of Comparative Neurology*, *169*(2), 221–261. https://doi.org/10.1002/cne.901690206

Coutinho, A. E., & Chapman, K. E. (2011). The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. *Molecular and Cellular Endocrinology*, *335*(1), 2–13. https://doi.org/10.1016/j.mce.2010.04.005

Creed, M., Pascoli, V. J., & Lüscher, C. (2015). Addiction therapy. Refining deep brain stimulation to emulate optogenetic treatment of synaptic pathology. *Science (New York, N.Y.)*, *347*(6222), 659–664. https://doi.org/10.1126/science.1260776

Cui, M., Yang, Y., Yang, J., Zhang, J., Han, H., Ma, W., Li, H., Mao, R., Xu, L., Hao, W., & Cao, J. (2006). Enriched environment experience overcomes the memory deficits and depressive-like behavior induced by early life stress. *Neuroscience Letters*, *404*(1–2), 208–212.

Cuneo, A. A., & Autieri, M. V. (2009). Expression and Function of Anti-Inflammatory Interleukins: The Other Side of the Vascular Response to Injury. *Current Vascular Pharmacology*, *7*(3), 267–276.

Cunningham, C., Campion, S., Teeling, J., Felton, L., & Perry, V. H. (2007). The sickness behaviour and CNS inflammatory mediator profile induced by systemic challenge of mice with synthetic double-stranded RNA (poly I:C). *Brain, Behavior, and Immunity*, *21*(4), 490–502. https://doi.org/10.1016/j.bbi.2006.12.007

Cuzzocrea, S., Genovese, T., Mazzon, E., Esposito, E., Di Paola, R., Muia, C., Crisafulli, C., Peli, A., Bramanti, P., & Chaudry, I. H. (2008). Effect of 17beta-estradiol on signal transduction pathways and secondary damage in experimental spinal cord trauma. *Shock*, *29*(3), 362–371. https://doi.org/10.1097/shk.0b013e31814545dc

Cymerblit-Sabba, A., Lasri, T., Gruper, M., Aga-Mizrachi, S., Zubedat, S., & Avital, A. (2013). Prenatal Enriched Environment improves emotional and attentional reactivity to adulthood stress. *Behavioural Brain Research*, *241*, 185–190. https://doi.org/10.1016/j.bbr.2012.12.019

Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W., & Kelley, K. W. (2008). From inflammation to sickness and depression: When the immune system subjugates the brain. [Review] [106 refs]. *Nature Reviews Neuroscience*, *9*(1), 46–56. https://doi.org/10.1038/nrn2297

D'Astous, M., Morissette, M., & Di Paolo, T. (2004). Effect of estrogen receptor agonists treatment in MPTP mice: Evidence of neuroprotection by an ER alpha agonist. *Neuropharmacology*, *47*(8), 1180–1188. https://doi.org/10.1016/j.neuropharm.2004.08.020

David, F., Farley, J., Huang, H., Lavoie, J.-P., & Laverty, S. (2007). Cytokine and chemokine gene expression of IL-1beta stimulated equine articular chondrocytes. *Veterinary Surgery: VS*, *36*(3), 221–227. https://doi.org/10.1111/j.1532-950X.2007.00253.x

Davis, M., Walker, D. L., Miles, L., & Grillon, C. (2010). Phasic vs sustained fear in rats and humans: Role of the extended amygdala in fear vs anxiety. *Neuropsychopharmacology: Official*

*Publication of the American College of Neuropsychopharmacology*, *35*(1), 105–135. https://doi.org/10.1038/npp.2009.109

Dawood, F. S., Hunt, D., Patel, A., Kittikraisak, W., Tinoco, Y., Kurhe, K., Soto, G., Hombroek, D., Garg, S., Chotpitayasunondh, T., Gonzales, O., Bhargav, S., Thompson, M. G., Chotpitayasunondh, B., Florian, R., Prakash, A., Arriola, S., Macareo, L., Das, P., … Azziz-Baumgartner, E. (2018). The Pregnancy and Influenza Multinational Epidemiologic (PRIME) study: A prospective cohort study of the impact of influenza during pregnancy among women in middle-income countries. *Reproductive Health*, *15*. https://doi.org/10.1186/s12978-018-0600-x

De Hertogh, R., Thomas, K., Bietlot, Y., Vanderheyden, I., & Ferin, J. (1975). Plasma Levels of Unconjugated Estrone, Estradiol and Estriol and of HCS Throughout Pregnancy in Normal Women. *The Journal of Clinical Endocrinology & Metabolism*, *40*(1), 93–101. https://doi.org/10.1210/jcem-40-1-93

Di Paolo, N. C., & Shayakhmetov, D. M. (2016). Interleukin 1α and the inflammatory process. *Nature Immunology*, *17*(8), 906–913. https://doi.org/10.1038/ni.3503

Diamond, M. C., Ingham, C. A., Johnson, R. E., Bennett, E. L., & Rosenzweig, M. R. (1976). Effects of environment on morphology of rat cerebral cortex and hippocampus. *Journal of Neurobiology*, *7*(1), 75–85.

Dishman, R. K., Bunnell, B. N., Youngstedt, S. D., Yoo, H. S., Mougey, E. H., & Meyerhoff, J. L. (1998). Activity wheel running blunts increased plasma adrenocorticotrophin (ACTH) after footshock and cage-switch stress. *Physiology & Behavior*, *63*(5), 911–917.

Donahue, J. G., Kieke, B. A., King, J. P., DeStefano, F., Mascola, M. A., Irving, S. A., Cheetham, T. C., Glanz, J. M., Jackson, L. A., Klein, N. P., Naleway, A. L., Weintraub, E., & Belongia, E. A. (2017). Association of spontaneous abortion with receipt of inactivated influenza vaccine

containing H1N1pdm09 in 2010–11 and 2011–12. *Vaccine*, *35*(40), 5314–5322. https://doi.org/10.1016/j.vaccine.2017.06.069

Dong, H. W., Petrovich, G. D., & Swanson, L. W. (2001). Topography of projections from amygdala to bed nuclei of the stria terminalis. *Brain Research. Brain Research Reviews*, *38*(1–2), 192–246.

Donzelli, A. (2019). Influenza vaccination in pregnancy: Careful assessment confirms safety concerns for the offspring. *Human Vaccines & Immunotherapeutics*, *1*, 1–3. https://doi.org/10.1080/21645515.2019.1605818

Dronjak, S., Gavrilović, L., Filipović, D., & Radojcić, M. B. (2004). Immobilization and cold stress affect sympatho-adrenomedullary system and pituitary-adrenocortical axis of rats exposed to long-term isolation and crowding. *Physiology & Behavior*, *81*(3), 409–415. https://doi.org/10.1016/j.physbeh.2004.01.011

Dubal, D. B., Zhu, H., Yu, J., Rau, S. W., Shughrue, P. J., Merchenthaler, I., Kindy, M. S., & Wise, P. M. (2001). Estrogen receptor alpha, not beta, is a critical link in estradiol-mediated protection against brain injury. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(4), 1952–1957. https://doi.org/10.1073/pnas.041483198

Dulac, C., O'Connell, L. A., & Wu, Z. (2014). Neural control of maternal and paternal behaviors. *Science*, *345*(6198), 765–770. https://doi.org/10.1126/science.1253291

Dumont, E. C., Mark, G. P., Mader, S., & Williams, J. T. (2005). Self-administration enhances excitatory synaptic transmission in the bed nucleus of the stria terminalis. *Nature Neuroscience*, *8*(4), 413–414. https://doi.org/10.1038/nn1414

Ehret, G., & Buckenmaier, J. (1994). Estrogen-receptor occurrence in the female mouse brain: Effects of maternal experience, ovariectomy, estrogen and anosmia. *Journal of Physiology*, *88*(5), 315–329.

Eid, R. S., Chaiton, J. A., Lieblich, S. E., Bodnar, T. S., Weinberg, J., & Galea, L. A. M. (2019). Early and late effects of maternal experience on hippocampal neurogenesis, microglia, and the circulating cytokine milieu. *Neurobiology of Aging*, *1*, 1–17. https://doi.org/10.1016/j.neurobiolaging.2019.01.021

Ekdahl, C. T., Claasen, J.-H., Bonde, S., Kokaia, Z., & Lindvall, O. (2003). Inflammation is detrimental for neurogenesis in adult brain. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(23), 13632–13637. https://doi.org/10.1073/pnas.2234031100

Elliott, M. J., Maini, R. N., Feldmann, M., Long-Fox, A., Charles, P., Katsikis, P., Brennan, F. M., Walker, J., Bijl, H., & Ghrayeb, J. (1993). Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor alpha. *Arthritis and Rheumatism*, *36*(12), 1681–1690. https://doi.org/10.1002/art.1780361206

Elloso, M. M., Phiel, K., Henderson, R. A., Harris, H. A., & Adelman, S. J. (2005). Suppression of experimental autoimmune encephalomyelitis using estrogen receptor-selective ligands. *The Journal of Endocrinology*, *185*(2), 243–252. https://doi.org/10.1677/joe.1.06063

Esser, E. S., Pulit-Penaloza, J. A., Kalluri, H., McAllister, D., Vassilieva, E. V., Littauer, E. Q., Lelutiu, N., Prausnitz, M. R., Compans, R. W., & Skountzou, I. (2017). Microneedle patch delivery of influenza vaccine during pregnancy enhances maternal immune responses promoting survival and long-lasting passive immunity to offspring. *Scientific Reports*, *7*(1), 5705. https://doi.org/10.1038/s41598-017-05940-7

Fahrbach, S. E., & Pfaff, D. W. (1986). Effect of preoptic region implants of dilute estradiol on the maternal behavior of ovariectomized, nulliparous rats. *Hormones and Behavior*, *20*(3), 354–363.

Fan, S.-J., Lee, F. Y., Cheung, M. M., Ding, A. Y., Yang, J., Ma, S. J., Khong, P.-L., & Wu, E. X. (2013). Bilateral substantia nigra and pyramidal tract changes following experimental

intracerebral hemorrhage: An MR diffusion tensor imaging study. *NMR in Biomedicine*, *26*(9), 1089–1095. https://doi.org/10.1002/nbm.2922

Fang, T. C., Schaefer, U., Mecklenbrauker, I., Stienen, A., Dewell, S., Chen, M. S., Rioja, I., Parravicini, V., Prinjha, R. K., Chandwani, R., MacDonald, M. R., Lee, K., Rice, C. M., & Tarakhovsky, A. (2012). Histone H3 lysine 9 di-methylation as an epigenetic signature of the interferon response. *The Journal of Experimental Medicine*, *209*(4), 661–669. https://doi.org/10.1084/jem.20112343

Feldmann, M., Brennan, F. M., Elliott, M., Katsikis, P., & Maini, R. N. (1994). TNF alpha as a therapeutic target in rheumatoid arthritis. *Circulatory Shock*, *43*(4), 179–184.

Felicio, L. F., Mann, P. E., & Bridges, R. S. (1991). Intracerebroventricular cholecystokinin infusions block beta-endorphin-induced disruption of maternal behavior. *Pharmacology Biochemistry and Behavior*, *39*(1), 201–204. https://doi.org/10.1016/0091-3057(91)90422-X

Fleming, A. S., Ruble, D., Krieger, H., & Wong, P. Y. (1997). Hormonal and experiential correlates of maternal responsiveness during pregnancy and the puerperium in human mothers. *Hormones & Behavior*, *31*(2), 145–158. https://doi.org/10.1006/hbeh.1997.1376

Fleming, Alison S., & Rosenblatt, J. S. (1974). Olfactory regulation of maternal behavior in rats: I. Effects of olfactory bulb removal in experienced and inexperienced lactating and cycling females. *Journal of Comparative and Physiological Psychology*, *86*(2), 221–232. https://doi.org/10.1037/h0035937

Fleming, Alison S., Vaccarino, F., Tambosso, L., & Chee, P. (1979). Vomeronasal and olfactory system modulation of maternal behavior in the rat. *Science*, *203*(4378), 372–374. https://doi.org/10.1126/science.760196

Fleming, J. G. (1967). The Role of Negligence in Modern Tort Law. *Virginia Law Review*, *53*(4), 815–846. https://doi.org/10.2307/1071415

Flynn, J. L., Goldstein, M. M., Chan, J., Triebold, K. J., Pfeffer, K., Lowenstein, C. J., Schreiber, R., Mak, T. W., & Bloom, B. R. (1995). Tumor necrosis factor-alpha is required in the protective immune response against Mycobacterium tuberculosis in mice. *Immunity*, *2*(6), 561–572.

Fox, C., Merali, Z., & Harrison, C. (2006). Therapeutic and protective effect of environmental enrichment against psychogenic and neurogenic stress. [Review] [108 refs]. *Behavioural Brain Research*, *175*(1), 1–8.

Francis, D. D., Diorio, J., Plotsky, P. M., & Meaney, M. J. (2002). Environmental enrichment reverses the effects of maternal separation on stress reactivity. *Journal of Neuroscience*, *22*(18), 7840–7843.

Galea, L. A. M., Ormerod, B. K., Sampath, S., Kostaras, X., Wilkie, D. M., & Phelps, M. T. (2000). Spatial Working Memory and Hippocampal Size across Pregnancy in Rats. *Hormones and Behavior*, *37*(1), 86–95. https://doi.org/10.1006/hbeh.1999.1560

Galea, L. A. M., Roes, M. M., Dimech, C. J., Chow, C., Mahmoud, R., Lieblich, S. E., & Duarte-Guterman, P. (2018). Premarin has opposing effects on spatial learning, neural activation, and serum cytokine levels in middle-aged female rats depending on reproductive history. *Neurobiology of Aging*, *70*, 291–307. https://doi.org/10.1016/j.neurobiolaging.2018.06.030

Galea, L. A. M., Spritzer, M. D., Barker, J. M., & Pawluski, J. L. (2006). Gonadal hormone modulation of hippocampal neurogenesis in the adult. [Review] [67 refs]. *Hippocampus*, *16*(3), 225–232.

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 Psychiatry*, *1*, e98. https://doi.org/10.1038/tp.2012.24

Garidou, L., Laffont, S., Douin-Echinard, V., Coureau, C., Krust, A., Chambon, P., & Guéry, J.-C. (2004). Estrogen receptor alpha signaling in inflammatory leukocytes is dispensable for 17beta-estradiol-mediated inhibition of experimental autoimmune encephalomyelitis. *Journal of Immunology (Baltimore, Md.: 1950)*, *173*(4), 2435–2442. https://doi.org/10.4049/jimmunol.173.4.2435

Ghalandari-Shamami, M., Nourizade, S., Yousefi, B., Vafaei, A. A., Pakdel, R., & Rashidy-Pour, A. (2019). Beneficial Effects of Physical Activity and Crocin Against Adolescent Stress Induced Anxiety or Depressive-Like Symptoms and Dendritic Morphology Remodeling in Prefrontal Cortex in Adult Male Rats. *Neurochemical Research*, *44*(4), 917–929. https://doi.org/10.1007/s11064-019-02727-2

Ghisletti, S., Meda, C., Maggi, A., & Vegeto, E. (2005). 17beta-estradiol inhibits inflammatory gene expression by controlling NF-kappaB intracellular localization. *Molecular and Cellular Biology*, *25*(8), 2957–2968. https://doi.org/10.1128/MCB.25.8.2957-2968.2005

Gibney, S. M., McGuinness, B., Prendergast, C., Harkin, A., & Connor, T. J. (2013). Poly I:C-induced activation of the immune response is accompanied by depression and anxiety-like behaviours, kynurenine pathway activation and reduced BDNF expression. *Brain, Behavior, and Immunity*, *28*, 170–181. https://doi.org/10.1016/j.bbi.2012.11.010

Giordano, A. L., Ahdieh, H. B., Mayer, A. D., Siegel, H. I., & Rosenblatt, J. S. (1990a). Cytosol and nuclear estrogen receptor binding in the preoptic area and hypothalamus of female rats during

pregnancy and ovariectomized, nulliparous rats after steroid priming: Correlation with maternal behavior. *Hormones & Behavior*, *24*(2), 232–255.

Giordano, A. L., Ahdieh, H. B., Mayer, A. D., Siegel, H. I., & Rosenblatt, J. S. (1990b). Cytosol and nuclear estrogen receptor binding in the preoptic area and hypothalamus of female rats during pregnancy and ovariectomized, nulliparous rats after steroid priming: Correlation with maternal behavior. *Hormones and Behavior*, *24*(2), 232–255.

Girard-Joyal, O., Faragher, A., Bradley, K., Kane, L., Hrycyk, L., & Ismail, N. (2015). Age and sex differences in c-Fos expression and serum corticosterone concentration following LPS treatment. *Neuroscience*, 293–301. https://doi.org/10.1016/j.neuroscience.2015.06.035

Girbovan, C., & Plamondon, H. (2013). Environmental enrichment in female rodents: Considerations in the effects on behavior and biochemical markers. *Behavioural Brain Research*, *253*, 178–190. https://doi.org/10.1016/j.bbr.2013.07.018

Glass, B. J. (2001). A comparative analysis of the right of a pregnant woman to refuse medical treatment for herself and her viable fetus: The United States and United Kingdom. *Indiana International & Comparative Law Review*, *11*(2), 507–541.

Gleeson, M., & Bishop, N. C. (2005). The T cell and NK cell immune response to exercise. [Review] [35 refs]. *Annals of Transplantation*, *10*(4), 43–48.

Glynn, L. M., Davis, E. P., Sandman, C. A., & Goldberg, W. A. (2016). Gestational hormone profiles predict human maternal behavior at 1-year postpartum. *Hormones and Behavior*, *85*, 19–25. https://doi.org/10.1016/j.yhbeh.2016.07.002

Godfraind, C., Louahed, J., Faulkner, H., Vink, A., Warnier, G., Grencis, R., & Renauld, J. C. (1998). Intraepithelial infiltration by mast cells with both connective tissue-type and mucosal-type

characteristics in gut, trachea, and kidneys of IL-9 transgenic mice. *Journal of Immunology (Baltimore, Md.: 1950)*, *160*(8), 3989–3996.

Goldstein, R. Z., & Volkow, N. D. (2002). Drug addiction and its underlying neurobiological basis: Neuroimaging evidence for the involvement of the frontal cortex. *The American Journal of Psychiatry*, *159*(10), 1642–1652. https://doi.org/10.1176/appi.ajp.159.10.1642

Goncalves, L. V., Herlinger, A. L., Ferreira, T. A. A., Coitinho, J. B., Pires, R. G. W., & Martins-Silva, C. (2018). Environmental enrichment cognitive neuroprotection in an experimental model of cerebral ischemia: Biochemical and molecular aspects. *Behavioural Brain Research*, *1*, 171–183. https://doi.org/10.1016/j.bbr.2018.04.023

Goodnight, W. H., & Soper, D. E. (2005). Pneumonia in pregnancy. *Critical Care Medicine*, *33*(10 Suppl), S390-397. https://doi.org/10.1097/01.ccm.0000182483.24836.66

Gracceva, G., Venerosi, A., Santucci, D., Calamandrei, G., & Ricceri, L. (2009). Early social enrichment affects responsiveness to different social cues in female mice. *Behavioural Brain Research*, *196*(2), 304–309. https://doi.org/10.1016/j.bbr.2008.09.023

Grace, A. A., Floresco, S. B., Goto, Y., & Lodge, D. J. (2007). Regulation of firing of dopaminergic neurons and control of goal-directed behaviors. *Trends in Neurosciences*, *30*(5), 220–227. https://doi.org/10.1016/j.tins.2007.03.003

Granucci, F., Petralia, F., Urbano, M., Citterio, S., Di Tota, F., Santambrogio, L., & Ricciardi-Castagnoli, P. (2003). The scavenger receptor MARCO mediates cytoskeleton rearrangements in dendritic cells and microglia. *Blood*, *102*(8), 2940–2947. https://doi.org/10.1182/blood-2002-12-3651

Gray, P., & Brooks, P. J. (1984). Effect of lesion location within the medial preoptic-anterior hypothalamic continuum on maternal and male sexual behaviors in female rats. *Behavioral Neuroscience*, *98*(4), 703–711. https://doi.org/10.1037/0735-7044.98.4.703

Greca, A. M. L., Swales, T., Klemp, S., Madigan, S., & Skyler, J. (1995). Adolescents With Diabetes: Gender Differences in Psychosocial Functioning and Glycemic Control. *Children's Health Care*, *24*(1), 61–78. https://doi.org/10.1207/s15326888chc2401_6

Green, E. J., Greenough, W. T., & Schlumpf, B. E. (1983). Effects of complex or isolated environments on cortical dendrites of middle-aged rats. *Brain Research*, *264*(2), 233–240.

Greenough, W. T., McDonald, J. W., Parnisari, R. M., & Camel, J. E. (1986). Environmental conditions modulate degeneration and new dendrite growth in cerebellum of senescent rats. *Brain Research*, *380*(1), 136–143.

Greenwood, B. N., & Fleshner, M. (2008). Exercise, learned helplessness, and the stress-resistant brain. [Review] [97 refs]. *NeuroMolecular Medicine*, *10*(2), 81–98. https://doi.org/10.1007/s12017-008-8029-y

Gruys, E., Toussaint, M. J. M., Niewold, T. A., & Koopmans, S. J. (2005). Acute phase reaction and acute phase proteins. *Journal of Zhejiang University. Science. B*, *6*(11), 1045–1056. https://doi.org/10.1631/jzus.2005.B1045

Guidotti, L. G., & Chisari, F. V. (2001). Noncytolytic control of viral infections by the innate and adaptive immune response. *Annual Review of Immunology*, *19*, 65–91. https://doi.org/10.1146/annurev.immunol.19.1.65

Haber, S. N., Groenewegen, H. J., Grove, E. A., & Nauta, W. J. (1985). Efferent connections of the ventral pallidum: Evidence of a dual striato pallidofugal pathway. *The Journal of Comparative Neurology*, *235*(3), 322–335. https://doi.org/10.1002/cne.902350304

Handa, R. J., Ogawa, S., Wang, J. M., & Herbison, A. E. (2012). Roles for Oestrogen Receptor β in Adult Brain Function. *Journal of Neuroendocrinology*, *24*(1), 160–173. https://doi.org/10.1111/j.1365-2826.2011.02206.x

Hantsoo, L., & Epperson, C. N. (2017). Anxiety Disorders Among Women: A Female Lifespan Approach. *Focus (American Psychiatric Publishing)*, *15*(2), 162–172. https://doi.org/10.1176/appi.focus.20160042

Harle, G., Lalonde, R., Fonte, C., Ropars, A., Frippiat, J.-P., & Strazielle, C. (2017). Repeated corticosterone injections in adult mice alter stress hormonal receptor expression in the cerebellum and motor coordination without affecting spatial learning. *Behavioural Brain Research*, *1*, 121–131. https://doi.org/10.1016/j.bbr.2017.02.035

Harris, G. C., & Aston-Jones, G. (2007). Activation in extended amygdala corresponds to altered hedonic processing during protracted morphine withdrawal. *Behavioural Brain Research*, *176*(2), 251–258. https://doi.org/10.1016/j.bbr.2006.10.012

Harris, L. H., & Paltrow, L. (2003). MSJAMA. The status of pregnant women and fetuses in US criminal law. *JAMA*, *289*(13), 1697–1699. https://doi.org/10.1001/jama.289.13.1697

Harvey, L., & Boksa, P. (2012). Prenatal and postnatal animal models of immune activation: Relevance to a range of neurodevelopmental disorders. *Developmental Neurobiology*, *72*(10), 1335–1348. https://doi.org/10.1002/dneu.22043

Hastings, M. H., Winn, P., & Dunnett, S. B. (1985). Neurotoxic amino acid lesions of the lateral hypothalamus: A parametric comparison of the effects of ibotenate, N-methyl-d,l-aspartate and quisqualate in the rat. *Brain Research*, *360*(1), 248–256. https://doi.org/10.1016/0006-8993(85)91240-5

Heckers, S. (2001). Neuroimaging studies of the hippocampus in schizophrenia. *Hippocampus*, *11*(5), 520–528. https://doi.org/10.1002/hipo.1068

Heckers, S., & Konradi, C. (2002). Hippocampal neurons in schizophrenia. *Journal of Neural Transmission (Vienna, Austria : 1996)*, *109*(0), 891–905. https://doi.org/10.1007/s007020200073

Heimer, L., Switzer, R. D., & Hoesen, G. W. V. (1982). Ventral striatum and ventral pallidum: Components of the motor system? *Trends in Neurosciences*, *5*, 83–87. https://doi.org/10.1016/0166-2236(82)90037-6

Heldring, N., Pike, A., Andersson, S., Matthews, J., Cheng, G., Hartman, J., Tujague, M., Strom, A., Treuter, E., Warner, M., & Gustafsson, J.-A. (2007). Estrogen receptors: How do they signal and what are their targets. [Review] [331 refs]. *Physiological Reviews*, *87*(3), 905–931. https://doi.org/10.1152/physrev.00026.2006

Hellemans, K. G. C., Nobrega, J. N., & Olmstead, M. C. (2005). Early environmental experience alters baseline and ethanol-induced cognitive impulsivity: Relationship to forebrain 5-HT1A receptor binding. *Behavioural Brain Research*, *159*(2), 207–220.

Hendriksen, H., Prins, J., Olivier, B., & Oosting, R. S. (2010). Environmental enrichment induces behavioral recovery and enhanced hippocampal cell proliferation in an antidepressant-resistant animal model for PTSD. *PLoS ONE [Electronic Resource]*, *5*(8), e11943. https://doi.org/10.1371/journal.pone.0011943

Heppner, F. L., Greter, M., Marino, D., Falsig, J., Raivich, G., Hövelmeyer, N., Waisman, A., Rülicke, T., Prinz, M., Priller, J., Becher, B., & Aguzzi, A. (2005). Experimental autoimmune encephalomyelitis repressed by microglial paralysis. *Nature Medicine*, *11*(2), 146–152. https://doi.org/10.1038/nm1177

Herman, J. P., & Mueller, N. K. (2006). Role of the ventral subiculum in stress integration. *Behavioural Brain Research*, *174*(2), 215–224. https://doi.org/10.1016/j.bbr.2006.05.035

Herrington, T. M., Cheng, J. J., & Eskandar, E. N. (2015). Mechanisms of deep brain stimulation. *Journal of Neurophysiology*, *115*(1), 19–38. https://doi.org/10.1152/jn.00281.2015

Ho, D. X. K., Tan, Y. C., Tan, J., Too, H. P., & Ng, W. H. (2014). High-frequency stimulation of the globus pallidus interna nucleus modulates GFRα1 gene expression in the basal ganglia. *Journal of Clinical Neuroscience: Official Journal of the Neurosurgical Society of Australasia*, *21*(4), 657–660. https://doi.org/10.1016/j.jocn.2013.05.024

Hoebe, K., Janssen, E., & Beutler, B. (2004). The interface between innate and adaptive immunity. *Nature Immunology*, *5*(10), 971–974. https://doi.org/10.1038/ni1004-971

Hoekzema, E., Barba-Müller, E., Pozzobon, C., Picado, M., Lucco, F., García-García, D., Soliva, J. C., Tobeña, A., Desco, M., Crone, E. A., Ballesteros, A., Carmona, S., & Vilarroya, O. (2017). Pregnancy leads to long-lasting changes in human brain structure. *Nature Neuroscience*, *20*(2), 287–296. https://doi.org/10.1038/nn.4458

Hoffmann, L. C., Schutte, S. R. M., Koch, M., & Schwabe, K. (2009). Effect of "enriched environment" during development on adult rat behavior and response to the dopamine receptor agonist apomorphine. *Neuroscience*, *158*(4), 1589–1598. https://doi.org/10.1016/j.neuroscience.2008.11.035

Holm, A., Andersson, K. E., Nordstrom, I., Hellstrand, P., & Nilsson, B.-O. (2010). Down-regulation of endothelial cell estrogen receptor expression by the inflammation promoter LPS. *Molecular & Cellular Endocrinology*, *319*(1–2), 8–13. https://doi.org/10.1016/j.mce.2010.01.002

Hoshino, K., Takeuchi, O., Kawai, T., Sanjo, H., Ogawa, T., Takeda, Y., Takeda, K., & Akira, S. (1999). Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to

lipopolysaccharide: evidence for TLR4 as the Lps gene product. *Journal of Immunology (Baltimore, Md.: 1950)*, *162*(7), 3749–3752.

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*(31), 12776–12781. https://doi.org/10.1073/pnas.1202556109

Htun, H., Holth, L. T., Walker, D., Davie, J. R., & Hager, G. L. (1999). Direct Visualization of the Human Estrogen Receptor α Reveals a Role for Ligand in the Nuclear Distribution of the Receptor. *Molecular Biology of the Cell*, *10*(2), 471–486.

Isaacs Alick, Lindenmann J., & Andrewes Christopher Howard. (1957). Virus interference. I. The interferon. *Proceedings of the Royal Society of London. Series B - Biological Sciences*, *147*(927), 258–267. https://doi.org/10.1098/rspb.1957.0048

Ismail, N., Kumlin, A. M., & Blaustein, J. D. (2013). A pubertal immune challenge alters the antidepressant-like effects of chronic estradiol treatment in inbred and outbred adult female mice. *Neuroscience*, 43–52. https://doi.org/10.1016/j.neuroscience.2012.09.047

Ismail, Nafissa, Garas, P., & Blaustein, J. D. (2011). Long-term effects of pubertal stressors on female sexual receptivity and estrogen receptor-α expression in CD-1 female mice. *Hormones and Behavior*, *59*(4), 565–571. https://doi.org/10.1016/j.yhbeh.2011.02.010

Ito, A., Bebo, B. F., Matejuk, A., Zamora, A., Silverman, M., Fyfe-Johnson, A., & Offner, H. (2001). Estrogen treatment down-regulates TNF-alpha production and reduces the severity of experimental autoimmune encephalomyelitis in cytokine knockout mice. *Journal of Immunology (Baltimore, Md.: 1950)*, *167*(1), 542–552. https://doi.org/10.4049/jimmunol.167.1.542

Ito, K., Ishikawa, Y., Skinner, R. D., Mrak, R. E., Morrison-Bogorad, M., Mukawa, J., & Griffin, W. S. T. (1997). Lesioning of the inferior olive using a ventral surgical approach. *Molecular and Chemical Neuropathology*, *31*(3), 245–264. https://doi.org/10.1007/BF02815128

Ivashkiv, L. B., & Donlin, L. T. (2014). Regulation of type I interferon responses. *Nature Reviews. Immunology*, *14*(1), 36–49. https://doi.org/10.1038/nri3581

Iwasaki, A. (2012). A Virological View of Innate Immune Recognition. *Annual Review of Microbiology*, *66*(1), 177–196. https://doi.org/10.1146/annurev-micro-092611-150203

Iwasaki, A., & Medzhitov, R. (2010). Regulation of adaptive immunity by the innate immune system. *Science (New York, N.Y.)*, *327*(5963), 291–295. https://doi.org/10.1126/science.1183021

Jacobson, C. D., Terkel, J., Gorski, R. A., & Sawyer, C. H. (1980). Effects of small medial preoptic area lesions on maternal behavior: Retrieving and nest building in the rat. *Brain Research*, *194*(2), 471–478. https://doi.org/10.1016/0006-8993(80)91226-3

Jalabert, M., Aston-Jones, G., Herzog, E., Manzoni, O., & Georges, F. (2009). Role of the bed nucleus of the stria terminalis in the control of ventral tegmental area dopamine neurons. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *33*(8), 1336–1346. https://doi.org/10.1016/j.pnpbp.2009.07.010

Jennings, J. H., Sparta, D. R., Stamatakis, A. M., Ung, R. L., Pleil, K. E., Kash, T. L., & Stuber, G. D. (2013). Distinct extended amygdala circuits for divergent motivational states. *Nature*, *496*(7444), 224–228. https://doi.org/10.1038/nature12041

Jonas, W., Atkinson, L., Steiner, M., Meaney, M. J., Wazana, A., Fleming, A. S., & team, M. research. (2015). Breastfeeding and maternal sensitivity predict early infant temperament. *Acta Paediatrica*, *104*(7), 678–686. https://doi.org/10.1111/apa.12987

Jurgens, H. A., & Johnson, R. W. (2012). Environmental enrichment attenuates hippocampal neuroinflammation and improves cognitive function during influenza infection. *Brain, Behavior, & Immunity*, *26*(6), 1006–1016. https://doi.org/10.1016/j.bbi.2012.05.015

Kalita, K., & Szymczak, S. (2003). [Estrogen receptors in the brain]. *Neurologia I Neurochirurgia Polska*, *37 Suppl 3*, 63–78.

Kassi, E., & Moutsatsou, P. (2010). *Estrogen Receptor Signaling and Its Relationship to Cytokines in Systemic Lupus Erythematosus* [Research article]. BioMed Research International. https://doi.org/10.1155/2010/317452

Kawai, T., & Akira, S. (2009). The roles of TLRs, RLRs and NLRs in pathogen recognition. *International Immunology*, *21*(4), 317–337. https://doi.org/10.1093/intimm/dxp017

Kawai, T., & Akira, S. (2010). The role of pattern-recognition receptors in innate immunity: Update on Toll-like receptors. *Nature Immunology*, *11*(5), 373–384. https://doi.org/10.1038/ni.1863

Kawashima, K., & Fujii, T. (2003). The lymphocytic cholinergic system and its contribution to the regulation of immune activity. *Life Sciences*, *74*(6), 675–696. https://doi.org/10.1016/j.lfs.2003.09.037

Kentner, A. C., Takeuchi, A., James, J. S., Miki, T., Seino, S., Hayley, S., & Bielajew, C. (2008). The effects of rewarding ventral tegmental area stimulation and environmental enrichment on lipopolysaccharide-induced sickness behavior and cytokine expression in female rats. *Brain Research*, *1*, 50–61. https://doi.org/10.1016/j.brainres.2008.04.041

Kentner, Amanda C. (2015). Neuroprotection and recovery from early-life adversity: Considerations for environmental enrichment. *Neural Regeneration Research*, *10*(10), 1545–1547. https://doi.org/10.4103/1673-5374.165315

Kentner, Amanda C., Bilbo, S. D., Brown, A. S., Hsiao, E. Y., McAllister, A. K., Meyer, U., Pearce, B. D., Pletnikov, M. V., Yolken, R. H., & Bauman, M. D. (2019). Maternal immune activation: Reporting guidelines to improve the rigor, reproducibility, and transparency of the model. [Review]. *Neuropsychopharmacology*, *44*(2), 245–258. https://doi.org/10.1038/s41386-018-0185-7

Kentner, Amanda C., Khoury, A., Lima Queiroz, E., & MacRae, M. (2016). Environmental enrichment rescues the effects of early life inflammation on markers of synaptic transmission and plasticity. *Brain, Behavior, & Immunity*, *1*, 151–160. https://doi.org/10.1016/j.bbi.2016.03.013

Kentner, Amanda C., Lima, E., Migliore, M. M., Shin, J., & Scalia, S. (2018a). Complex Environmental Rearing Enhances Social Salience and Affects Hippocampal Corticotropin Releasing Hormone Receptor Expression in a Sex-Specific Manner. *Neuroscience*, *1*, 399–411. https://doi.org/10.1016/j.neuroscience.2017.11.035

Kentner, Amanda C., Lima, E., Migliore, M. M., Shin, J., & Scalia, S. (2018b). Complex Environmental Rearing Enhances Social Salience and Affects Hippocampal Corticotropin Releasing Hormone Receptor Expression in a Sex-Specific Manner. *Neuroscience*, *1*, 399–411. https://doi.org/10.1016/j.neuroscience.2017.11.035

Kentner, Amanda C., Scalia, S., Shin, J., Migliore, M. M., & Rondon-Ortiz, A. N. (2018). Targeted sensory enrichment interventions protect against behavioral and neuroendocrine consequences of early life stress. *Psychoneuroendocrinology*, *1*, 74–85. https://doi.org/10.1016/j.psyneuen.2018.07.029

Kettenmann, H., Hanisch, U.-K., Noda, M., & Verkhratsky, A. (2011). Physiology of microglia. [Review]. *Physiological Reviews*, *91*(2), 461–553. https://doi.org/10.1152/physrev.00011.2010

Khan, D., & Ansar Ahmed, S. (2016). The Immune System Is a Natural Target for Estrogen Action: Opposing Effects of Estrogen in Two Prototypical Autoimmune Diseases. *Frontiers in Immunology*, *6*. https://doi.org/10.3389/fimmu.2015.00635

Khansari, N., Shakiba, Y., & Mahmoudi, M. (2009). Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. *Recent Patents on Inflammation & Allergy Drug Discovery*, *3*(1), 73–80.

Kinsley, C. H., & Amory-Meyer, E. (2011). Why the maternal brain?. [Review]. *Journal of Neuroendocrinology*, *23*(11), 974–983. https://doi.org/10.1111/j.1365-2826.2011.02194.x

Kinsley, Craig Howard, & Lambert, K. G. (2006). The maternal brain. *Scientific American*, *294*(1), 72–79. https://doi.org/10.1038/scientificamerican0106-72

Kohman, R. A., & Rhodes, J. S. (2013). Neurogenesis, inflammation and behavior. [Review]. *Brain, Behavior, & Immunity*, *27*(1), 22–32. https://doi.org/10.1016/j.bbi.2012.09.003

Konkle, A. T. M., Baker, S. L., Kentner, A. C., Barbagallo, L. S.-M., Merali, Z., & Bielajew, C. (2003). Evaluation of the effects of chronic mild stressors on hedonic and physiological responses: Sex and strain compared. *Brain Research*, *992*(2), 227–238.

Konkle, A. T. M., Kentner, A. C., Baker, S. L., Stewart, A., & Bielajew, C. (2010). Environmental-enrichment-related variations in behavioral, biochemical, and physiologic responses of Sprague-Dawley and Long Evans rats. *Journal of the American Association for Laboratory Animal Science*, *49*(4), 427–436.

Konkle, A. T. M., & McCarthy, M. M. (2011). Developmental time course of estradiol, testosterone, and dihydrotestosterone levels in discrete regions of male and female rat brain. *Endocrinology*, *152*(1), 223–235. https://doi.org/10.1210/en.2010-0607

Konsman, J. P., Parnet, P., & Dantzer, R. (2002). Cytokine-induced sickness behaviour: Mechanisms and implications. *Trends in Neurosciences*, *25*(3), 154–159.

Kopitar-Jerala, N. (2017). The Role of Interferons in Inflammation and Inflammasome Activation. *Frontiers in Immunology*, *8*. https://doi.org/10.3389/fimmu.2017.00873

Kreutzberg, G. W. (1996). Principles of Neuronal Regeneration. In A. Baethmann, O. S. Kempski, N. Plesnila, & F. Staub (Eds.), *Mechanisms of Secondary Brain Damage in Cerebral Ischemia and Trauma* (pp. 103–106). Springer Vienna.

Krężel, W., Dupont, S., Krust, A., Chambon, P., & Chapman, P. F. (2001). Increased anxiety and synaptic plasticity in estrogen receptor β-deficient mice. *Proceedings of the National Academy of Sciences*, *98*(21), 12278–12282. https://doi.org/10.1073/pnas.221451898

Krishnan, L., Guilbert, L. J., Russell, A. S., Wegmann, T. G., Mosmann, T. R., & Belosevic, M. (1996). Pregnancy impairs resistance of C57BL/6 mice to Leishmania major infection and causes decreased antigen-specific IFN-gamma response and increased production of T helper 2 cytokines. *Journal of Immunology (Baltimore, Md.: 1950)*, *156*(2), 644–652.

Kumar, H., Kawai, T., & Akira, S. (2009). Pathogen recognition in the innate immune response. *The Biochemical Journal*, *420*(1), 1–16. https://doi.org/10.1042/BJ20090272

Kumar, H., Kawai, T., & Akira, S. (2011). Pathogen recognition by the innate immune system. *International Reviews of Immunology*, *30*(1), 16–34. https://doi.org/10.3109/08830185.2010.529976

Kumar, R., Zakharov, M. N., Khan, S. H., Miki, R., Jang, H., Toraldo, G., Singh, R., Bhasin, S., & Jasuja, R. (2011). The dynamic structure of the estrogen receptor. *Journal of Amino Acids*. https://doi.org/10.4061/2011/812540

Laibl, V. R., & Sheffield, J. S. (2005). Influenza and pneumonia in pregnancy. *Clinics in Perinatology*, *32*(3), 727–738. https://doi.org/10.1016/j.clp.2005.04.009

Lambert, T. J., Fernandez, S. M., & Frick, K. M. (2005). Different types of environmental enrichment have discrepant effects on spatial memory and synaptophysin levels in female mice. *Neurobiology of Learning and Memory*, *83*(3), 206–216. https://doi.org/10.1016/j.nlm.2004.12.001

Laviola, G., Rea, M., Morley-Fletcher, S., Di Carlo, S., Bacosi, A., De Simone, R., Bertini, M., & Pacifici, R. (2004). Beneficial effects of enriched environment on adolescent rats from stressed pregnancies. *Journal of Neuroscience*, *20*(6), 1655–1664.

Law, R. O. (1994). Regulation of mammalian brain cell volume. *Journal of Experimental Zoology*, *268*(2), 90–96. https://doi.org/10.1002/jez.1402680204

Le Belle, J. E., Sperry, J., Ngo, A., Ghochani, Y., Laks, D. R., López-Aranda, M., Silva, A. J., & Kornblum, H. I. (2014). Maternal inflammation contributes to brain overgrowth and autism-associated behaviors through altered redox signaling in stem and progenitor cells. *Stem Cell Reports*, *3*(5), 725–734. https://doi.org/10.1016/j.stemcr.2014.09.004

Lebow, M. A., & Chen, A. (2016). Overshadowed by the amygdala: The bed nucleus of the stria terminalis emerges as key to psychiatric disorders. *Molecular Psychiatry*, *21*(4), 450–463. https://doi.org/10.1038/mp.2016.1

Lee, P., Son, D., Lee, J., Kim, Y. S., Kim, H., & Kim, S. Y. (2003). Excessive production of nitric oxide induces the neuronal cell death in lipopolysaccharide-treated rat hippocampal slice culture. *Neuroscience Letters*, *349*(1), 33–36. https://doi.org/10.1016/s0304-3940(03)00697-9

Leggio, M. G., Mandolesi, L., Federico, F., Spirito, F., Ricci, B., Gelfo, F., & Petrosini, L. (2005). Environmental enrichment promotes improved spatial abilities and enhanced dendritic growth in the rat. *Behavioural Brain Research*, *163*(1), 78–90.

Lernmark, B., Persson, B., Fisher, L., & Rydelius, P. A. (1999). Symptoms of depression are important to psychological adaptation and metabolic control in children with diabetes mellitus. *Diabetic Medicine: A Journal of the British Diabetic Association*, *16*(1), 14–22. https://doi.org/10.1046/j.1464-5491.1999.00008.x

Leuner, B., & Gould, E. (2010). Dendritic Growth in Medial Prefrontal Cortex and Cognitive Flexibility Are Enhanced during the Postpartum Period. *Journal of Neuroscience*, *30*(40), 13499–13503. https://doi.org/10.1523/JNEUROSCI.3388-10.2010

Li, M., Wang, M., Ding, S., Li, C., & Luo, X. (2012). Environmental Enrichment during Gestation Improves Behavior Consequences and Synaptic Plasticity in Hippocampus of Prenatal-Stressed Offspring Rats. *Acta Histochemica Et Cytochemica*, *45*(3), 157–166. https://doi.org/10.1267/ahc.11054

Limosin, F., Rouillon, F., Payan, C., Cohen, J.-M., & Strub, N. (2003). Prenatal exposure to influenza as a risk factor for adult schizophrenia. *Acta Psychiatrica Scandinavica*, *107*(5), 331–335. https://doi.org/10.1034/j.1600-0447.2003.00052.x

Littauer, E. Q., Esser, E. S., Antao, O. Q., Vassilieva, E. V., Compans, R. W., & Skountzou, I. (2017). H1N1 influenza virus infection results in adverse pregnancy outcomes by disrupting tissue-specific hormonal regulation. *PLoS Pathogens*, *13*(11), e1006757. https://doi.org/10.1371/journal.ppat.1006757

Liu, X., Fan, X.-L., Zhao, Y., Luo, G.-R., Li, X.-P., Li, R., & Le, W.-D. (2005). Estrogen provides neuroprotection against activated microglia-induced dopaminergic neuronal injury through both

estrogen receptor-alpha and estrogen receptor-beta in microglia. *Journal of Neuroscience Research*, *81*(5), 653–665. https://doi.org/10.1002/jnr.20583

Lombardo, M. V., Moon, H. M., Su, J., Palmer, T. D., Courchesne, E., & Pramparo, T. (2017a). Maternal immune activation dysregulation of the fetal brain transcriptome and relevance to the pathophysiology of autism spectrum disorder. *Molecular Psychiatry*, *1*. https://doi.org/10.1038/mp.2017.15

Lombardo, M. V., Moon, H. M., Su, J., Palmer, T. D., Courchesne, E., & Pramparo, T. (2017b). Maternal immune activation dysregulation of the fetal brain transcriptome and relevance to the pathophysiology of autism spectrum disorder. *Molecular Psychiatry*. https://doi.org/10.1038/mp.2017.15

Lonstein, J. S., Greco, B., De Vries, G. J., Stern, J. M., & Blaustein, J. D. (2000). Maternal behavior stimulates c-fos activity within estrogen receptor alpha-containing neurons in lactating rats. *Neuroendocrinology*, *72*(2), 91–101.

Luheshi, G. N., Stefferl, A., Turnbull, A. V., Dascombe, M. J., Brouwer, S., Hopkins, S. J., & Rothwell, N. J. (1997). Febrile response to tissue inflammation involves both peripheral and brain IL-1 and TNF-alpha in the rat. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *272*(3), R862–R868. https://doi.org/10.1152/ajpregu.1997.272.3.R862

Luyck, K., Tambuyzer, T., Deprez, M., Rangarajan, J., Nuttin, B., & Luyten, L. (2017). Electrical stimulation of the bed nucleus of the stria terminalis reduces anxiety in a rat model. *Translational Psychiatry*, *7*(2), e1033. https://doi.org/10.1038/tp.2017.2

Luyten, L., Hendrickx, S., Raymaekers, S., Gabriëls, L., & Nuttin, B. (2016). Electrical stimulation in the bed nucleus of the stria terminalis alleviates severe obsessive-compulsive disorder. *Molecular Psychiatry*, *21*(9), 1272–1280. https://doi.org/10.1038/mp.2015.124

Mackinnon, D. A., & Stern, J. M. (1977). Pregnancy duration and fetal number: Effects on maternal behavior in rats. *Physiology & Behavior*, *18*(5), 793–797. https://doi.org/10.1016/0031-9384(77)90185-8

Madhi, S. A., Cutland, C. L., Kuwanda, L., Weinberg, A., Hugo, A., Jones, S., Adrian, P. V., van Niekerk, N., Treurnicht, F., Ortiz, J. R., Venter, M., Violari, A., Neuzil, K. M., Simões, E. A. F., Klugman, K. P., Nunes, M. C., & Maternal Flu Trial (Matflu) Team. (2014). Influenza vaccination of pregnant women and protection of their infants. *The New England Journal of Medicine*, *371*(10), 918–931. https://doi.org/10.1056/NEJMoa1401480

Maesawa, S., Kaneoke, Y., Kajita, Y., Usui, N., Misawa, N., Nakayama, A., & Yoshida, J. (2004). Long-term stimulation of the subthalamic nucleus in hemiparkinsonian rats: Neuroprotection of dopaminergic neurons. *Journal of Neurosurgery*, *100*(4), 679–687. https://doi.org/10.3171/jns.2004.100.4.0679

Maggi, A., Ciana, P., Belcredito, S., & Vegeto, E. (2004). Estrogens in the nervous system: Mechanisms and nonreproductive functions. *Annual Review of Physiology*, *66*, 291–313. https://doi.org/10.1146/annurev.physiol.66.032802.154945

Maggioli, E., McArthur, S., Mauro, C., Kieswich, J., Kusters, D. H. M., Reutelingsperger, C. P. M., Yaqoob, M., & Solito, E. (2016). Estrogen protects the blood-brain barrier from inflammation-induced disruption and increased lymphocyte trafficking. *Brain, Behavior, & Immunity*, 212–222. https://doi.org/10.1016/j.bbi.2015.08.020

Maini, R N, Elliott, M. J., Brennan, F. M., & Feldmann, M. (1995). Beneficial effects of tumour necrosis factor-alpha (TNF-alpha) blockade in rheumatoid arthritis (RA). *Clinical and Experimental Immunology*, *101*(2), 207–212.

Maini, Ravinder N., Elliott, M. J., Brennan, E. M., Williams, R. O., Chu, C. Q., Paleolog, E., Charles, P. J., Taylor, P. C., & Feldmann, M. (1995). Monoclonal anti-TNFα Antibody as a Probe of Pathogenesis and Therapy of Rheumatoid Disease. *Immunological Reviews*, *144*(1), 195–223. https://doi.org/10.1111/j.1600-065X.1995.tb00070.x

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*(4), 607–616. https://doi.org/10.1016/j.bbi.2012.01.011

Mann, P. E., & Babb, J. A. (2005a). Neural steroid hormone receptor gene expression in pregnant rats. *Brain Research*, *142*(1), 39–46. https://doi.org/10.1016/j.molbrainres.2005.09.001

Mann, P. E., & Babb, J. A. (2005b). Neural steroid hormone receptor gene expression in pregnant rats. *Brain Research. Molecular Brain Research*, *142*(1), 39–46. https://doi.org/10.1016/j.molbrainres.2005.09.001

Mann, P. E., & Bridges, R. S. (2001). Chapter 18 Lactogenic hormone regulation of maternal behavior. In *Progress in Brain Research* (Vol. 133, pp. 251–262). Elsevier. https://doi.org/10.1016/S0079-6123(01)33019-4

Mao, K., Chen, S., Chen, M., Ma, Y., Wang, Y., Huang, B., He, Z., Zeng, Y., Hu, Y., Sun, S., Li, J., Wu, X., Wang, X., Strober, W., Chen, C., Meng, G., & Sun, B. (2013). Nitric oxide suppresses NLRP3 inflammasome activation and protects against LPS-induced septic shock. *Cell Research*, *23*(2), 201–212. https://doi.org/10.1038/cr.2013.6

Marashi, V., Barnekow, A., Ossendorf, E., & Sachser, N. (2003). Effects of different forms of environmental enrichment on behavioral, endocrinological, and immunological parameters in male mice. *Hormones and Behavior*, *43*(2), 281–292.

Markham, J. A., & Koenig, J. I. (2011). Prenatal stress: Role in psychotic and depressive diseases. *Psychopharmacology*, *214*(1), 89–106. https://doi.org/10.1007/s00213-010-2035-0

Marriott, L. K., McGann-Gramling, K. R., Hauss-Wegrzyniak, B., Sheldahl, L. C., Shapiro, R. A., Dorsa, D. M., & Wenk, G. L. (2007). Estrogen replacement regimen and brain infusion of lipopolysaccharide differentially alter steroid receptor expression in the uterus and hypothalamus. *Endocrine*, *32*(3), 317–328. https://doi.org/10.1007/s12020-008-9038-1

Martin, A., Cox, S., Jamieson, D. J., Whiteman, M. K., Kulkarni, A., & Tepper, N. K. (2013). Respiratory illness hospitalizations among pregnant women during influenza season, 1998-2008. *Maternal and Child Health Journal*, *17*(7), 1325–1331. https://doi.org/10.1007/s10995-012-1135-3

Matejuk, A., Adlard, K., Zamora, A., Silverman, M., Vandenbark, A. A., & Offner, H. (2001). 17β-estradiol inhibits cytokine, chemokine, and chemokine receptor mRNA expression in the central nervous system of female mice with experimental autoimmune encephalomyelitis. *Journal of Neuroscience Research*, *65*(6), 529–542. https://doi.org/10.1002/jnr.1183

Mayer, A. D., & Rosenblatt, J. S. (1977). Effects of intranasal zinc sulfate on open field and maternal behavior in female rats. *Physiology & Behavior*, *18*(1), 101–109. https://doi.org/10.1016/0031-9384(77)90100-7

McCullough, L. B., & Chervenak, F. A. (2008). A critical analysis of the concept and discourse of "unborn child." *The American Journal of Bioethics: AJOB*, *8*(7), 34–39. https://doi.org/10.1080/15265160802248161

McDonald, A. J., Shammah-Lagnado, S. J., Shi, C., & Davis, M. (1999). Cortical afferents to the extended amygdala. *Annals of the New York Academy of Sciences*, *877*, 309–338. https://doi.org/10.1111/j.1749-6632.1999.tb09275.x

McHenry, J. A., Rubinow, D. R., & Stuber, G. D. (2015). Maternally responsive neurons in the bed nucleus of the stria terminalis and medial preoptic area: Putative circuits for regulating anxiety and reward. *Frontiers in Neuroendocrinology*, *38*, 65–72. https://doi.org/10.1016/j.yfrne.2015.04.001

McLean, C. P., Asnaani, A., Litz, B. T., & Hofmann, S. G. (2011). Gender Differences in Anxiety Disorders: Prevalence, Course of Illness, Comorbidity and Burden of Illness. *Journal of Psychiatric Research*, *45*(8), 1027–1035. https://doi.org/10.1016/j.jpsychires.2011.03.006

McNab, F., Mayer-Barber, K., Sher, A., Wack, A., & O'Garra, A. (2015). Type I interferons in infectious disease. *Nature Reviews. Immunology*, *15*(2), 87–103. https://doi.org/10.1038/nri3787

McNeil, S. A., Dodds, L. A., Fell, D. B., Allen, V. M., Halperin, B. A., Steinhoff, M. C., & MacDonald, N. E. (2011). Effect of respiratory hospitalization during pregnancy on infant outcomes. *American Journal of Obstetrics and Gynecology*, *204*(6 Suppl 1), S54-57. https://doi.org/10.1016/j.ajog.2011.04.031

McQuaid, R. J., Audet, M.-C., Jacobson-Pick, S., & Anisman, H. (2013). Environmental enrichment influences brain cytokine variations elicited by social defeat in mice. *Psychoneuroendocrinology*, *38*(7), 987–996. https://doi.org/10.1016/j.psyneuen.2012.10.003

Medzhitov, R. (2007). Recognition of microorganisms and activation of the immune response. *Nature*, *449*(7164), 819–826. https://doi.org/10.1038/nature06246

Medzhitov, R. (2008). Origin and physiological roles of inflammation. *Nature*, *454*(7203), 428–435. https://doi.org/10.1038/nature07201

Merchenthaler, I., Lane, M. V., Numan, S., & Dellovade, T. L. (2004). Distribution of estrogen receptor alpha and beta in the mouse central nervous system: In vivo autoradiographic and immunocytochemical analyses. *The Journal of Comparative Neurology*, *473*(2), 270–291. https://doi.org/10.1002/cne.20128

Meyer, U. (2014). Prenatal poly(i:C) exposure and other developmental immune activation models in rodent systems. *Biological Psychiatry*, *75*(4), 307–315. https://doi.org/10.1016/j.biopsych.2013.07.011

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*(3), 1308–1321. https://doi.org/10.1016/j.neuropharm.2011.01.009

Meyer, U., Feldon, J., Schedlowski, M., & Yee, B. K. (2005). Towards an immuno-precipitated neurodevelopmental animal model of schizophrenia. *Neuroscience and Biobehavioral Reviews*, *29*(6), 913–947. https://doi.org/10.1016/j.neubiorev.2004.10.012

Meyer, U., Nyffeler, M., Engler, A., Urwyler, A., Schedlowski, M., Knuesel, I., Yee, B. K., & Feldon, J. (2006). The time of prenatal immune challenge determines the specificity of inflammation-mediated brain and behavioral pathology. *Journal of Neuroscience*, *26*(18), 4752–4762.

Miller, N. R., Jover, T., Cohen, H. W., Zukin, R. S., & Etgen, A. M. (2005). Estrogen can act via estrogen receptor alpha and beta to protect hippocampal neurons against global ischemia-induced cell death. *Endocrinology*, *146*(7), 3070–3079. https://doi.org/10.1210/en.2004-1515

Minkoff, H., Marshall, M. F., & Liaschenko, J. (2014). The fetus, the "potential child," and the ethical obligations of obstetricians. *Obstetrics and Gynecology*, *123*(5), 1100–1103. https://doi.org/10.1097/AOG.0000000000000225

Mishra, B. B., Rathinam, V. A. K., Martens, G. W., Martinot, A. J., Kornfeld, H., Fitzgerald, K. A., & Sassetti, C. M. (2013). Nitric oxide controls the immunopathology of tuberculosis by inhibiting NLRP3 inflammasome-dependent processing of IL-1β. *Nature Immunology*, *14*(1), 52–60. https://doi.org/10.1038/ni.2474

Mitra, S. W., Hoskin, E., Yudkovitz, J., Pear, L., Wilkinson, H. A., Hayashi, S., Pfaff, D. W., Ogawa, S., Rohrer, S. P., Schaeffer, J. M., McEwen, B. S., & Alves, S. E. (2003). Immunolocalization of estrogen receptor beta in the mouse brain: Comparison with estrogen receptor alpha. *Endocrinology*, *144*(5), 2055–2067. https://doi.org/10.1210/en.2002-221069

Mizel, S. B. (1989). The interleukins. *The FASEB Journal*, *3*(12), 2379–2388. https://doi.org/10.1096/fasebj.3.12.2676681

Molgaard-Nielsen, D., Fischer, T. K., Krause, T. G., & Hviid, A. (2019). Effectiveness of maternal immunization with trivalent inactivated influenza vaccine in pregnant women and their infants. *Journal of Internal Medicine*, *1*. https://doi.org/10.1111/joim.12947

Moncek, F., Duncko, R., Johansson, B. B., & Jezova, D. (2004). Effect of environmental enrichment on stress related systems in rats. *Journal of Neuroendocrinology*, *16*(5), 423–431.

Mor, G., & Cardenas, I. (2010). The Immune System in Pregnancy: A Unique Complexity. *American Journal of Reproductive Immunology (New York, N.Y. : 1989)*, *63*(6), 425–433. https://doi.org/10.1111/j.1600-0897.2010.00836.x

Moradi-Kor, N., Ghanbari, A., Rashidipour, H., Yousefi, B., Bandegi, A. R., & Rashidy-Pour, A. (2019). Beneficial effects of Spirulina platensis, voluntary exercise and environmental enrichment against adolescent stress induced deficits in cognitive functions, hippocampal BDNF and morphological remolding in adult female rats. *Hormones & Behavior*, *1*, 20–31. https://doi.org/10.1016/j.yhbeh.2019.03.004

Morales, L. B. J., Loo, K. K., Liu, H.-B., Peterson, C., Tiwari-Woodruff, S., & Voskuhl, R. R. (2006). Treatment with an estrogen receptor alpha ligand is neuroprotective in experimental autoimmune encephalomyelitis. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *26*(25), 6823–6833. https://doi.org/10.1523/JNEUROSCI.0453-06.2006

Morley-Fletcher, S., Rea, M., Maccari, S., & Laviola, G. (2003). Environmental enrichment during adolescence reverses the effects of prenatal stress on play behaviour and HPA axis reactivity in rats. *European Journal of Neuroscience*, *18*(12), 3367–3374. https://doi.org/10.1111/j.1460-9568.2003.03070.x

Mosmann, T. R., & Coffman, R. L. (1989). TH1 and TH2 cells: Different patterns of lymphokine secretion lead to different functional properties. *Annual Review of Immunology*, *7*, 145–173. https://doi.org/10.1146/annurev.iy.07.040189.001045

Moya, J., Phillips, L., Sanford, J., Wooton, M., Gregg, A., & Schuda, L. (2014). A review of physiological and behavioral changes during pregnancy and lactation: Potential exposure factors and data gaps. *Journal of Exposure Science and Environmental Epidemiology*, *24*(5), 449–458. https://doi.org/10.1038/jes.2013.92

Muanda, F. T., Sheehy, O., & Bérard, A. (2017). Use of antibiotics during pregnancy and risk of spontaneous abortion. *CMAJ*, *189*(17), E625–E633. https://doi.org/10.1503/cmaj.161020

Munoz, F. M., Greisinger, A. J., Wehmanen, O. A., Mouzoon, M. E., Hoyle, J. C., Smith, F. A., & Glezen, W. P. (2005). Safety of influenza vaccination during pregnancy. *American Journal of Obstetrics and Gynecology*, *192*(4), 1098–1106. https://doi.org/10.1016/j.ajog.2004.12.019

Murray, C., Sanderson, D. J., Barkus, C., Deacon, R. M. J., Rawlins, J. N. P., Bannerman, D. M., & Cunningham, C. (2012). Systemic inflammation induces acute working memory deficits in the

primed brain: Relevance for delirium. *Neurobiology of Aging*, *33*(3), 603–616. https://doi.org/10.1016/j.neurobiolaging.2010.04.002

Mychasiuk, R., Muhammad, A., & Kolb, B. (2014). Environmental enrichment alters structural plasticity of the adolescent brain but does not remediate the effects of prenatal nicotine exposure. *Synapse*, *68*(7), 293–305. https://doi.org/10.1002/syn.21737

Nauta, W. J., Smith, G. P., Faull, R. L., & Domesick, V. B. (1978). Efferent connections and nigral afferents of the nucleus accumbens septi in the rat. *Neuroscience*, *3*(4–5), 385–401. https://doi.org/10.1016/0306-4522(78)90041-6

Nelson, L. R., & Bulun, S. E. (2001). Estrogen production and action. [Review] [63 refs]. *Journal of the American Academy of Dermatology*.

Neuwald, M. F., Agranonik, M., Portella, A. K., Fleming, A., Wazana, A., Steiner, M., Levitan, R. D., Meaney, M. J., Silveira, P. P., & Team, M. S. (2014). Transgenerational effects of maternal care interact with fetal growth and influence attention skills at 18 months of age. *Early Human Development*, *90*(5), 241–246. https://doi.org/10.1016/j.earlhumdev.2014.01.015

Nishijima, T., Kamidozono, Y., Ishiizumi, A., Amemiya, S., & Kita, I. (2017). Negative rebound in hippocampal neurogenesis following exercise cessation. *Journal of Physiology - Regulatory Integrative*, *312*(3), R347–R357. https://doi.org/10.1152/ajpregu.00397.2016

Nizri, E., Hamra-Amitay, Y., Sicsic, C., Lavon, I., & Brenner, T. (2006). Anti-inflammatory properties of cholinergic up-regulation: A new role for acetylcholinesterase inhibitors. *Neuropharmacology*, *50*(5), 540–547. https://doi.org/10.1016/j.neuropharm.2005.10.013

Numan, M. (1988). Neural basis of maternal behavior in the rat. *Psychoneuroendocrinology*, *13*(1–2), 47–62.

Numan, M., Morrell, J. I., & Pfaff, D. W. (1985). Anatomical identification of neurons in selected brain regions associated with maternal behavior deficits induced by knife cuts of the lateral hypothalamus in rats. *The Journal of Comparative Neurology*, *237*(4), 552–564. https://doi.org/10.1002/cne.902370411

Numan, Michael. (1974). Medial preoptic area and maternal behavior in the female rat. *Journal of Comparative and Physiological Psychology*, *87*(4), 746–759. https://doi.org/10.1037/h0036974

Numan, Michael, & Callahan, E. C. (1980). The connections of the medial preoptic region and maternal behavior in the rat. *Physiology & Behavior*, *25*(5), 653–665. https://doi.org/10.1016/0031-9384(80)90367-4

Numan, Michael, Corodimas, K. P., Numan, M. J., Factor, E. M., & Piers, W. D. (1988). Axon-sparing lesions of the preoptic region and substantia innominata disrupt maternal behavior in rats. *Behavioral Neuroscience*, *102*(3), 381–396. https://doi.org/10.1037/0735-7044.102.3.381

Numan, Michael, & Insel, T. R. (2006). *The Neurobiology of Parental Behavior*. Springer Science & Business Media.

Numan, Michael, Rosenblatt, J. S., & Komisaruk, B. R. (1977). Medial preoptic area and onset of maternal behavior in the rat. *Journal of Comparative and Physiological Psychology*, *91*(1), 146–164. https://doi.org/10.1037/h0077304

Nunes, M. C., & Madhi, S. A. (2015). Review on the effects of influenza vaccination during pregnancy on preterm births. *Human Vaccines & Immunotherapeutics*, *11*(11), 2538–2548. https://doi.org/10.1080/21645515.2015.1059976

Nyhuis, T. J., Masini, C. V., Sasse, S. K., Day, H. E. W., & Campeau, S. (2010). Physical activity, but not environmental complexity, facilitates HPA axis response habituation to repeated audiogenic

stress despite neurotrophin mRNA regulation in both conditions. *Brain Research*, *1*, 68–77. https://doi.org/10.1016/j.brainres.2010.09.038

Oatridge, A., Holdcroft, A., Saeed, N., Hajnal, J. V., Puri, B. K., Fusi, L., & Bydder, G. M. (2002). Change in brain size during and after pregnancy: Study in healthy women and women with preeclampsia. *AJNR. American Journal of Neuroradiology*, *23*(1), 19–26.

O'Garra, A., & Arai, N. (2000). The molecular basis of T helper 1 and T helper 2 cell differentiation. *Trends in Cell Biology*, *10*(12), 542–550.

Ogawa, S., Inoue, S., Watanabe, T., Hiroi, H., Orimo, A., Hosoi, T., Ouchi, Y., & Muramatsu, M. (1998). The complete primary structure of human estrogen receptor beta (hER beta) and its heterodimerization with ER alpha in vivo and in vitro. *Biochemical & Biophysical Research Communications*, *243*(1), 122–126. https://doi.org/10.1006/bbrc.1997.7893

Oguma, Y., & Shinoda-Tagawa, T. (2004). Physical activity decreases cardiovascular disease risk in women: Review and meta-analysis. [Review] [85 refs]. *Journal of Preventive Medicine*, *26*(5), 407–418.

Olesen, K. M., Ismail, N., Merchasin, E. D., & Blaustein, J. D. (2011). Long-term alteration of anxiolytic effects of ovarian hormones in female mice by a peripubertal immune challenge. *Hormones and Behavior*, *60*(4), 318–326. https://doi.org/10.1016/j.yhbeh.2011.06.005

Oskvig, D. B., Elkahloun, A. G., Johnson, K. R., Phillips, T. M., & Herkenham, M. (2012). Maternal immune activation by LPS selectively alters specific gene expression profiles of interneuron migration and oxidative stress in the fetus without triggering a fetal immune response. *Brain, Behavior, and Immunity*, *26*(4), 623–634. https://doi.org/10.1016/j.bbi.2012.01.015

Otmishi, P., Gordon, J., El-Oshar, S., Li, H., Guardiola, J., Saad, M., Proctor, M., & Yu, J. (2008). Neuroimmune Interaction in Inflammatory Diseases. *Clinical Medicine. Circulatory, Respiratory and Pulmonary Medicine*, *2*, 35–44.

Paltrow, L. M., & Flavin, J. (2013). Arrests of and forced interventions on pregnant women in the United States, 1973-2005: Implications for women's legal status and public health. *Journal of Health Politics, Policy and Law*, *38*(2), 299–343. https://doi.org/10.1215/03616878-1966324

Parameswaran, N., & Patial, S. (2010). Tumor necrosis factor-α signaling in macrophages. *Critical Reviews in Eukaryotic Gene Expression*, *20*(2), 87–103.

Park, H., Li, Z., Yang, X. O., Chang, S. H., Nurieva, R., Wang, Y.-H., Wang, Y., Hood, L., Zhu, Z., Tian, Q., & Dong, C. (2005). A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nature Immunology*, *6*(11), 1133–1141. https://doi.org/10.1038/ni1261

Parkin, J., & Cohen, B. (2001). An overview of the immune system. *Lancet (London, England)*, *357*(9270), 1777–1789. https://doi.org/10.1016/S0140-6736(00)04904-7

Pasantes-Morales, H., & Schousboe, A. (1997). Role of taurine in osmoregulation in brain cells: Mechanisms and functional implications. *Amino Acids*, *12*(3), 281–292. https://doi.org/10.1007/BF01373008

Pasco, A., Minassian, A. T., Chapon, C., Lemaire, L., Franconi, F., Darabi, D., Caron, C., Benoit, J.-P., & Le Jeune, J.-J. (2006). Dynamics of cerebral edema and the apparent diffusion coefficient of water changes in patients with severe traumatic brain injury. A prospective MRI study. *European Radiology*, *16*(7), 1501. https://doi.org/10.1007/s00330-005-0086-0

Patten, A. R., Sickmann, H., Hryciw, B. N., Kucharsky, T., Parton, R., Kernick, A., & Christie, B. R. (2013). Long-term exercise is needed to enhance synaptic plasticity in the hippocampus. *Learning & Memory*, *20*(11), 642–647. https://doi.org/10.1101/lm.030635.113

Patterson, P. H. (2009). Immune involvement in schizophrenia and autism: Etiology, pathology and animal models. *Behavioural Brain Research*, *204*(2), 313–321. https://doi.org/10.1016/j.bbr.2008.12.016

Patterson, P. H. (2011). Maternal infection and immune involvement in autism. *Trends in Molecular Medicine*, *17*(7), 389–394. https://doi.org/10.1016/j.molmed.2011.03.001

Patterson, S., Moran, P., Epel, E., Sinclair, E., Kemeny, M. E., Deeks, S. G., Bacchetti, P., Acree, M., Epling, L., Kirschbaum, C., & Hecht, F. M. (2013). Cortisol Patterns Are Associated with T Cell Activation in HIV. *PLoS ONE*, *8*(7). https://doi.org/10.1371/journal.pone.0063429

Paul, W. E. (1991). Interleukin-4: A prototypic immunoregulatory lymphokine. *Blood*, *77*(9), 1859–1870.

Perel, P., Roberts, I., Sena, E., Wheble, P., Briscoe, C., Sandercock, P., Macleod, M., Mignini, L. E., Jayaram, P., & Khan, K. S. (2007). Comparison of treatment effects between animal experiments and clinical trials: Systematic review. *BMJ*, *334*(7586), 197. https://doi.org/10.1136/bmj.39048.407928.BE

Perkel, J. M. (2001). Distinguishing Th1 and Th2 cells by. *Scientist (Philadelphia, Pa.)*, *15*, 22–23.

Pernis, A. B. (2007). Estrogen and CD4+ T cells. *Current Opinion in Rheumatology*, *19*(5), 414–420. https://doi.org/10.1097/BOR.0b013e328277ef2a

Perretti, M., & Ahluwalia, A. (2000). The microcirculation and inflammation: Site of action for glucocorticoids. *Microcirculation (New York, N.Y.: 1994)*, *7*(3), 147–161.

Perry, V. H., Cunningham, C., & Holmes, C. (2007). Systemic infections and inflammation affect chronic neurodegeneration. [Review] [90 refs]. *Nature Reviews*, *7*(2), 161–167. https://doi.org/10.1038/nri2015

Pfaff, D., & Keiner, M. (1973). Atlas of estradiol-concentrating cells in the central nervous system of the female rat. *The Journal of Comparative Neurology*, *151*(2), 121–158. https://doi.org/10.1002/cne.901510204

Pfeffer, K., Matsuyama, T., Kündig, T. M., Wakeham, A., Kishihara, K., Shahinian, A., Wiegmann, K., Ohashi, P. S., Krönke, M., & Mak, T. W. (1993). Mice deficient for the 55 kd tumor necrosis factor receptor are resistant to endotoxic shock, yet succumb to L. monocytogenes infection. *Cell*, *73*(3), 457–467. https://doi.org/10.1016/0092-8674(93)90134-c

Phillipson, O. T. (1979). Afferent projections to the ventral tegmental area of Tsai and interfascicular nucleus: A horseradish peroxidase study in the rat. *The Journal of Comparative Neurology*, *187*(1), 117–143. https://doi.org/10.1002/cne.901870108

Pires, P. W., Dams Ramos, C. M., Matin, N., & Dorrance, A. M. (2013). The effects of hypertension on the cerebral circulation. *American Journal of Physiology. Heart and Circulatory Physiology*, *304*(12), H1598-1614. https://doi.org/10.1152/ajpheart.00490.2012

Polanczyk, M. J., Hopke, C., Huan, J., Vandenbark, A. A., & Offner, H. (2005). Enhanced FoxP3 expression and Treg cell function in pregnant and estrogen-treated mice. *Journal of Neuroimmunology*, *170*(1–2), 85–92. https://doi.org/10.1016/j.jneuroim.2005.08.023

Poltorak, A., He, X., Smirnova, I., Liu, M. Y., Van Huffel, C., Du, X., Birdwell, D., Alejos, E., Silva, M., Galanos, C., Freudenberg, M., Ricciardi-Castagnoli, P., Layton, B., & Beutler, B. (1998). Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: Mutations in Tlr4 gene. *Science (New York, N.Y.)*, *282*(5396), 2085–2088. https://doi.org/10.1126/science.282.5396.2085

Poobalan, A. S., Aucott, L. S., Ross, L., Smith, W. C. S., Helms, P. J., & Williams, J. H. G. (2007). Effects of treating postnatal depression on mother-infant interaction and child development:

Systematic review. *The British Journal of Psychiatry*, *191*(5), 378–386. https://doi.org/10.1192/bjp.bp.106.032789

PrabhuDas, M., Bonney, E., Caron, K., Dey, S., Erlebacher, A., Fazleabas, A., Fisher, S., Golos, T., Matzuk, M., McCune, J. M., Mor, G., Schulz, L., Soares, M., Spencer, T., Strominger, J., Way, S. S., & Yoshinaga, K. (2015). Immune mechanisms at the maternal-fetal interface: Perspectives and challenges. *Nature Immunology*, *16*, 328–334. https://doi.org/10.1038/ni.3131

Prescott, I. A. (n.d.). *SYNAPTIC PLASTICITY IN BASAL GANGLIA OUTPUT NEURONS IN PARKINSON'S DISEASE PATIENTS*. 104.

*Prescottâ□"SYNAPTIC PLASTICITY IN BASAL GANGLIA OUTPUT NEURON.pdf*. (n.d.). Retrieved March 3, 2020, from https://tspace.library.utoronto.ca/bitstream/1807/18986/1/Prescott_Ian_A_200911_MSc_thesis.pdf

Purohit, A., Newman, S. P., & Reed, M. J. (2002). The role of cytokines in regulating estrogen synthesis: Implications for the etiology of breast cancer. [Review] [40 refs]. *Breast Cancer Research*, *4*(2), 65–69.

Quan, N., Sundar, S. K., & Weiss, J. M. (1994). Induction of interleukin-1 in various brain regions after peripheral and central injections of lipopolysaccharide. *Journal of Neuroimmunology*, *49*(1), 125–134. https://doi.org/10.1016/0165-5728(94)90188-0

Rainbow, T. C., Parsons, B., MacLusky, N. J., & McEwen, B. S. (1982). Estradiol receptor levels in rat hypothalamic and limbic nuclei. *Journal of Neuroscience*, *2*(10), 1439–1445. https://doi.org/10.1523/JNEUROSCI.02-10-01439.1982

Raisman, G. (1972). An experimental study of the projection of the amygdala to the accessory olfactory bulb and its relationship to the concept of a dual olfactory system. *Experimental Brain Research*, *14*(4), 395–408. https://doi.org/10.1007/BF00235035

Ransohoff, R. M., & Brown, M. A. (2012). Innate immunity in the central nervous system. [Review]. *Journal of Clinical Investigation*, *122*(4), 1164–1171. https://doi.org/10.1172/JCI58644

Rathgeber, E. M., & Vlassoff, C. (1993). Gender and tropical diseases: A new research focus. *Social Science & Medicine (1982)*, *37*(4), 513–520. https://doi.org/10.1016/0277-9536(93)90286-d

Ravenelle, R., Byrnes, E. M., Byrnes, J. J., McInnis, C., Park, J. H., & Donaldson, S. T. (2013). Environmental enrichment effects on the neurobehavioral profile of selective outbred trait anxiety rats. *Behavioural Brain Research*, *252*, 49–57. https://doi.org/10.1016/j.bbr.2013.05.041

Regan, A. K., Moore, H. C., Sullivan, S. G., DE Klerk, N., & Effler, P. V. (2017). Epidemiology of seasonal influenza infection in pregnant women and its impact on birth outcomes. *Epidemiology and Infection*, *145*(14), 2930–2939. https://doi.org/10.1017/S0950268817001972

Research, C. for D. E. and. (2019). Pregnancy and Lactation Labeling (Drugs) Final Rule. *FDA*. http://www.fda.gov/drugs/labeling/pregnancy-and-lactation-labeling-drugs-final-rule

Rickwood, D. J., & Braithwaite, V. A. (1994). Social-psychological factors affecting help-seeking for emotional problems. *Social Science & Medicine (1982)*, *39*(4), 563–572. https://doi.org/10.1016/0277-9536(94)90099-x

Roderick, R. C., & Kentner, A. C. (2019). Building a framework to optimize animal models of maternal immune activation: Like your ongoing home improvements, it's a work in progress. *Brain, Behavior, & Immunity*, *1*, 6–7. https://doi.org/10.1016/j.bbi.2018.10.011

Ronovsky, M., Berger, S., Molz, B., Berger, A., & Pollak, D. D. (2016). Animal Models of Maternal Immune Activation in Depression Research. *Current Neuropharmacology*, *14*(7), 688–704.

Rosenfeld, A., & Weller, A. (2012). Behavioral effects of environmental enrichment during gestation in WKY and Wistar rats. *Behavioural Brain Research*, *233*(2), 245–255. https://doi.org/10.1016/j.bbr.2012.05.006

Rosenzweig, M. R., & Bennett, E. L. (1996). Psychobiology of plasticity: Effects of training and experience on brain and behavior. [Review] [79 refs]. *Behavioural Brain Research*, *78*(1), 57–65.

Ryan, K. J. (1982). Biochemistry of aromatase: Significance to female reproductive physiology. *Cancer Research*.

Sale, A., Berardi, N., & Maffei, L. (2014). Environment and brain plasticity: Towards an endogenous pharmacotherapy. *Physiological Reviews*, *94*(1), 189–234. https://doi.org/10.1152/physrev.00036.2012

Sasse, S. K., Greenwood, B. N., Masini, C. V., Nyhuis, T. J., Fleshner, M., Day, H. E. W., & Campeau, S. (2008). Chronic voluntary wheel running facilitates corticosterone response habituation to repeated audiogenic stress exposure in male rats. *Stress*, *11*(6), 425–437. https://doi.org/10.1080/10253890801887453

Saunders, N. R., Liddelow, S. A., & Dziegielewska, K. M. (2012). Barrier mechanisms in the developing brain. *Frontiers in Pharmacology*, *3*, 46. https://doi.org/10.3389/fphar.2012.00046

Scheller, J., Chalaris, A., Schmidt-Arras, D., & Rose-John, S. (2011). The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochimica Et Biophysica Acta*, *1813*(5), 878–888. https://doi.org/10.1016/j.bbamcr.2011.01.034

Scherer, I. J., Holmes, P. V., & Harris, R. B. S. (2011). The importance of corticosterone in mediating restraint-induced weight loss in rats. *Physiology & Behavior*, *102*(2), 225–233. https://doi.org/10.1016/j.physbeh.2010.11.014

Schloesser, R. J., Lehmann, M., Martinowich, K., Manji, H. K., & Herkenham, M. (2010). Environmental enrichment requires adult neurogenesis to facilitate the recovery from psychosocial stress. *Molecular Psychiatry*, *15*(12), 1152–1163. https://doi.org/10.1038/mp.2010.34

Schneider, W. M., Chevillotte, M. D., & Rice, C. M. (2014). Interferon-stimulated genes: A complex web of host defenses. *Annual Review of Immunology*, *32*, 513–545. https://doi.org/10.1146/annurev-immunol-032713-120231

Schrijver, N. C. A., Bahr, N. I., Weiss, I. C., & Wurbel, H. (2002). Dissociable effects of isolation rearing and environmental enrichment on exploration, spatial learning and HPA activity in adult rats. *Pharmacology, Biochemistry & Behavior*, *73*(1), 209–224.

Scott, J. W., McBride, R. L., & Schneider, S. P. (1980). The organization of projections from the olfactory bulb to the piriform cortex and olfactory tubercle in the rat. *The Journal of Comparative Neurology*, *194*(3), 519–534. https://doi.org/10.1002/cne.901940304

Seo, D. Y., Lee, S. R., Kwak, H.-B., Seo, K. W., McGregor, R. A., Yeo, J. Y., Ko, T. H., Bolorerdene, S., Kim, N., Ko, K. S., Rhee, B. D., & Han, J. (2016). Voluntary stand-up physical activity enhances endurance exercise capacity in rats. *Journal of Physiology*, *20*(3), 287–295. https://doi.org/10.4196/kjpp.2016.20.3.287

Sheehan, T., & Numan, M. (2002). Estrogen, progesterone, and pregnancy termination alter neural activity in brain regions that control maternal behavior in rats. *Neuroendocrinology*, *75*(1), 12–23.

Shi, L., Smith, S. E. P., Malkova, N., Tse, D., Su, Y., & Patterson, P. H. (2009). Activation of the maternal immune system alters cerebellar development in the offspring. *Brain, Behavior, and Immunity*, *23*(1), 116–123. https://doi.org/10.1016/j.bbi.2008.07.012

Short, S. J., Lubach, G. R., Karasin, A. I., Olsen, C. W., Styner, M., Knickmeyer, R. C., Gilmore, J. H., & Coe, C. L. (2010). Maternal Influenza Infection During Pregnancy Impacts Postnatal Brain Development in the Rhesus Monkey. *Biological Psychiatry*, *67*(10), 965–973. https://doi.org/10.1016/j.biopsych.2009.11.026

Simerly, R. B., & Swanson, L. W. (1986). The organization of neural inputs to the medial preoptic nucleus of the rat. *The Journal of Comparative Neurology*, *246*(3), 312–342. https://doi.org/10.1002/cne.902460304

Simpson, J., & Kelly, J. P. (2011). The impact of environmental enrichment in laboratory ratsâ□”Behavioural and neurochemical aspects. [Review]. *Behavioural Brain Research*, *222*(1), 246–264. https://doi.org/10.1016/j.bbr.2011.04.002

Singleton, J. M., & Garland, T. J. (2019). Influence of corticosterone on growth, home-cage activity, wheel running, and aerobic capacity in house mice selectively bred for high voluntary wheel-running behavior. *Physiology & Behavior*, *1*, 27–41. https://doi.org/10.1016/j.physbeh.2018.10.001

Skaper, S. D., Giusti, P., & Facci, L. (2012). Microglia and mast cells: Two tracks on the road to neuroinflammation. [Review]. *FASEB Journal*, *26*(8), 3103–3117. https://doi.org/10.1096/fj.11-197194

Skelly, D. T., Hennessy, E., Dansereau, M.-A., & Cunningham, C. (2013). A systematic analysis of the peripheral and CNS effects of systemic LPS, IL-1beta, [corrected] TNF-alpha and IL-6 challenges in C57BL/6 mice.[Erratum appears in PLoS One. 2013;8(12). Doi:10.1371/annotation/90c76048-2edd-4315-8404-4d9d8cbd411e]. *PLoS ONE [Electronic Resource]*, *8*(7). https://doi.org/10.1371/journal.pone.0069123

Smith, C. L., & O'Malley, B. W. (2004). Coregulator function: A key to understanding tissue specificity of selective receptor modulators. [Review] [337 refs]. *Endocrine Reviews*, *25*(1), 45–71. https://doi.org/10.1210/er.2003-0023

Smith, S. E. P., Li, J., Garbett, K., Mirnics, K., & Patterson, P. H. (2007a). Maternal Immune Activation Alters Fetal Brain Development through Interleukin-6. *Journal of Neuroscience*, *27*(40), 10695–10702. https://doi.org/10.1523/JNEUROSCI.2178-07.2007

Smith, S. E. P., Li, J., Garbett, K., Mirnics, K., & Patterson, P. H. (2007b). Maternal Immune Activation Alters Fetal Brain Development through Interleukin-6. *Journal of Neuroscience*, *27*(40), 10695–10702. https://doi.org/10.1523/JNEUROSCI.2178-07.2007

Soucy, G., Boivin, G., Labrie, F., & Rivest, S. (2005). Estradiol is required for a proper immune response to bacterial and viral pathogens in the female brain. *Journal of Immunology*, *174*(10), 6391–6398.

Spieles-Engemann, A. L., Steece-Collier, K., Behbehani, M. M., Collier, T. J., Wohlgenant, S. L., Kemp, C. J., Cole-Strauss, A., Levine, N. D., Gombash, S. E., Thompson, V. B., Lipton, J. W., & Sortwell, C. E. (2011). Subthalamic nucleus stimulation increases brain derived neurotrophic factor in the nigrostriatal system and primary motor cortex. *Journal of Parkinson's Disease*, *1*(1), 123–136.

Sroga, J. M., Jones, T. B., Kigerl, K. A., McGaughy, V. M., & Popovich, P. G. (2003). Rats and mice exhibit distinct inflammatory reactions after spinal cord injury. *The Journal of Comparative Neurology*, *462*(2), 223–240. https://doi.org/10.1002/cne.10736

Stark, G. R., Kerr, I. M., Williams, B. R. G., Silverman, R. H., & Schreiber, R. D. (1998). How Cells Respond to Interferons. *Annual Review of Biochemistry*, *67*(1), 227–264. https://doi.org/10.1146/annurev.biochem.67.1.227

Streit, W. J., & Kincaid-Colton, C. A. (1995). The brain's immune system. *Scientific American*, *273*(5), 54–55, 58–61. https://doi.org/10.1038/scientificamerican1195-54

Streit, Wolfgang J, Mrak, R. E., & Griffin, W. S. T. (2004). Microglia and neuroinflammation: A pathological perspective. *Journal of Neuroinflammation*, *1*, 14. https://doi.org/10.1186/1742-2094-1-14

Streit, Wolfgang J., Mrak, R. E., & Griffin, W. S. T. (2004). Microglia and neuroinflammation: A pathological perspective. *Journal of Neuroinflammation*, *1*(1), 14. https://doi.org/10.1186/1742-2094-1-14

Strzelewicz, A. R., Ordones Sanchez, E., Rondon-Ortiz, A. N., Raneri, A., Famularo, S. T., Bangasser, D. A., & Kentner, A. C. (2019). Access to a high resource environment protects against accelerated maturation following early life stress: A translational animal model of high, medium and low security settings. *Hormones & Behavior*, *1*, 46–59. https://doi.org/10.1016/j.yhbeh.2019.01.003

Su, X., Maguire-Zeiss, K. A., Giuliano, R., Prifti, L., Venkatesh, K., & Federoff, H. J. (2008). Synuclein activates microglia in a model of Parkinson's disease. *Neurobiology of Aging*, *29*(11), 1690–1701. https://doi.org/10.1016/j.neurobiolaging.2007.04.006

Subramanian, S., Matejuk, A., Zamora, A., Vandenbark, A. A., & Offner, H. (2003). Oral feeding with ethinyl estradiol suppresses and treats experimental autoimmune encephalomyelitis in SJL mice and inhibits the recruitment of inflammatory cells into the central nervous system. *Journal of Immunology (Baltimore, Md.: 1950)*, *170*(3), 1548–1555. https://doi.org/10.4049/jimmunol.170.3.1548

Sugino, K., Dohi, K., Yamada, K., & Kawasaki, T. (1987). The role of lipid peroxidation in endotoxin-induced hepatic damage and the protective effect of antioxidants. *Surgery*, *101*(6), 746–752.

Swanson, L. W. (1976). An autoradiographic study of the efferent connections of the preoptic region in the rat. *Journal of Comparative Neurology*, *167*(2), 227–256. https://doi.org/10.1002/cne.901670207

Swanson, L. W. (1982). The projections of the ventral tegmental area and adjacent regions: A combined fluorescent retrograde tracer and immunofluorescence study in the rat. *Brain Research Bulletin*, *9*(1–6), 321–353. https://doi.org/10.1016/0361-9230(82)90145-9

Swanson, L. W., & Cowan, W. M. (1979). The connections of the septal region in the rat. *The Journal of Comparative Neurology*, *186*(4), 621–655. https://doi.org/10.1002/cne.901860408

Swanson, L. W., Mogenson, G. J., Gerfen, C. R., & Robinson, P. (1984). Evidence for a projection from the lateral preoptic area and substantia innominata to the 'mesencephalic locomotor region' in the rat. *Brain Research*, *295*(1), 161–178. https://doi.org/10.1016/0006-8993(84)90827-8

Takai, D., Abe, A., Miura, H., Tanaka, S., & Komura, J.-I. (2019). Minimum environmental enrichment is effective in activating antitumor immunity to transplanted tumor cells in mice. *Experimental Animals*, *1*. https://doi.org/10.1538/expanim.19-0055

Takeuchi, O., & Akira, S. (2010). Pattern recognition receptors and inflammation. *Cell*, *140*(6), 805–820. https://doi.org/10.1016/j.cell.2010.01.022

Tamma, P. D., Ault, K. A., del Rio, C., Steinhoff, M. C., Halsey, N. A., & Omer, S. B. (2009). Safety of influenza vaccination during pregnancy. *American Journal of Obstetrics and Gynecology*, *201*(6), 547–552. https://doi.org/10.1016/j.ajog.2009.09.034

Tanaka, S., Ide, M., Shibutani, T., Ohtaki, H., Numazawa, S., Shioda, S., & Yoshida, T. (2006). Lipopolysaccharide-induced microglial activation induces learning and memory deficits without neuronal cell death in rats. *Journal of Neuroscience Research*, *83*(4), 557–566. https://doi.org/10.1002/jnr.20752

Tapia, M. D., Sow, S. O., Tamboura, B., Tégueté, I., Pasetti, M. F., Kodio, M., Onwuchekwa, U., Tennant, S. M., Blackwelder, W. C., Coulibaly, F., Traoré, A., Keita, A. M., Haidara, F. C., Diallo, F., Doumbia, M., Sanogo, D., DeMatt, E., Schluterman, N. H., Buchwald, A., … Levine, M. M. (2016). Maternal immunisation with trivalent inactivated influenza vaccine for prevention of influenza in infants in Mali: A prospective, active-controlled, observer-blind, randomised phase 4 trial. *The Lancet. Infectious Diseases*, *16*(9), 1026–1035. https://doi.org/10.1016/S1473-3099(16)30054-8

Temel, Y., Visser-Vandewalle, V., Kaplan, S., Kozan, R., Daemen, M. A. R. C., Blokland, A., Schmitz, C., & Steinbusch, H. W. M. (2006). Protection of nigral cell death by bilateral subthalamic nucleus stimulation. *Brain Research*, *1120*(1), 100–105. https://doi.org/10.1016/j.brainres.2006.08.082

Teske, J. A., Perez-Leighton, C. E., Noble, E. E., Wang, C., Billington, C. J., & Kotz, C. M. (2016). Effect of Housing Types on Growth, Feeding, Physical Activity, and Anxiety-Like Behavior in Male Sprague-Dawley Rats. *Frontiers in Nutrition*, *3*. https://doi.org/10.3389/fnut.2016.00004

Tonelli, L., Kramer, P., Webster, J. I., Wray, S., Listwak, S., & Sternberg, E. (2002). Lipopolysaccharide-induced oestrogen receptor regulation in the paraventricular hypothalamic nucleus of lewis and Fischer rats. *Journal of Neuroendocrinology*, *14*(11), 847–852.

Trinchieri, G. (2010). Type I interferon: Friend or foe? *The Journal of Experimental Medicine*, *207*(10), 2053–2063. https://doi.org/10.1084/jem.20101664

Tyagi, E., Agrawal, R., Nath, C., & Shukla, R. (2008). Influence of LPS-induced neuroinflammation on acetylcholinesterase activity in rat brain. *Journal of Neuroimmunology*, *205*(1–2), 51–56. https://doi.org/10.1016/j.jneuroim.2008.08.015

Uchide, N., Ohyama, K., Bessho, T., Takeichi, M., & Toyoda, H. (2012). Possible roles of proinflammatory and chemoattractive cytokines produced by human fetal membrane cells in the pathology of adverse pregnancy outcomes associated with influenza virus infection. *Mediators of Inflammation*, *2012*, 270670. https://doi.org/10.1155/2012/270670

Valero, J., Mastrella, G., Neiva, I., Sanchez, S., & Malva, J. O. (2014). Long-term effects of an acute and systemic administration of LPS on adult neurogenesis and spatial memory. *Frontiers in Neuroscience*. https://doi.org/10.3389/fnins.2014.00083

van den Boorn, J. G., Barchet, W., & Hartmann, G. (2012). Nucleic acid adjuvants: Toward an educated vaccine. *Advances in Immunology*, *114*, 1–32. https://doi.org/10.1016/B978-0-12-396548-6.00001-9

van Praag, H., Kempermann, G., & Gage, F. H. (2000). Neural consequences of environmental enrichment. [Review] [129 refs]. *Nature Reviews Neuroscience*, *1*(3), 191–198.

Vega-Rivera, N. M., Ortiz-Lopez, L., Gomez-Sanchez, A., Oikawa-Sala, J., Estrada-Camarena, E. M., & Ramirez-Rodriguez, G. B. (2016). The neurogenic effects of an enriched environment and its protection against the behavioral consequences of chronic mild stress persistent after enrichment cessation in six-month-old female Balb/C mice. *Behavioural Brain Research*, *1*, 72–83. https://doi.org/10.1016/j.bbr.2015.12.028

Vegeto, E., Bonincontro, C., Pollio, G., Sala, A., Viappiani, S., Nardi, F., Brusadelli, A., Viviani, B., Ciana, P., & Maggi, A. (2001). Estrogen prevents the lipopolysaccharide-induced inflammatory

response in microglia. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *21*(6), 1809–1818.

Vegeto, Elisabetta, Belcredito, S., Etteri, S., Ghisletti, S., Brusadelli, A., Meda, C., Krust, A., Dupont, S., Ciana, P., Chambon, P., & Maggi, A. (2003). Estrogen receptor-alpha mediates the brain antiinflammatory activity of estradiol. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(16), 9614–9619. https://doi.org/10.1073/pnas.1531957100

Vegeto, Elisabetta, Benedusi, V., & Maggi, A. (2008). Estrogen anti-inflammatory activity in brain: A therapeutic opportunity for menopause and neurodegenerative diseases. *Frontiers in Neuroendocrinology*, *29*(4), 507–519. https://doi.org/10.1016/j.yfrne.2008.04.001

Vertes, R. P. (2006). Interactions among the medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive processing in the rat. *Neuroscience*, *142*(1), 1–20. https://doi.org/10.1016/j.neuroscience.2006.06.027

Vivinetto, A. L., Suárez, M. M., & Rivarola, M. A. (2013). Neurobiological effects of neonatal maternal separation and post-weaning environmental enrichment. *Behavioural Brain Research*, *240*, 110–118. https://doi.org/10.1016/j.bbr.2012.11.014

Vlahopoulos, S., Boldogh, I., Casola, A., & Brasier, A. R. (1999). Nuclear factor-kappaB-dependent induction of interleukin-8 gene expression by tumor necrosis factor alpha: Evidence for an antioxidant sensitive activating pathway distinct from nuclear translocation. *Blood*, *94*(6), 1878–1889.

Vlassoff, C. (2007). Gender Differences in Determinants and Consequences of Health and Illness. *Journal of Health, Population, and Nutrition*, *25*(1), 47–61.

Walker, D. L., Toufexis, D. J., & Davis, M. (2003). Role of the bed nucleus of the stria terminalis versus the amygdala in fear, stress, and anxiety. *European Journal of Pharmacology*, *463*(1–3), 199–216. https://doi.org/10.1016/s0014-2999(03)01282-2

Walkinshaw, E. (2011). Mandatory vaccinations: The Canadian picture. *CMAJ : Canadian Medical Association Journal*, *183*(16), e1165–e1166. https://doi.org/10.1503/cmaj.109-3992

Wallace, H. A. C., Marques-Kranc, F., Richardson, M., Luna-Crespo, F., Sharpe, J. A., Hughes, J., Wood, W. G., Higgs, D. R., & Smith, A. J. H. (2007). Manipulating the mouse genome to engineer precise functional syntenic replacements with human sequence. *Cell*, *128*(1), 197–209. https://doi.org/10.1016/j.cell.2006.11.044

Weir, R. K., Forghany, R., Smith, S. E. P., Patterson, P. H., McAllister, A. K., Schumann, C. M., & Bauman, M. D. (2015). Preliminary evidence of neuropathology in nonhuman primates prenatally exposed to maternal immune activation. *Brain, Behavior, and Immunity*, *48*, 139–146. https://doi.org/10.1016/j.bbi.2015.03.009

Welberg, L., Thrivikraman, K. V., & Plotsky, P. M. (2006). Combined pre- and postnatal environmental enrichment programs the HPA axis differentially in male and female rats. *Psychoneuroendocrinology*, *31*(5), 553–564. https://doi.org/10.1016/j.psyneuen.2005.11.011

Wendland, B. E., Atkinson, L., Steiner, M., Fleming, A. S., Pencharz, P., Moss, E., Gaudreau, H., Silveira, P. P., Arenovich, T., Matthews, S. G., Meaney, M. J., Levitan, R. D., & Team, M. S. (2014). Low maternal sensitivity at 6 months of age predicts higher BMI in 48 month old girls but not boys. *Appetite*, 97–102. https://doi.org/10.1016/j.appet.2014.07.012

Whitacre, C. C., Reingold, S. C., & O'Looney, P. A. (1999). A gender gap in autoimmunity. *Science (New York, N.Y.)*, *283*(5406), 1277–1278. https://doi.org/10.1126/science.283.5406.1277

Wilkins, C., & Gale, M. (2010). Recognition of viruses by cytoplasmic sensors. *Current Opinion in Immunology*, *22*(1), 41–47. https://doi.org/10.1016/j.coi.2009.12.003

Williamson, L. L., Chao, A., & Bilbo, S. D. (2012). Environmental enrichment alters glial antigen expression and neuroimmune function in the adult rat hippocampus. *Brain, Behavior, & Immunity*, *26*(3), 500–510. https://doi.org/10.1016/j.bbi.2012.01.003

Workman, J. L., Fonken, L. K., Gusfa, J., Kassouf, K. M., & Nelson, R. J. (2011). Post-weaning environmental enrichment alters affective responses and interacts with behavioral testing to alter nNOS immunoreactivity. *Pharmacology Biochemistry and Behavior*, *100*(1), 25–32. https://doi.org/10.1016/j.pbb.2011.07.008

Wright, R. W. (1985). Causation in Tort Law. *California Law Review*, *73*, 1735.

Wrona, D., Sukiennik, L., Jurkowski, M. K., Jurkowlaniec, E., Glac, W., & Tokarski, J. (2005). Effects of amphetamine on NK-related cytotoxicity in rats differing in locomotor reactivity and social position. *Brain, Behavior, & Immunity*, *19*(1), 69–77.

Wu, R., Yuan, A., Yuan, Q., Guo, R., Tai, F., Song, Z., & Yu, C. (2011). Comparison of sociability, parental care and central estrogen receptor alpha expression between two populations of mandarin voles (Microtus mandarinus). *Journal of Comparative Physiology A-Sensory Neural*, *197*(3), 267–277. https://doi.org/10.1007/s00359-010-0609-2

Wusteman, M., Wight, D. G., & Elia, M. (1990). Protein metabolism after injury with turpentine: A rat model for clinical trauma. *The American Journal of Physiology*, *259*(6 Pt 1), E763-769. https://doi.org/10.1152/ajpendo.1990.259.6.E763

Xia, Y., Qi, F., Zou, J., Yang, J., & Yao, Z. (2014). Influenza vaccination during early pregnancy contributes to neurogenesis and behavioral function in offspring. *Brain, Behavior, & Immunity*, *1*, 212–221. https://doi.org/10.1016/j.bbi.2014.06.202

Xu, H., Gelyana, E., Rajsombath, M., Yang, T., Li, S., & Selkoe, D. (2016). Environmental Enrichment Potently Prevents Microglia-Mediated Neuroinflammation by Human Amyloid beta-Protein Oligomers. *Journal of Neuroscience*, *36*(35), 9041–9056. https://doi.org/10.1523/JNEUROSCI.1023-16.2016

Yaghmaie, F., Saeed, O., Garan, S. A., Freitag, W., Timiras, P. S., & Sternberg, H. (2005). Caloric restriction reduces cell loss and maintains estrogen receptor-alpha immunoreactivity in the pre-optic hypothalamus of female B6D2F1 mice. *Neuro Endocrinology Letters*, *26*(3), 197–203.

Yamawaki, H., Kameshima, S., Usui, T., Okada, M., & Hara, Y. (2012). A novel adipocytokine, chemerin exerts anti-inflammatory roles in human vascular endothelial cells. *Biochemical and Biophysical Research Communications*, *423*(1), 152–157. https://doi.org/10.1016/j.bbrc.2012.05.103

Yarilina, A., Park-Min, K.-H., Antoniv, T., Hu, X., & Ivashkiv, L. B. (2008). TNF activates an IRF1-dependent autocrine loop leading to sustained expression of chemokines and STAT1-dependent type I interferon-response genes. *Nature Immunology*, *9*(4), 378–387. https://doi.org/10.1038/ni1576

Young, W. S., Alheid, G. F., & Heimer, L. (1984). The ventral pallidal projection to the mediodorsal thalamus: A study with fluorescent retrograde tracers and immunohistofluorescence. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *4*(6), 1626–1638.

Zaman, K., Roy, E., Arifeen, S. E., Rahman, M., Raqib, R., Wilson, E., Omer, S. B., Shahid, N. S., Breiman, R. F., Breiman, R. E., & Steinhoff, M. C. (2008). Effectiveness of maternal influenza immunization in mothers and infants. *The New England Journal of Medicine*, *359*(15), 1555–1564. https://doi.org/10.1056/NEJMoa0708630

Zaretsky, M. V., Alexander, J. M., Byrd, W., & Bawdon, R. E. (2004). Transfer of inflammatory cytokines across the placenta. *Obstetrics and Gynecology*, *103*(3), 546–550. https://doi.org/10.1097/01.AOG.0000114980.40445.83

Zerbo, O., Iosif, A.-M., Walker, C., Ozonoff, S., Hansen, R. L., & Hertz-Picciotto, I. (2013). Is Maternal Influenza or Fever During Pregnancy Associated with Autism or Developmental Delays? Results from the CHARGE (CHildhood Autism Risks from Genetics and Environment) Study. *Journal of Autism and Developmental Disorders*, *43*(1), 25–33. https://doi.org/10.1007/s10803-012-1540-x

Zhou, I. Y., Chan, R. W., Ho, L. C., & Wu, E. X. (2013). Longitudinal metabolic changes in the hippocampus and thalamus of the maternal brain revealed by proton magnetic resonance spectroscopy. *Neuroscience Letters*, *553*, 170–175. https://doi.org/10.1016/j.neulet.2013.08.041

Zhu, B. T., Han, G.-Z., Shim, J.-Y., Wen, Y., & Jiang, X.-R. (2006). Quantitative structure-activity relationship of various endogenous estrogen metabolites for human estrogen receptor alpha and beta subtypes: Insights into the structural determinants favoring a differential subtype binding. *Endocrinology*, *147*(9), 4132–4150. https://doi.org/10.1210/en.2006-0113