Maternal choline supplementation modulates cognition and induces anti-inflammatory signaling in the prefrontal cortex of adolescent rats exposed to maternal immune activation

by

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Abstract

Maternal infection has long been described as a risk factor for neurodevelopmental disorders, especially autism spectrum disorders (ASD) and schizophrenia. Although many pathogens do not cross the placenta and infect the developing fetus directly, the maternal immune response to them is sufficient to alter fetal neurodevelopment, a phenomenon termed maternal immune activation (MIA). Low maternal choline is also a risk factor for neurodevelopmental disorders, and most pregnant people do not receive enough of it. In addition to its role in neurodevelopment, choline is capable of inducing anti-inflammatory signaling through a nicotinic pathway. Therefore, it was hypothesized that maternal choline supplementation would blunt the neurodevelopmental impact of MIA in offspring through long-term instigation of cholinergic anti-inflammatory signaling.

To model MIA in rats, the viral mimetic polyinosinic:polycytidylic acid (poly(I:C)) was used to elicit a maternal antiviral innate immune response in dams both with and without choline supplementation. Offspring were reared to both early and late adolescent stages (postnatal days 28 and 50, respectively), where cognition and anxiety-related behaviors were examined. After behavioral testing, animals were euthanized, and their prefrontal cortices (PFCs) were collected for analysis. MIA offspring demonstrated sex-specific patterns of altered cognition and repetitive behaviors, which were modulated by maternal choline supplementation. Choline supplementation also bolstered anti-inflammatory signaling in the PFCs of MIA animals at both early and late adolescent stages. These findings suggest that maternal choline supplementation may be sufficient to blunt some of the behavioral and neurobiological impacts of inflammatory exposures *in utero*, indicating that it may be a cheap, safe, and effective intervention for neurodevelopmental disorders.

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Dedication

I dedicate this research and its results to the animals whose lives were sacrificed in the collection of these data. Conducting research with animal subjects is a privilege and a solemn responsibility, and it is not lost on me the sacrifices that the animals used in these experiments were forced to make. Let us not forget these sacrifices in the pursuit of ethical and principled science that minimizes the suffering of those subjects entrusted into our care.

Chapter 1 - Introduction

Epidemiological Basis

The psychiatric sequelae of influenza infection have been known for more than a century, with reports of the "psychoses of influenza" populating British medical journals as early as the 1890s Russian Influenza pandemic (Honigsbaum, 2013). In fact, famed Kansas psychiatrist Karl Menninger directed substantial attention toward these psychoses, reporting 100 cases of "mental disturbances" in 1919, most notably including 25 cases of dementia praecox, now known as schizophrenia (Kępińska et al., 2020; Menninger, 1919). Years later, several ecologic and observational studies identified a relationship between birth immediately after influenza epidemics in the 1950s and schizophrenia risk, renewing interest in Meninger's hypothesis (W. Adams et al., 1993; Barr et al., 1990; Kunugi et al., 1995; McGrath et al., 1994; Mednick et al., 1988; Menninger, 1926; O'Callaghan et al., 1991). Further supporting the hypothesis of a possible infectious origin of schizophrenia were ecologic studies that identified a relationship between birth season and schizophrenia, with late winter and early spring births associated with excess schizophrenia risk (Davies et al., 2003; Kępińska et al., 2020; Torrey et al., 1997; Zerbo et al., 2011).

Despite compelling epidemiological evidence of a link between maternal influenza infection and schizophrenia, mechanisms of this association remained elusive for many years. Influenza does not commonly transfer from mother to fetus, unlike the canonical "TORCH" pathogens (*Toxoplasma gondii*, rubella virus, cytomegalovirus, herpes simplex virus), which are also associated with the development of schizophrenia (Brown & Derkits, 2010; Devaraju et al., 2023; Massrali et al., 2022). This suggests that these various pathogens may alter neurodevelopment through similar mechanisms, such as instigation of the maternal innate

immune response through microbe-associated molecular patterns (MAMPs) and damage-associated molecular patterns (DAMPs). In line with this hypothesis, animal models of maternal immune activation via Toll-like receptor pathways induce both behavioral and biological changes consistent with neurodevelopmental disorders (Brown & Conway, 2019; Estes & McAllister, 2016; Meyer, 2014). Therefore, the teratogenic effects of prenatal infection are likely not explained solely by direct pathogen interactions with the developing brain. Rather, they may be a consequence of the maternal immune response "spilling over" into the fetal compartment, a phenomenon known as maternal immune activation (MIA).

Although maternal infection during pregnancy has been most reliably described as a risk factor for schizophrenia, it has also been identified as a risk factor for other neurodevelopmental conditions like autism spectrum disorders (ASD). Specifically, an exploratory study in 2010 found an association between ASD and severe infections requiring hospitalization during the first and second trimesters (Atladóttir et al., 2010). Since the publication of this study, several others have replicated the relationship between maternal infection or fever and ASD using both prospective and retrospective study designs with birth cohorts in Europe and the United States (Atladóttir et al., 2012; Hornig et al., 2018; Lee et al., 2015; Zerbo et al., 2013). What is less clear, however, is the significance of the gestational timing of infection and ASD risk. Although evidence links severe maternal febrile episodes during any stage of pregnancy with ASD diagnosis, researchers in Denmark only found statistical associations between viral infections and ASD in the first trimester, while an association for bacterial infections was found only in the second trimester (Atladóttir et al., 2010). However, other studies have found associations between maternal infection or prolonged febrile episodes and neurodevelopmental disorders in

all trimesters, despite greater vulnerabilities in the first and second trimesters (Hornig et al., 2018; Zerbo et al., 2013).

Beyond schizophrenia and ASD, a link between MIA and attention deficit hyperactivity disorder (ADHD) has also been uncovered regardless of the timing of infection (Pineda et al., 2007; Silva et al., 2014). However, other epidemiological work has identified temporality in this association, with maternal fever between gestational weeks 9-12 and genitourinary infections between weeks 33-36 linked to ADHD development (Werenberg Dreier et al., 2016). In addition, maternal levels of C-reactive protein, a marker of systemic inflammation, have been associated with ADHD diagnosis by age 10, and maternal autoimmune disease has also been associated with ADHD diagnosis in a large Australian birth cohort (Nielsen et al., 2021). MIA has also been tenuously linked to bipolar disorder (BD), anxiety disorders, and depressive disorders (Fig. 1.1). A 2013 case-control study identified that maternal influenza infection may be associated with the development of BD, regardless of trimester (Parboosing et al., 2013). However, a second case-control study by the same research group in 2014 replicated the association only for BD with accompanying psychotic features, suggesting that the link may be more related to psychosis than BD per se (Canetta et al., 2014). In 2019, Al-Haddad et al. found that maternal infection was not related to BD diagnosis, but that it was linked to an increased risk of depression diagnosis (Al-Haddad et al., 2019). The evidence linking MIA to depressive disorders, as well as anxiety disorders, is generally weak and poorly replicated. It is also unclear whether these complications are themselves linked to MIA or simply more likely to co-occur in people with ASD, schizophrenia, and ADHD (Machón et al., 1997; Mednick et al., 1988; Murphy et al., 2017).

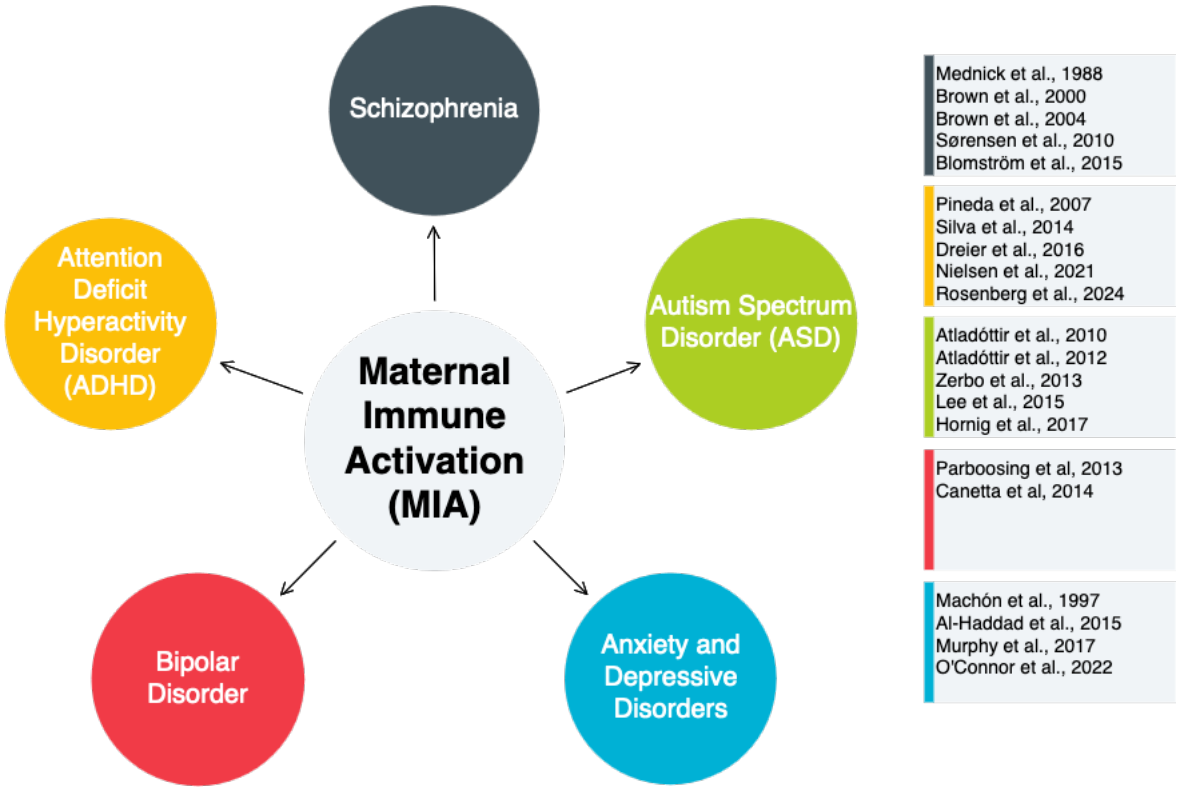

Figure 1.1: Maternal Immune Activation in the Epidemiology of Psychiatric Conditions

Maternal immune activation is strongly implicated in the etiology of ASD and schizophrenia, and it is moderately implicated in the development of ADHD. Its relationship with bipolar disorder, anxiety disorders, and depressive disorders is tenuous, especially since these often co-occur in ASD, schizophrenia, and ADHD.

Consistent with epidemiological findings, animal studies have uncovered that the immune system is central in regulating brain development (Comer et al., 2020; Erta et al., 2012; Goines & Ashwood, 2013). Resident immune cells in the brain are responsible for proper refinement of synaptic wiring, and several immune signaling molecules direct neurons to grow and differentiate (Comer et al., 2020; Erta et al., 2012). Some of these molecules—whose levels are significantly elevated during infections—can cross the placental barrier from mother to fetus, such as the front-line cytokine IL-6 (Zaretsky et al., 2004). Animal studies have discovered that, indeed, the rush of cytokines into the fetal compartment from maternal infections can alter brain development long-term at the molecular, cellular, and circuit levels (Garay et al., 2013; Wei et

al., 2012). Most importantly, though, these changes precipitate behavioral changes that closely resemble those observed in ASD and schizophrenia (Careaga et al., 2017).

In humans, key immune modulators are often dysregulated across the lifespan in individuals with neurodevelopmental disorders, supporting the hypothesis that they are linked. Several studies have characterized dysregulation of inflammatory cytokines, namely IL-1β, IL-6, TNF-α, and IFN-γ, in the brain, gut, and periphery of patients with ASD (Goines & Ashwood, 2013; Smith et al., 2010; Xie et al., 2017). These cytokine imbalances are posited to impact neurodevelopment and mediate some behavioral aspects of ASD (Goines & Ashwood, 2013). In addition to ASD, several studies have associated cytokine imbalances with schizophrenia, and a 2018 systematic review correlated dysregulation of specific cytokines with hallmark characteristics of schizophrenia (Rodrigues-Amorim et al., 2018). Specifically, IL-2, IL-6, IL-8, and TNF-α were correlated with impairments in cognitive performance, pointing to these pro-inflammatory cytokines as key modulators of neurodevelopmental processes that lead to cognitive outcomes later in life.

Mechanisms of Maternal Immune Activation *in Utero*

Crosstalk between the maternal and fetal immune systems is currently an active area of research, and little is known about the many complicated processes by which this crosstalk occurs. It has been shown that some pro-inflammatory cytokines can cross the placenta and impact fetal development (Zaretsky et al., 2004). However, the mechanisms by which these cytokines may do so are unclear, and many candidate mechanisms have been put forward. What most of these hypotheses share is that they suggest a key role of IL-6, a generally pro-inflammatory cytokine that is critical for the generation of innate immune responses instigated

by pattern recognition receptors (Ratnayake et al., 2013). Experiments with the three key front-line pro-inflammatory cytokines (IL-1, IL-6, and TNF-α) showed that IL-6 is capable of bidirectional transfer across the placenta, while IL-1α and TNF-α showed minimal transfer, indicating that the closely related IL-1β likely minimally transfers as well (Zaretsky et al., 2004). In addition, MIA experiments using a viral mimetic with either an anti-IL-6 antibody or an IL-6 knockout mouse strain did not find the same behavioral abnormalities and transcriptional changes seen in offspring without IL-6 blocked (Smith et al., 2007). This same study found no behavioral effects of antibody blockades of IL-1β or IFN-γ.

In addition to its direct role in pro-inflammatory signaling, IL-6 also has downstream effects that result in the release of IL-17A from differentiated T-helper 17 (Th17) cells. Th17 cells and IL-17A have been reported to cross the placenta, but this has not been widely demonstrated (Wienecke et al., 2012). What is known, however, is that maternal IL-17A induces a trans-placental signaling cascade that results in its production and release in the periphery of the fetus. In rodent models, it is suggested that the fetus becomes capable of producing Th17 cells endogenously as T-cell hematopoiesis shifts to the thymus at around gestational day (GD) 13 (Crawford et al., 2010; Wong & Hoeffer, 2018). Supporting the downstream role of IL-17A in pathological brain development are studies that show, as with IL-6, that an antibody blockade against it can rescue behavioral phenotypes in MIA mice (Choi et al., 2016).

Supporting its role as the key pro-inflammatory cytokine in MIA, IL-6 acts directly on the developing brain. In addition to its role in inflammatory responses, IL-6 also plays key roles in the differentiation of neurons and glial cells in both the central and peripheral nervous systems (Islam et al., 2009). Further, IL-6 regulates many important neurodevelopmental processes like axonal pathfinding, synaptic connection, and neural cell proliferation (Wong & Hoeffer, 2018;

Islam et al., 2009). IL-6 is also a neurotrophic factor that acts synergistically with neural growth factor (NGF) co-signaling to regulate cell proliferation and development (Kummer et al., 2021; Reeh et al., 2019). Although IL-6 is involved in many critical neurodevelopmental processes, its levels in the brain are tightly regulated through a network that is still not fully understood. Therefore, one main hypothesis is that MIA causes drastic changes in the levels of IL-6 in the fetal brain from baseline, leading to a disruption of the intricately timed processes that it regulates (Fig. 1.2).

Downstream of IL-6, IL-17A also acts directly on the fetal brain through its receptor, IL-17AR, in response to MIA (Wong & Hoeffer, 2018). The receptor is expressed at low levels in the cortex, but its expression has not yet been isolated to specific cell types (Choi et al., 2016). What is known, though, is that its expression significantly increases in response to MIA (Choi et al., 2016; Wong & Hoeffer, 2018). Although it is not clear which cell populations are most affected by IL-17A signaling, there are two main mechanisms by which it may affect cortical development in MIA. First, IL-17AR expression increases from baseline in cultured embryonic neurons following ischemic stress, and it has been found in culture to inhibit the differentiation of neural stem cells (Z. Li et al, 2013; Wang et al., 2009). Therefore, the upregulation of IL-17A signaling in response to MIA may tap into similar mechanisms to cause cortical dysplasia and behavioral changes associated with ASD (Wong & Hoeffer, 2018). The second key mechanism through which IL-17A may affect brain development is through the activation of microglia. In addition to their role in immunity, microglia regulate neural stem cell development and vascularization in the brain (Antony et al., 2011). It is possible that IL-17A may also contribute to aberrant brain development by dysregulating these microglia, providing another mechanism

by which MIA-induced IL-17A may lead to structural brain changes later in life (Wong & Hoeffer, 2018) (Fig. 1.2).

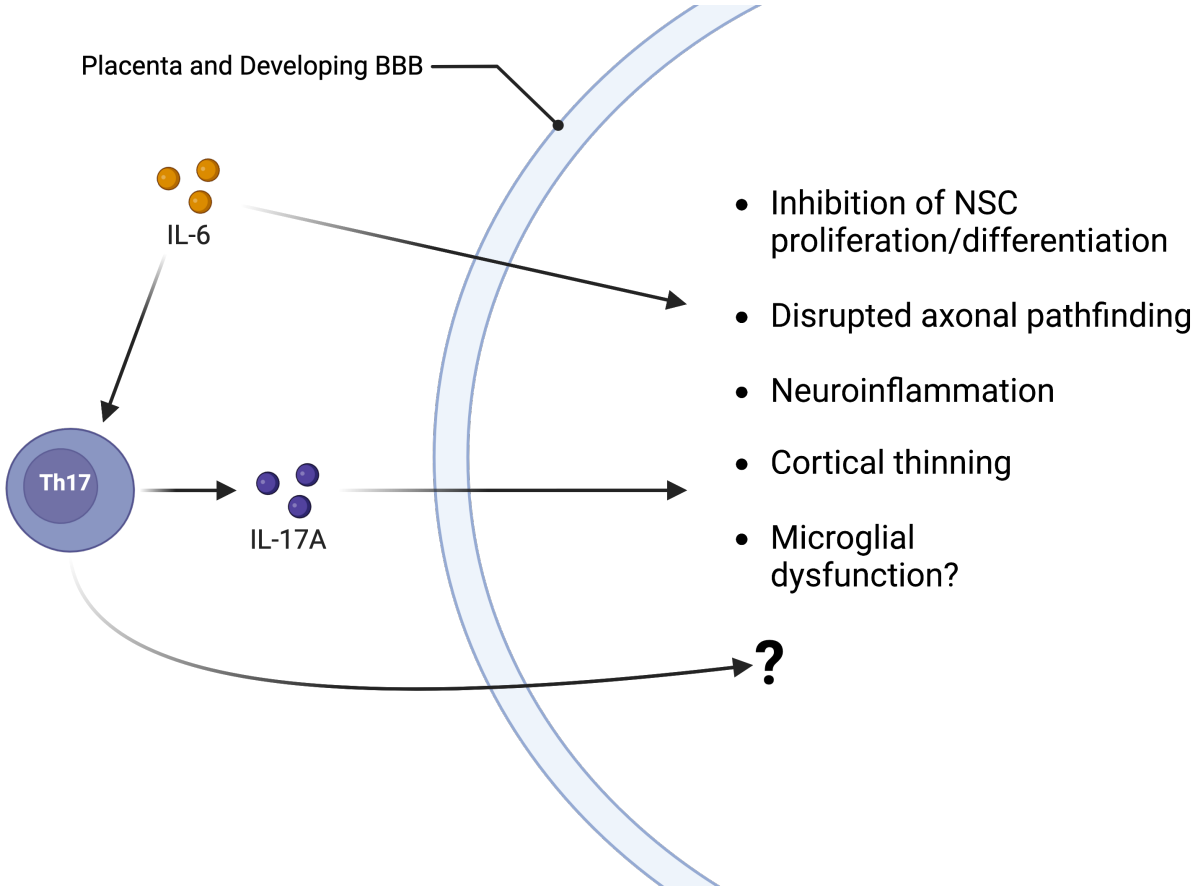

Figure 1.2: Synergistic Actions of IL-6 and IL-17A in MIA

IL-6 polarizes CD4+ T-cell development to the Th17 phenotype and acts in conjunction with IL-17A, the key Th17 cytokine, to induce inflammatory signaling across the placental barrier. This pro-inflammatory signaling disrupts corticogenesis and is implicated in several pathologies consistent with neurodevelopmental disorders. The mechanisms of IL-17A signaling across the placenta are not well understood, but it has been suggested that Th17 cells themselves may cross, especially when the placental barrier is disrupted, and follow chemotactic signals to take up long-term residence in the developing nervous system (L. Osborne et al., 2019).

In sum, IL-6 and IL-17A can act both independently and synergistically to cause developmental abnormalities during fetal development. Given the dynamic nature of the fetal immune system and brain, as well as the placenta, the exact mechanisms by which these cytokines act may differ depending on the gestational timing of MIA. However, these two

cytokines are inextricably linked, and they are both directly involved in the immune response to MIA that takes place in the brain of the developing fetus. Therefore, they are considered the primary drivers of altered neurodevelopment in response to prenatal immune challenge.

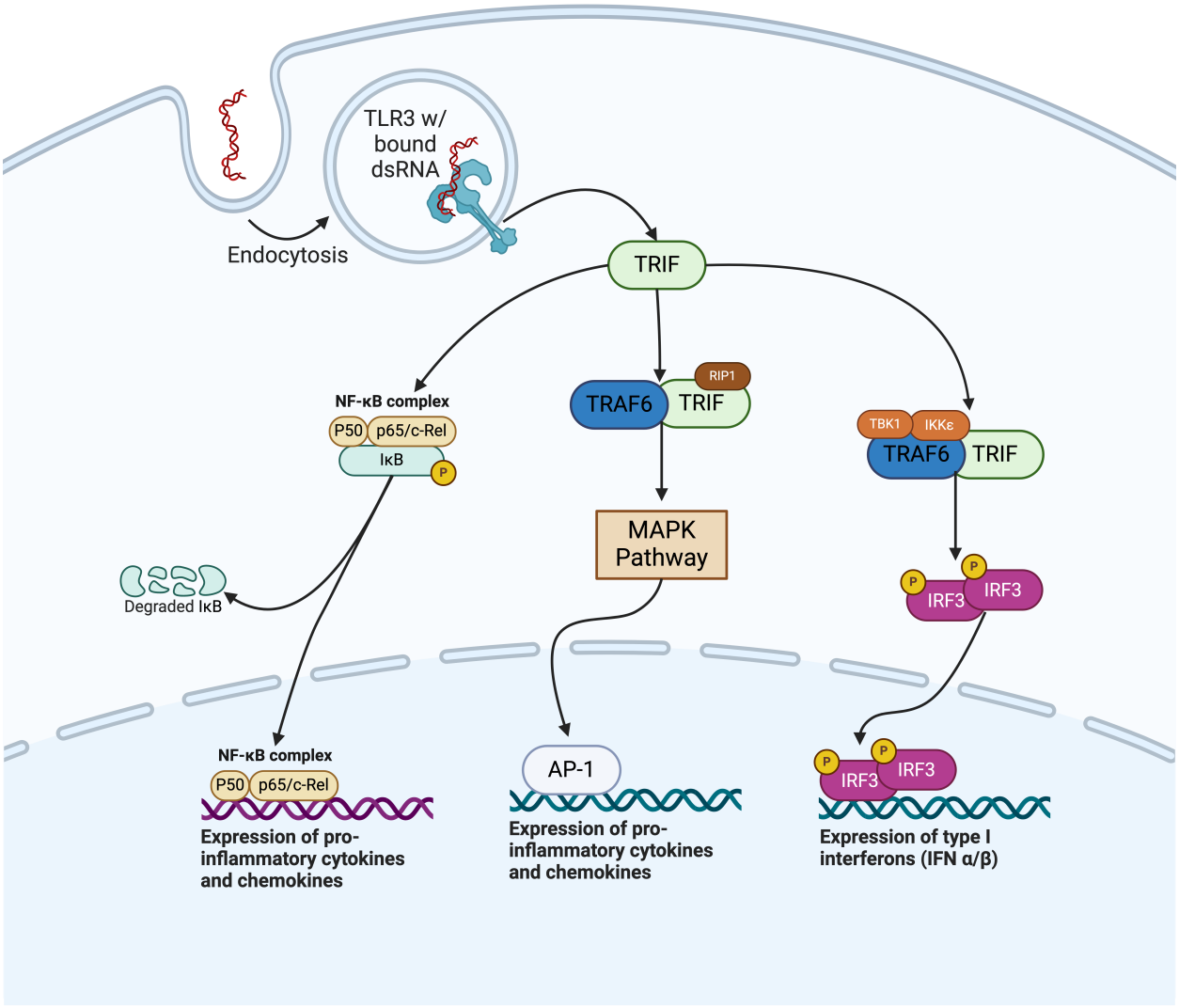

Figure 1.3: Mechanism of Poly(I:C)-Instigated Innate Immune Signaling

Poly(I:C), a dsRNA analog, binds TLR3 on the endosomal membrane. The poly(I:C)-bound TLR3 instigates TRIF signaling, which promotes the degradation of the negative NF-κB regulator IκB, causing the transcription factor NF-κB to translocate to the nucleus. TRIF also stimulates Tumor Necrosis Factor-Associated Factor 6 (TRAF), leading to the translocation of Interferon Regulatory Factor 3 (IRF3) to the nucleus and activation of the Mitogen-Activated Protein Kinase (MAPK) pathway, which causes the downstream translocation of Activator Protein 1 (AP-1) to the nucleus. Together, these transcription factors induce the expression of innate viral defense genes like proinflammatory cytokines, type I interferons, and chemokines.

To induce MIA in a similar manner to viral pathogens, the synthetic double-stranded RNA (dsRNA) analog polyinosinic-polycytidylic acid (Poly(I:C)) may be injected into the pregnant dam, eliciting a pro-inflammatory innate immune response. Poly(I:C) binds Toll-Like Receptor 3 (TLR3), a pattern recognition receptor that recognizes dsRNA, on the endosomal membrane (Reisinger et al., 2015). Once activated, TLR3 induces TIR-domain-containing adapter-inducing interferon-β (TRIF) signaling that leads to downstream activation of the transcription factors IRF3, NF-κB, and AP-1, which act on genes related to the innate immune response (Vercammen et al., 2008). IRF-3 activates genes encoding antiviral interferons, and NF-κB and AP-1 activate genes encoding pro-inflammatory cytokines like IL-6 (Reisinger et al., 2015) (Fig. 1.3).

Behavioral Alterations in MIA Models

Rodent MIA studies have reliably identified behavioral phenotypes consistent with schizophrenia. In particular, MIA consistently produces deficits in pre-pulse inhibition of the acoustic startle response, a classical conditioning paradigm to study sensorimotor processing, a deficit commonly observed in humans with schizophrenia. These findings have been reliably replicated in the poly(I:C) model by several research groups and across different poly(I:C) administration paradigms, indicating that the model captures similar behavioral abnormalities to humans with schizophrenia (Garay et al., 2013; Garcia-Partida et al., 2022; Li et al., 2021; Smith et al., 2007; Su et al., 2022; Vorhees et al., 2015). Similarly, disruptions in latent inhibition, a form of memory interference, have been found in poly(I:C)-exposed offspring (Smith et al., 2007; Zuckerman & Weiner, 2005). Further, these disruptions in latent inhibition can be ameliorated by clozapine, an antipsychotic drug used to treat schizophrenia (Zuckerman & Weiner, 2005).

Beyond pre-pulse inhibition and latent inhibition, other sensorimotor deficits have been observed in MIA models from the beginning of postnatal life, where MIA offspring have deficient reflexes compared to control animals (Arsenault et al., 2014; Haida et al., 2019). Animals exposed to MIA have displayed impairments in the rotarod test, which is commonly used to test motor coordination (Monteiro et al., 2022; Naviaux et al., 2013; X. Zhang et al., 2021; Z. Zhang & van Praag, 2015). The motor deficits observed in the MIA model with the rotarod test have been replicated across a wide range of rodent models of ASD and schizophrenia, replicating similar findings in humans (Fatemi et al., 2012; Walther & Strik, 2012). One limitation of the current literature, however, is that most animal studies examining this type of coordination have been conducted in adulthood, leaving open questions about these deficits in adolescence, especially given that the MIA model has primarily been used to model schizophrenia. Schizophrenia does not typically present until early adulthood; however, ASD typically presents much earlier, so further study of motor coordination in adolescence is warranted in the MIA model given its increasing use as a model of ASD.

In addition to the common behavioral deficits associated with schizophrenia, exposure to MIA may also lead to disrupted anxiety-like behaviors in offspring. However, like the epidemiological literature on anxiety disorders, there is considerable heterogeneity to these findings. Some studies have reported increased anxiety behaviors in the elevated plus maze (EPM) test, which is commonly considered indicative of anxiety-like behaviors in rodents, in late adolescence following prenatal exposure to poly(I:C) (Li et al., 2021; Su et al., 2022). Other work using the open field test (OFT) is more consistent, with MIA offspring generally displaying increased anxiety-like behaviors in adolescence and adulthood (Guerrin et al., 2022; Shi et al., 2003; Su et al., 2022; W. Wu et al., 2015). These increases in anxiety-like behaviors are

consistent with the human literature's general findings of increased anxiety in individuals with neurodevelopmental disorders (Hollocks et al., 2019; Koyuncu et al., 2022; Temmingh & Stein, 2015).

In addition to anxiety-like behaviors, MIA models have identified vast and varied cognitive deficits consistent with several neurodevelopmental disorders. First, several MIA studies have identified alterations in object recognition and memory using the novel object recognition test (NORT), with some studies reporting deficiencies in novel object recognition and others reporting enhancements (Gray et al., 2019; Guerrin et al., 2022; Ito et al., 2010; A. Osborne et al., 2017; Shi et al., 2003; Su et al., 2022). To account for this heterogeneity, it has been suggested that MIA may selectively enhance object recognition in NORT paradigms with no spatial memory component, as MIA animals often display impairments in spatial working memory in the Morris water maze, T-maze, and Y-maze tests (Ito et al., 2010; Li et al., 2021; Schaafsma et al., 2017). MIA animals also consistently display preferences for objects in three-chamber social tests where they are presented with the choice between interacting with another animal in one chamber or an object in another, suggesting possible ASD-like phenotypes (Butera et al., 2024; Naviaux et al., 2013; Smith et al., 2007).

Although object recognition is largely hippocampus-dependent, MIA animals also display deficits in cognitive tasks dependent on prefrontal regions. In particular, MIA animals display impaired reversal learning, which tests animals' ability to flexibly adjust reward seeking behavior when reward contingencies are reversed in a discrimination test. Impairments in this type of learning have been found in adulthood on many reversal learning task variants (Amodeo et al., 2019; Han et al., 2011; Lins et al., 2019). These findings are consistent with those from other animal models and human studies, where difficulties in flexibly adapting to changing

reward contingencies are observed in schizophrenia and ASD (Reddy et al., 2016; Yerys et al., 2009). These reversal learning deficits are also suggestive of perseverative and repetitive behaviors, which are particularly evident in ASD (D'Cruz et al., 2013). Indeed, MIA studies have also identified increases in perseverative and repetitive behaviors, providing further face validity to MIA as a model of ASD in addition to schizophrenia (Estes et al., 2020; Vigli et al., 2020).

Neuroimmune Dysfunction in MIA

Neurodevelopmental disorders and intellectual disabilities are often characterized by dysfunction in the prefrontal cortex (PFC). The PFC is critical for higher-order cognition, information synthesis, and goal-directed behavior (Birrell & Brown, 2000; Ragozzino et al., 1999). It also modulates many cognitive processes through its wide array of connections throughout the brain (Ragozzino, 2007). Common PFC changes in neurodevelopmental disorders include disrupted functional connectivity to other brain regions, excitatory/inhibitory imbalances, loss of extracellular matrix (perineuronal nets), and dysregulated astrocytic and microglial activation (Mittli, 2023; Paylor et al., 2016; Tendilla-Beltrán et al., 2021). Though diverse processes, they are all regulated to varying degrees by the immune system. Further, the immune functions disrupted in the MIA model parallel human findings, correlate with core behavioral changes in neurodevelopmental disorders, and represent a translationally valid model to study them (Bergdolt & Dunaevsky, 2019).

Altered excitatory/inhibitory balance in the PFC is a feature common to both ASD and schizophrenia that has been widely replicated in animal models (Gao & Penzes, 2015; Wei et al., 2012). In MIA, gestational poly(I:C) treatment alters the excitatory/inhibitory balance of the PFC

at the synaptic level (L. Zhang et al., 2023). Further, these changes can be prevented by administering ibudilast, an anti-inflammatory drug that modulates microglial and astrocytic function, to the dam during lactation (Coiro et al., 2015). Supporting these findings are studies that observed reduced inhibitory interneuron density in the PFC following MIA (Canetta et al., 2016; Wischhof et al., 2015). Reduced inhibitory interneuron counts and/or disrupted perineuronal nets surrounding them are consistently implicated in excitatory/inhibitory imbalances in both animal models and is considered a shared characteristic of both ASD and schizophrenia (Kaar et al., 2019; Paylor et al., 2016). Studies beyond the MIA model have identified immune processes that mediate excitatory/inhibitory balance, suggesting that chronic disruptions in immune signaling may underpin the imbalances seen in ASD and schizophrenia (Wei et al., 2011; L. Yang et al., 2021). Furthermore, acute administration of LPS or the pro-inflammatory cytokine IL-1β causes increased susceptibility to seizures, which occur as a result of hyperexcitability due to excitatory/inhibitory imbalances (Rana & Musto, 2018; Semple et al., 2017). Interestingly, patients with ASD and schizophrenia are far more susceptible to seizures than the general population, further implicating excitatory/inhibitory imbalances in their development (Besag, 2017; Hyde & Weinberger, 1997).

A putative causative agent of the synaptic dysregulations observed in MIA is the microglia. Microglia support growing neurons with trophic factors required for growth and myelination and refine synaptic wiring through complement-mediated phagocytosis of weak synapses (Loewen et al., 2023). Thus, microglia are critical for orchestrating and coordinating the intricate development of the PFC *in utero* (Antony et al., 2011). In addition, as the brain's resident immune cells, microglia respond strongly to proinflammatory signals such as those induced by MIA. Indeed, microglia are highly reactive to both IL-6 and IL-17A, the key MIA

cytokines, releasing pro-inflammatory cytokines and interferons when stimulated by them (Iitani et al., 2023; West et al., 2019). Therefore, infiltration of these cytokines into the fetal compartment during vulnerable periods of development may represent the beginning of a mechanism by which they induce long-term PFC changes. Consistent with this idea, studies have found altered microglial phenotypes in the PFC of MIA offspring (Cieślik et al., 2020; Loayza et al., 2023). It has also been suggested that the microglia are at least partially responsible for the cortical gray matter losses observed in schizophrenia, where increased microglial populations and activation have been correlated with this loss (Hirayasu et al., 2001; Rački et al., 2016; Uranova et al., 2021). These findings have been replicated in MIA models. Rodent models have demonstrated global decreases in PFC volume, and non-human primate models have found persistent grey matter reduction from six months of age through adulthood (Garcia-Partida et al., 2022; Hanson et al., 2022). In sum, the microglia represent a strong candidate mechanism by which MIA exerts its teratogenic effects and represent an explanation as to why these deficits may be latent until late in development.

In addition to the microglia, several inflammatory cytokines exert impacts on developing neurons in the PFC. First, beyond its critical role in MIA, IL-6 is highly pleiotropic. It is expressed in the central nervous system (CNS) and acts as a neurotrophic factor. As such, IL-6 is considered a neuropoietin and is the founding member of the neuropoietic cytokine family. It directs neuron migration, enhances cell survival, and acts in concert with other neurotrophic factors to promote gliogenesis and neurogenesis (Erta et al., 2012; Goines & Ashwood, 2013). Distinct behavioral alterations occur when IL-6 is underexpressed in an IL-6 knockout model, and ASD-like brain volume alterations occur when it is overexpressed, suggesting that IL-6 is involved in regulating both behavioral and neurobiological aspects of neurodevelopmental

disorders (Erta et al., 2015; Wei et al., 2012). While critical for regulating neurodevelopment and the response to injury, overexpression of IL-6 during vulnerable windows, as in MIA, may skew neuronal differentiation in such a way that facilitates excitatory/inhibitory imbalances (Erta et al., 2012).

Beyond its direct action on neurons, IL-6 also polarizes CD4+ T-cell development to the Th17 phenotype (Wong & Hoeffer, 2018). IL-17A, a main Th17 cytokine, is linked to increases in neuroinflammation and disrupted blood-brain barrier integrity in multiple sclerosis, and it has recently been implicated in neurodevelopmental disorders like ASD and schizophrenia (Milovanovic et al., 2020; Reale et al., 2021). Evidence also suggests that IL-6 and IL-17A may together shift chemokine production by astrocytes to facilitate infiltration of T-cells into the CNS, potentially causing a positive feedback loop wherein IL-6 facilitates Th17 differentiation and increases IL-17A levels, which precipitates CNS injury, further stimulating increases in IL-6 (Erta et al., 2012; Meares et al., 2012).

In addition to IL-6 and IL-17A, TNF-α also plays a large role in CNS health and disease. At normal physiological levels, TNF-α promotes oligodendrogenesis and myelination, and it also regulates excitatory/inhibitory synaptic balance by downregulating the expression of surface GABA receptors (Arnett et al., 2001; Gonzalez Caldito, 2023). Thus, its perturbation by chronic inflammation, such as that which is caused by MIA, likely contributes to the excitatory/inhibitory imbalances seen in ASD and schizophrenia. Pathological increases in TNF-α, such as those observed in ASD and schizophrenia, also facilitate breakdown of the blood-brain barrier (Goldsmith et al., 2016; Gonzalez Caldito, 2023; Xie et al., 2017). Breakdown of the blood-brain barrier promotes neuroinflammation and is a shared pathology of neurodegenerative conditions (Gonzalez Caldito, 2023). MIA models have found increases in

both CNS and peripheral TNF-α, suggesting that MIA may facilitate blood-brain barrier breakdown as is seen in ASD and schizophrenia (Cieślik et al., 2020; Garay et al., 2013; MacDowell et al., 2017).

Several other cytokines, both pro- and anti-inflammatory, have been associated with ASD and schizophrenia, including IL-1β, IFN-γ, IL-4, and IL-10, in pathological or protective roles (Comer et al., 2020; Goines & Ashwood, 2013). For example, IL-1β exhibits extraordinarily complex regional effects in the developing brain and has been linked to varying conditions like depression and seizures, but its exact roles in neurodevelopmental pathology and protection have not been well-described (Park et al., 2015; Semple et al., 2017). IFN-γ and IL-4 counteract to balance type I and II immune responses, but neither crosses the blood-brain barrier under normal physiological conditions, so their direct effects on the CNS are largely inferred from their effects on macrophages, although it is known that they affect neural growth and synapse formation *in vitro* (Onore et al., 2014; Park et al., 2015). However, whether increases or decreases of these two represent pathological features or reflexive immune responses to injury is unclear. Levels of these cytokines have all been found altered, with varying degrees of replication, either peripherally, centrally, or both, in ASD and schizophrenia, so further investigation into their roles in neurodevelopment is warranted.

In sum, the nervous and immune systems intricately coordinate to regulate brain formation and differentiation of neurons and glia to fulfill distinct niches. Neuroimmune processes are especially important for the development of higher-order brain regions like the PFC, and infiltration of pro-inflammatory cytokines from MIA during corticogenesis represents a major risk factor for altered PFC development. These alterations closely parallel human findings in a variety of psychiatric and neuroimmune conditions, making the MIA model an indispensable

tool to understand the psychoneuroimmune basis of neurodevelopmental disorders like ASD and schizophrenia.

Choline and Neuroimmune Function

Choline is a micronutrient similar to the B vitamins that has been recognized as an essential nutrient by the Institute of Medicine of the National Academy of Sciences since 1998 (Institute of Medicine, 1998). Choline is a major component of the cell membrane, a key methyl group donor, and has anti-inflammatory properties (Fig. 1.4). Current guidelines for choline intake in humans are 425 mg/day for females over 19 years of age, 450 mg/day for pregnant females, and 550 mg/day for males over 19 years of age and lactating females. However, it is likely that the overwhelming majority of pregnant and lactating females do not achieve these levels, and mounting evidence suggests that the recommended intakes themselves are insufficient (Derbyshire & Obeid, 2020; Yan et al., 2012). Choline specifically plays a key role in neural tube formation, hippocampal development, and epigenetic regulation, making its inclusion into prenatal vitamins also necessary (Zeisel & Da Costa, 2009). Thus, since choline intake in pregnant people is far below adequate levels, characterizing it's potential ability to bolster key neurodevelopmental processes in the face of immune challenge is critical (J. B. Adams et al., 2022; Korsmo et al., 2019).

Although choline can be synthesized endogenously, the developing fetus' demand for choline exceeds this capacity (Derbyshire & Obeid, 2020; Yan et al., 2012). Therefore, exogenous choline is essential for proper nervous system development, with mounting evidence suggesting that it can protect the developing fetus from alcohol exposure and infections (Derbyshire & Obeid, 2020). Further research into choline's neuroprotective effects is needed to

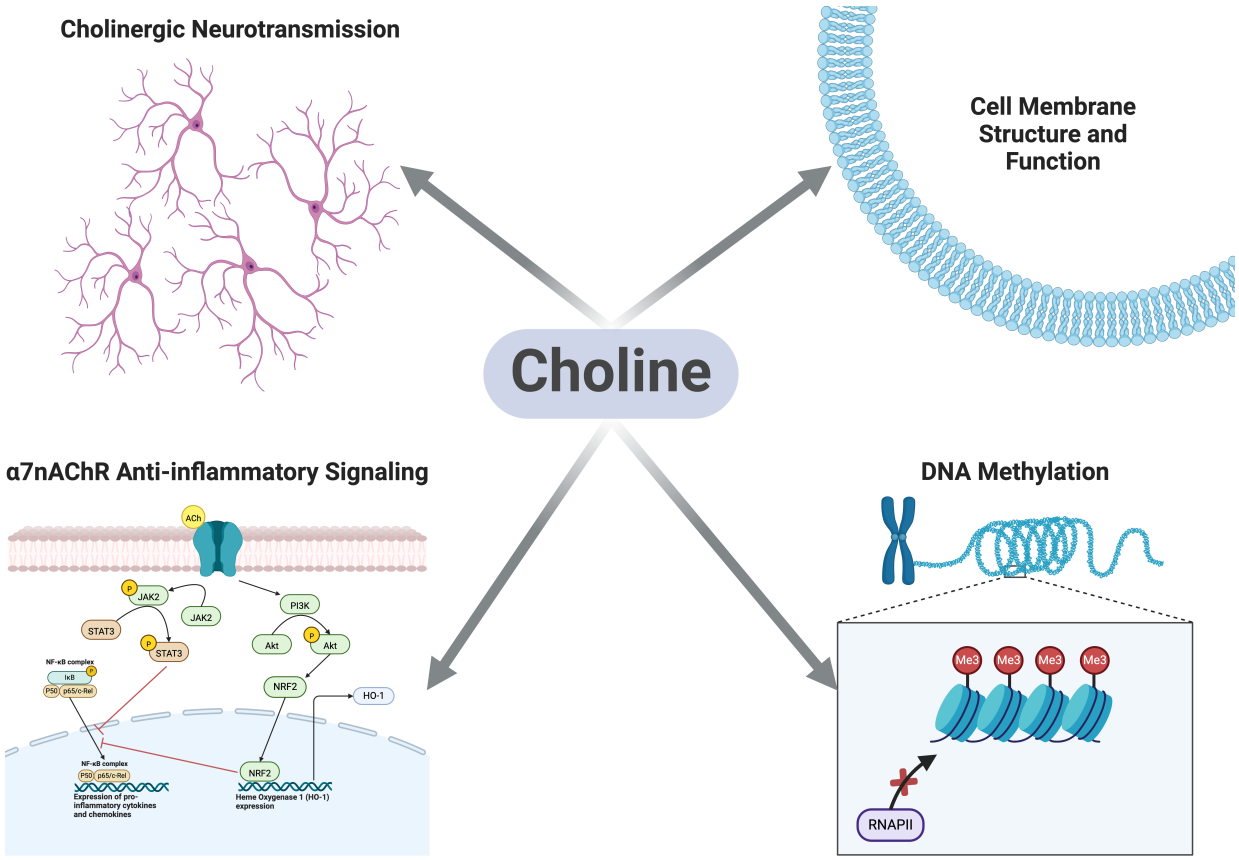

Figure 1.4: Effects of Choline on the Mammalian Nervous System

Choline's wide-ranging effects on the mammalian nervous system include modulation of cholinergic neurotransmission, structural contributions to the cell membrane, chromatin-mediated gene regulation via DNA methylation, and cholinergic anti-inflammatory signaling in glial cells.

expand on current epidemiological and experimental studies, which have found that choline supplementation during pregnancy and lactation is associated with improved cognitive, language, and motor development at 18 months of age, as well as improved attention and sociability (Korsmo et al., 2019; R. Ross et al., 2016; B. Wu et al., 2012). Furthermore, higher maternal levels of choline have been correlated with improved self-regulation and attention in children exposed to SARS-CoV-2, the virus that causes COVID-19, *in utero*, indicating that choline or its metabolites likely have some effect that can blunt the neurodevelopmental impacts of MIA fromo respiratory viruses (Freedman et al., 2020).

Perhaps the most unique role of choline is that it is highly involved in neuroimmune signaling through the alpha-7 nicotinic acetylcholine receptor (α7nAChR). The α7nAChR is comprised of five identical subunits and is highly expressed in the microglia of the CNS (Egea et al., 2015). Further, the α7nAChR is agonized by acetylcholine, a key neurotransmitter in the CNS, and free choline with high specificity (Alkondon et al., 1997). When bound to acetylcholine or free choline, the α7nAChR activates both JAK2/STAT3 (Janus Kinase 2/Signal Transducer and Activator of Transcription 3) and PI3K/Akt (Phosphoinositide-3-Kinase/Akt or Protein Kinase B) signaling, both of which play a role in quenching pro-inflammatory immune responses by activating antioxidant defenses and inhibiting the nuclear translocation of the pro-inflammatory transcription factor NF-κB (Carnac, 2022; Egea et al., 2015; Youssef et al., 2020) (Fig. 1.5). These responses inhibit the release of pro-inflammatory cytokines and promote a shift from microglia in the pro-inflammatory M1 state to the anti-inflammatory M2 state (Egea et al., 2015). Experimental studies using cultured microglia have identified this shift through the administration of nicotine, a potent and selective α7nAChR agonist, to microglia. When treated with nicotine, the microglia shift cytokine production away from the pro-inflammatory IL-1β and TNF-α and to the anti-inflammatory IL-4, IL-10, and TGF-β (Rock et al., 2008; Suzuki et al., 2006). These effects have also been replicated *in vivo* (Egea et al., 2015; Loram et al., 2010). The switch to the M2 phase is then triggered in other microglia, and possibly even astrocytes, by these cytokines, thereby quenching the pro-inflammatory state.

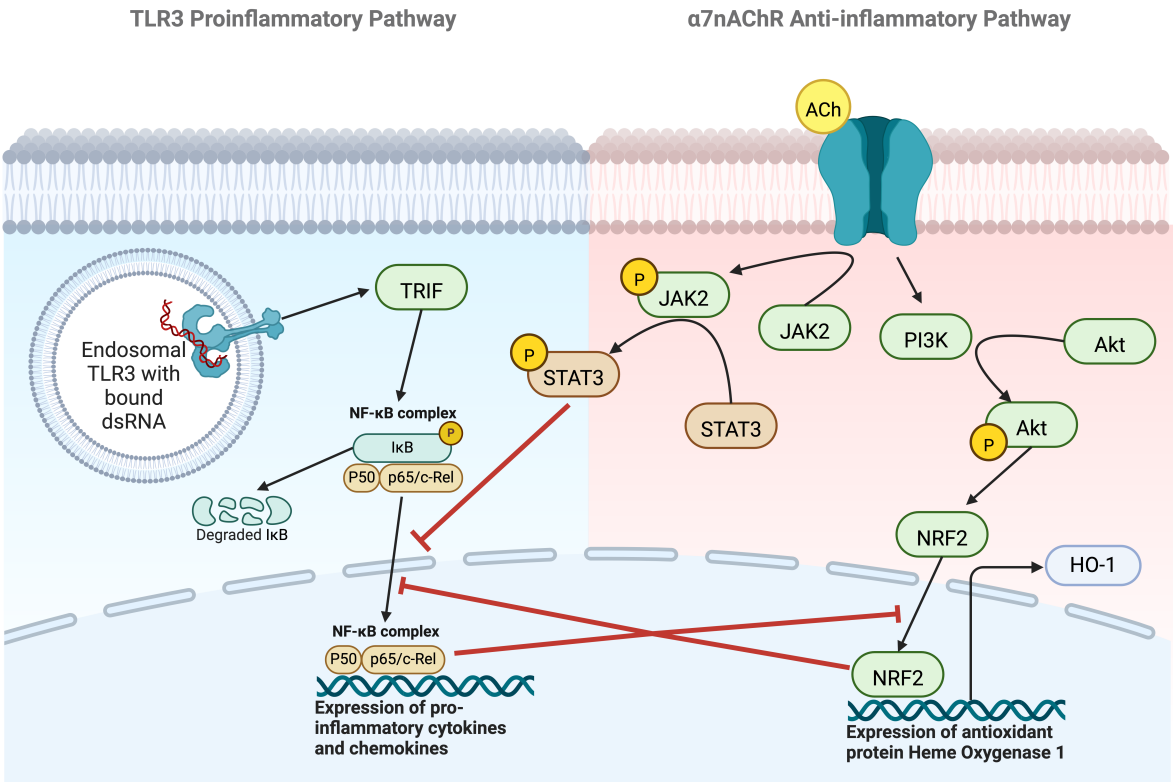

Figure 1.5: Antagonism between α7nAChR and TLR3 Signaling

Signaling downstream of the alpha-7 nicotinic acetylcholine receptor inhibits NF-κB-mediated pro-inflammatory signaling via two main mechanisms: JAK/STAT3 signaling and PI3K/Akt signaling. JAK/STAT3 signaling inhibits the degradation of the IκB complex, thereby preventing NF-κB translocation to the nucleus. PI3K/Akt signaling induces antioxidant defense mechanisms in an Nrf2-dependent manner whereby Nrf2 competes with NF-κB for binding sites and inhibits pro-inflammatory responses.

Studies in animal models of neurodevelopmental disorders have replicated the association between choline and positive neurodevelopmental outcomes. For example, in the MECP2 knockdown model of Rett Syndrome, a form of intellectual disability often co-occuring with ASD, both prenatal and postnatal choline supplementation have been found to modulate behavioral outcomes, including motor coordination and anxiety behaviors (Chin et al., 2019; Ricceri et al., 2011). These same studies also found that choline supplementation improves synaptic plasticity and increases neurotrophic factor expression in the cortex. Mutations in the

gene encoding MECP2 are also considered risk factors for schizophrenia, so these findings have relevance to multiple psychiatric conditions (Chen et al., 2020). Similar findings have been replicated in the BTBR + Itpr3tf/J (Black and Tan Brachyury bred with inserted *tufted* (*Itpr3*^{tf})) mouse model of ASD, where gestational choline supplementation was associated with decreases in anxiety behaviors, increased sociability, and reductions in repetitive behavior in ASD-modeled mice (Langley et al., 2015). In a different mouse model specific to schizophrenia, gestational choline supplementation improved sensory inhibition despite not changing the levels of the α7nAChR in the hippocampus or any of its subregions (Stevens et al., 2014). These results from non-MIA animal models suggest that the beneficial effects of choline may be seen in the MIA model, especially due to the α7nAChR's specificity for free choline and its anti-inflammatory properties (Alkondon et al., 1997).

Within the context of MIA, choline's benefits are understudied. Since MIA clearly recapitulates several inflammatory components of ASD and schizophrenia, the anti-inflammatory properties of choline must be studied within this paradigm. Indeed, limited studies in MIA and other models of neurodevelopmental disorders suggest that choline may modulate behavioral and neurological outcomes. The only known study to date of maternal choline supplementation in the MIA model found that gestational choline ameliorated anxiety-like and repetitive behaviors, both common in ASD and schizophrenia, in adolescent mice (W. Wu et al., 2015). Further, mice exposed to MIA with a full knockout or haploinsufficiency of the α7nAChR were more susceptible to the impacts of MIA, strongly implicating cholinergic anti-inflammatory signaling in resistance to MIA. This study also found differences in α7nAChR expression between choline-supplemented and non-choline-supplemented offspring, but only the hippocampus was examined (W. Wu et al., 2015). Therefore, maternal choline's impact on the developing PFC in

the context of MIA is still relatively unknown. Supporting the concept of a cholinergic anti-inflammatory pathway through the α7nAChR is another study showing that nicotine, a potent α7nAChR agonist, blunted inflammatory signaling induced by LPS in the pregnant dam and elevated the number of live pups born to the dams to near-control levels (J. Yang et al., 2014).

In sum, evidence from epidemiological, clinical, and animal studies indicates a critical role of choline in nervous system development and neuroimmune functioning. Emerging evidence supports the role of the cholinergic anti-inflammatory pathway as a promising therapeutic target for both neurodegenerative and neurodevelopmental disorders. Choline's specific anti-inflammatory effects suggest that maternal supplementation may be a cost-effective and simple way to protect developing fetuses from immune insults in the age of emerging and re-emerging infectious diseases. By testing this strategy in animal models of MIA, the effectiveness of this simple intervention can be examined in a controlled and cost-effective manner.

Rationale and Hypothesis

Although the impacts of MIA have been widely studied in adulthood, relatively few studies have focused on behavioral alterations in adolescence. Additionally, cost-effective and simple interventions to blunt the impacts of MIA are gaining attention in the age of emerging infectious diseases like COVID-19 and Zika, where large birth cohorts are now regularly exposed to novel viral infections. Based on the need for a more robust characterization of MIA in adolescence and the need to test new interventions to bolster fetal neurodevelopment, this study examines the impact of maternal choline supplementation on behavioral and inflammatory outcomes in adolescence within the MIA model. It was hypothesized that, behaviorally, MIA-exposed animals would exhibit cognitive dysfunction, increased anxiety behaviors, and poor

motor coordination at juvenile and adolescent stages and that maternal choline supplementation would modulate these changes to an intermediate level between non-choline MIA offspring and saline controls.

At the molecular level, it was hypothesized that increases in pro-inflammatory cytokines (e.g., IL-1β, IL-6, TNF-α) would be found in the PFCs of juvenile and adolescent rats exposed to MIA. Regarding maternal choline supplementation, it was hypothesized that MIA rats from dams supplemented with choline would either have reduced expression of pro-inflammatory markers or a reflexive increase in anti-inflammatory ones (e.g., IL-4, IL-10) in the PFC to counteract the pro-inflammatory impacts of MIA. It was also hypothesized that MIA exposure would lead to downregulation of the PI3K/Akt cell survival pathway and decreased expression of the downstream antioxidant enzyme heme oxygenase 1 (HO-1), and that maternal choline supplementation would bring the activity of this pathway closer to control levels. The increased activation of the PI3K/Akt/HO-1 axis by maternal choline, especially if accompanied by changes in downstream cytokines, would be indicative of an anti-inflammatory response through the α7nAChR-mediated cholinergic anti-inflammatory pathway.

Chapter 2 - Materials and Methods

Animals and Maternal Immune Activation

Timed pregnant rat dams were ordered from Charles River and arrived at the facility on gestational day (GD) 7. Immediately after arrival, dams were assigned to one of three conditions: saline control, poly(I:C) with normal food, and poly(I:C) with choline-supplemented food. Those in the choline group were administered chow supplemented with choline chloride at a dose of 5 g/kg from arrival on GD 7 through weaning on postnatal day (PND) 21. On GD 15, all dams were injected intraperitoneally with either high molecular weight poly(I:C) (InvivoGen, catalog number tlrl-pic-5) at a dose of 10 mg/kg or saline. This dosage is consistent with previous MIA studies using the intraperitoneal route in rats (Gilmore et al., 2005; Monteiro et al., 2022; Su et al., 2022; Vorhees et al., 2015). Investigators were blind to condition and treatment. In total, 5 dams were assigned to the control condition, 7 to the poly(I:C) only condition, and 6 to the poly(I:C) plus maternal choline condition. All injections were carried out between the hours of 9:30-10:30 a.m. to control for circadian fluctuations in immune responses in the dams. On PND 21, pups were weaned, sexed, and separated into double or triple housing with same-sex littermates, depending on numbers of each sex in the litter. Whenever possible, subjects were pair housed. All animal care, testing, and procedures were conducted in accordance with the requirements and guidelines of the Kansas State University Institutional Animal Care and Use Committee (IACUC).

To verify maternal illness post-injection, a sickness score for each dam was calculated based on the presence or absence of four symptoms: piloerection, ptosis, lethargy, and huddling. Symptoms were observed in the home cage 0.5, 2, 4, 6, 24, and 48 hours post-injection without disturbing the dam. This is in line with other studies that have found significant differences in

symptom profiles between poly(I:C)-injected mice and rats at these time points, thus allowing for verification of the maternal immune response (Kolmogorova et al., 2017). Additionally, maternal weight was measured 6, 24, and 48 hours post-injection, as it has repeatedly been reported that dams injected with poly(I:C) display either slight weight loss or a lack of weight gain in this period, whereas control dams continue to gain weight (Kolmogorova et al., 2017). This allows for secondary verification that the dosage and route of poly(I:C) injection are sufficient to elicit maternal immune responses.

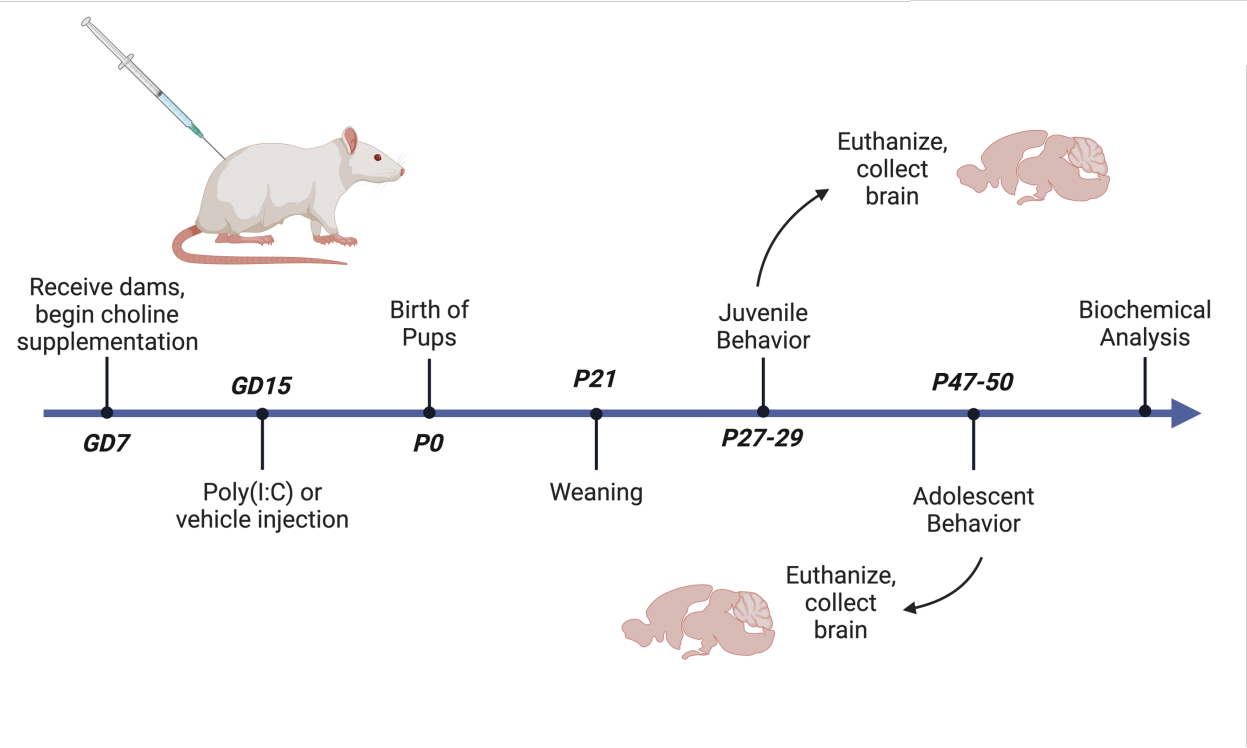

Figure 2.1: Study Timeline

Timed pregnant dams arrived to the facility on GD 7 and were assigned a food condition. Dams were injected with 10 mg/kg poly(I:C) or saline on GD 15 and were then left undisturbed until weaning. Offspring were weaned on PND 21 and behavioral data were collected between PND 27-29 for the juvenile cohort and P47-50 for the adolescent cohort. PFC samples were taken at the conclusion of behavioral testing and frozen at -80°C for later use.

Behavioral Tests

Object Memory: Novel Object Recognition Test (NORT)

To assess object memory, the novel object recognition test (NORT) was conducted. Testing occurred over two consecutive days. On the first day, animals were placed in a 65 x 50-centimeter arena for five minutes to habituate. Then, animals were removed and two identical objects (candle holder or wrench socket) were placed in opposite corners of the box. Animals were then placed back in the arena to explore both objects for ten minutes. The next day, animals were again placed in the same arena, except with one identical object to those which it had encountered the day before and another, novel object. Objects were counterbalanced in their presentation and location to control for preference for one or the other location or object. Time spent investigating each object was recorded manually by blind-to-condition researchers in Actimetrics Limelight video coding software. Data were analyzed as percent exploration time spent with the novel object.

Cognitive Flexibility: Reversal Learning

Apparatus and Stimuli

Reversal learning was assessed at PND 50 only to examine cognitive flexibility. Animals were placed in a plexiglass arena (50 x 37 x 25 cm) with a sliding door between the holding chamber and testing arena. The door was lifted at the beginning of each trial to allow access to two flowerpots with opposing odor cues and digging media. Odor cues presented were vanilla/rum or cinnamon/anise, and media presented were aspen/shredded manila folders or sequins/buttons. These odor and media combinations were selected based on the results of the lab's previous studies with similar tasks (Mali et al., 2023). Both the presentation order of the

stimuli and which stimulus (odor or medium) constituted the relevant dimension were counterbalanced via Latin square. Digging media were recycled between rats to disseminate scent cues, and pots were only ever scented with the same odor. Honey Nut Cheerios™ (General Mills) powder was dusted on all pots, and investigators touched each pot before the start of each trial to ensure that rats were discriminating on the basis of the odors and media presented to them, rather than based on outside cues.

Training

About seven days before the full task, rats were food restricted to 12 grams per day to ensure motivation for the food reward. Weights were taken daily for these animals to ensure that they did not fall below 80% of their free feeding-weight. If an animal was nearing 80% of its free-feeding weight, the animal was given more food, and its weight was recorded again the following day. After about five days of food restriction, rats were trained in basic digging and simple discrimination over two consecutive days before moving on to the full task. On day one of training, rats were trained to dig in flowerpots to receive food rewards, which were 1/4 to 1/3 pieces of Honey Nut Cheerios™ (General Mills). Training began with the cheerio pieces placed on top of the digging media, and they were buried progressively deeper with each consecutive trial. Once fully buried, the animal was required to retrieve fully buried food rewards with an average latency under one minute to pass on to the second day of training. On the second day of training, rats were presented two simple discriminations (i.e., only odor or media cues presented): one odor (lavender vs nutmeg) in regular bedding, and one digging media (shredded paper vs pine shavings) in unscented pots. If the rat was able to complete these simple

discriminations with six consecutive correct choices within 25 trials, it was allowed to continue to the full task the following day.

Reversal Learning Task

The reversal learning task was presented as a series of four phases, all of which were compound discriminations. That is, both different odors and different media were presented simultaneously. Rats were required to learn which dimension (odor or medium) to attend to, as well as which of the two exemplars was rewarded. For each phase, the first four trials were considered "discovery trials" where the animal was allowed to explore both pots, even if it made an incorrect choice. These trials were counted in the six consecutive correct trials, if applicable. After the first compound discrimination was completed with six trials to criterion, a reversal phase was presented where the rewarded exemplar was switched. After this, an intradimensional (ID) shift was presented, where the reward dimension stayed the same, but the exemplars were changed. After the ID shift, a second reversal was presented. After the animals completed each phase with six consecutive correct trials, the task was completed, the animal was returned to its home cage, and food restriction was immediately lifted.

Motor Coordination/Learning: Rotarod Test

Animals were placed on a rod rotating at 4 rpm, facing the same direction each time. Once the animal was securely on the rod, the trial began. Throughout the trial, the rod constantly accelerated to 40 rpm over the course of five minutes. Latency to fall off the rod was measured, except for those trials lasting the maximum five minutes. This was done a total of three times on the same day, with at least ten minutes of rest between trials. To control for fatigue effects, the

maximum latency between trials two and three was considered and analyzed in reference to baseline performance, as it has been suggested that rapid short-term increases in latency may be reflective of repetitive motor behavior acquisition (Fuccillo et al., 2014).

Anxiety-Like Behaviors: Open Field Test (OFT)

For the OFT, animals were placed in a clear Plexiglass enclosure measuring 60 centimeters long on each side. Animals were placed in the center of the enclosure facing the same direction each time, after which they were free to explore the arena for 10 minutes. For analysis, the enclosure was divided into nine 20 x 20-centimeter zones. Time spent in the center zone, as well as crossings through the center zone were taken as measures of anxiety-like behaviors. Total locomotor activity, measured by distance traveled, was also assessed.

Anxiety-Like Behaviors: Elevated Plus Maze (EPM)

To further assess anxiety behaviors, animals were placed on an elevated four-armed maze with alternating open and closed arms, such that the open arms were opposite each other. Animals were placed on the maze facing the same direction at the start of each trial, after which they were free to explore the maze for five minutes. Videos of trials were collected and animal tracking was done with ANY-maze™ (Stoelting) software. Time spent in the center zone, open arms, and closed arms was assessed, as were the number of entries into the open and closed arms. If an animal fell off the maze during the trial, video acquisition was paused, the animal was placed back on the maze as at the beginning, and the trial was resumed. If the animal fell off a second time, it was excluded from analysis.

Tissue Harvest and Preparation

On PND 30 or 53, depending on age cohort, animals were deeply anesthetized under 5% isoflurane gas and euthanized by decapitation. Brains were immediately removed, medial prefrontal cortices were quickly dissected, placed into polypropylene microcentrifuge tubes, and flash frozen on dry ice or in liquid nitrogen. After freezing, tissue samples were stored at -80ºC until further use. All euthanasia was completed between the hours of 10:30 a.m. and 2:30 p.m. to control for circadian fluctuations in immune markers.

After all samples were collected, tissues were homogenized in different cell lysis buffers depending on downstream application. Left medial prefrontal cortices were taken for Milliplex cytokine and Akt/phospho-Akt assays and were homogenized in cell lysis buffer (Milliplex, catalog number 43-040) containing phosphatase and protease inhibitors (Roche) via trituration through a 1000 µL pipette tip. Then, samples were incubated on an orbital plate shaker at 500 rpm for two hours at 4ºC to maximize cell lysis. After two hours, samples were centrifuged at 4500 x *g* for 15 minutes, supernatants were collected, and aliquots were frozen at -80ºC for later use. The same process was followed for right medial prefrontal cortices, except that they were homogenized in another cell lysis buffer (Abcam Cell Extraction Buffer PTR, catalog number ab193170) for Heme Oxygenase 1 ELISA. To account for differences in total protein load between samples, a Qubit™ (Invitrogen) fluorometric assay was performed on an aliquot of each sample to determine total protein concentration. The results of each assay were normalized to their respective total protein concentrations for direct comparison.

Biochemical Data Collection

Milliplex Cytokine Assay

The cytokines IL-1β, IL-4, IL-6, IL-10, IL-17A, IFN-γ, and TNF-α were assayed with a magnetic bead panel (Milliplex®, catalog number RECYTMAG-65K). Samples were diluted to a concentration between 500-750 µg/mL (1,000-1,500 µg/mL in lysis buffer and then 1:2 in assay buffer) before assay per the manufacturer's recommendations. Samples and standards were then incubated with capture antibodies immobilized on magnetic beads in assay buffer at room temperature with agitation for 2 hours. Samples were then removed and wells were washed, after which beads were incubated with detection antibodies for 1 hour. Streptavidin-phycoerythrin was then added, and beads were then incubated with agitation for another 30 minutes. Liquid was then decanted, wells were washed again, and beads were suspended in xMAP™ Sheath Fluid Plus. Median fluorescent intensity was then read for each analyte bead on a Luminex 200™ plate reader using Luminex xMAP™ technology. The median fluorescent intensity was then analyzed via a five-parameter standard curve fit by Intelliflex® software.

Milliplex Akt/phospo-Akt Assay

Phosphorylation of Akt at Ser473 and total Akt were assayed with a Milliplex® Cell Signaling kit (Milliplex®, catalog number 48-618MAG). Samples were diluted to a concentration between 300-500 µg/mL (600-1,000 µg/mL in lysis buffer and then 1:2 in assay buffer) per the manufacturer's recommendations. Samples were incubated with capture antibodies immobilized on magnetic beads at 4ºC with agitation for 18 hours overnight. Samples were removed and wells were washed, after which beads were incubated with detection antibodies for 1 hour (after which detection antibodies were removed), streptavidin-

phycoerythrin for 15 minutes, and amplification buffer for 15 more minutes. Liquid was then decanted from the wells, after which beads were suspended in assay buffer and median fluorescent intensity was read on a Luminex 200™ plate reader using Luminex xMAP™ technology. Results were analyzed as both total protein and the proportion of fluorescent intensity attributable to the phosphorylated form of Akt as a means to determine pathway activation under basal conditions.

Heme Oxygenase 1 (HO-1) ELISA

Heme oxygenase 1 was assayed using a commercially available ELISA kit (Abcam SimpleStep®, catalog number ab279414). Samples were diluted to a concentration between 700-1,000 µg/mL concentration (determined by a pilot experiment) in lysis buffer and incubated on an immobilization antibody-coated plate coated with immunoaffinity-tagged detection antibodies and capture antibodies for one hour with agitation. Wells were then washed and incubated with TMB substrate for ten minutes in the dark, followed by stop solution for one minute. Optical densities were then read at 450 nm. Standard curves were fit using online GainData® ELISA analysis software, and the average of the two replicates was used to calculate analyte concentration in each well.

Statistical Analysis

Behavioral data were analyzed with generalized linear mixed models in R with appropriate distributional assumptions selected based upon data type and structure. Normal-, Gamma-, and Poisson-distributed data were analyzed using the lme4 package and beta-distributed data were analyzed using the glmmTMB package. Condition, sex, and the interaction

between them were included as fixed-effect predictors, and litter was included as a random effect where appropriate to control for pseudoreplication due to multiple littermates within groups. *P*-values were adjusted to account for multiple comparisons using the Bonferroni method where appropriate. Biochemical data were analyzed nonparametrically using a Wilcoxon sign-ranked test followed by Dunn's test for pairwise comparisons. Each litter was represented by at least one member of each sex at each age, and no more than two littermates of the same sex were included at each age to control for litter-to-litter variability. No significant or near-significant effects of sex were observed in these data, so those data were analyzed with both sexes combined by condition.

Chapter 3 - Results

Maternal Illness and Offspring Numbers

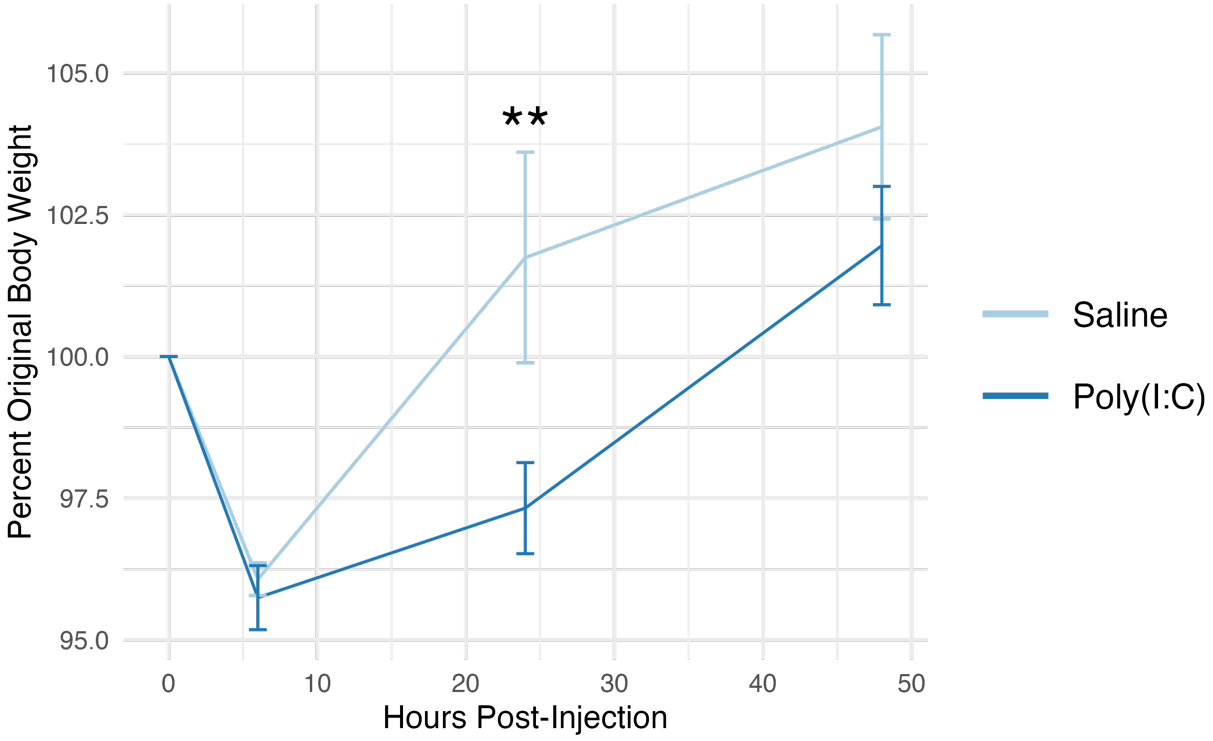

Figure 3.1: Maternal Post-Injection Weight Loss

Weight trajectory of dams post-injection reported as percentage of weight at injection. Poly(I:C)-treated dams had significant weight loss at 24h post-injection compared to controls (p=0.003). Though not significant, these weights were still reduced compared with controls at 48h post-injection (p=0.14). Error bars represent SEM.

Maternal weight was analyzed with a linear mixed model with a random effect of rat ID to account for repeated measures. Following injection, both saline and poly(I:C) dams exhibited initial weight loss after six hours ($t_{48} = 5.359$, $p < 0.001$) (Fig. 3.1). However, there was a significant difference between saline and poly(I:C) dam weight 24 hours post-injection ($t_{44} = 3.187$, $p = 0.003$). At 48 hours post-injection, though, poly(I:C) dams had generally recovered, and their weights were not significantly different from saline dams ($t_{44} = 1.511$, $p = 0.138$). There were no discernable differences in sickness behavior profiles between saline and poly(I:C)

dams (supplemental figure A.1). Numbers of offspring from dams used in behavioral and biochemical experiments are displayed in Tables 3.1 and 3.2, respectively.

	P28 Cohort		P50 Cohort	
	Males	**Females**	**Males**	**Females**
Saline	8	12	9 (8)	13 (10)
Poly(I:C)	14	11	15 (13)	10 (10)
Poly(I:C) + Choline	16	14	16 (13)	13 (12)

Table 3.1: Number of Animals Included in Behavioral Experiments

These animals are derived from 5 saline dams and 13 poly(I:C) dams, 6 of which received gestational choline supplementation. To account for group imbalances, mixed-effects modeling was used for behavioral experiments to account for litter-to-litter variability in behavior. Numbers in parentheses indicate animals that passed training for the reversal learning task and whose data were used for analysis.

	P28 Cohort						P50 Cohort					
	Males			Females			Males			Females		
	Cyto-kines	**Akt**	**HO-1**	**Cyto-kines**	**Akt**	**HO-1**	**Cyto-kines**	**Akt**	**HO-1**	**Cyto-kines**	**Akt**	**HO-1**
Saline	7	7	7	7	7	9	6	7	9	7	6	9
Poly(I:C)	7	7	9	7	7	9	7	7	9	7	7	9
Poly(I:C) + Choline	7	7	9	7	7	9	7	7	9	7	7	9

Table 3.2: Number of Animals Included in Biochemical Experiments

As with behavioral experiments, these animals are derived from 5 saline dams and 13 poly(I:C) dams, 6 of which received gestational choline supplementation. Since group sizes are relatively balanced with respect to these experiments, mixed-effects modeling was not used. Instead, nonparametric statistical methods were used.

Behavioral Results

Object Memory: Novel Object Recognition Task (NORT)

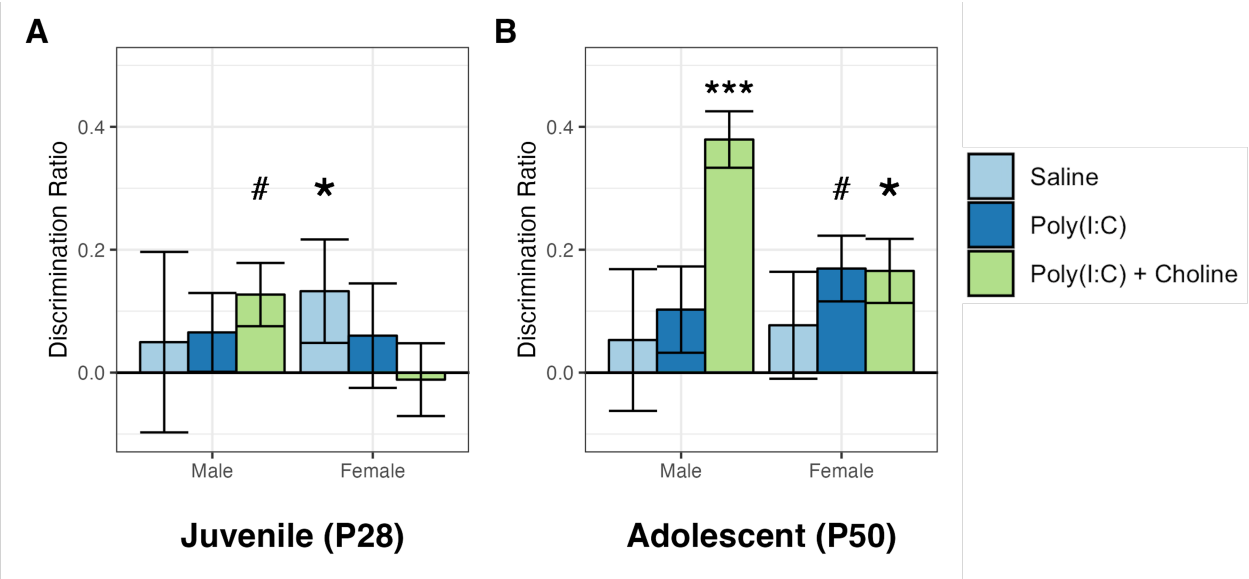

Figure 3.2: Novel Object Discrimination Ratio

The novel object discrimination ratio reflects the difference in time spent with the novel object compared with the familiar object, divided by the total amount of time spent with either object, thus providing a standardized value centered around zero, which represents discrimination at the level of chance (50%). **(A)** *At P28, only control females discriminated the novel object above chance levels ($p = 0.007$), although poly(I:C) + choline males trended toward the same ($p = 0.055$).* **(B)** *At P50, however, poly(I:C)-only animals discriminated above choice levels regardless of sex ($p = 0.024$), though this was not significant for males ($p = 0.149$) or females ($p = 0.082$) alone. For poly(I:C) + choline animals, males ($p < 0.001$) and females ($p = 0.034$) both demonstrated greater than chance discrimination of the novel object. Error bars represent SEM. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, #$p < 0.10$.*

The NORT was conducted at both P28 and P50. Data were analyzed as percentage of total interaction time using a generalized linear mixed model fit with a beta distribution. A random effect of litter was included to account for the nested structure of the data. At P28, there were no significant effects of condition, sex, or the interaction between them. When examined as discrimination above chance levels (50% of time with each object, regardless of familiarity), only control females displayed novel object recognition significantly above chance levels ($z = 2.696$, $p = 0.007$). However, poly(I:C) + choline males trended toward above-chance

performance ($z = 1.920$, $p = 0.055$) (Fig. 3.2). At P50, there were likewise no significant effects of condition, sex, or the interaction between them. However, when analyzed as discrimination above chance, both poly(I:C)-only ($z = 2.260$, $p = 0.024$) and poly(I:C) + choline ($z = 4.146$, $p < 0.001$) animals discriminated significantly above chance levels regardless of sex (Fig. 3.2). There was no main effect of age on novel object discrimination, but there was a trend-level interaction between condition and age ($\chi^2_2 = 5.036$, $p = 0.081$). Pairwise analysis revealed that poly(I:C) + choline animals, regardless of sex, had improved novel object discrimination at P50 over P28, thus driving the interaction. However, this effect was only trend-level after adjustment for multiple comparisons ($z = 2.322$, $p = 0.061$). Point estimates are given in table 3.3.

P28 Novel Object Recognition

	Male				Female			
	Percent Time	*Lower CI*	*Upper CI*	*p*-value	*Percent Time*	*Lower CI*	*Upper CI*	*p*-value
Saline	51.8	43.2	60.4	0.681	59.8	52.7	66.5	0.007
Poly(I:C)	54.7	47.9	61.4	0.177	48.9	41.6	51.3	0.779
Poly(I:C) + Choline	56.0	49.9	62.0	0.055	49.4	42.9	56.0	0.859

P50 Novel Object Recognition

	Male				Female			
	Percent Time	*Lower CI*	*Upper CI*	*p*-value	*Percent Time*	*Lower CI*	*Upper CI*	*p*-value
Saline	51.0	42.1	59.8	0.830	53.8	46.4	61.1	0.317
Poly(I:C)	55.1	48.2	61.8	0.149	58.0	49.0	66.4	0.082
Poly(I:C) + Choline	63.1	56.5	69.3	0.0001	58.1	50.6	65.2	0.034

Table 3.3: Percent Time Spent with Novel Object Compared to Chance

All values are given as percentages. Point estimates for the mean percent time spent with the novel object, lower and upper bounds of the 95% confidence interval on that estimate, and the p-value associated with the estimate.

Cognitive Flexibility: Reversal Learning

Reversal learning was only examined at P50 due to the difficulty of the task, as the lab has found that rats younger than P40 do not pass at sufficient rates for analysis. It was expected that MIA animals would be impaired on this task. Trials to criterion and errors were analyzed via mixed-effects generalized linear models fit with a Poisson distribution and random effects of litter and rat ID to account for repeated measures and nested subjects within litters. Latencies were analyzed using the average latency for each animal, a Gamma distribution to account for positive skew, and a random effect of litter. Sexes were analyzed separately based on the lab's previous findings with similar tasks (Mali et al., 2023). For males, there was a significant condition*phase interaction (χ^2_6= 35.196, $p < 0.001$). Planned pairwise comparisons to examine initial learning during the compound discrimination phase revealed that poly(I:C) males took significantly fewer trials to learn than saline controls, though this was only significant for the poly(I:C) + choline group (estimate = 0.566, *SE* = 0.182, z = 3.110, $p_{adj} = 0.009$; non-choline estimate = 0.415, *SE* = 0.179, $z = 2.317$, $p_{adj} = 0.082$). However, when the first reversal was presented, poly(I:C) males took significantly more trials to reach criterion than their initial learning (non-choline estimate = 0.337, *SE* = 0.101, $z = 3.340$, $p_{adj} = 0.005$; choline estimate = 0.440, *SE* = 0.107, $z = 4.114$, $p_{adj} < 0.001$), while control animals did not (Fig. 3.3A). Despite no differences on the ID shift, the poly(I:C)-only males took more trials to complete the second reversal relative to ID performance, while control and poly(I:C) + choline males did not, indicating a continued reversal impairment (estimate = 0.288, *SE* = 0.106, $z = 2.724$, $p_{adj} = 0.045$). Control males also trended in the same direction, but this appears to be more due to improved ID shifting than a reversal learning impairment per se (estimate = 0.340, *SE* = 0.138, $z = 2.456$, $p_{adj} = 0.084$). In female poly(I:C) animals, there was also a significant poly(I:C)*phase

interaction (χ^2_6= 14.457, p = 0.025), but there were no significant pairwise comparisons after correction for multiple comparisons (Fig. 3.3B).

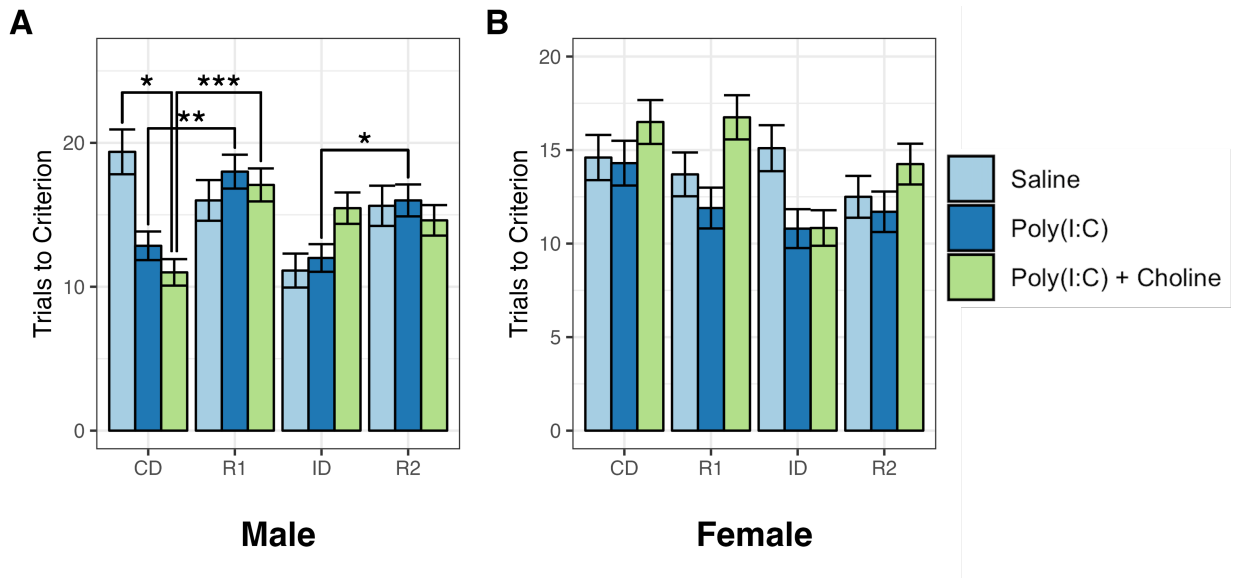

Figure 3.3: Reversal Learning Trials to Criterion

(A) *On the reversal learning task, male poly(I:C) animals displayed improved basic learning on the first compound discrimination compared to controls (choline p_{adj} = 0.013, non-choline p_{adj} = 0.123). However, they performed worse on the first reversal phase with baseline performance taken into account. This reversal learning deficit was present in the second compound discrimination-reversal combination only for the non-choline poly(I:C) males (p = 0.045).* **(B)** *There were no differences in performance in the female group. Error bars represent SEM. ***p < 0.001, **p < 0.01, *p < 0.05, #p < 0.10. CD = compound discrimination, R1 = reversal 1, ID = intradimensional shift, R2 = reversal 2.*

In spite of the relatively subtle group differences, a significant sex effect (χ^2_1 = 10.087, p = 0.001) and a sex*condition interaction (χ^2_2 = 7.778, p = 0.034) on total errors made during the task were observed. When split into errors committed during the reversal phases and the non-reversal phases, there were only significant differences by sex (χ^2_1 = 8.763, p = 0.003) and sex*condition (χ^2_2 = 8.958, p = 0.011) for errors on the reversal phases (non-reversal sex χ^2_1 = 0.013, p = 0.909, sex*condition χ^2_2 = 0.551, p = 0.759). Based on this, errors committed during reversals were further classified into two subtypes: perseverative and regressive. Perseverative

errors were defined as all incorrect responses, excluding the first trial of a new phase (if applicable), until the animal achieved three consecutive correct responses. All errors on the same phase after three consecutive correct responses were classified as regressive. This analysis found that males committed more perseverative errors during the reversal phases than females (χ^2_1 = 14.863, $p < 0.001$). Pairwise comparisons revealed that the sex-mediated increase in perseverative errors was only significant in the control group (estimate = 0.736, SE = 0.234, z = 3.140, p_{adj} = 0.015) (Fig. 3.4B). For regressive errors, a main effect of sex was found where males committed more regressive errors on the reversal phases of the task (χ^2_1= 7.602, p = 0.006). A significant condition*sex interaction was observed (χ^2_2= 6.976, p = 0.031), and

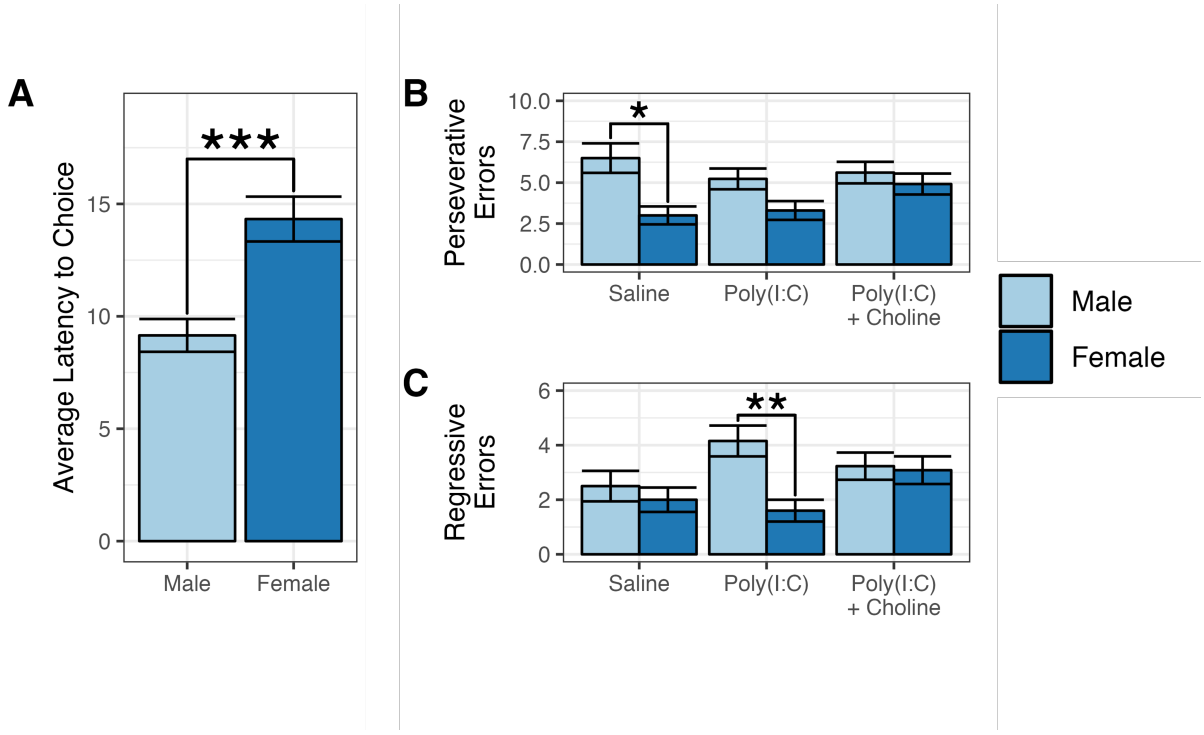

Figure 3.4: Sex Differences on the Reversal Learning Task

(A) *Males had significantly shorter latencies to choice than females (p < 0.001) and* **(B)** *made significantly more perseverative errors (p < 0.001), especially in the control group (p = 0.007) on the reversal phases of the task. However,* **(C)** *for regressive errors, there was only a sex effect in the poly(I:C) group where males made significantly more regressive errors than females on the reversal phases (p = 0.004). Error bars represent SEM. ***p < 0.001, **p < 0.01, *p < 0.05, #p < 0.10.*

pairwise analysis revealed that only in the poly(I:C)-only group did males commit more regressive errors than females (estimate = 1.014, SE = 0.283, z = 3.579, p_{adj} = 0.003) (Fig. 3.4C). In addition to these sex differences in error rates and types, males had much shorter latencies to choice than females across the whole task (χ^2_1= 21.196, $p < 0.001$) (Fig. 3.4A).

Motor Coordination/Learning: Rotarod Test

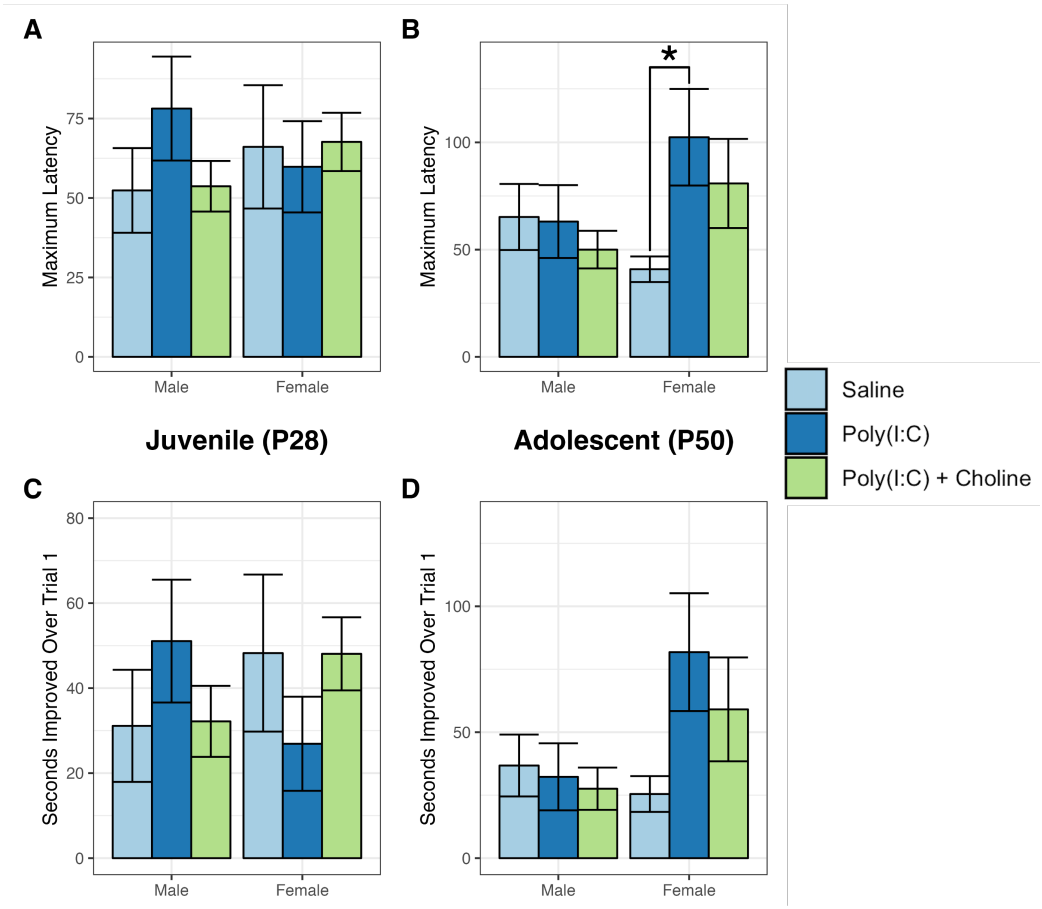

Figure 3.5: Maximum Latency on the Rotarod Test and Improvement Over Baseline

On the rotarod test, **(A)** *juvenile animals displayed no condition or sex differences in maximum latency on the rotarod, but* **(B)** *at P50, poly(I:C)-only females had longer maximum latencies than controls (p = 0.027).* **(C)** *As with maximum latencies, there were no differences in improvement over baseline performance between groups or sexes at P28, but* **(D)** *poly(I:C)-only females had improved performance relative to baseline at P50 compared to controls, but this did not survive adjustment for multiple comparisons. Error bars represent SEM. ***p < 0.001, **p < 0.01, *p < 0.05, #p < 0.10.*

To determine if MIA impacted motor performance on the rotarod, data were analyzed with mixed-effects linear models with a random effect of litter to account for the nested structure of the data. Maximum latencies were analyzed with a generalized linear model with a Gamma distribution to account for skewness. Improvement over baseline was analyzed by subtracting trial one latency from the maximum latency of trials two and three and square root transforming to adhere to the Gaussian assumption of normally distributed residuals. These data were analyzed with a linear mixed model. There were no differences in either maximum latency to fall off the rod or improvement over baseline performance in either sex at P28 (Fig. 3.5A). At P50, however, a condition*sex interaction was found for maximum latency to fall off the rod ($\chi^2_2 = 8.242$, $p = 0.016$). Pairwise comparisons revealed that, after adjustment for multiple comparisons, only poly(I:C) females had significantly longer latencies to fall off the rod, though this only survived correction for multiple comparisons in the non-choline group (non-choline estimate = 0.874, $SE = 0.307$, $z = 2.844$, $p = 0.027$; choline estimate = 0.681, $SE = 0.292$, z = 2.330, $p = 0.099$) (Fig. 3.5B).

When considered as improvement over baseline performance, poly(I:C) females, regardless of maternal choline, trended toward improved performance over controls (estimate = 2.963, $SE = 1.421$, $t_{35.04} = 2.086$, $p_{adj} = 0.089$) (Fig. 3.5D). Pairwise comparisons revealed that this improvement was only significant in the non-choline poly(I:C) females, though this did not survive adjustment for multiple comparisons (estimate = 3.693, $SE = 1.720$, $t_{40.23} = 2.146$, $p_{adj} = 0.228$). Surprisingly, there was no difference in maximum latency with age ($\chi^2_1 = 0.007$, $p = 0.935$). There were also no significant interactions between age, sex, and condition for improvement over baseline. Individual trial data are summarized in supplemental figure A.2.

Anxiety-Like Behavior: Open Field Test (OFT)

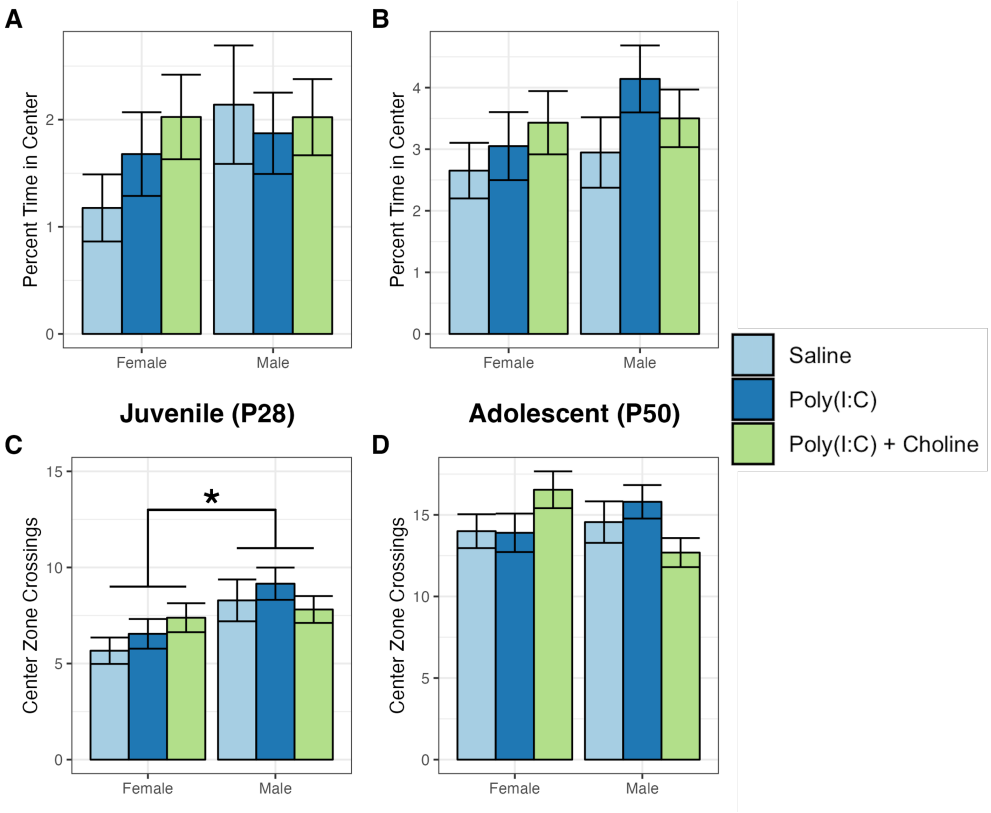

Figure 3.6: Center Zone Time and Crossings in the Open Field

(A, B) *Percent time spent in the center zone of the open field. There were no significant differences in time spent in the center zone of the open field at either P28 or P50. However, P50 animals as a whole did spend more time in the center zone than P28 animals ($p < 0.001$).* **(C)** *P28 center zone crossings. At P28, males crossed through the center zone more frequently than females, but* **(D)** *there was no such effect at P50. As with time spent in the center zone, P50 animals crossed through the center zone significantly more frequently than P28 animals ($p < 0.001$). Error bars represent SEM. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, #$p < 0.10$.*

For the OFT, which tests anxiety-like behaviors and general activity, total distance traveled, time in the center zone, and crossings through the center zone were analyzed for group and sex differences. Data were analyzed with generalized linear mixed models containing a random effect of litter to account for nested data structure. Total distance traveled was analyzed with a Gamma distribution, time spent in the center zone was analyzed as percent time spent in the center with a beta distribution, and crossings through the center zone were analyzed using a

Poisson distribution. There were no group or sex differences in total distance traveled at either age (supplemental figure A.3). There were also no group or sex differences in time spent in the center zone at either age when analyzed as percentage of time spent in the center (Fig. 3.6A and B). Regarding crossings through the center zone, there were no group differences, but males crossed through the center zone more than females at P28 only ($\chi^2_1 = 7.623$, $p = 0.006$) (Fig. 3.6C). Despite very few differences within age groups, the older cohort of animals traveled farther ($\chi^2_1 = 88.883$, $p < 0.001$), spent more time in the center zone ($\chi^2_1 = 32.543$, $p < 0.001$), and crossed through it more often ($\chi^2_1 = 153.185$, $p < 0.001$) than did the younger cohort.

Anxiety-Like Behavior: Elevated Plus Maze (EPM)

EPM data were analyzed as both time spent in the open, closed, and center zones, as well as entries into each zone, via generalized linear mixed models. Time spent in each zone was analyzed as a percentage of total time with a beta distribution, and zone crossings were analyzed with a Poisson distribution. All models contained a random effect of litter to account for the nested structure of the data. Entries were defined as crossing of the anterior half of the body into a zone, the default setting for the ANY-maze™ (Stoelting) software. There were no group or sex differences at either age for time spent in any zone (supplemental figures A.4 and A.5). For zone entries, there were no group differences, but there were sex effects in the number of entries into the center zone at P28 only, where males entered the center zone more times than females did ($\chi^2_1 = 6.522$, $p = 0.011$). In the control group only at P28, males also made more entries into the open arms than females, though this did not survive correction for multiple comparisons (estimate = 0.307, $SE = 0.120$, $z = 2.563$, $p_{adj} = 0.093$). There were no group or sex differences at P50. Despite few differences within age groups, P50 animals spent more time in the open arms

of the maze ($\chi^2_1 = 131.808$, $p < 0.001$) and had more zone entries ($\chi^2_1 = 19.483$, $p < 0.001$) than P28 animals in all groups.

Biochemical Results

Cytokine Expression

Cytokine expression was analyzed via Kruskal-Wallis tests with a predictor of condition, and post-hoc testing was conducted with Dunn's test. Sex was initially included as a factor, but no significant effect of sex was observed for any molecules, so data are presented by condition only. At P28, a significant condition effect was found only for IL-4 ($\chi^2_2 = 13.969$, $p < 0.001$). Pairwise comparisons via Dunn's test revealed that poly(I:C)-only animals at P28 had reduced IL-4 expression compared to controls (estimate = 2.095, $p = 0.036$) and poly(I:C) + choline animals (estimate = 3.728, $p < 0.001$). Despite no significant differences, trend-level differences were observed in IL-6, where poly(I:C) + choline animals had higher PFC expression compared to poly(I:C)-only animals (estimate = 1.679, $p = 0.093$), and IFN-γ, where poly(I:C) + choline animals had higher levels than both control (estimate = 1.680, $p = 0.093$) and poly(I:C)-only (estimate = 1.742, $p = 0.081$) offspring. No significant or trend-level differences were observed for IL-1β, IL-10, IL-17A, and TNF-α (Fig. 3.7A). At P50, as with P28, a significant condition effect was found for only IL-4 ($\chi^2_2 = 13.423$, $p < 0.001$). Similar to P28, pairwise comparisons with Dunn's test showed that poly(I:C) + choline animals had higher IL-4 expression than poly(I:C)-only animals (estimate = 2.146, $p = 0.032$) and controls (estimate = 3.640, $p < 0.001$). Unlike P28, however, there were no trend-level differences in the levels of the other cytokines at this age (Fig. 3.7B). Mean values and standard deviations for each cytokine at each age are given in Table 3.4 for all groups.

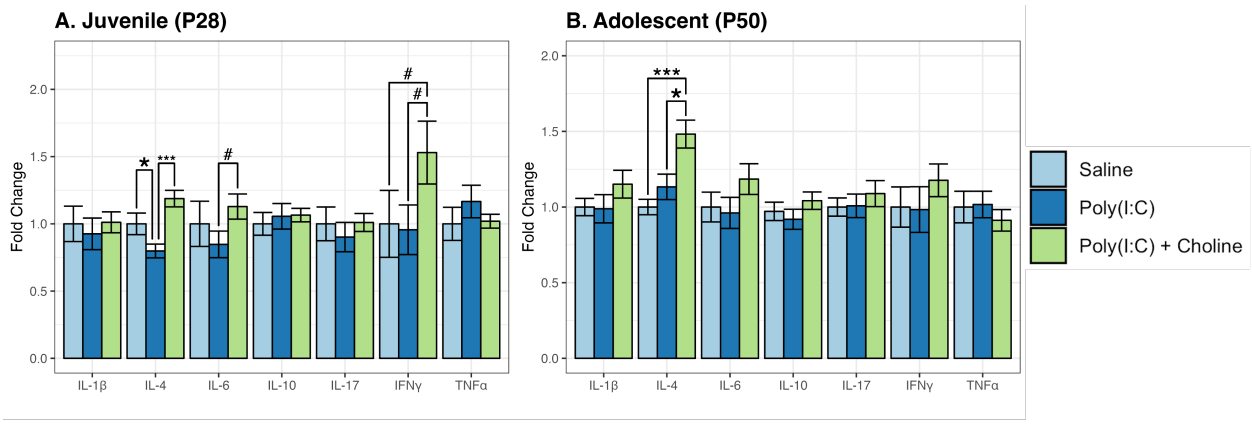

Figure 3.7: Relative Cytokine Expression in the Prefrontal Cortex

Fold changes in cytokine expression at P28 and P50, combined sexes. **(A)** *At P28, poly(I:C)-only animals had significantly lowered expression of IL-4 compared to controls ($p = 0.036$) and poly(I:C) + choline ($p < 0.001$) animals. Maternal choline also tended to increase IL-6 levels in the poly(I:C) group ($p = 0.093$), and the poly(I:C) + choline group had trend-level increases in IFN-γ expression compared to both controls ($p = 0.093$) and poly(I:C)-only ($p = 0.081$) animals.* **(B)** *At P50, only IL-4 was significantly increased in the poly(I:C) + choline animals compared to both controls ($p < 0.001$) and poly(I:C)-only ($p = 0.032$) animals. Error bars represent SEM. ***p < 0.001, **p < 0.01, *p < 0.05, #p < 0.10.*

Despite the general lack of group differences within ages, there were some notable differences in the expression of cytokines between P28 and P50. First, there were several main effects and trends of age where, regardless of condition, the expression of IL-1β ($\chi^2_5 = 9.689$, $p = 0.085$), IL-17A ($\chi^2_5 = 13.546$, $p = 0.02$), and IFN-γ ($\chi^2_5 = 14.961$, $p = 0.011$) increased from P28 to P50 across all conditions. For IL-6, P50 animals had elevated IL-6 across all groups ($\chi^2_5 = 14.245$, $p = 0.014$), but this was only significant in the poly(I:C)-only group ($p = 0.047$), with a trend-level increase in the poly(I:C) + choline group (estimate = 1.717, $p = 0.086$). Similarly, IL-4 expression significantly increased from P28 to P50 ($\chi^2_5 = 33.47$, $p < 0.001$), but this elevation was only significant in the poly(I:C)-only (estimate = 3.473, $p = 0.001$) and poly(I:C) + choline (estimate = 2.046, $p = 0.041$) animals, but not in controls (estimate = 0.170, $p = 0.865$). Lastly, for IL-10, P50 animals displayed increased expression compared to P28 animals ($\chi^2_5 = 13.267$, $p = 0.021$). However, this effect was only significant for the poly(I:C) + choline (estimate = 2.329,

p = 0.020) and control (estimate = 2.209, $p = 0.027$) groups, but not the poly(I:C)-only group (estimate = 0.643, $p = 0.520$). The only cytokine that did not have increased elevation at P50 over P28 was TNF-α ($\chi^2_5 = 5.446$, $p = 0.364$), which was not significantly different in any group between the two ages.

	P28						P50					
	Saline		Poly(I:C)		Poly(I:C) + Choline		Saline		Poly(I:C)		Poly(I:C) + Choline	
	pg/mg	*SE*	*pg/mg*	*SE*	*pg/mg*	*SE*	*pg/mg*	*SE*	*pg/mg*	*SE*	*pg/mg*	*SE*
IL-1β	308	41.1	287	36.8	314	24.9	364	21.2	361	34.6	421	33.4
IL-4	186	14.6	149	9.33	220	11.2	194	9.70	219	16.2	286	17.7
IL-6	3340	548	2836	324	3754	302	4181	403	4008	422	4936	417
IL-10	352	29.2	371	33.2	374	17.4	437	25.5	392	27.7	459	22.7
IL-17A	803	113	728	94.3	830	62.3	989	65.7	1016	81.8	1081	91.2
IFN-γ	5762	1126	4779	896	7577	1136	8045	1069	9210	866	9474	873
TNF-α	50.8	6.26	59.2	6.14	51.8	2.61	46.9	4.89	47.7	4.10	42.8	3.33

Table 3.4: Cytokine Levels in the Prefrontal Cortex

Although there were relatively few group differences within each age cohort, there were several changes between ages. Most cytokines increased in expression between the two ages, although there were some group-specific differences in the magnitude of this trend for some cytokines. TNF-α was the only cytokine without significantly different expression between P28 and P50.

Protein Kinase B (Akt) Phosphorylation

Protein Kinase B (Akt) phosphorylation downstream of the α7nAChR was analyzed as the percentage of total fluorescent intensity attributable to the phosphorylated form of the protein (pAkt) at Ser473 without stimulation. As with the cytokine data, no sex effects were found, so data were analyzed by condition with both sexes combined. At P28, there was a significant condition effect ($\chi^2_2 = 11.098$, $p = 0.003$), and pairwise analysis via Dunn's test revealed that

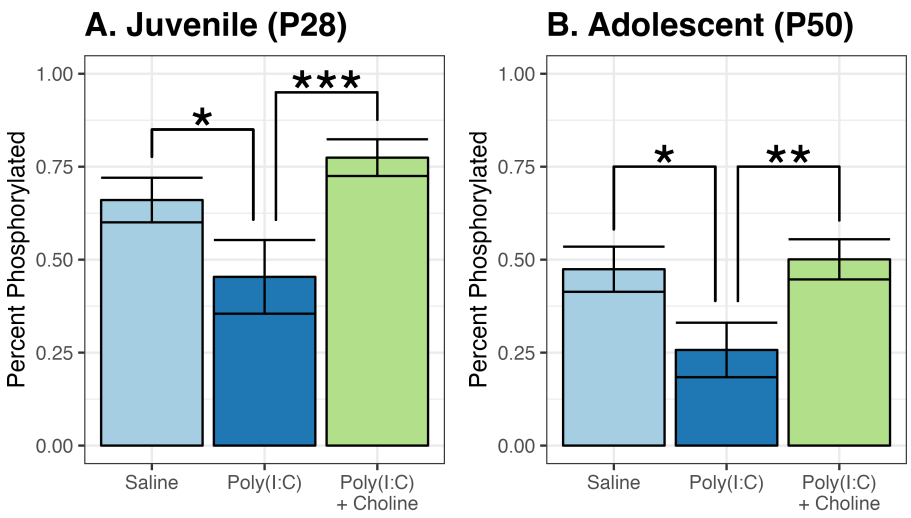

Figure 3.8: Akt Phosphorylation in the Prefrontal Cortex

Results are presented as the percentage of fluorescent intensity attributable to pAkt relative to the total Akt fluorescent signal. **(A)** *At P28, poly(I:C)-only animals had reduced pAkt expression relative to both control ($p = 0.039$) and poly(I:C) + choline ($p < 0.001$) animals.* **(B)** *At P50, the same pattern held where poly(I:C) animals had reduced pAkt expression compared to both control ($p = 0.018$) and poly(I:C) + choline ($p = 0.010$) animals. There was a significant decrease in relative pAkt expression between P28 and P50. Error bars represent SEM. ***p < 0.001, **p < 0.01, *p < 0.05, #p < 0.10.*

poly(I:C)-only animals had significantly reduced pAkt expression than poly(I:C) + choline ($p < 0.001$) and control ($p = 0.039$) animals (Fig. 3.8A). Likewise, there was also a significant condition effect at P50 ($\chi^2_2 = 8.207$, $p = 0.017$). Pairwise comparisons showed that, as with the P28 animals, poly(I:C)-only animals had reduced pAkt expression compared to poly(I:C) + choline ($p = 0.010$) and control ($p = 0.018$) animals (Fig. 3.8B). Beyond the increased pAkt expression relative to total Akt protein, poly(I:C)-only animals at both ages had decreased total Akt protein expression compared with poly(I:C) + choline animals (P28 estimate = 2.819, $p = 0.005$; P50 estimate = 3.204, $p = 0.001$). To examine the effect of age on pAkt expression, a Wilcoxon rank sum test comparing the two age groups found that the P50 animals had reduced pAkt expression relative to total Akt compared to the P28 group ($W = 1249$, $p = 0.001$). P50

animals also had reduced total Akt protein expression than P28 animals, indicating decreased reservoirs at this age in addition to decreased phosphorylation ($W = 1638$, $p < 0.001$).

Heme Oxygenase 1 (HO-1) Expression

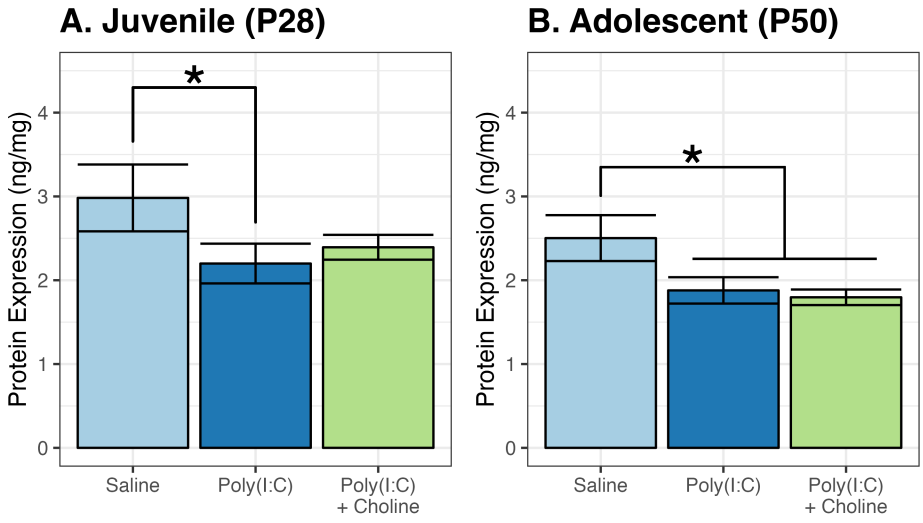

Figure 3.9: Heme Oxygenase 1 Protein Expression in the Prefrontal Cortex

(A) *At P28, poly(I:C) animals had reduced HO-1 expression relative to controls (p = 0.041), while the poly(I:C) + choline group did not.* **(B)** *At P50, despite no significant difference between groups, poly(I:C) animals, taken together regardless of maternal choline supplementation, displayed reduced HO-1 expression compared to controls (p = 0.048). ***p < 0.001, **p < 0.01, *p < 0.05, #p < 0.10.*

Heme oxygenase 1 (HO-1) ELISA data were analyzed similarly to the cytokine data: by Kruskal-Wallis tests followed by Dunn's test post hoc. As with the other molecules, no significant sex effects were observed, so data were analyzed by condition with both sexes combined. At P28, poly(I:C)-only animals had reduced HO-1 expression compared to controls (estimate = 2.046, $p = 0.041$), despite no overall significant effect of condition ($\chi^2_2 = 4.436$, $p = 0.109$) (Fig. 3.9A). At P50, though, there was no effect of condition on HO-1 expression ($\chi^2_2 = 4.125$, $p = 0.127$) or any significant pairwise results, though both poly(I:C) groups trended toward decreased HO-1 expression compared with controls (poly(I:C) + choline estimate =

1.939, $p = 0.053$; poly(I:C) only estimate = 1.494, $p = 0.135$). Noticing this, a Wilcoxon ranked sum test with both poly(I:C) groups combined was conducted which found that, regardless of maternal choline, poly(I:C) animals had reduced HO-1 expression compared to controls ($W = 432$, $p = 0.048$) (Fig. 3.9B). As with pAkt expression, HO-1 expression declined with age across all groups ($W = 1863$, $p = 0.004$).

Chapter 4 - Discussion

Behavioral

Object Memory: Novel Object Recognition Test (NORT)

The increased preference for the novel object found in this study replicates previous findings of improved object memory in the MIA model during adolescence, despite commonly observed impairments in adults (Gray et al., 2019; Guerrin et al., 2022; Ito et al., 2010; A. Osborne et al., 2017; Shi et al., 2003; Su et al., 2022). In line with the findings here, a study using both the NORT and an object location recognition task found that young adult MIA mice demonstrated heightened sensitivity to novel objects compared to control mice, but not objects placed in novel locations (Ito et al., 2010). This suggests that the MIA may have altered object information processing, but not spatial processing. Further, they found decreased expression of c-Fos, an activity-dependent neuronal gene product, in CA1 (a hippocampal subregion) pyramidal neurons when mice were exposed to novel objects in the home cage, but not in mice exposed to a novel environment. These results were obtained from animals between six and eleven weeks old, suggesting that they may be generalizable to MIA studies in adolescence like this one. Indeed, a different study identified enhanced novel object recognition in MIA rats at P45 (mid-adolescence), more closely aligning with the paradigm of this study (Su et al., 2022). However, no spatial processing was assessed in that study or the present one, so future research should examine both of these domains of memory to replicate the results of Ito et al. and to more firmly establish their biological underpinnings. Further, future research should examine the effect of age on novel object recognition and hippocampal function in the MIA model to reconcile the disparate findings between adolescents and adults.

Prenatal choline supplementation, as in this study, has been found to improve hippocampus-dependent memory processes, such as those used in the NORT, in a variety of animal models (Meck et al., 1988; Waddell et al., 2020; Zeisel, 2006a). For example, in a rat model of fetal-neonatal iron deficiency, choline supplementation to iron-deficient dams ameliorated novel object recognition deficits in young adult offspring (Kennedy et al., 2014). Rats from these dams also had increased *bdnf-IV* and myelin basic protein (*mbp*) gene expression in the hippocampus to a level comparable with iron-sufficient control rats. The beneficial effects of prenatal choline supplementation also extend to aging and are protective against age-related declines in NORT performance (Glenn et al., 2008). This protection is associated with increased expression of vascular endothelial growth factor (VEGF) in the female hippocampus and increased cell proliferation in the male hippocampus. Since the maternal choline supplementation paradigm of this study spanned E11-E18, the key gestational period for hippocampal development in rodents, similar neuroprotective mechanisms likely acted synergistically with MIA to produce strong object memory in the NORT used in this study (Bayer, 1980; Zeisel, 2006b). Based on the considerable body of literature suggesting that prenatal choline supplementation is protective against age-related memory decline in rodent models of Alzheimer's disease, it is possible that prenatal choline supplementation taps into similar protective mechanisms in neurodevelopment (Chartampila et al., 2023; Glenn et al., 2008; Moreno et al., 2013). Future studies should aim to identify shared mechanisms of choline's neuroprotective effects between development and aging.

Cognitive Flexibility: Reversal Learning

Reversal learning, which requires flexible adaptation to changing reward contingencies, is often impaired in MIA animals (Amodeo et al., 2019; Han et al., 2011; Lins et al., 2018; Meyer et al., 2005; Wallace et al., 2014; Y. Zhang et al., 2012). However, there are many types of reversal learning tasks, many of which incorporate spatial learning components, and there is thus considerable heterogeneity in reversal learning findings. Studies using the T-maze, Y-maze, and Morris Water Maze for reversal learning often report conflicting findings, with some finding reversal learning impairments, others reporting enhancements, and others reporting differences by sex (Amodeo et al., 2019; Gogos et al., 2020; Han et al., 2011; Kleinmans & Bilkey, 2018; Meyer et al., 2005). These versions of the task require spatial working memory, which appears to be less affected by MIA, at least in adolescence (see discussion above). MIA animals are impaired in reversal learning tasks without large spatial working memory components, like the one in this study (Lins et al., 2019; Wallace et al., 2014). However, few MIA studies have used the digging version of the task used here, and very few have examined reversal learning in adolescence. The digging version of the task used in this study is a suitable way to examine reversal learning without introducing a large spatial working memory component, meaning that this version of the task can more easily isolate PFC-dependent reversal learning processes from hippocampus-mediated spatial working memory (Tait et al., 2014, 2021). This version of the reversal learning task is sufficient to recapitulate reversal learning impairments in adult MIA rats, suggesting that it can also assay the same underlying cognitive processes in adolescents (Wallace et al., 2014).

In the first compound discrimination phase of the task, male poly(I:C) animals, regardless of maternal choline, demonstrated improved basic learning, but these animals took more trials to

complete the reversal phase in reference to baseline, while saline controls took fewer. This may be evidence of differential strategy in the task. Perhaps control male animals spent more trials sampling each pot to learn the rule, and their subsequent improvement on the reversal is more indicative of a flexible sampling strategy when reward conditions changed in the reversal phase, whereas the poly(I:C) animals had difficulty in adapting to the rule change. This same impairment was found on the second reversal only for the poly(I:C)-only animals. Especially since the poly(I:C) + choline animals did not display the same impairment on the second reversal phase, this suggests that prenatal choline supplementation may have modulated the reversal learning impairment such that the animals are better able to learn over successive reversals. Indeed, maternal choline supplementation has been shown to facilitate reversal learning in non-MIA animal studies (Thomas et al., 2004; Waddell et al., 2020). Further lending credence to this possibility, cholinergic circuits involving the basal forebrain, dorsal striatum, and prefrontal cortex are known to function in cognitive flexibility (Bell et al., 2019; Prado et al., 2017; Tait et al., 2021). Further, these cholinergic circuits are specifically engaged during reversal learning, and not during initial rule learning (Bell et al., 2018). However, the exact role of these circuits in cognitive flexibility is controversial, but the results observed here suggest that these circuits may facilitate rule learning when successive reversals are presented.

In females, no group differences in reversal learning were found. The lack of female impairments observed here may be due to a strain-specific sex effect in reversal learning, which has been observed Long-Evans rats (the strain used in this study), regardless of poly(I:C) exposure (Gogos et al., 2020). Further, a lack of modulation by maternal choline may be because females, from development through reproductive age, have vastly different choline metabolism than males (Tessitore et al., 1995; Zeisel, 2006b). This difference appears to extend to CNS

cholinergic neurotransmission, as female rodents have differential cholinergic signaling in the hippocampus and frontal cortex, as well as differential sensitivity to transgenic manipulation of cholinergic tone (Kipp et al., 2021; Mitsushima, 2011; Tryon et al., 2023). Based on the historical underrepresentation of female subjects in preclinical research, these sex effects should be further examined in other strains and in clinical populations to determine if they are persistent across different paradigms.

Motor Coordination/Learning: Rotarod Test

On the rotarod test, poly(I:C)-only females demonstrated significantly longer latencies to fall off the accelerating rod compared to controls at P50, and they also trended toward improved performance over successive trials. This contrasts with findings that people with ASD and schizophrenia generally display impaired motor coordination, which has been replicated in MIA studies (Fatemi et al., 2012; Monteiro et al., 2022; Walther & Strik, 2012; Z. Zhang & van Praag, 2015). However, many research groups using rotarod tests implement training over several days so that all groups of animals learn the repetitive motor routines necessary to stay on the rod before implementing tests of motor control specifically (Buitrago et al., 2004; Naviaux et al., 2013; X. Zhang et al., 2021). The present study conducted trials successively on the same day with only short breaks between trials, thus preventing long-term improvements in performance due to motor learning. This suggests that these results may be more indicative of repetitive behavior acquisition than motor learning, which may explain the blunting of MIA's effect by maternal choline.

Supporting the notion that short-term variants of the rotarod test like the one in this study may be due to repetitive behavior acquisition, an elegant 2014 study by Fuccillo et al.

demonstrated that improved performance on the rotarod over time is modulated by neural circuits involved in repetitive and stereotyped behaviors instead of motor learning ones (Fuccillo et al., 2014). Using conditional and total knockouts of neuroligin-3, a risk gene for ASD, Fucillo et al. observed that total knockout mice and those with conditional knockouts in only ventral striatal dopaminergic neurons had improved rotarod performance, which was correlated with repetitive gait patterns on the rod. In contrast, those with conditional knockouts in cerebellar Purkinje cells did not display improved performance or enhanced acquisition of repetitive gait, despite the role of these cells in motor coordination and learning. Since striatal circuits are involved in the acquisition of repetitive and stereotyped behaviors, and because they seem to be involved in rotarod learning, it is feasible that the poly(I:C)-only females' improved performance on the rotarod is indicative of enhanced repetitive behavior acquisition (Abbott et al., 2018; Curry-Pochy et al., 2020; Soghomonian, 2023). Especially since many striatal circuits involved in repetitive behaviors are cholinergic, the modulation of rotarod performance in the poly(I:C) + choline group may be indicative that, indeed, this study's MIA paradigm acts upon these circuits specifically, rather than on the classical motor learning ones, and that maternal choline supplementation modulates the impact of MIA on those circuits (Bell et al., 2019; Williams & Christakou, 2022). Future studies should examine gait patterns on the rotarod and separate out the roles of cholinergic striatal circuitry and motor learning circuitry in facilitating acquisition of the repetitive behaviors necessary for success on the rotarod.

Anxiety-Like Behaviors: Elevated Plus Maze (EPM) and Open Field Test (OFT)

Strikingly few behavioral differences were observed in the EPM or OFT at either age. There are a few explanations why this might be. First, these tests are locomotion-dependent; that

is, the amount that the animal moves greatly influences the measurable outcomes of these tests (Börchers et al., 2022). Compared to younger animals, older animals explore and move more in the EPM and OFT, leading to more opportunities to cross into open/center zones, which are the most commonly reported outcomes of these tasks (Börchers et al., 2022; Lynn & Brown, 2010). These trends were indeed seen between the two age groups where older animals traveled more distance and crossed through the center zone more. However, the older animals may not have been old enough for group differences, like those reported in other MIA studies in adult animals, to become apparent, although some did find increased anxiety-like behavior in adolescent MIA offspring with different gestational timing or OFT procedures (Li et al., 2021; Su et al., 2022; W. Wu et al., 2015). Second, these tests, particularly the EPM, were originally developed to identify and manipulate brain regions implicated in anxiety and to assess pharmacologic treatments (La-Vu et al., 2020; Walf & Frye, 2007). Since this study used an environmental model with no brain manipulations or drugs, it is possible that this further reduces the sensitivity of these assays. Together, these explanations may account for this study's general lack of replication of anxiety-like behaviors in the MIA model.

Biological

Cytokines

There were few changes in PFC cytokine expression at either P28 or P50. Though unexpected, this finding supports the idea that cytokine expression in the PFC is highly region- and time-specific throughout development. Indeed, in a poly(I:C) model similar to the one used here, Garay et al. identified decreases in PFC IL-1β, IL-6, and IL-17A expression at P30 in poly(I:C)-only animals (Garay et al., 2013). However, the observed fold-changes in their study

were small, meaning that these subtle differences may be difficult to replicate statistically, although the present study did replicate the directionality of the relationships they observed. Notably, the changes in PFC cytokine levels in poly(I:C) animals found by Garay et al. were largely opposite from the periphery at P30, where pro-inflammatory cytokines were generally downregulated in the PFC and upregulated in the periphery (Garay et al., 2013). Therefore, the lack of strong changes in the PFC cannot necessarily be interpreted as a failure to replicate MIA and human studies that have observed increased levels of circulating pro-inflammatory cytokines in adolescents (Cieślik et al., 2020; Rodrigues-Amorim et al., 2018; Zhao et al., 2021).

Despite the lack of differences in many cytokines' expression, one cytokine whose expression was persistently elevated at both P28 and P50 by maternal choline supplementation was IL-4. IL-4 is a canonical anti-inflammatory cytokine that drives the polarization of macrophages and microglia from the pro-inflammatory M1 state to the anti-inflammatory M2 state (He et al., 2020; Pu et al., 2023; Yu et al., 2020). M2 microglia scavenge debris, including amyloid protein aggregates, and further secrete IL-4 and IL-10 to maintain homeostasis (Muto et al., 2023; Onore et al., 2014). IL-4 is also upregulated following cholinergic stimulation of the α7nAChR, suggesting that maternal choline supplementation was sufficient to activate the cholinergic anti-inflammatory pathway (Q. Zhang et al., 2017). It is also known that choline metabolism is critical for shifts between the M1 and M2 phenotype in macrophages and microglia (Ghorbani et al., 2023; Okada et al., 2022). Although the mechanism has not been fully worked out, it has been proposed that this is due to choline's ubiquitous presence in membrane lipids. Since cellular membranes must expand for effective phagocytosis, choline metabolism dynamically changes based on the needs of phagocytic cells (Snider et al., 2018). Furthermore, microglial phagocytosis plays a critical role in neurodevelopment through synaptic pruning and

debris scavenging (He et al., 2020; Neumann et al., 2009). Therefore, the putative shift to an M2 phenotype by cholinergic signaling through the α7nAChR is consistent with the changes in IL-4 observed here and other studies using maternal choline supplementation (M. Zhang et al., 2016, 2018).

Interestingly, PFC IL-4 expression increased between P28 and P50 in the poly(I:C) groups, regardless of maternal choline, but not in controls. Since poly(I:C)-only animals had decreased IL-4 expression relative to other groups at P28, this increase only brought IL-4 to control levels. Poly(I:C) + choline animals, on the other hand, further increased their already elevated IL-4 levels between P28 and P50, suggesting a persistent, long-term anti-inflammatory impact of maternal choline supplementation. Beyond IL-4, there were also a few noteworthy trends in cytokine expression between the P28 and P50 poly(I:C)-only animals. This group displayed significant increases in IL-6 expression between P28 and P50, while the control and poly(I:C) + choline groups did not. Though not increased to levels above controls, this replicates previously observed increases in IL-6 from P30 through development (Garay et al., 2013). IL-6 is a front-line pro-inflammatory cytokine that underpins the neurodevelopmental impacts of MIA (Smith et al., 2007). It has also been found upregulated in humans with ASD and schizophrenia, and it is associated with several neuroinflammatory pathologies (Goines & Ashwood, 2013; Goldsmith et al., 2016; Wei et al., 2012). However, it has been suggested that the amount of IL-6 alone is not what drives neuroinflammation per se, but rather its amount relative to canonical anti-inflammatory cytokines like IL-10 (Meyer et al., 2009; H. Ross et al., 2013). Supporting this, while increases in IL-6 between P28 and P50 were observed in the poly(I:C)-only group, this group was the only one not to have increased IL-10 expression at P50 relative to P28. This suggests that, though there were not significant differences in the expression of IL-6 and IL-10

between the poly(I:C)-only and control groups, an underlying process may have been driving changes that would have been observable at later ages.

Cholinergic Anti-Inflammatory Pathway

Protein Kinase B (Akt) was assayed in the PFC as a measure of PI3K/Akt cell-survival pathway activation. This pathway is also activated by cholinergic signaling through the α7nAChR (Youssef et al., 2020). However, the PI3K/Akt pathway is activated by several receptors and ligands, so the downstream product heme oxygenase 1 (HO-1) was assayed as well to more specifically isolate the cholinergic anti-inflammatory pathway. As expected, the phosphorylated (i.e., active) form of Akt was significantly decreased in poly(I:C)-only animals at both P28 and P50 but was boosted to control levels by maternal choline supplementation. Akt-mediated pathways have been implicated in schizophrenia and have been found downregulated in the PFC of poly(I:C)-exposed mice (Bitanihirwe et al., 2010). Akt is also highly involved in regulating the development of dopaminergic circuitry in the PFC, and its phosphorylation is modulated by the antipsychotic drug haloperidol, further implicating it in schizophrenia (Emamian et al., 2004). Furthermore, due to the role of PFC dopaminergic signaling in ASD and schizophrenia, association studies have identified structural and functional differences in the PFCs of people with polymorphisms in the *AKT1* gene (Mandic-Maravic et al., 2022; Tan et al., 2008). As such, future studies should work to identify and clarify the role of Akt-mediated processes in the development of schizophrenia, especially in the context of cholinergic signaling. This study provides evidence that dysfunctions in Akt-mediated processes may be evident as early as adolescence, which carries implications for it as a candidate biomarker for disturbed dopaminergic signaling, especially since its levels are also altered in the periphery of people with schizophrenia (Emamian et al., 2004). Further, Akt's modulation in adolescence by maternal

choline supplementation is an exciting finding that further supports choline's critical role in proper neurodevelopment and anti-inflammatory signaling.

Downstream of Akt, HO-1 is an inducible antioxidant enzyme that converts heme into bilirubin, free iron, and carbon monoxide. Bilirubin is a strong antioxidant, and carbon monoxide exerts other protective effects (Salinas et al., 2004). In the context of cholinergic anti-inflammatory signaling, HO-1 gene expression is upregulated by the PI3K/Akt pathway through the transcription factor NRF2, which binds antioxidant response element enhancer binding sites, whose downstream gene products negatively regulate NF-κB-driven inflammatory responses (Saha et al., 2020). Since cholinergic anti-inflammatory signaling through the α7nAChR inhibits NF-κB nuclear translocation through the JAK/STAT3 pathway and positively regulates NRF2 translocation via PI3K/Akt signaling, it was hypothesized that the basal expression of HO-1 in the PFC would be decreased by MIA and modulated nearer to controls in the MIA + choline group (Youssef et al., 2020). This was indeed observed, although marginally, at P28. As with Akt, HO-1 protein expression was significantly decreased at P28 in poly(I:C)-only animals but was modulated by maternal choline supplementation. At P50, however, the poly(I:C) animals, regardless of maternal choline, had decreased PFC HO-1 protein expression with no evidence of modulation by maternal choline.

The decreased expression of HO-1 in the PFC does not necessarily discount the role of cholinergic anti-inflammatory signaling. HO-1 transcription is regulated by a number of transcription factors, namely NRF2, AP-1, HSF, and NF-κB (Medina et al., 2020). Therefore, other transcription factors beyond the control of the cholinergic pathway may have modulated the relationship between the cholinergic anti-inflammatory signaling observed here and HO-1 protein expression. Akt can also phosphorylate HO-1 directly, thereby mobilizing a rapid cellular

response to oxidative stress using existing stores of the enzyme, meaning that upregulation of HO-1 gene expression per se may not be necessary for an antioxidant effect (Salinas et al., 2004). Further, HO-1 can also translocate to the nucleus and to other cellular locations to coordinate other antioxidant responses (Y. Wu & Hsieh, 2022). These mechanisms were unexplored in this study but would be consistent with the increase in Akt and pAkt expression in the poly(I:C) + maternal choline group and may explain the lack of difference between the poly(I:C)-only and poly(I:C) + maternal choline group. Future studies should examine HO-1 activity and phosphorylation beyond what was conducted here to examine this noncanonical mechanism, which may reconcile some of the disparate findings regarding the role of HO-1 in neuroprotection and injury.

Conclusion

This study contributes to the understanding of the biological underpinnings of neurodevelopmental disorders like ASD and schizophrenia in a number of ways. First, previous behavioral findings observed in the poly(I:C) MIA model were replicated that are consistent with neurodevelopmental disorders. Secondly, this study identified inflammatory outcomes in the PFC that are consistent with those observed in ASD and schizophrenia, further highlighting the MIA model's translational validity for those conditions. The main contribution of this study to the extant literature, however, is the evidence supporting maternal choline supplementation as an effective means to counteract the neurodevelopmental insult of MIA. At the behavioral level, maternal choline supplementation boosted object recognition memory in both sexes, modulated reversal learning in males, and blunted the acquisition of repetitive behaviors in females compared to non-choline supplemented MIA adolescents. Furthermore, maternal choline

supplementation boosted the levels of IL-4 in the PFC and protected against significant age-related increases in IL-6 while facilitating age-related increases in IL-10. This study also provides evidence that this anti-inflammatory impact was exerted through the cholinergic anti-inflammatory pathway via the recovery of Akt and pAkt expression, which is downstream of the α7nAChR, to control levels in the PFC, although this was not strongly reflected by the further downstream marker HO-1.

This study has several strengths. First, MIA and euthanasia procedures were standardized to the same time of day so that circadian fluctuations in immune responses would not confound the results. These measures are not widely reported in the literature. Second, this study controlled the molecular weight of the poly(I:C) used to induce MIA, using only a high molecular weight form, a measure only taken by a minority of studies despite evidence that it influences the vigorousness of the maternal immune response (Kentner et al., 2019). Third, although within-subject comparisons with this type of biological data are impossible, all P28 and P50 offspring came from the same litters, such that all litters were represented in both groups. Furthermore, the confounding effect of litter was controlled through mixed-effects modeling for behavioral data and non-parametric comparisons in the biological data, thus limiting type I errors.

Despite the strengths of this study, there are also limitations. First, all data was collected during development, making generalization to the larger body of literature using adult animals difficult. Second, the strength of the maternal immune response or baseline immunoreactivity were not considered here. Future studies should take this into account, as it has been demonstrated that these factors greatly influence MIA outcomes (Estes et al., 2020). Third, the maternal choline supplementation regimen used in this study did not begin until GD7 since animals were not bred in-house. Therefore, maternal choline supplementation, though begun

more than one week before MIA induction, was not truly conducted throughout pregnancy. Lastly, no positive control group was included in this study due to practical constraints, making it difficult to isolate the impact of maternal choline supplementation on MIA specifically from its general impacts in development. However, there is considerable literature on this topic with which the results of this study align.

In sum, this study provides strong evidence that maternal choline supplementation modulates the impacts of MIA at both the behavioral level and biologically in the PFC. By studying adolescent animals, this study also identifies developmental trajectories that add to the current understanding of the impacts of MIA during adolescence. This is only the second study to our knowledge that examines maternal choline supplementation in the MIA model, and the first in rats. Therefore, more work must be done to identify the specific mechanisms by which maternal choline supplementation bolsters neurodevelopment and counteracts the pathological processes induced by MIA, especially at the structural and circuit levels. All told, the findings of this study, in conjunction with the existing literature, provide strong evidence that maternal choline supplementation is a cheap, simple, and effective intervention that bolsters neurodevelopment *in utero*, especially in the face of inflammatory insults. Regulatory bodies should carefully consider this evidence and develop public health guidance and interventions to increase choline intake in children and the pregnant population.

References

Abbott, A. E., Linke, A. C., Nair, A., Jahedi, A., Alba, L. A., Keown, C. L., Fishman, I., & Müller, R.-A. (2018). Repetitive behaviors in autism are linked to imbalance of corticostriatal connectivity: A functional connectivity MRI study. *Social Cognitive and Affective Neuroscience*, *13*(1), 32–42. https://doi.org/10.1093/scan/nsx129

Adams, J. B., Kirby, J. K., Sorensen, J. C., Pollard, E. L., & Audhya, T. (2022). Evidence based recommendations for an optimal prenatal supplement for women in the US: Vitamins and related nutrients. *Maternal Health, Neonatology and Perinatology*, *8*(1), 4. https://doi.org/10.1186/s40748-022-00139-9

Adams, W., Kendell, R. E., Hare, E. H., & Munk-Jørgensen, P. (1993). Epidemiological evidence that maternal influenza contributes to the aetiology of schizophrenia. An analysis of Scottish, English, and Danish data. *The British Journal of Psychiatry: The Journal of Mental Science*, *163*, 522–534. https://doi.org/10.1192/bjp.163.4.522

Al-Haddad, B. J. S., Jacobsson, B., Chabra, S., Modzelewska, D., Olson, E. M., Bernier, R., Enquobahrie, D. A., Hagberg, H., Östling, S., Rajagopal, L., Adams Waldorf, K. M., & Sengpiel, V. (2019). Long-term Risk of Neuropsychiatric Disease After Exposure to Infection In Utero. *JAMA Psychiatry*, *76*(6), 594–602. https://doi.org/10.1001/jamapsychiatry.2019.0029

Alkondon, M., Pereira, E. F., Cortes, W. S., Maelicke, A., & Albuquerque, E. X. (1997). Choline is a selective agonist of alpha7 nicotinic acetylcholine receptors in the rat brain neurons. *The European Journal of Neuroscience*, *9*(12), 2734–2742. https://doi.org/10.1111/j.1460-9568.1997.tb01702.x

Amodeo, D. A., Lai, C. Y., Hassan, O., Mukamel, E. A., Behrens, M. M., & Powell, S. B. (2019). Maternal immune activation impairs cognitive flexibility and alters transcription in frontal cortex. *Neurobiology of Disease*, *125*, 211–218. https://doi.org/10.1016/j.nbd.2019.01.025

Antony, J. M., Paquin, A., Nutt, S. L., Kaplan, D. R., & Miller, F. D. (2011). Endogenous microglia regulate development of embryonic cortical precursor cells. *Journal of Neuroscience Research*, *89*(3), 286–298. https://doi.org/10.1002/jnr.22533

Arnett, H. A., Mason, J., Marino, M., Suzuki, K., Matsushima, G. K., & Ting, J. P. (2001). TNF alpha promotes proliferation of oligodendrocyte progenitors and remyelination. *Nature Neuroscience*, *4*(11), 1116–1122. https://doi.org/10.1038/nn738

Arsenault, D., St-Amour, I., Cisbani, G., Rousseau, L. S., & Cicchetti, F. (2014). The different effects of LPS and poly I: C prenatal immune challenges on the behavior, development and inflammatory responses in pregnant mice and their offspring. *Brain, Behavior, and Immunity*, *38*, 77–90. https://doi.org/10.1016/j.bbi.2013.12.016

Atladóttir, H. Ó., 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). https://doi.org/10.1542/peds.2012-1107

Atladóttir, H. Ó., Thorsen, P., Østergaard, L., Schendel, D. E., Lemcke, S., Abdallah, M., & Parner, E. T. (2010). Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders. *Journal of Autism and Developmental Disorders*, *40*(12), 1423–1430. https://doi.org/10.1007/s10803-010-1006-y

Barr, C. E., Mednick, S. A., & Munk-Jorgensen, P. (1990). Exposure to influenza epidemics during gestation and adult schizophrenia. A 40-year study. *Archives of General Psychiatry*, *47*(9), 869–874. https://doi.org/10.1001/archpsyc.1990.01810210077012

Bayer, S. A. (1980). Development of the hippocampal region in the rat I. Neurogenesis examined with 3H-thymidine autoradiography. *Journal of Comparative Neurology*, *190*(1), 87–114. https://doi.org/10.1002/cne.901900107

Bell, T., Lindner, M., Langdon, A., Mullins, P. G., & Christakou, A. (2019). Regional Striatal Cholinergic Involvement in Human Behavioral Flexibility. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *39*(29), 5740–5749. https://doi.org/10.1523/JNEUROSCI.2110-18.2019

Bell, T., Lindner, M., Mullins, P. G., & Christakou, A. (2018). Functional neurochemical imaging of the human striatal cholinergic system during reversal learning. *The European Journal of Neuroscience*, *47*(10), 1184–1193. https://doi.org/10.1111/ejn.13803

Bergdolt, L., & Dunaevsky, A. (2019). Brain changes in a maternal immune activation model of neurodevelopmental brain disorders. *Progress in Neurobiology*, *175*, 1–19. https://doi.org/10.1016/j.pneurobio.2018.12.002

Besag, F. M. (2017). Epilepsy in patients with autism: Links, risks and treatment challenges. *Neuropsychiatric Disease and Treatment*, *14*, 1–10. https://doi.org/10.2147/NDT.S120509

Birrell, J. M., & Brown, V. J. (2000). Medial frontal cortex mediates perceptual attentional set shifting in the rat. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *20*(11), 4320–4324. https://doi.org/10.1523/JNEUROSCI.20-11-04320.2000

Bitanihirwe, B. K. Y., Weber, L., Feldon, J., & Meyer, U. (2010). Cognitive impairment following prenatal immune challenge in mice correlates with prefrontal cortical AKT1 deficiency. *International Journal of Neuropsychopharmacology*, *13*(8), 981–996. Scopus. https://doi.org/10.1017/S1461145710000192

Börchers, S., Krieger, J.-P., Asker, M., Maric, I., & Skibicka, K. P. (2022). Commonly-used rodent tests of anxiety-like behavior lack predictive validity for human sex differences. *Psychoneuroendocrinology*, *141*, 105733. https://doi.org/10.1016/j.psyneuen.2022.105733

Brown, A. S., & Conway, F. (2019). Maternal immune activation and related factors in the risk of offspring psychiatric disorders. *Frontiers in Psychiatry*, *10*(MAY). https://doi.org/10.3389/fpsyt.2019.00430

Brown, A. S., & Derkits, E. J. (2010). Prenatal infection and schizophrenia: A review of epidemiologic and translational studies. *American Journal of Psychiatry*, *167*(3), 261–280. https://doi.org/10.1176/appi.ajp.2009.09030361

Buitrago, M. M., Schulz, J. B., Dichgans, J., & Luft, A. R. (2004). Short and long-term motor skill learning in an accelerated rotarod training paradigm. *Neurobiology of Learning and Memory*, *81*(3), 211–216. https://doi.org/10.1016/j.nlm.2004.01.001

Butera, A., De Simone, R., Potenza, R. L., Sanchez, M., Armida, M., Campanile, D., Di Carlo, N., Trenta, F., Boirivant, M., & Ricceri, L. (2024). Effects of a gut-selective integrin-targeted therapy in male mice exposed to early immune activation, a model for the study of autism spectrum disorder. *Brain, Behavior, and Immunity*, *115*, 89–100. https://doi.org/10.1016/j.bbi.2023.09.024

Canetta, S., Bao, Y., Co, M. D. T., Ennis, F. A., Cruz, J., Terajima, M., Shen, L., Kellendonk, C., Schaefer, C. A., & Brown, A. S. (2014). Serological documentation of maternal influenza exposure and bipolar disorder in adult offspring. *The American Journal of Psychiatry*, *171*(5), 557–563. https://doi.org/10.1176/appi.ajp.2013.13070943

Canetta, S., Bolkan, S., Padilla-Coreano, N., Song, L. J., Sahn, R., Harrison, N. L., Gordon, J. A., Brown, A., & Kellendonk, C. (2016). Maternal immune activation leads to selective functional deficits in offspring parvalbumin interneurons. *Molecular Psychiatry*, *21*(7), Article 7. https://doi.org/10.1038/mp.2015.222

Careaga, M., Murai, T., & Bauman, M. D. (2017). Maternal Immune Activation and Autism Spectrum Disorder: From Rodents to Nonhuman and Human Primates. *Biological Psychiatry*, *81*(5), 391–401. https://doi.org/10.1016/j.biopsych.2016.10.020

Carnac, T. (2022). Schizophrenia Hypothesis: Autonomic Nervous System Dysregulation of Fetal and Adult Immune Tolerance. *Frontiers in Systems Neuroscience*, *16*, 844383. https://doi.org/10.3389/fnsys.2022.844383

Chartampila, E., Elayouby, K. S., Leary, P., LaFrancois, J. J., Alcantara-Gonzalez, D., Jain, S., Gerencer, K., Botterill, J. J., Ginsberg, S. D., & Scharfman, H. E. (2023). Choline supplementation in early life improves and low levels of choline can impair outcomes in a mouse model of Alzheimer's disease. *bioRxiv*, 2023.05.12.540428. https://doi.org/10.1101/2023.05.12.540428

Chen, C., Cheng, M., Huang, A., Hu, T., Ping, L., & Chang, Y. (2020). Detection of Rare Methyl-CpG Binding Protein 2 Gene Missense Mutations in Patients With Schizophrenia. *Frontiers in Genetics*, *11*, 476. https://doi.org/10.3389/fgene.2020.00476

Chin, E. W. M., Lim, W. M., Ma, D., Rosales, F. J., & Goh, E. L. K. (2019). Choline Rescues Behavioural Deficits in a Mouse Model of Rett Syndrome by Modulating Neuronal Plasticity. *Molecular Neurobiology*, *56*(6), 3882–3896. https://doi.org/10.1007/s12035-018-1345-9

Choi, G. B., Yim, Y. S., Wong, H., Kim, S., Kim, H., Kim, S. V., Hoeffer, C. A., Littman, D. R., & Huh, J. R. (2016). *The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring*. https://www.science.org

Cieślik, M., Gąssowska-Dobrowolska, M., Jęśko, H., Czapski, G. A., Wilkaniec, A., Zawadzka, A., Dominiak, A., Polowy, R., Filipkowski, R. K., Boguszewski, P. M., Gewartowska, M., Frontczak-Baniewicz, M., Sun, G. Y., Beversdorf, D. Q., & Adamczyk, A. (2020). Maternal immune activation induces neuroinflammation and cortical synaptic deficits in the adolescent rat offspring. *International Journal of Molecular Sciences*, *21*(11), 1–29. https://doi.org/10.3390/ijms21114097

Coiro, P., Padmashri, R., Suresh, A., Spartz, E., Pendyala, G., Chou, S., Jung, Y., Meays, B., Roy, S., Gautam, N., Alnouti, Y., Li, M., & Dunaevsky, A. (2015). Impaired synaptic development in a maternal immune activation mouse model of neurodevelopmental disorders. *Brain, Behavior, and Immunity*, *50*, 249–258. https://doi.org/10.1016/j.bbi.2015.07.022

Comer, A. L., Carrier, M., Tremblay, M. È., & Cruz-Martín, A. (2020). The Inflamed Brain in Schizophrenia: The Convergence of Genetic and Environmental Risk Factors That Lead to Uncontrolled Neuroinflammation. *Frontiers in Cellular Neuroscience*, *14*. https://doi.org/10.3389/fncel.2020.00274

Crawford, L. W., Foley, J. F., & Elmore, S. A. (2010). Histology Atlas of the Developing Mouse Hepatobiliary System with Emphasis on Embryonic Days 9.5-18.5. *Toxicologic Pathology*, *38*(6), 872–906. https://doi.org/10.1177/0192623310374329

Curry-Pochy, L. S., Kravetz, Z., Feinstein, J., Yaffe, B., Tanios, V., Makar, J., & Lewis, M. H. (2020). Differential consequences of habitual responding in a mouse model of repetitive behavior. *Behavioral Neuroscience*, *134*(1), 21–33. https://doi.org/10.1037/bne0000348

Davies, G., by, Welham, J., Chant, D., Fuller Torrey, E., & McQrath, J. (2003). *A Systematic Review and Meta-analysis of Northern Hemisphere Season of Birth Studies in Schizophrenia* (pp. 587–593). https://academic.oup.com/schizophreniabulletin/article/29/3/587/1928787

D'Cruz, A.-M., Ragozzino, M. E., Mosconi, M. W., Shrestha, S., Cook, E. H., & Sweeney, J. A. (2013). Reduced behavioral flexibility in autism spectrum disorders. *Neuropsychology*, *27*(2), 152–160. https://doi.org/10.1037/a0031721

Derbyshire, E., & Obeid, R. (2020). Choline, neurological development and brain function: A systematic review focusing on the first 1000 days. *Nutrients*, *12*(6), 1–32. https://doi.org/10.3390/nu12061731

Devaraju, M., Li, A., Ha, S., Li, M., Shivakumar, M., Li, H., Nishiguchi, E. P., Gérardin, P., Waldorf, K. A., & Al-Haddad, B. J. S. (2023). Beyond TORCH: A narrative review of the impact of antenatal and perinatal infections on the risk of disability. *Neuroscience and Biobehavioral Reviews*, *153*, 105390. https://doi.org/10.1016/j.neubiorev.2023.105390

Egea, J., Buendia, I., Parada, E., Navarro, E., León, R., & Lopez, M. G. (2015). Anti-inflammatory role of microglial alpha7 nAChRs and its role in neuroprotection. *Biochemical Pharmacology*, *97*(4), 463–472. https://doi.org/10.1016/j.bcp.2015.07.032

Emamian, E. S., Hall, D., Birnbaum, M. J., Karayiorgou, M., & Gogos, J. A. (2004). Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia. *Nature Genetics*, *36*(2), 131–137. https://doi.org/10.1038/ng1296

Erta, M., Giralt, M., Esposito, F. L., Fernandez-Gayol, O., & Hidalgo, J. (2015). Astrocytic IL-6 mediates locomotor activity, exploration, anxiety, learning and social behavior. *Hormones and Behavior*, *73*, 64–74. https://doi.org/10.1016/j.yhbeh.2015.06.016

Erta, M., Quintana, A., & Hidalgo, J. (2012). Interleukin-6, a Major Cytokine in the Central Nervous System. *International Journal of Biological Sciences*, *8*(9), 1254–1266. https://doi.org/10.7150/ijbs.4679

Estes, M. L., & McAllister, A. K. (2016). Maternal immune activation: Implications for neuropsychiatric disorders. *Science (New York, N.Y.)*, *353*(6301), 772–777. https://doi.org/10.1126/science.aag3194

Estes, M. L., Prendergast, K., MacMahon, J. A., Cameron, S., Paul Aboubechara, J., Farrelly, K., Sell, G. L., Haapanen, L., Schauer, J. D., Horta, A., Shaffer, I. C., Le, C. T., Kincheloe, G. N., Tan, D. J., van der List, D., Bauman, M. D., Carter, C. S., Van de Water, J., & McAllister, A. K. (2020). Baseline immunoreactivity before pregnancy and poly(I:C) dose combine to dictate susceptibility and resilience of offspring to maternal immune activation. *Brain, Behavior, and Immunity*, *88*, 619–630. https://doi.org/10.1016/j.bbi.2020.04.061

Fatemi, S. H., Aldinger, K. A., Ashwood, P., Bauman, M. L., Blaha, C. D., Blatt, G. J., Chauhan, A., Chauhan, V., Dager, S. R., Dickson, P. E., Estes, A. M., Goldowitz, D., Heck, D. H., Kemper, T. L., King, B. H., Martin, L. A., Millen, K. J., Mittleman, G., Mosconi, M. W.,

… Welsh, J. P. (2012). Consensus Paper: Pathological Role of the Cerebellum in Autism. *The Cerebellum*, *11*(3), 777–807. https://doi.org/10.1007/s12311-012-0355-9

Freedman, R., Hunter, S. K., Law, A. J., D'Alessandro, A., Noonan, K., Wyrwa, A., & Camille Hoffman, M. (2020). Maternal choline and respiratory coronavirus effects on fetal brain development: Prenatal coronavirus and choline fetal brain effects. *Journal of Psychiatric Research*, *128*, 1–4. https://doi.org/10.1016/j.jpsychires.2020.05.019

Fuccillo, M. V., Rothwell, P. E., Maxeiner, S., Hayton, S. J., Gokce, O., Lim, B. K., Fowler, S. C., Malenka, R. C., & Südhof, T. C. (2014). Autism-Associated Neuroligin-3 Mutations Commonly Impair Striatal Circuits to Boost Repetitive Behaviors. *Cell*, *158*(1), 198–212. https://doi.org/10.1016/j.cell.2014.04.045

Gao, R., & Penzes, P. (2015). Common Mechanisms of Excitatory and Inhibitory Imbalance in Schizophrenia and Autism Spectrum Disorders. *Current Molecular Medicine*, *15*(2), 146–167. https://doi.org/10.2174/1566524015666150303003028

Garay, P. A., Hsiao, E. Y., Patterson, P. H., & McAllister, A. K. (2013). Maternal immune activation causes age- and region-specific changes in brain cytokines in offspring throughout development. *Brain, Behavior, and Immunity*, *31*, 54–68. https://doi.org/10.1016/j.bbi.2012.07.008

Garcia-Partida, J. A., Torres-Sanchez, S., MacDowell, K., Fernández-Ponce, M. T., Casas, L., Mantell, C., Soto-Montenegro, M. L., Romero-Miguel, D., Lamanna-Rama, N., Leza, J. C., Desco, M., & Berrocoso, E. (2022). The effects of mango leaf extract during adolescence and adulthood in a rat model of schizophrenia. *Frontiers in Pharmacology*, *13*, 886514. https://doi.org/10.3389/fphar.2022.886514

Ghorbani, P., Kim, S. Y., Smith, T. K. T., Minarrieta, L., Robert-Gostlin, V., Kilgour, M. K., Ilijevska, M., Alecu, I., Snider, S. A., Margison, K. D., Nunes, J. R. C., Woo, D., Pember, C., O'Dwyer, C., Ouellette, J., Kotchetkov, P., St-Pierre, J., Bennett, S. A. L., Lacoste, B., … Fullerton, M. D. (2023). Choline metabolism underpins macrophage IL-4 polarization and RELMα up-regulation in helminth infection. *PLoS Pathogens*, *19*(9), e1011658. https://doi.org/10.1371/journal.ppat.1011658

Gilmore, J. H., Jarskog, L. F., & Vadlamudi, S. (2005). Maternal poly I:C exposure during pregnancy regulates TNFα, BDNF, and NGF expression in neonatal brain and the maternal-fetal unit of the rat. *Journal of Neuroimmunology*, *159*(1–2), 106–112. https://doi.org/10.1016/j.jneuroim.2004.10.008

Glenn, M. J., Kirby, E. D., Gibson, E. M., Wong-Goodrich, S., Mellott, T. J., Blusztajn, J. K., & Williams, C. L. (2008). Age-related declines in exploratory behavior and markers of hippocampal plasticity are attenuated by prenatal choline supplementation in rats. *Brain Research*, *1237*, 110–123. https://doi.org/10.1016/j.brainres.2008.08.049

Gogos, A., Sbisa, A., Witkamp, D., & van den Buuse, M. (2020). Sex differences in the effect of maternal immune activation on cognitive and psychosis-like behaviour in Long Evans rats. *The European Journal of Neuroscience*, *52*(1), 2614–2626. https://doi.org/10.1111/ejn.14671

Goines, P. E., & Ashwood, P. (2013). Cytokine dysregulation in autism spectrum disorders (ASD): Possible role of the environment. *Neurotoxicology and Teratology*, *36*, 67–81. https://doi.org/10.1016/j.ntt.2012.07.006

Goldsmith, D. R., Rapaport, M. H., & Miller, B. J. (2016). A meta-analysis of blood cytokine network alterations in psychiatric patients: Comparisons between schizophrenia, bipolar

disorder and depression. *Molecular Psychiatry*, *21*(12), 1696–1709. https://doi.org/10.1038/mp.2016.3

Gonzalez Caldito, N. (2023). Role of tumor necrosis factor-alpha in the central nervous system: A focus on autoimmune disorders. *Frontiers in Immunology*, *14*. https://www.frontiersin.org/articles/10.3389/fimmu.2023.1213448

Gray, A., Tattoli, R., Dunn, A., Hodgson, D. M., Michie, P. T., & Harms, L. (2019). Maternal immune activation in mid-late gestation alters amphetamine sensitivity and object recognition, but not other schizophrenia-related behaviours in adult rats. *Behavioural Brain Research*, *356*, 358–364. https://doi.org/10.1016/j.bbr.2018.08.016

Guerrin, C. G. J., Shoji, A., Doorduin, J., & de Vries, E. F. J. (2022). Immune Activation in Pregnant Rats Affects Brain Glucose Consumption, Anxiety-like Behaviour and Recognition Memory in their Male Offspring. *Molecular Imaging and Biology*, *24*(5), 740–749. https://doi.org/10.1007/s11307-022-01723-3

Haida, O., Al Sagheer, T., Balbous, A., Francheteau, M., Matas, E., Soria, F., Fernagut, P. O., & Jaber, M. (2019). Sex-dependent behavioral deficits and neuropathology in a maternal immune activation model of autism. *Translational Psychiatry*, *9*(1), Article 1. https://doi.org/10.1038/s41398-019-0457-y

Han, X., Li, N., Meng, Q., Shao, F., & Wang, W. (2011). Maternal Immune Activation Impairs Reversal Learning and Increases Serum Tumor Necrosis Factor-α in Offspring. *Neuropsychobiology*, *64*(1), 9–14. https://doi.org/10.1159/000322455

Hanson, K. L., Grant, S. E., Funk, L. H., Schumann, C. M., & Bauman, M. D. (2022). Impact of Maternal Immune Activation on Nonhuman Primate Prefrontal Cortex Development: Insights for Schizophrenia. *Biological Psychiatry*, *92*(6), 460–469. https://doi.org/10.1016/j.biopsych.2022.04.004

He, Y., Gao, Y., Zhang, Q., Zhou, G., Cao, F., & Yao, S. (2020). IL-4 Switches Microglia/macrophage M1/M2 Polarization and Alleviates Neurological Damage by Modulating the JAK1/STAT6 Pathway Following ICH. *Neuroscience*, *437*, 161–171. https://doi.org/10.1016/j.neuroscience.2020.03.008

Hirayasu, Y., Tanaka, S., Shenton, M. E., Salisbury, D. F., DeSantis, M. A., Levitt, J. J., Wible, C., Yurgelun-Todd, D., Kikinis, R., Jolesz, F. A., & McCarley, R. W. (2001). Prefrontal Gray Matter Volume Reduction in First Episode Schizophrenia. *Cerebral Cortex*, *11*(4), 374–381. https://doi.org/10.1093/cercor/11.4.374

Hollocks, M. J., Lerh, J. W., Magiati, I., Meiser-Stedman, R., & Brugha, T. S. (2019). Anxiety and depression in adults with autism spectrum disorder: A systematic review and meta-analysis. *Psychological Medicine*, *49*(4), 559–572. https://doi.org/10.1017/S0033291718002283

Honigsbaum, M. (2013). "An inexpressible dread": Psychoses of influenza at fin-de-siècle. *The Lancet*, *381*(9871), 988–989. https://doi.org/10.1016/S0140-6736(13)60701-1

Hornig, M., Bresnahan, M. A., Che, X., Schultz, A. F., Ukaigwe, J. E., Eddy, M. L., Hirtz, D., Gunnes, N., Lie, K. K., Magnus, P., Mjaaland, S., Reichborn-Kjennerud, T., Schjølberg, S., Øyen, A.-S., Levin, B., Susser, E. S., Stoltenberg, C., & Lipkin, W. I. (2018). Prenatal fever and autism risk. *Molecular Psychiatry*, *23*(3), Article 3. https://doi.org/10.1038/mp.2017.119

Hyde, T. M., & Weinberger, D. R. (1997). Seizures and schizophrenia. *Schizophrenia Bulletin*, *23*(4), 611–622. https://doi.org/10.1093/schbul/23.4.611

Iitani, Y., Miki, R., Imai, K., Fuma, K., Ushida, T., Tano, S., Yoshida, K., Yokoi, A., Kajiyama, H., & Kotani, T. (2023). Interleukin-17A stimulation induces alterations in Microglial microRNA expression profiles. *Pediatric Research*. https://doi.org/10.1038/s41390-023-02825-6

Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline. (1998). *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline*. National Academies Press (US). http://www.ncbi.nlm.nih.gov/books/NBK114310/

Islam, O., Gong, X., Rose-John, S., & Heese, K. (2009). Interleukin-6 and Neural Stem Cells: More Than Gliogenesis. *Molecular Biology of the Cell*, *20*, 188–199. https://doi.org/10.1091/mbc.E08

Ito, H. T., Smith, S. E. P., Hsiao, E., & Patterson, P. H. (2010). Maternal immune activation alters nonspatial information processing in the hippocampus of the adult offspring. *Brain, Behavior, and Immunity*, *24*(6), 930–941. https://doi.org/10.1016/j.bbi.2010.03.004

Kaar, S. J., Angelescu, I., Marques, T. R., & Howes, O. D. (2019). Pre-frontal parvalbumin interneurons in schizophrenia: A meta-analysis of post-mortem studies. *Journal of Neural Transmission*, *126*(12), 1637–1651. https://doi.org/10.1007/s00702-019-02080-2

Kennedy, B. C., Dimova, J. G., Siddappa, A. J. M., Tran, P. V., Gewirtz, J. C., & Georgieff, M. K. (2014). Prenatal Choline Supplementation Ameliorates the Long-Term Neurobehavioral Effects of Fetal-Neonatal Iron Deficiency in Rats. *The Journal of Nutrition*, *144*(11), 1858–1865. https://doi.org/10.3945/jn.114.198739

Kentner, A. 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. *Neuropsychopharmacology*, *44*(2), 245–258. https://doi.org/10.1038/s41386-018-0185-7

Kępińska, A. P., Iyegbe, C. O., Vernon, A. C., Yolken, R., Murray, R. M., & Pollak, T. A. (2020). Schizophrenia and Influenza at the Centenary of the 1918-1919 Spanish Influenza Pandemic: Mechanisms of Psychosis Risk. *Frontiers in Psychiatry*, *11*, 72. https://doi.org/10.3389/fpsyt.2020.00072

Kipp, B. T., Nunes, P. T., & Savage, L. M. (2021). Sex Differences in Cholinergic Circuits and Behavioral Disruptions Following Chronic Ethanol Exposure with and without Thiamine Deficiency. *Alcoholism, Clinical and Experimental Research*, 10.1111/acer.14594. https://doi.org/10.1111/acer.14594

Kleinmans, M., & Bilkey, D. K. (2018). Reversal learning impairments in the maternal immune activation rat model of schizophrenia. *Behavioral Neuroscience*, *132*(6), 520–525. https://doi.org/10.1037/bne0000275

Kolmogorova, D., Murray, E., & Ismail, N. (2017). Monitoring Pathogen-Induced Sickness in Mice and Rats. *Current Protocols in Mouse Biology*, *7*, 65–76. https://doi.org/10.1002/.cpmo.27

Korsmo, H. W., Jiang, X., & Caudill, M. A. (2019). Choline: Exploring the growing science on its benefits for moms and babies. *Nutrients*, *11*(8). https://doi.org/10.3390/nu11081823

Koyuncu, A., Ayan, T., Ince Guliyev, E., Erbilgin, S., & Deveci, E. (2022). ADHD and Anxiety Disorder Comorbidity in Children and Adults: Diagnostic and Therapeutic Challenges.

Current Psychiatry Reports, *24*(2), 129–140. https://doi.org/10.1007/s11920-022-01324-5

Kummer, K. K., Zeidler, M., Kalpachidou, T., & Kress, M. (2021). Role of IL-6 in the regulation of neuronal development, survival and function. *Cytokine*, *144*, 155582. https://doi.org/10.1016/j.cyto.2021.155582

Kunugi, H., Nanko, S., Takei, N., Saito, K., Hayashi, N., & Kazamatsuri, H. (1995). Schizophrenia following in utero exposure to the 1957 influenza epidemics in Japan. *The American Journal of Psychiatry*, *152*(3), 450–452. https://doi.org/10.1176/ajp.152.3.450

Langley, E. A., Krykbaeva, M., Blusztajn, J. K., & Mellott, T. J. (2015). High maternal choline consumption during pregnancy and nursing alleviates deficits in social interaction and improves anxiety-like behaviors in the BTBR T+Itpr3tf/J mouse model of autism. *Behavioural Brain Research*, *278*, 210–220. https://doi.org/10.1016/j.bbr.2014.09.043

La-Vu, M., Tobias, B. C., Schuette, P. J., & Adhikari, A. (2020). To Approach or Avoid: An Introductory Overview of the Study of Anxiety Using Rodent Assays. *Frontiers in Behavioral Neuroscience*, *14*. https://www.frontiersin.org/articles/10.3389/fnbeh.2020.00145

Lee, B. K., Magnusson, C., Gardner, R. M., Blomström, Å., Newschaffer, C. J., Burstyn, I., Karlsson, H., & Dalman, C. (2015). Maternal hospitalization with infection during pregnancy and risk of autism spectrum disorders. *Brain, Behavior, and Immunity*, *44*, 100–105. https://doi.org/10.1016/j.bbi.2014.09.001

Li, W., Chen, M., Feng, X., Song, M., Shao, M., Yang, Y., Zhang, L., Liu, Q., Lv, L., & Su, X. (2021). Maternal immune activation alters adult behavior, intestinal integrity, gut microbiota and the gut inflammation. *Brain and Behavior*, *11*(5). https://doi.org/10.1002/brb3.2133

Lins, B. R., Hurtubise, J. L., Roebuck, A. J., Marks, W. N., Zabder, N. K., Scott, G. A., Greba, Q., Dawicki, W., Zhang, X., Rudulier, C. D., Gordon, J. R., & Howland, J. G. (2018). Prospective analysis of the effects of maternal immune activation on rat cytokines during pregnancy and behavior of the male offspring relevant to Schizophrenia. *eNeuro*, *5*(4). https://doi.org/10.1523/ENEURO.0249-18.2018

Lins, B. R., Marks, W. N., Zabder, N. K., Greba, Q., & Howland, J. G. (2019). Maternal immune activation during pregnancy alters the behavior profile of female offspring of sprague dawley rats. *eNeuro*, *6*(2). https://doi.org/10.1523/ENEURO.0437-18.2019

Loayza, M., Lin, S., Carter, K., Ojeda, N., Fan, L.-W., Ramarao, S., Bhatt, A., & Pang, Y. (2023). Maternal immune activation alters fetal and neonatal microglia phenotype and disrupts neurogenesis in mice. *Pediatric Research*, *93*(5), Article 5. https://doi.org/10.1038/s41390-022-02239-w

Loewen, S. M., Chavesa, A. M., Murray, C. J., Traetta, M. E., Burns, S. E., Pekarik, K. H., & Tremblay, M.-È. (2023). The Outcomes of Maternal Immune Activation Induced with the Viral Mimetic Poly I:C on Microglia in Exposed Rodent Offspring. *Developmental Neuroscience*, *45*(4), 191–209. https://doi.org/10.1159/000530185

Loram, L. C., Harrison, J. A., Chao, L., Taylor, F. R., Reddy, A., Travis, C. L., Giffard, R., Al-Abed, Y., Tracey, K., Maier, S. F., & Watkins, L. R. (2010). Intrathecal injection of an alpha seven nicotinic acetylcholine receptor agonist attenuates gp120-induced mechanical allodynia and spinal pro-inflammatory cytokine profiles in rats. *Brain, Behavior, and Immunity*, *24*(6), 959–967. https://doi.org/10.1016/j.bbi.2010.03.008

Lynn, D. A., & Brown, G. R. (2010). The ontogeny of anxiety-like behavior in rats from adolescence to adulthood. *Developmental Psychobiology*, *52*(8), 731–739. https://doi.org/10.1002/dev.20468

MacDowell, K. S., Munarriz-Cuezva, E., Caso, J. R., Madrigal, J. L. M., Zabala, A., Meana, J. J., García-Bueno, B., & Leza, J. C. (2017). Paliperidone reverts Toll-like receptor 3 signaling pathway activation and cognitive deficits in a maternal immune activation mouse model of schizophrenia. *Neuropharmacology*, *116*, 196–207. https://doi.org/10.1016/j.neuropharm.2016.12.025

Machón, R. A., Mednick, S. A., & Huttunen, M. O. (1997). Adult Major Affective Disorder After Prenatal Exposure to an Influenza Epidemic. *Archives of General Psychiatry*, *54*(4), 322–328. https://doi.org/10.1001/archpsyc.1997.01830160040006

Mali, I., Payne, M., King, C., Maze, T. R., Davison, T., Challans, B., Bossmann, S. H., & Plakke, B. (2023). Adolescent female valproic acid rats have impaired extra-dimensional shifts of attention and enlarged anterior cingulate cortices. *Brain Research*, *1800*, 148199. https://doi.org/10.1016/j.brainres.2022.148199

Mandic-Maravic, V., Grujicic, R., Milutinovic, L., Munjiza-Jovanovic, A., & Pejovic-Milovancevic, M. (2022). Dopamine in Autism Spectrum Disorders—Focus on D2/D3 Partial Agonists and Their Possible Use in Treatment. *Frontiers in Psychiatry*, *12*, 787097. https://doi.org/10.3389/fpsyt.2021.787097

Massrali, A., Adhya, D., Srivastava, D. P., Baron-Cohen, S., & Kotter, M. R. (2022). Virus-Induced Maternal Immune Activation as an Environmental Factor in the Etiology of Autism and Schizophrenia. *Frontiers in Neuroscience*, *16*. https://www.frontiersin.org/articles/10.3389/fnins.2022.834058

McGrath, J. J., Pemberton, M. R., Welham, J. L., & Murray, R. M. (1994). Schizophrenia and the influenza epidemics of 1954, 1957 and 1959: A southern hemisphere study. *Schizophrenia Research*, *14*(1), 1–8. https://doi.org/10.1016/0920-9964(94)90002-7

Meares, G. P., Ma, X., Qin, H., & Benveniste, E. N. (2012). Regulation of CCL20 expression in astrocytes by IL-6 and IL-17. *Glia*, *60*(5), 771–781. https://doi.org/10.1002/glia.22307

Meck, W. H., Smith, R. A., & Williams, C. L. (1988). Pre- and postnatal choline supplementation produces long-term facilitation of spatial memory. *Developmental Psychobiology*, *21*(4), 339–353. https://doi.org/10.1002/dev.420210405

Medina, M. V., Sapochnik, D., Garcia Solá, M., & Coso, O. (2020). Regulation of the Expression of Heme Oxygenase-1: Signal Transduction, Gene Promoter Activation, and Beyond. *Antioxidants & Redox Signaling*, *32*(14), 1033–1044. https://doi.org/10.1089/ars.2019.7991

Mednick, S. A., Machon, R. A., Huttunen, M. O., & Bonett, D. (1988). Adult schizophrenia following prenatal exposure to an influenza epidemic. *Archives of General Psychiatry*, *45*(2), 189–192. https://doi.org/10.1001/archpsyc.1988.01800260109013

Menninger, K. A. (1919). PSYCHOSES ASSOCIATED WITH INFLUENZA: I. GENERAL DATA: STATISTICAL ANALYSIS. *Journal of the American Medical Association*, *72*(4), 235–241. https://doi.org/10.1001/jama.1919.02610040001001

Menninger, K. A. (1926). Influenza and schizophrenia. *American Journal of Psychiatry*, *82*(4), 469–529. https://doi.org/10.1176/ajp.82.4.469

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., & Fatemi, S. H. (2009). In-vivo rodent models for the experimental investigation of prenatal immune activation effects in neurodevelopmental brain disorders. *Neuroscience & Biobehavioral Reviews*, *33*(7), 1061–1079. https://doi.org/10.1016/j.neubiorev.2009.05.001

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

Milovanovic, J., Arsenijevic, A., Stojanovic, B., Kanjevac, T., Arsenijevic, D., Radosavljevic, G., Milovanovic, M., & Arsenijevic, N. (2020). Interleukin-17 in Chronic Inflammatory Neurological Diseases. *Frontiers in Immunology*, *11*. https://www.frontiersin.org/articles/10.3389/fimmu.2020.00947

Mitsushima, D. (2011). Sex differences in the septo-hippocampal cholinergic system in rats: Behavioral consequences. *Current Topics in Behavioral Neurosciences*, *8*, 57–71. https://doi.org/10.1007/7854_2010_95

Mittli, D. (2023). Inflammatory processes in the prefrontal cortex induced by systemic immune challenge: Focusing on neurons. *Brain, Behavior, & Immunity - Health*, *34*, 100703. https://doi.org/10.1016/j.bbih.2023.100703

Monteiro, V. R. S., Andrade, C. B. V., Gomes, H. R., Reginatto, M. W., Império, G. E., Fontes, K. N., Spiess, D. A., Rangel-Junior, W. S., Nascimento, V. M. O., Lima, C. O. S., Sousa, R. P. C., Bloise, F. F., Matthews, S. G., Bloise, E., Pimentel-Coelho, P. M., & Ortiga-Carvalho, T. M. (2022). Mid-pregnancy poly(I:C) viral mimic disrupts placental ABC transporter expression and leads to long-term offspring motor and cognitive dysfunction. *Scientific Reports*, *12*(1). https://doi.org/10.1038/s41598-022-14248-0

Moreno, H. C., de Brugada, I., Carias, D., & Gallo, M. (2013). Long-lasting effects of prenatal dietary choline availability on object recognition memory ability in adult rats. *Nutritional Neuroscience*, *16*(6), 269–274. https://doi.org/10.1179/1476830513Y.0000000055

Murphy, S. K., Fineberg, A. M., Maxwell, S. D., Alloy, L. B., Zimmermann, L., Krigbaum, N. Y., Cohn, B. A., Drabick, D. A. G., & Ellman, L. M. (2017). Maternal infection and stress during pregnancy and depressive symptoms in adolescent offspring. *Psychiatry Research*, *257*, 102–110. https://doi.org/10.1016/j.psychres.2017.07.025

Muto, E., Okada, T., Yamanaka, T., Uchino, H., & Inazu, M. (2023). Licochalcone E, a β-Amyloid Aggregation Inhibitor, Regulates Microglial M1/M2 Polarization via Inhibition of CTL1-Mediated Choline Uptake. *Biomolecules*, *13*(2), 191. https://doi.org/10.3390/biom13020191

Naviaux, R. K., Zolkipli, Z., Wang, L., Nakayama, T., Naviaux, J. C., Le, T. P., Schuchbauer, M. A., Rogac, M., Tang, Q., Dugan, L. L., & Powell, S. B. (2013). Antipurinergic therapy corrects the autism-like features in the poly(IC) mouse model. *PloS One*, *8*(3), e57380. https://doi.org/10.1371/journal.pone.0057380

Neumann, H., Kotter, M. R., & Franklin, R. J. M. (2009). Debris clearance by microglia: An essential link between degeneration and regeneration. *Brain*, *132*(2), 288–295. https://doi.org/10.1093/brain/awn109

Nielsen, T. C., Nassar, N., Shand, A. W., Jones, H., Guastella, A. J., Dale, R. C., & Lain, S. J. (2021). Association of Maternal Autoimmune Disease With Attention-Deficit/Hyperactivity Disorder in Children. *JAMA Pediatrics*, *175*(3), e205487. https://doi.org/10.1001/jamapediatrics.2020.5487

O'Callaghan, E., Sham, P., Takei, N., Glover, G., & Murray, R. M. (1991). Schizophrenia after prenatal exposure to 1957 A2 influenza epidemic. *Lancet (London, England)*, *337*(8752), 1248–1250. https://doi.org/10.1016/0140-6736(91)92919-s

Okada, T., Muto, E., Yamanaka, T., Uchino, H., & Inazu, M. (2022). Functional Expression of Choline Transporters in Microglia and Their Regulation of Microglial M1/M2 Polarization. *International Journal of Molecular Sciences*, *23*(16), 8924. https://doi.org/10.3390/ijms23168924

Onore, C. E., Schwartzer, J. J., Careaga, M., Berman, R. F., & Ashwood, P. (2014). Maternal immune activation leads to activated inflammatory macrophages in offspring. *Brain, Behavior, and Immunity*, *38*, 220–226. https://doi.org/10.1016/j.bbi.2014.02.007

Osborne, A., Solowij, N., Babic, I., Huang, X.-F., & Weston-Green, K. (2017). Improved Social Interaction, Recognition and Working Memory with Cannabidiol Treatment in a Prenatal Infection (poly I:C) Rat Model. *Neuropsychopharmacology*, *42*(7), Article 7. https://doi.org/10.1038/npp.2017.40

Osborne, L., Brar, A., & Klein, S. (2019). The role of Th17 cells in the pathophysiology of pregnancy and perinatal mood and anxiety disorders. *Brain, Behavior, and Immunity*, *76*, 7–16. https://doi.org/10.1016/j.bbi.2018.11.015

Parboosing, R., Bao, Y., Shen, L., Schaefer, C. A., & Brown, A. S. (2013). Gestational influenza and bipolar disorder in adult offspring. *JAMA Psychiatry*, *70*(7), 677–685. https://doi.org/10.1001/jamapsychiatry.2013.896

Park, H.-J., Shim, H.-S., An, K., Starkweather, A., Kim, K. S., & Shim, I. (2015). IL-4 Inhibits IL-1β-Induced Depressive-Like Behavior and Central Neurotransmitter Alterations. *Mediators of Inflammation*, *2015*, 941413. https://doi.org/10.1155/2015/941413

Paylor, J. W., Lins, B. R., Greba, Q., Moen, N., De Moraes, R. S., Howland, J. G., & Winship, I. R. (2016). Developmental disruption of perineuronal nets in the medial prefrontal cortex after maternal immune activation. *Scientific Reports*, *6*. Scopus. https://doi.org/10.1038/srep37580

Pineda, D. A., Palacio, L. G., Puerta, I. C., Merchán, V., Arango, C. P., Galvis, A. Y., Gómez, M., Aguirre, D. C., Lopera, F., & Arcos-Burgos, M. (2007). Environmental influences that affect attention deficit/hyperactivity disorder. *European Child & Adolescent Psychiatry*, *16*(5), 337–346. https://doi.org/10.1007/s00787-007-0605-4

Prado, V. F., Janickova, H., Al-Onaizi, M. A., & Prado, M. A. M. (2017). Cholinergic circuits in cognitive flexibility. *Neuroscience*, *345*, 130–141. https://doi.org/10.1016/j.neuroscience.2016.09.013

Pu, H., Wang, Y., Yang, T., Leak, R. K., Stetler, R. A., Yu, F., Zhang, W., Shi, Y., Hu, X., Yin, K., Hitchens, T. K., Dixon, C. E., Bennett, M. V. L., & Chen, J. (2023). Interleukin-4 mitigates anxiety-like behavior and loss of neurons and fiber tracts in limbic structures in a microglial PPARγ-dependent manner after traumatic brain injury. *Neurobiology of Disease*, *180*, 106078. https://doi.org/10.1016/j.nbd.2023.106078

Rački, V., Petrić, D., Kučić, N., Gržeta, N., Jurdana, K., & Rončević-Gržeta, I. (2016). Cortical gray matter loss in schizophrenia: Could microglia be the culprit? *Medical Hypotheses*, *88*, 18–21. https://doi.org/10.1016/j.mehy.2015.12.021

Ragozzino, M. E. (2007). The Contribution of the Medial Prefrontal Cortex, Orbitofrontal Cortex, and Dorsomedial Striatum to Behavioral Flexibility. *Annals of the New York Academy of Sciences*, *1121*(1), 355–375. https://doi.org/10.1196/annals.1401.013

Ragozzino, M. E., Detrick, S., & Kesner, R. P. (1999). Involvement of the Prelimbic–Infralimbic Areas of the Rodent Prefrontal Cortex in Behavioral Flexibility for Place and Response Learning. *The Journal of Neuroscience*, *19*(11), 4585–4594. https://doi.org/10.1523/JNEUROSCI.19-11-04585.1999

Rana, A., & Musto, A. E. (2018). The role of inflammation in the development of epilepsy. *Journal of Neuroinflammation*, *15*(1), 144. https://doi.org/10.1186/s12974-018-1192-7

Ratnayake, U., Quinn, T., Walker, D. W., & Dickinson, H. (2013). Cytokines and the neurodevelopmental basis of mental illness. *Frontiers in Neuroscience*, *7 OCT*. https://doi.org/10.3389/fnins.2013.00180

Reale, M., Costantini, E., & Greig, N. H. (2021). Cytokine Imbalance in Schizophrenia. From Research to Clinic: Potential Implications for Treatment. *Frontiers in Psychiatry*, *12*. https://www.frontiersin.org/articles/10.3389/fpsyt.2021.536257

Reddy, L. F., Waltz, J. A., Green, M. F., Wynn, J. K., & Horan, W. P. (2016). Probabilistic Reversal Learning in Schizophrenia: Stability of Deficits and Potential Causal Mechanisms. *Schizophrenia Bulletin*, *42*(4), 942–951. https://doi.org/10.1093/schbul/sbv226

Reeh, H., Rudolph, N., Billing, U., Christen, H., Streif, S., Bullinger, E., Schliemann-Bullinger, M., Findeisen, R., Schaper, F., Huber, H. J., & Dittrich, A. (2019). Response to IL-6 trans- and IL-6 classic signalling is determined by the ratio of the IL-6 receptor α to gp130 expression: Fusing experimental insights and dynamic modelling. *Cell Communication and Signaling : CCS*, *17*, 46. https://doi.org/10.1186/s12964-019-0356-0

Reisinger, S., Khan, D., Kong, E., Berger, A., Pollak, A., & Pollak, D. D. (2015). The Poly(I:C)-induced maternal immune activation model in preclinical neuropsychiatric drug discovery. *Pharmacology and Therapeutics*, *149*, 213–226. https://doi.org/10.1016/j.pharmthera.2015.01.001

Ricceri, L., De Filippis, B., Fuso, A., & Laviola, G. (2011). Cholinergic hypofunction in MeCP2-308 mice: Beneficial neurobehavioural effects of neonatal choline supplementation. *Behavioural Brain Research*, *221*(2), 623–629. https://doi.org/10.1016/j.bbr.2011.03.051

Rock, R. B., Gekker, G., Aravalli, R. N., Hu, S., Sheng, W. S., & Peterson, P. K. (2008). Potentiation of HIV-1 Expression in Microglial Cells by Nicotine: Involvement of Transforming Growth Factor-β1. *Journal of Neuroimmune Pharmacology*, *3*(3), 143–149. https://doi.org/10.1007/s11481-007-9098-7

Rodrigues-Amorim, D., Rivera-Baltanás, T., Spuch, C., Caruncho, H. J., González-Fernandez, Á., Olivares, J. M., & Agís-Balboa, R. C. (2018). Cytokines dysregulation in schizophrenia: A systematic review of psychoneuroimmune relationship. *Schizophrenia Research*, *197*, 19–33. https://doi.org/10.1016/j.schres.2017.11.023

Ross, H., Guo, Y., Coleman, K., Ousley, O., & Miller, A. (2013). Association of IL-12p70 and IL-6:IL-10 ratio with autism-related behaviors in 22q11.2 deletion syndrome: A preliminary report. *Brain, Behavior, and Immunity*, *31*, 76–81. https://doi.org/10.1016/j.bbi.2012.12.021

Ross, R., Hunter, S., Hoffman, M., McCarthy, L., Chambers, B. M., Law, A., Leonard, S., Zerbe, G., & Freedman, R. (2016). Perinatal phosphatidylcholine supplementation and early childhood behavior problems: Evidence for CHRNA7 moderation. *American Journal of Psychiatry*, *173*(5), 509–516. https://doi.org/10.1176/appi.ajp.2015.15091188

Saha, S., Buttari, B., Panieri, E., Profumo, E., & Saso, L. (2020). An Overview of Nrf2 Signaling Pathway and Its Role in Inflammation. *Molecules*, *25*(22), 5474. https://doi.org/10.3390/molecules25225474

Salinas, M., Wang, J., Rosa de Sagarra, M., Martín, D., Rojo, A. I., Martin-Perez, J., Ortiz de Montellano, P. R., & Cuadrado, A. (2004). Protein kinase Akt/PKB phosphorylates heme oxygenase-1 in vitro and in vivo. *FEBS Letters*, *578*(1–2), 90–94. https://doi.org/10.1016/j.febslet.2004.10.077

Schaafsma, W., Basterra, L. B., Jacobs, S., Brouwer, N., Meerlo, P., Schaafsma, A., Boddeke, E. W. G. M., & Eggen, B. J. L. (2017). Maternal inflammation induces immune activation of fetal microglia and leads to disrupted microglia immune responses, behavior, and learning performance in adulthood. *Neurobiology of Disease*, *106*, 291–300. https://doi.org/10.1016/j.nbd.2017.07.017

Semple, B. D., O'Brien, T. J., Gimlin, K., Wright, D. K., Kim, S. E., Casillas-Espinosa, P. M., Webster, K. M., Petrou, S., & Noble-Haeusslein, L. J. (2017). Interleukin-1 Receptor in Seizure Susceptibility after Traumatic Injury to the Pediatric Brain. *The Journal of Neuroscience*, *37*(33), 7864–7877. https://doi.org/10.1523/JNEUROSCI.0982-17.2017

Shi, L., Fatemi, S. H., Sidwell, R. W., & Patterson, P. H. (2003). Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *23*(1), 297–302. https://doi.org/10.1523/JNEUROSCI.23-01-00297.2003

Silva, D., Colvin, L., Hagemann, E., & Bower, C. (2014). Environmental risk factors by gender associated with attention-deficit/hyperactivity disorder. *Pediatrics*, *133*(1), e14-22. https://doi.org/10.1542/peds.2013-1434

Smith, S. E. P., Hsiao, E., & Patterson, P. H. (2010). Activation of the Maternal Immune System as a Risk Factor for Neuropsychiatric Disorders. In A. W. Zimmerman & S. L. Connors (Eds.), *Maternal Influences on Fetal Neurodevelopment: Clinical and Research Aspects* (pp. 97–115). Springer. https://doi.org/10.1007/978-1-60327-921-5_7

Smith, S. E. P., Li, J., Garbett, K., Mirnics, K., & Patterson, P. H. (2007). Maternal immune activation alters fetal brain development through interleukin-6. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *27*(40), 10695–10702. https://doi.org/10.1523/JNEUROSCI.2178-07.2007

Snider, S. A., Margison, K. D., Ghorbani, P., LeBlond, N. D., O'Dwyer, C., Nunes, J. R. C., Nguyen, T., Xu, H., Bennett, S. A. L., & Fullerton, M. D. (2018). Choline transport links macrophage phospholipid metabolism and inflammation. *Journal of Biological Chemistry*, *293*(29), 11600–11611. https://doi.org/10.1074/jbc.RA118.003180

Soghomonian, J.-J. (2023). The cortico-striatal circuitry in autism-spectrum disorders: A balancing act. *Frontiers in Cellular Neuroscience*, *17*, 1329095. https://doi.org/10.3389/fncel.2023.1329095

Stevens, K. E., Choo, K. S., Stitzel, J. A., Marks, M. J., & Adams, C. E. (2014). Long-term improvements in sensory inhibition with gestational choline supplementation linked to α7 nicotinic receptors through studies in Chrna7 null mutation mice. *Brain Research*, *1552*, 26–33. https://doi.org/10.1016/j.brainres.2014.01.022

Su, Y., Lian, J., Hodgson, J., Zhang, W., & Deng, C. (2022). Prenatal Poly I:C Challenge Affects Behaviors and Neurotransmission via Elevated Neuroinflammation Responses in Female Juvenile Rats. *International Journal of Neuropsychopharmacology*, *25*(2), 160–171. https://doi.org/10.1093/ijnp/pyab087

Suzuki, T., Hide, I., Matsubara, A., Hama, C., Harada, K., Miyano, K., Andrä, M., Matsubayashi, H., Sakai, N., Kohsaka, S., Inoue, K., & Nakata, Y. (2006). Microglial α7 nicotinic acetylcholine receptors drive a phospholipase C/IP3 pathway and modulate the cell activation toward a neuroprotective role. *Journal of Neuroscience Research*, *83*(8), 1461–1470. https://doi.org/10.1002/jnr.20850

Tait, D., Bowman, E., Miller, S., Dovlatyan, M., Sanchez, C., & Brown, V. (2021). Escitalopram Restores Reversal Learning Impairments in Rats with Lesions of Orbital Frontal Cortex. In S. Löbner, T. Gamerschlag, T. Kalenscher, M. Schrenk, & H. Zeevat (Eds.), *Concepts, Frames and Cascades in Semantics, Cognition and Ontology* (pp. 389–409). Springer International Publishing. https://doi.org/10.1007/978-3-030-50200-3_18

Tait, D., Chase, E., & Brown, V. (2014). Attentional Set-Shifting in Rodents: A Review of Behavioural Methods and Pharmacological Results. *Current Pharmaceutical Design*, *20*(31), 5046–5059. https://doi.org/10.2174/1381612819666131216115802

Tan, H.-Y., Nicodemus, K., Chen, Q., Li, Z., Brooke, J., Honea, R., Kolachana, B., Straub, R., Meyer-Lindenberg, A., Sei, Y., Mattay, V., Callicott, J., & Weinberger, D. (2008). Genetic variation in AKT1 is linked to dopamine-associated prefrontal cortical structure and function in humans. *The Journal of Clinical Investigation*, *118*(6), 2200–2208. https://doi.org/10.1172/JCI34725

Temmingh, H., & Stein, D. J. (2015). Anxiety in Patients with Schizophrenia: Epidemiology and Management. *CNS Drugs*, *29*(10), 819–832. https://doi.org/10.1007/s40263-015-0282-7

Tendilla-Beltrán, H., Sanchez-Islas, N. del C., Marina-Ramos, M., Leza, J. C., & Flores, G. (2021). The prefrontal cortex as a target for atypical antipsychotics in schizophrenia, lessons of neurodevelopmental animal models. *Progress in Neurobiology*, *199*, 101967. https://doi.org/10.1016/j.pneurobio.2020.101967

Tessitore, L., Sesca, E., Greco, M., Pani, P., & Dianzani, M. U. (1995). Sexually differentiated response to choline in choline deficiency and ethionine intoxication. *International Journal of Experimental Pathology*, *76*(2), 125–129.

Thomas, J. D., Garrison, M., & O'Neill, T. M. (2004). Perinatal choline supplementation attenuates behavioral alterations associated with neonatal alcohol exposure in rats. *Neurotoxicology and Teratology*, *26*(1), 35–45. https://doi.org/10.1016/j.ntt.2003.10.002

Torrey, E. F., Miller, J., Rawlings, R., & Yolken, R. H. (1997). Seasonality of births in schizophrenia and bipolar disorder: A review of the literature. *Schizophrenia Research*, *28*(1), 1–38. https://doi.org/10.1016/S0920-9964(97)00092-3

Tryon, S. C., Sakamoto, I. M., Kaigler, K. F., Gee, G., Turner, J., Bartley, K., Fadel, J. R., & Wilson, M. A. (2023). ChAT::Cre transgenic rats show sex-dependent altered fear behaviors, ultrasonic vocalizations and cholinergic marker expression. *Genes, Brain, and Behavior*, *22*(1), e12837. https://doi.org/10.1111/gbb.12837

Uranova, N. A., Vikhreva, O. V., & Rakhmanova, V. I. (2021). Abnormal microglial reactivity in gray matter of the prefrontal cortex in schizophrenia. *Asian Journal of Psychiatry*, *63*, 102752. https://doi.org/10.1016/j.ajp.2021.102752

Vercammen, E., Staal, J., & Beyaert, R. (2008). Sensing of Viral Infection and Activation of Innate Immunity by Toll-Like Receptor 3. *Clinical Microbiology Reviews*, *21*(1), 13–25. https://doi.org/10.1128/cmr.00022-07

Vigli, D., Palombelli, G., Fanelli, S., Calamandrei, G., Canese, R., Mosca, L., Scattoni, M. L., & Ricceri, L. (2020). Maternal Immune Activation in Mice Only Partially Recapitulates the

Autism Spectrum Disorders Symptomatology. *Neuroscience*, *445*, 109–119. https://doi.org/10.1016/j.neuroscience.2020.05.009

Vorhees, C. V., Graham, D. L., Braun, A. A., Schaefer, T. L., Skelton, M. R., Richtand, N. M., & Williams, M. T. (2015). Prenatal immune challenge in rats: Effects of polyinosinic-polycytidylic acid on spatial learning, prepulse inhibition, conditioned fear, and responses to MK-801 and amphetamine. *Neurotoxicology and Teratology*, *47*, 54–65. https://doi.org/10.1016/j.ntt.2014.10.007

Waddell, J., Hill, E., Tang, S., Jiang, L., Xu, S., & Mooney, S. M. (2020). Choline Plus Working Memory Training Improves Prenatal Alcohol-Induced Deficits in Cognitive Flexibility and Functional Connectivity in Adulthood in Rats. *Nutrients*, *12*(11), 3513. https://doi.org/10.3390/nu12113513

Walf, A. A., & Frye, C. A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nature Protocols*, *2*(2), 322–328. https://doi.org/10.1038/nprot.2007.44

Wallace, J., Marston, H. M., McQuade, R., & Gartside, S. E. (2014). Evidence that aetiological risk factors for psychiatric disorders cause distinct patterns of cognitive deficits. *European Neuropsychopharmacology*, *24*(6), 879–889. https://doi.org/10.1016/j.euroneuro.2013.12.005

Walther, S., & Strik, W. (2012). Motor Symptoms and Schizophrenia. *Neuropsychobiology*, *66*(2), 77–92. https://doi.org/10.1159/000339456

Wei, H., Chadman, K. K., McCloskey, D. P., Sheikh, A. M., Malik, M., Brown, W. T., & Li, X. (2012). Brain IL-6 elevation causes neuronal circuitry imbalances and mediates autism-like behaviors. *Biochimica Et Biophysica Acta*, *1822*(6), 831–842. https://doi.org/10.1016/j.bbadis.2012.01.011

Wei, H., Zou, H., Sheikh, A. M., Malik, M., Dobkin, C., Brown, W. T., & Li, X. (2011). IL-6 is increased in the cerebellum of autistic brain and alters neural cell adhesion, migration and synaptic formation. *Journal of Neuroinflammation*, *8*, 52. https://doi.org/10.1186/1742-2094-8-52

Werenberg Dreier, J., Nybo Andersen, A.-M., Hvolby, A., Garne, E., Kragh Andersen, P., & Berg-Beckhoff, G. (2016). Fever and infections in pregnancy and risk of attention deficit/hyperactivity disorder in the offspring. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *57*(4), 540–548. https://doi.org/10.1111/jcpp.12480

West, P. K., Viengkhou, B., Campbell, I. L., & Hofer, M. J. (2019). Microglia responses to interleukin-6 and type I interferons in neuroinflammatory disease. *Glia*, *67*(10), 1821–1841. https://doi.org/10.1002/glia.23634

Wienecke, J., Hebel, K., Hegel, K. J., Pierau, M., Brune, T., Reinhold, D., Pethe, A., & Brunner-Weinzierl, M. C. (2012). Pro-inflammatory effector Th cells transmigrate through anti-inflammatory environments into the murine fetus. *Placenta*, *33*(1), 39–46. https://doi.org/10.1016/j.placenta.2011.10.014

Williams, B., & Christakou, A. (2022). Dissociable roles for the striatal cholinergic system in different flexibility contexts. *IBRO Neuroscience Reports*, *12*, 260–270. https://doi.org/10.1016/j.ibneur.2022.03.007

Wischhof, L., Irrsack, E., Osorio, C., & Koch, M. (2015). Prenatal LPS-exposure – a neurodevelopmental rat model of schizophrenia – differentially affects cognitive functions, myelination and parvalbumin expression in male and female offspring.

Progress in Neuro-Psychopharmacology and Biological Psychiatry, *57*, 17–30. https://doi.org/10.1016/j.pnpbp.2014.10.004

Wong, H., & Hoeffer, C. (2018). Maternal IL-17A in autism. *Experimental Neurology*, *299*, 228–240. https://doi.org/10.1016/j.expneurol.2017.04.010

Wu, B., Dyer, R., King, D. J., Richardson, K., & Innis, S. (2012). Early second trimester maternal plasma choline and betaine are related to measures of early cognitive development in term infants. *PLoS ONE*, *7*(8). https://doi.org/10.1371/journal.pone.0043448

Wu, W., Adams, C., Stevens, K., Chow, K., Freedman, R., & Patterson, P. (2015). The interaction between maternal immune activation and alpha 7 nicotinic acetylcholine receptor in regulating behaviors in the offspring. *Brain, Behavior, and Immunity*, *46*, 192–202. https://doi.org/10.1016/j.bbi.2015.02.005

Wu, Y., & Hsieh, H. (2022). Roles of Heme Oxygenase-1 in Neuroinflammation and Brain Disorders. *Antioxidants*, *11*(5), 923. https://doi.org/10.3390/antiox11050923

Xie, J., Huang, L., Li, X., Li, H., Zhou, Y., Zhu, H., Pan, T., Kendrick, K. M., & Xu, W. (2017). Immunological cytokine profiling identifies TNF-α as a key molecule dysregulated in autistic children. *Oncotarget*, *8*(47), 82390–82398. https://doi.org/10.18632/oncotarget.19326

Yan, J., Jiang, X., West, A. A., Perry, C. A., Malysheva, O. V., Devapatla, S., Pressman, E., Vermeylen, F., Stabler, S. P., Allen, R. H., & Caudill, M. A. (2012). Maternal choline intake modulates maternal and fetal biomarkers of choline metabolism in humans. *American Journal of Clinical Nutrition*, *95*(5), 1060–1071. https://doi.org/10.3945/ajcn.111.022772

Yang, J., Shi, S. Q., Shi, L., Fang, D., Liu, H., & Garfield, R. E. (2014). Nicotine, an α7 nAChR agonist, reduces lipopolysaccharide-induced inflammatory responses and protects fetuses in pregnant rats. *American Journal of Obstetrics and Gynecology*, *211*(5), 538.e1-538.e7. https://doi.org/10.1016/j.ajog.2014.04.026

Yang, L., Liu, C., Li, W., Ma, Y., Huo, S., Ozathaley, A., Ren, J., Yuan, W., Ni, H., Li, D., Zhang, J., & Liu, Z. (2021). Depression-like behavior associated with E/I imbalance of mPFC and amygdala without TRPC channels in mice of knockout IL-10 from microglia. *Brain, Behavior, and Immunity*, *97*, 68–78. https://doi.org/10.1016/j.bbi.2021.06.015

Yerys, B. E., Wallace, G. L., Harrison, B., Celano, M. J., Giedd, J. N., & Kenworthy, L. E. (2009). Set-shifting in children with autism spectrum disorders: Reversal shifting deficits on the Intradimensional/Extradimensional Shift Test correlate with repetitive behaviors. *Autism*, *13*(5), 523–538. https://doi.org/10.1177/1362361309335716

Youssef, M. E., Moustafa, Y., & Abdelrazek, H. (2020). Molecular mechanisms of α7-nAchR-mediated anti-inflammatory effects. *Indian Journal of Physiology and Pharmacology*, *64*(3), 158–173. https://doi.org/10.25259/IJPP_129_2020

Yu, L., Miao, J., Li, W., Chen, N., & Chen, Q. (2020). Intranasal IL-4 Administration Alleviates Functional Deficits of Periventricular Leukomalacia in Neonatal Mice. *Frontiers in Neurology*, *11*, 930. https://doi.org/10.3389/fneur.2020.00930

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

Zeisel, S. H. (2006a). Choline: Critical role during fetal development and dietary requirements in adults. *Annual Review of Nutrition*, *26*, 229–250. https://doi.org/10.1146/annurev.nutr.26.061505.111156

Zeisel, S. H. (2006b). The fetal origins of memory: The role of dietary choline in optimal brain development. *The Journal of Pediatrics*, *149*(5 Suppl), S131-136. https://doi.org/10.1016/j.jpeds.2006.06.065

Zeisel, S. H., & Da Costa, K. A. (2009). Choline: An essential nutrient for public health. *Nutrition Reviews*, *67*(11), 615–623. https://doi.org/10.1111/j.1753-4887.2009.00246.x

Zerbo, O., Iosif, A. M., Delwiche, L., Walker, C., & Hertz-Picciotto, I. (2011). Month of conception and risk of Autism. *Epidemiology*, *22*(4), 469–475. https://doi.org/10.1097/EDE.0b013e31821d0b53

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

Zhang, L., Lin, C., Zhu, J., He, Y., Zhan, M., Xia, X., Yang, N., Yang, K., Wang, B., Zhong, Z., Wang, Y., Ding, W., & Yang, Y. (2023). Restoring prefrontal cortical excitation-inhibition balance with cannabidiol ameliorates neurobehavioral abnormalities in a mouse model of neurodevelopmental disorders. *Neuropharmacology*, *240*, 109715. https://doi.org/10.1016/j.neuropharm.2023.109715

Zhang, M., Bao, J., Liu, Y., Yang, J., Zhang, G., & Liu, H. (2016). 40 Gestational choline supplementation prevents inflammatory responses of lipopolysaccharide-induced preeclampsia in pregnant rats: Immune and inflammatory mechanisms. *Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health*, *6*(3), 197. https://doi.org/10.1016/j.preghy.2016.08.122

Zhang, M., Han, X., Bao, J., Yang, J., Shi, S.-Q., Garfield, R. E., & Liu, H. (2018). Choline Supplementation During Pregnancy Protects Against Gestational Lipopolysaccharide-Induced Inflammatory Responses. *Reproductive Sciences*, *25*(1), 74–85. https://doi.org/10.1177/1933719117702247

Zhang, Q., Lu, Y., Bian, H., Guo, L., & Zhu, H. (2017). Activation of the α7 nicotinic receptor promotes lipopolysaccharide-induced conversion of M1 microglia to M2. *American Journal of Translational Research*, *9*(3), 971–985.

Zhang, X., Ibi, M., Haga, R., Iwata, K., Matsumoto, M., Asaoka, N., Liu, J., Katsuyama, M., & Yabe-Nishimura, C. (2021). NOX1/NADPH oxidase affects the development of autism-like behaviors in a maternal immune activation model. *Biochemical and Biophysical Research Communications*, *534*, 59–66. https://doi.org/10.1016/j.bbrc.2020.11.070

Zhang, Y., Cazakoff, B. N., Thai, C. A., & Howland, J. G. (2012). Prenatal exposure to a viral mimetic alters behavioural flexibility in male, but not female, rats. *Neuropharmacology*, *62*(3), 1299–1307. https://doi.org/10.1016/j.neuropharm.2011.02.022

Zhang, Z., & van Praag, H. (2015). Maternal immune activation differentially impacts mature and adult-born hippocampal neurons in male mice. *Brain, Behavior, and Immunity*, *45*, 60–70. https://doi.org/10.1016/j.bbi.2014.10.010

Zhao, H., Zhang, H., Liu, S., Luo, W., Jiang, Y., & Gao, J. (2021). Association of Peripheral Blood Levels of Cytokines With Autism Spectrum Disorder: A Meta-Analysis. *Frontiers*

in Psychiatry, *12*.
https://www.frontiersin.org/journals/psychiatry/articles/10.3389/fpsyt.2021.670200
Zuckerman, L., & Weiner, I. (2005). Maternal immune activation leads to behavioral and pharmacological changes in the adult offspring. *Journal of Psychiatric Research*, *39*(3), 311–323. https://doi.org/10.1016/j.jpsychires.2004.08.008

Appendix A - Supplemental Figures

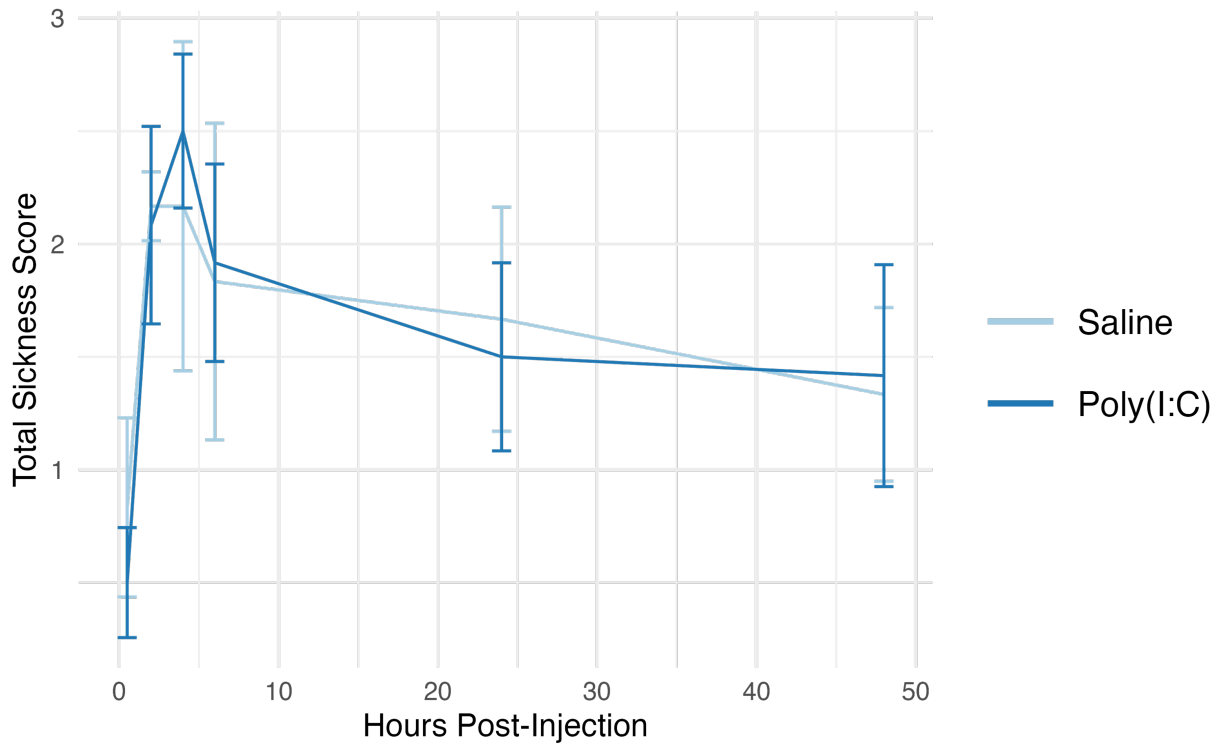

Figure A.1: Maternal Post-Injection Sickness Scores

Despite differences in maternal weight post-injection, there were no differences in symptom scores between saline and poly(I:C) dams at either 0.5, 1, 2, 4, 6, 24, and 48 hours post-injection. Both groups had increases in sickness behaviors (ptosis, lethargy, huddling, and piloerection) in the first six hours post-injection, and both gradually decreased over the next 42 hours. Since injections were given late in gestation (GD15), it is possible that typical pregnancy-related behaviors (e.g., lethargy) hampered our ability to detect differences in sickness behaviors. Error bars represent SEM.

Figure A.2: Rotarod Latencies by Trial

Although differences were found in maximum latency on the rotarod, there were no significant differences in latencies within each trial block. That is, there were no differences in latency on trials one, two, or three when compared to other groups on that same trial. The lack of differences between trials may be due to fatigue for those animals that performed better on trial two. Therefore, maximum latencies and improvement over trial one performance were analyzed instead. Error bars represent SEM.

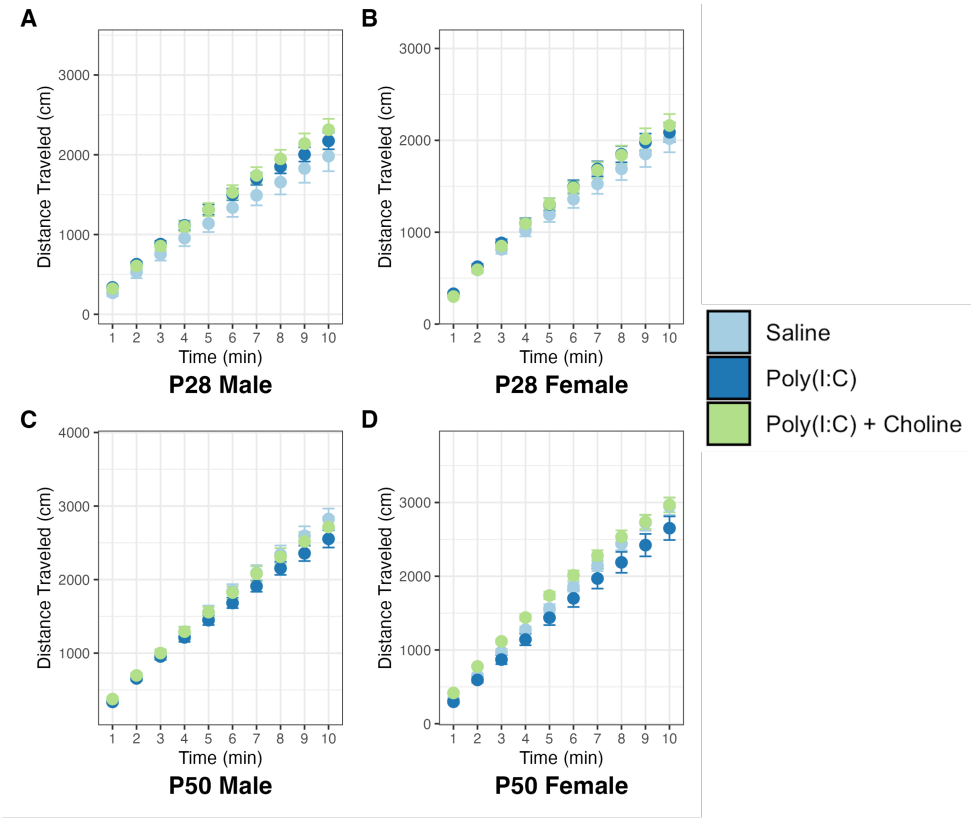

Figure A.3: Distance Traveled in the Open Field

There were no significant differences between groups in either sex in total distance traveled in the open field at either P28 or P50. However, P50 animals did travel significantly farther than P28 animals ($\chi^2_1 = 88.883$, $p < 0.001$), and there was a visual trend where poly(I:C)-only animals of both sexes traveled less total distance. Perhaps differences would be detectable on a variant of the task where animals spend more than 10 minutes in the open field. Error bars represent SEM.

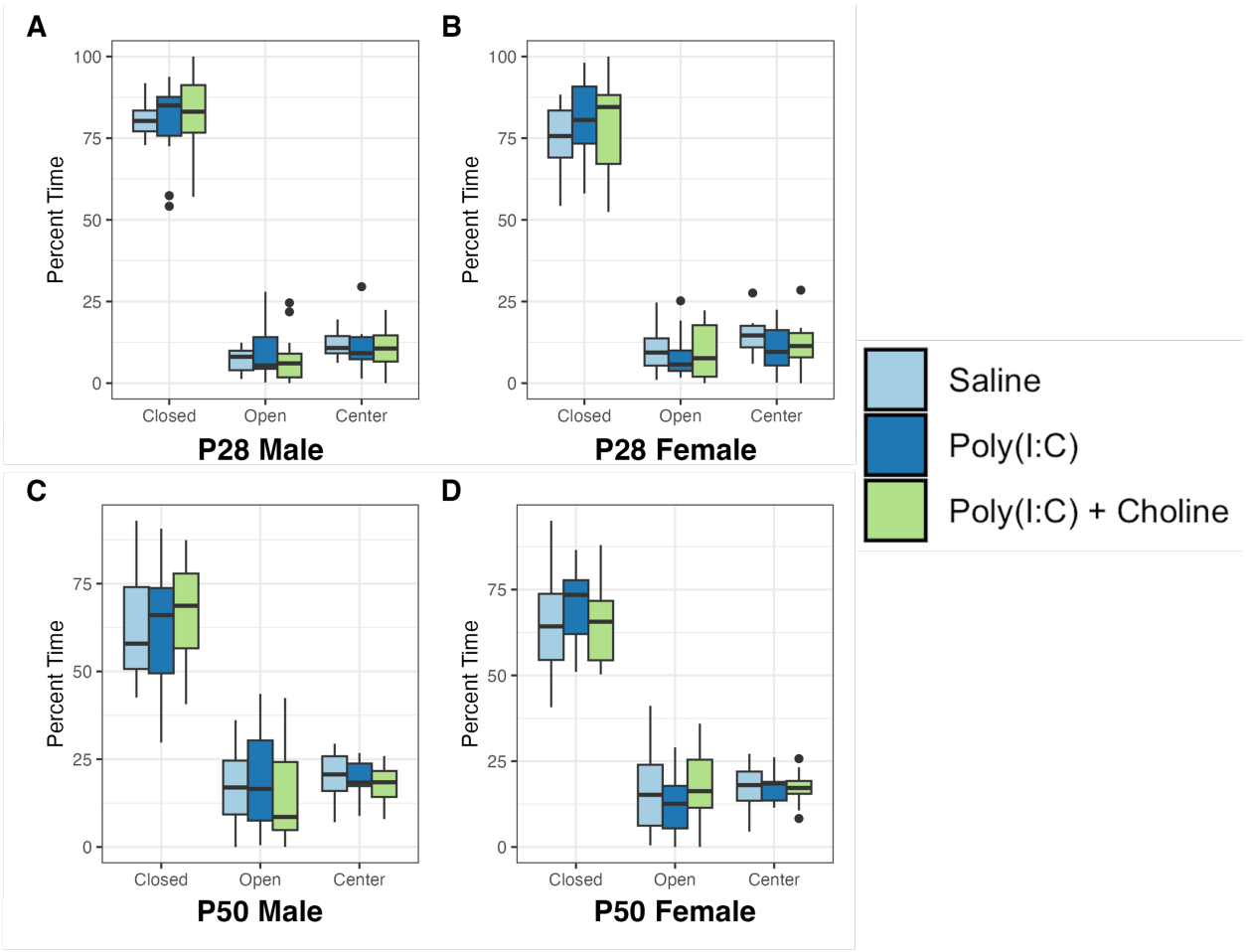

Figure A.4: Time Spent in Elevated Plus Maze Zones

There were no differences in time spent in either zone of the EPM at either age group, although differences between ages in these parameters were observed (see chapter 3). Error bars represent SEM.

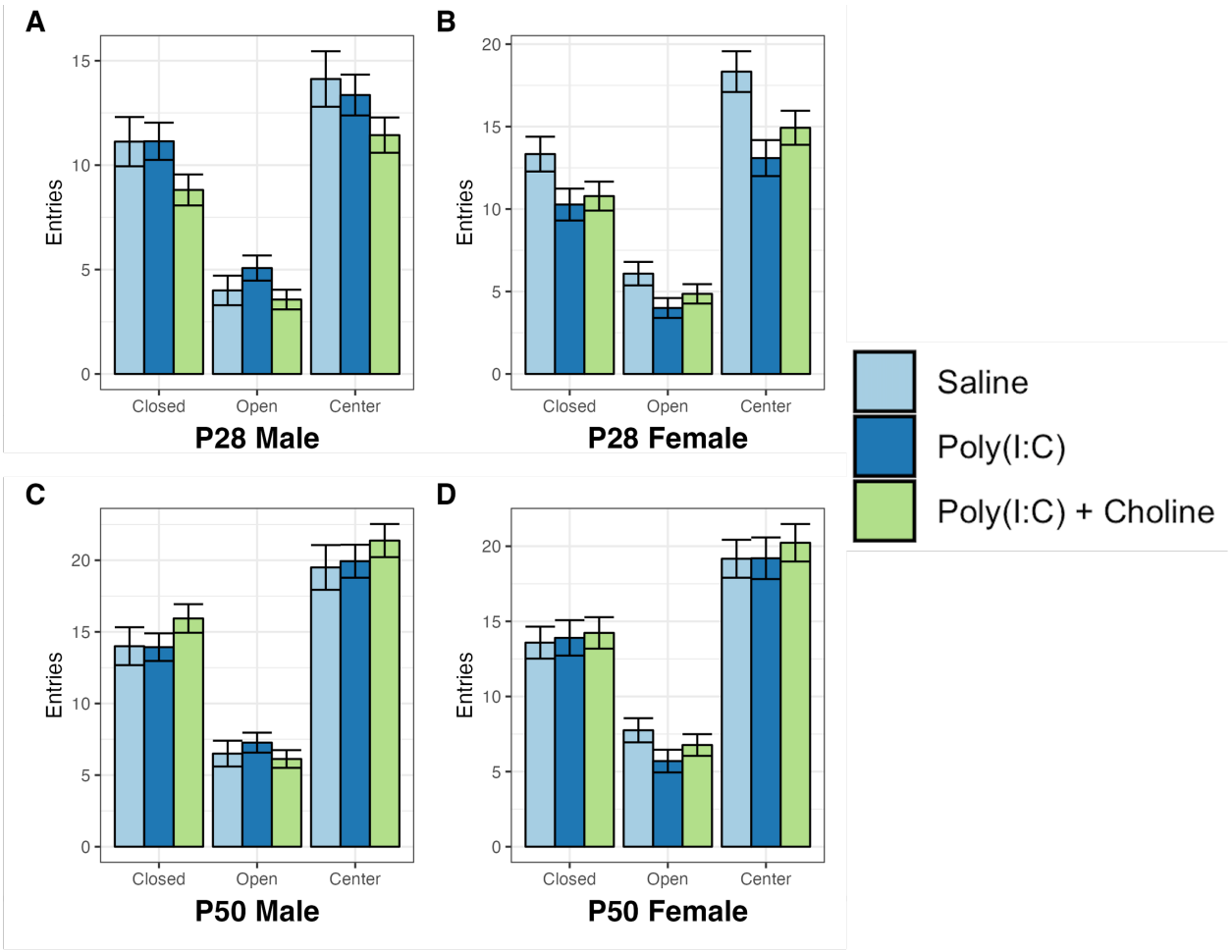

Figure A.5: Elevated Plus Maze Zone Entries

Although there were limited sex differences in entries into EPM zones, there were no differences by group at either P28 or P50 in either sex. Error bars represent SEM.