Prenatal Zinc Supplementation Ameliorates Hippocampal Astrocytes Activation and Inflammatory Cytokines Expression Induced by Lipopolysaccharide in a Rat Model of Maternal Immune Activation

Ebrahim Savareh1, Nahid Davoodian2,3*, Ronak Mousaviyan1, Maryam Ghasemi-Kasman4,5, Ali Atashabparvar6, Ebrahim Eftekhar1

1. Molecular Medicine Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.
2. Endocrinology and Metabolism Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.
3. Department of Clinical Biochemistry, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.
4. Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Science, Babol, Iran.
5. Neuroscience Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran.
6. Department of Pathology, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.

* Corresponding Author:
Nahid Davoodian, PhD.
Address: Department of Biochemistry, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.
Tel: +98 (917) 322 9106
E-mail: Nahid_Davoodian@yahoo.com

**Abstract**

**Introduction:** Evidence suggests that gestational exposure to Lipopolysaccharide (LPS) results in fetal zinc deficiency and eventually neurodevelopmental abnormalities. In this study, we utilized a rat model of Maternal Immune Activation (MIA) to investigate the possible neuroprotective effects of zinc supplementation during pregnancy on hippocampal astrocytes activation as well as inflammatory cytokines expression in adult offspring.

**Methods:** Pregnant rats received intraperitoneal injections of either LPS (0.5 mg/kg) or saline on Gestational Days (GD) 15 and 16, and orally gavaged with zinc sulfate (30 mg/kg) during pregnancy. Astrocyte density and histological assessment were evaluated in the hippocampus of adult offspring on Postnatal Days (PND) 60 to 62. Also, the mRNA levels of IL-6, TNF-α, IL-1β, NF-κB, and GFAP were measured using qPCR analysis.

**Results:** Prenatal exposure to LPS resulted in upregulated expression levels of IL-6, TNF-α, NF-κB, and GFAP in the hippocampus of adult pups. Moreover, the offspring from the LPS group showed an increased astrocyte density in the CA1 region with no histological alterations in CA1 and CA3 areas. However, maternal zinc supplementation ameliorated the LPS-induced inflammatory alterations.

**Conclusion:** This study supports the premise that zinc supplementation during pregnancy might be an early treatment option to inhibit hippocampal inflammation induced by the maternal immune response to infectious agents.
1. Introduction

Schizophrenia is a psychiatric disorder of neurodevelopmental origin. An interaction between genetic and environmental risk factors induces the development of this mental disease. Based on the neurodevelopmental theory, a widely considered theory of schizophrenia, stressful events during specific stages of embryogenesis lead to disturbance in normal brain development with deleterious and long-lasting impact on the brain function and structure later in life (Lang, Puls, Müller, Strutz-Seebohm, & Gallinat, 2007). The gestational infection has gained particular interest among researchers. It is a prenatal risk factor that increases the incidence of schizophrenia in the progeny. Numerous epidemiological and experimental studies support this theory (Boksa, 2010; Khandaker, Zimbron, Lewis, & Jones, 2013; Markham & Koenig, 2011; Meyer, Feldon, & Yee, 2009; Meyer, Yee, & Feldon, 2007). In this regard, maternal immune response to pathogenic agents results in cytokine imbalance in the fetal brain and eventually the manifestation of mental disorders such as schizophrenia (Solek, Farooqi, Verly, Lim, & Ruthazer, 2018). Although the exact mechanism of these detrimental alterations in the brain is unclear, animal models of Maternal Immune Activation (MIA) have been established based on the administration of immunogenic agents such as Lipopolysaccharide (LPS) to pregnant rodents at specific neurodevelopmental phases. Indeed, several studies have supported the face validity of this model to represent several phenotypes related to schizophrenia by detailing the use of the MIA model with various protocols (Alizadeh, Davoodian, Kazemi, Ghasemi-Kasman, & Shaerzadeh, 2020; de Souza et al., 2015; Hao, Hao, Li, & Li, 2010; Mattei et al., 2014; Waterhouse, Roper, Brennan, & Ellenbroek, 2016; Wischhof, Irrsack, Ossorio, & Koch, 2015).

Several human studies have provided evidence that the etiology of schizophrenia involves neuroinflammation, abnormal morphology, and functionality of glial cells (Fillman et al., 2013; Müller, 2018; Potvin et al., 2008). Recently, a postmortem study has highlighted a significant upregulation in cellular pathways associated with inflammation in the dorsolateral prefrontal cortex, striatum, and particularly hippocampus of subjects with schizophrenia (Lanz et al., 2019). Consistently, the association between schizophrenia and immune dysregulation has been further supported by numerous MIA animal model studies, which dominantly evaluated the density or activity of microglia and astrocytes in different brain regions (Solek et al., 2018). Although microglia are the principal innate immunity component in the brain, astrocytes, the most numerous cell in the Central Nervous System (CNS), are also crucial in the brain’s immune activity (Farina, Aloisi, & Meinl, 2007). As such, remarkable astrogliosis, in addition to neuroinflammation, has been detected in the hippocampus of pups with prenatal exposure to LPS or IL-6 (Hao et al., 2010; Samuelsson, Jennische, Hansson, & Holmang, 2006). This finding is further supported by a recent study demonstrating the significant astrogliosis in both the prefrontal cortex and hippocampus of adult pups born...
to dams treated with polyriboinosinic–polyriboceytidylic acid (poly[I:C]) (Ding et al., 2019). These results indicate that maternal infection leads to long-lasting alterations in the offspring’s brain. However, there is a controversy surrounding the changes in the density and activity of astrocytes in both postmortem brains of schizophrenic patients (Trepanier, Hopperton, Mizrahi, Mechawar, & Bazinet, 2016) and MIA animal models (de Souza et al., 2015). This controversy might be because of different experimental designs.

Epidemiological studies have documented that the response to MIA, and not a specific kind of pathogen, is involved in the etiology of neurodevelopmental disorders, including schizophrenia (Brown et al., 2004; Solek et al., 2018; Yolken, Dickerson, & Fuller Torrey, 2009). The obvious main response to maternal immune challenge is the increased production of pro-inflammatory cytokines; however, the secondary consequences of systemic immune activation also need to be considered (Reisinger et al., 2015). Accordingly, gestational exposure to LPS acts as a potent inducer of maternal Metallothionein (MT), resulting in maternal hypozincemia and, ultimately, fetal zinc deficiency (Carey, Berbée, Coyle, Philcox, & Rofe, 2003; Coyle, Tran, Fung, Summers, & Rofe, 2009). Zinc is an essential and abundant trace element required for the proper function of numerous proteins. Also, it greatly influences a broad spectrum of cellular processes, including immunity, wound healing, and normal brain functions (Chasapis, Loutsidou, Spiliopoulou, & Stefanidou, 2012). Zinc dyshomeostasis contributes to a range of psychiatric diseases, such as depression and schizophrenia (Portbury & Adlard, 2017). This issue is further supported by recent human studies demonstrating a significant reduction in the serum concentration of zinc in schizophrenic subjects (Cai et al., 2015; Cao et al., 2019). In addition, zinc supplementation during pregnancy is confirmed to reduce neurobehavioral alterations and teratogenicity, as well as fetal death induced by LPS in MIA animal model (Alizadeh et al., 2020; Carey et al., 2003; Coyle et al., 2009; Kirsten et al., 2015). However, the cellular mechanism underlying the protective effect of zinc supplementation against LPS-induced impairments is yet to be fully clarified.

Because of the putative role of the hippocampus in neurogenesis and learning, as well as its involvement in neuropsychological impairments associated with schizophrenia (Ewing & Winter, 2013), in this study, we utilized the MIA animal model to evaluate the possible neuroprotective effect of zinc supplementation during pregnancy on astrocyte activation and several inflammatory mediators in the hippocampus of adult pups.

2. Materials and Methods

Study animal

Female and male Wistar rats (female: 200-230 g; male: 250-300 g) were obtained from the animal house of Hormozgan University of Medical Sciences. Animals were kept under standard environmental conditions (temperature: 22°C, humidity: 60%-70%, 12 h light-dark cycle), with unlimited access to food and tap water. In the present study, we used 8-week-old male offspring selected based on the results of our previous study (Alizadeh et al., 2020). All experimental procedures were based on the National Institutes of Health guide for the care and use of laboratory animals and approved by the Ethics Committee of Hormozgan University of Medical Sciences (HUMS) (IR.HUMS.REC.1397.276). To minimize the number and the suffering of animals based on the Three Rs principle, we shared the treated animals in two other published articles (Alizadeh et al., 2020; Mousaviyan et al., 2021).

Study treatment

Adult male and female rats were housed overnight, and the first day of pregnancy was confirmed by the presence of spermatozoa in vaginal smears, which was designated as gestational day 1 (GD1). Pregnant dams were randomly assigned into four treatment conditions with 6 litters per group: control, pregnant dams received Intraperitoneal (IP) injections of saline at GD 15 and 16; LPS, pregnant dams received LPS injections (0.5 mg/kg, IP, Escherichia coli L2630) (Waterhouse et al., 2016; Wischhof et al., 2015) at GD15 and 16; LPS+Zinc, pregnant dams received LPS injections (0.5 mg/kg, IP) at GD15 and 16 and orally gavaged with zinc sulfate (30 mg/kg) (Mozzadi, Ghobbeddin, & Parham, 2007) during pregnancy; and Zinc, pregnant dams received IP injections of saline at GD15 and 16 and orally gavaged with zinc sulfate (30 mg/kg) during pregnancy. The control and LPS groups were administered with an equal volume of water during pregnancy by gavage. The experimental timeline is provided in Figure 1. Based on sex and treatment, the resulting offspring were weaned on postnatal day 21 (PND21) and maintained undisturbed until PND60. For sample size calculation, we conducted the resource equation method (Charan & Kantharia, 2013). To minimize the litter effects, one male offspring from each litter was randomly selected for future analysis. The remaining pups were used for other experiments that are not reported here.
Tissue collection, RNA isolation, and qPCR analysis

At PND 60, six male pups from each group (n=6, one pup per litter and 6 litters per group) were sacrificed using Carbon Dioxide (CO2) euthanasia. The whole brain was rapidly removed, and placed on ice, followed by the microdissection of the hippocampus (Chiu, Lau, Lau, So, & Chang, 2007). The tissues were immediately snap-frozen and kept at -80°C.

The following experimental procedures were carried out based on MIQE guidelines (Bustin et al., 2009). Total RNA was extracted using TRIzol™ Reagent (Sigma-Aldrich, USA), based on the manufacturer’s instructions. After assessing the RNA integrity using agarose gel electrophoresis, RNA (1 μg) of each sample was reverse-transcribed using the cDNA synthesis kit (Thermo Ficher, USA), as described by the manufacturer’s protocol. qPCR reactions were performed using a Mic qPCR system (Australia) with the primer sets for IL-6, TNF-α, Nuclear Factor Kappa B (NF-κB), GFAP, and IL-1 β as the target genes and GAPDH as a reference gene (Table 1). The reactions were conducted in SYBR Premix Ex Taq II (Takara, Japan) with a three-step protocol, as described previously (Alizadeh et al., 2020). After normalization with GAPDH, relative gene expression analysis was calculated using the 2−ΔΔCt method.

Immunostaining

Immunofluorescence staining was carried out, as described previously (Mousaviyan et al., 2021). After anesthetization with ketamine/xylazine (100 mg/kg - 10 mg/kg), animals (n=4/group) were transcardially perfused with Phosphate-Buffered Saline (PBS) and 4% paraformaldehyde (PFA). The brain tissues were harvested, post-fixed in PFA overnight, and finally immersed in sucrose solution (30%) for 48 h. After freezing in the O.C.T compound, coronal sections of the hippocampus (6 μm) were obtained by a cryostat apparatus (MICROM HM 525, Germany) and mounted on charged slides.

For immunostaining, tissue sections were washed with PBS for three 5-min, followed by blocking in 10% normal goat serum and 0.3% Triton X-100 for 1h. The slides were then incubated overnight with rabbit Anti-Glial Fibrillary Acidic Protein (GFAP) (1:400, Z0334, Dako) at 4°C. Afterward, the slides were washed with PBS for three 10-min periods and incubated with Goat anti-rabbit Alexa Fluor® 594 (1:1000 dilution, ab150080) secondary antibody for 1 h, followed by staining with 4’,6-diamidino-2-phenylindole (DAPI) for 10 min. Subsequently, the images were obtained by a fluorescence microscope (Nikon, Japan). The fluorescence images were manually quantified as the number of GFAP positive cells soma/total cells using ImageJ 1.42 software (NIH, USA).

Histopathological examination

Similar to the immunofluorescence staining procedure, animals (n=4/group) were perfused with PBS and 4% PFA. Then, the cerebral samples were rapidly harvested and stored in 4% PFA overnight. After dehydration with a series of alcohol and paraffin embedding, the tissues were cut into 6-μm thick coronal sections. Finally, H&E staining was performed, and the slides were evaluated under a microscope.

Statistical analysis

Data analysis was conducted by GraphPad Prism 6 (GraphPad Software Inc. San Diego, CA, USA). The data from qPCR and immunostaining were analyzed by a 2-way analysis of variance (ANOVA), followed by Tukey’s multiple comparison test. Prenatal treatment (saline vs LPS) and maternal supplementation (vehicle vs zinc) was considered between-group variables. The data are presented as Mean±SD, and P≤0.05 was considered significant.

3. Results

Prenatal zinc supplementation suppressed the up-regulation of pro-inflammatory markers induced by LPS

To evaluate the LPS-induced inflammatory reaction in the hippocampus of the offspring and the possible protective effect of prenatal zinc supplementation, we measured the expression levels of IL-6, TNF-α, NF-κB, GFAP, and IL-1 β by the qPCR technique. As illustrated in Figure 2a, the expression level of IL-6 was differentially affected by LPS, with a moderate increase in the hippocampus of the offspring prenatally exposed to LPS compared to the corresponding control (the main effect of prenatal treatment, F1, 20=8.829, P=0.0075; LPS group vs control group Tukey’s multiple comparison, P<0.01), and this effect was prevented by zinc supplementation in the LPS+Zinc group (interaction of main effects, F1, 20=9.403, P=0.0061; LPS+Zinc group vs. LPS group Tukey’s multiple comparison, P<0.05) (Figure 2a). Furthermore, a significant effect of prenatal treatment was detected for tumor necrosis factor-alpha (TNF-α) mRNA level, reflected by a slight increase in the hippocampus of LPS-exposed pups related to the control group (main effect of prenatal treatment, F1, 16=8.577, P=0.0083; LPS group vs. control group...
Tukey’s multiple comparison, P<0.01), that returned to the control level upon maternal zinc supplementation in the LPS+Zinc group (interaction of main effects, F1, 16=8.401, P=0.0089; main effect of maternal supplementation, F1, 16=4.754, P=0.0413; LPS+Zinc group vs. LPS group Tukey’s multiple comparison, P<0.01) (Figure 2c). However, gene expression analysis revealed no significant effects of prenatal treatment (F1, 17=0.7645, P=0.3923), maternal supplementation (F1, 17=0.1752, P=0.6800), and their interaction (F1, 17=1.507, P=0.2339) for IL-1β in the experimental groups (Figure 2b). Conversely, LPS treatment had a moderate but significant effect on the expression level of NF-κB, depending on the prenatal supplementation. NF-κB expression level was significantly upregulated in the offspring from LPS-treated mothers compared to the control pups (main effect of the prenatal treatment, F1, 17=6.305, P=0.0207; LPS group vs. control group Tukey’s multiple comparison, P<0.01), whereas this increase was suppressed in the LPS+Zinc group (interaction of main effects, F1, 17=14.12, P=0.0012; main effect of the maternal supplementation, F1, 17=5.549, P=0.0288; LPS+Zinc group vs. LPS group Tukey’s multiple comparison, P<0.01) (Figure 2d). Similarly, there was a significant increase in the GFAP mRNA level in the pups of LPS-exposed mothers related to the control group (main effect of the prenatal treatment, F1, 20=4.822, P=0.0401; LPS group vs. control group Tukey’s multiple comparison, P<0.05), while in the offspring of the LPS+Zinc group, GFAP expression level was approximately back to the control level (LPS+Zinc group vs. LPS group Tukey’s multiple comparison, P=0.05). However, no significant interaction or main effect of the maternal supplementation were found for GFAP expression level (interaction of main effects, F1, 20=4.157, P=0.0549; main effect of maternal zinc supplementation, F1, 16=0.330, P=0.5746; LPS+Zinc group vs. LPS group Tukey’s multiple comparison, P=0.4377).

**Table 1. Primer sets applied for qPCR**

| Gene      | Accession Number | Sequences                      |
|-----------|------------------|--------------------------------|
| IL-6      | NM_012589.2      | 5′-TGATGATGCCTCAACTG-3′         |
|           |                   | 5′-GACATGGAAATGCTGGT-3′         |
| IL-1β     | NM_031512.2      | 5′-GCTGTGCCAGCCTACTATGTGTTG-3′ |
|           |                   | 5′-AGGTGTGCTCATCCACAGAG-3′      |
| TNF-A     | NM_012675.3      | 5′-AAATGGGCTCCCTCTACAGTTG-3′   |
|           |                   | 5′-TCTGCTTTGTTGCTGCTACGAG-3′   |
| nf-κB     | XM_006233360.3   | 5′-TGACAGAAGAGACATGGAGTTG-3′   |
|           |                   | 5′-AGGTATGCTACGCTATGG-3′       |
| GFAP      | NM_017009.2      | 5′-TGACACAGAATGAACTGCAAG-3′    |
|           |                   | 5′-CAGTTGCGGCGGATGATCAT-3′     |
| GAPDH     | XM_017593963.1   | 5′-AGCAGAAGATGCGAGCGACAG-3′    |
|           |                   | 5′-GACATATGACAGCAGACATACC-3′   |

**Figure 1.** A schematic diagram describing the experimental timeline

On Gestation Days (GD) 15 and 16, pregnant dams were intraperitoneally administered with either LPS (500 µg/kg) or saline and gavaged with zinc sulfate (30 mg/kg)/vehicle. The resulting offspring were submitted to qPCR, immunostaining, and morphological analysis at PND60.
main effect of maternal supplementation, $F_{1, 12}=3.139$, $P=0.0917$) (Figure 2e).

Prenatal zinc supplementation alleviation effect on the increased density of astrocytes induced by LPS in the CA1 hippocampus

To examine whether the alterations in the mRNA levels of GFAP and pro-inflammatory markers were associated with the changes in astrocyte density, we carried out immunostaining in CA1 and CA3 areas (Figures 3 and 4). In the CA1 region of the hippocampus, a significant effect of prenatal treatment was revealed (main effect of prenatal treatment, $F_{1, 12}=13.86$, $P=0.0029$), with pups born to LPS-exposed dams exhibiting a marked increase in GFAP+ cells compared to the control group (LPS group vs. control group Tukey’s multiple comparison, $P<0.01$). This effect was restrained by the maternal zinc supplementation in the LPS+Zinc group (interaction of main effects, $F_{1, 12}=11.32$, $P=0.0056$; LPS+Zinc group vs LPS group Tukey’s multiple comparison, $P<0.05$), with no main effect of the maternal supplementation (main effect of maternal supplementation, $F_{1, 12}=3.382$, $P=0.0908$) (Figure 3b). However, in CA3 area of the hippocampus, no statistically significant effect for prenatal treatment, maternal supplementation, as well as their interaction was detected on the astrocyte density (interaction of main effects, $F_{1, 12}=0.0$, $P>0.9999$; main effect of prenatal treatment, $F_{1, 12}=0.2297$, $P=0.6404$; main effect of maternal supplementation) $F_{1, 1}=0.2297$, $P=0.6404$) (Figure 4b).

No histological changes were observed in CA1 and CA3 hippocampus of offspring

H&E staining was performed to examine the possible histological alterations, including changes in the arrangement and morphology structure of cells in both CA1 and CA3 hippocampus of offspring. As depicted in Figure 5a, the CA1 area of the hippocampus from all groups

---

**Figure 2.** Prenatal LPS treatment enhanced the expression of pro-inflammatory mediators in the hippocampus of offspring at PND60, suppressed by maternal zinc supplementation

Prenatal LPS exposure significantly induced the expression of IL-6 (a), TNF-α (c), NF-κB (d), and GFAP (e) in the hippocampus of pups, which was mitigated by maternal zinc supplementation. The data are presented as mean±SD, n=6 per group. *$P<0.05$, **$P<0.01$, and ***$P<0.001$ compared to the control group. #$P<0.05$, ##$P<0.01$, and ###$P<0.001$ compared to the LPS group.
exhibited arranged neurons with normally rounded nuclei (Figure 5a). Similarly, we detected no evidence of histological changes in the CA3 hippocampus of pups in all experimental groups (Figure 5b).

4. Discussion

Maternal infection is considered a prenatal risk factor associated with deleterious effects on the brain function and structure in the progeny (Garay, Hsiao, Patterson, & McAllister, 2013). In our previous study, we demonstrated that prenatal exposure to LPS on GD15 and 16 results in significant behavioral impairments in adult offspring as a phenotype associated with schizophrenia (Alizadeh et al., 2020). Remarkably, these behavioral deficits were observed only among male pups, which were selected for further investigation. In this study, our findings revealed the long-lasting alterations in the hippocampus of adult offspring prenatally exposed to LPS, characterized by the enhanced expression levels of IL-6, TNF-α, NF-κB, and GFAP as well as increased astrocyte density in the CA1 region of the hippocampus. Furthermore, the mentioned changes were alleviated by maternal zinc supplementation.

Figure 3. Immunostaining for GFAP in the CA1 hippocampus of MIA offspring

Immunofluorescence images (a) and the quantified graph (b) of GFAP in the CA1 area of pups at PND60. The data are presented as Mean±SD, n=4 per group. Scale bar: 50 μm, **P<0.01 compared to the control group and #P<0.05 compared to the LPS group.
Hippocampus is a region in the brain with a prominent role in memory, learning, and neurogenesis. Accordingly, both human and animal studies have repeatedly highlighted the importance of the hippocampus as one of the main areas involved in the pathophysiology of schizophrenia (Antoniades et al., 2018; de Souza et al., 2015; Hao et al., 2010; Lieberman et al., 2018). This issue is further evidenced by the well-described reduction in hippocampal volume in schizophrenic patients and MIA offspring (Adriano, Caltagirone, & Spalletta, 2012; van Erp et al., 2016; Zhou, 2015). Furthermore, a recent postmortem study has detected a robust upregulation of inflammatory pathways, especially the IL-6 pathway, in the hippocampus of subjects with schizophrenia. This finding supports the persistent neuroinflammation in the brain of schizophrenic patients (Lanz et al., 2019). However, in MIA model studies, contradictory findings have been reported regarding the alterations in hippocampal pro-inflammatory markers of resulting offspring. Herein, we detected the increased mRNA levels of NF-κB, TNF-α, and IL-6 with an unchanged expression level of IL-1β in the hippocampus of the offspring born to LPS-treated mothers. In this regard, Pups exposed in utero to poly I:C (4 mg/kg, IV) exhibited the upregulation in the expression of both TNF-α and IL-1β in the hippocampus at PND120 (Mattei et al., 2014). In the same region,
another study showed the elevated mRNA level of *IL-6* in adult offspring prenatally exposed to *IL-6* (9 μg/kg, IP) (Samuelsson et al., 2006). However, protein levels of *IL-6, IL-1β*, and *TNF-α* were reported unchanged in the hippocampus of pups prenatally exposed to poly I:C (5 mg/kg, IV) (Giovanoli et al., 2015). Similar results were demonstrated by a recent study using a different dose of poly I:C (10 mg/kg, IV) (Ding et al., 2019). In addition, in the only MIA study, according to our knowledge, that examined the expression levels of different inflammatory genes, the mRNA level of *NF-κB* was found to be unchanged in the hippocampus of offspring at PND21 (Zhou, 2015). Methodological differences, including the difference in dose, immunogenic substances, and the gestational stage during which the MIA is induced, might be the reasons behind the discrepancies in the reported results. Notably, our study revealed the upregulation in GFAP expression level and the increased GFAP positive cells in the CA1 hippocampus of offspring prenatally exposed to LPS with no alteration in the CA3 region. This discrepancy observed in hippocampal CA1 and CA3 is probably because of the difference in astrocyte sensitivity and vulnerability of these regions, which is reported to be responsible for the selective neural death in CA1 after forebrain ischemia (Sun, Fukushima, Wang, & Yamamoto, 2018). Using a similar protocol, de Souza et al. noted the increased level of GFAP protein (measured by ELISA) and GFAP immunocontent in the CA1 hippocampus of adult rats born to LPS-challenged mothers (0.5 mg/kg, IP) (de Souza et al., 2015). Consequently, adult offspring of dams treated with LPS (0.79 mg/kg, IP) exhibited higher numbers of GFAP immunoreactive cells related to the control (Hao et al., 2010). In contrast, mice exposed prenatally to poly I:C (5 mg/kg, IV) showed no alterations in the density of GFAP-positive astrocytes in CA1, CA3, and dentate gyrus areas at PND28 and 140 (Giovanoli et al., 2015). Different infectious agents and their concentrations might explain these conflicting results. Considerably, human studies have also highlighted the pivotal role of astrocytes in the etiology and pathophysiology of schizophrenia (Catts, Wong, Fillman, Fung, & Shannon Weickert, 2014; Tarasov et al., 2020). Therefore, dysregulation of astrocytes function, which are morphologically and functionally associated with neurons, might be a new point of view to illustrate metabolic and transmitter alterations in the brain of schizophrenic patients (Tarasov et al., 2020). Moreover, we performed histological evaluation in the CA1 and CA2 areas of hippocampus of adult offspring. In contrast to the previous study in which a disordered and reduced number of nuclei were reported in the CA1 hippocampus of pups born to LPS-challenged mothers (0.79 mg/kg, IP) (Hao et al., 2010), we detected no significant histological changes in both areas. Similarly, the number of neurons is unchanged in the hippocampus of schizophrenic patients (Heckers & Konradi, 2002), as well as in the prenatally immune-challenged offspring in the schizophrenic MIA model (de Souza et al., 2015). This finding supports the hypothesis that disruption in neural connectivity (Ruiz, Birbaumer, & Sitaram, 2013), instead of reduced neuron number, might be involved in the development of this mental disease.

**Figure 5.** Histological assessment of CA1 and CA3 hippocampus

No histological alterations were observed in both CA1 (a) and CA3 (b) areas for the offspring in all experimental groups. Black arrows represent the normal (N) neurocyte. Scale bar: 50μm, (n=4).
As a complex mental health disease, schizophrenia is characterized by three major manifestations: positive, negative, and cognitive symptoms. Currently, the available pharmacological treatment options mainly provide relief for positive symptoms with no effective treatment for negative and cognitive symptoms (Patel, Cherian, Gohil, & Atkinson, 2014). With this background, the discovery of satisfactory pharmacological options and early therapeutic interventions is of interest. Using an MIA animal model with well-established face and predictive validity (Reisinger et al., 2015), we found that maternal zinc supplementation alleviated LPS-induced increased expression of IL-6, TNF-α, NF-κB, GFAP, and also GFAP-positive cells in the hippocampus of male pups. It has been reported that gestational infection with LPS is correlated with maternal and fetal hypozincemia mainly because of the stimulation of metallothionein synthesis in the maternal liver (Carey et al., 2003; Coyle et al., 2009). In addition to being involved in innate and adaptive immunity, zinc is strongly required for normal brain functions. Meanwhile, fetal hypozincemia can eventually lead to neurodevelopmental damage in the offspring (Chua et al., 2012; Coyle et al., 2012; Coyle et al., 2012). One possible mechanism is that zinc supplementation during pregnancy might counteract the LPS-induced reduction of zinc availability to the fetus and ultimately prevent the abnormal neurodevelopment in the progeny. Another mechanism can be explained by the antioxidant and anti-inflammatory properties of this element. In support of this, numerous studies have consistently documented the link between zinc deficiency and increased production of oxidative stress and inflammatory markers (Jarosz, Olbert, Wyszogrodzka, Młyniec, & Librowski, 2017). The literature demonstrates the negative regulatory effect of zinc on NF-κB as one of the main inflammatory pathways (Jarosz et al., 2017). This signaling cascade positively regulates the expression of pro-inflammatory genes IL-6, TNF-α, IL-1β, and so on (Lawrence, 2009). In this context, it has been suggested that zinc exerts an inhibitory effect on LPS-induced NF-κB and eventually suppresses the expression of inflammatory mediators, which further supports our findings (Jarosz et al., 2017).

As mentioned earlier, we also found that maternal zinc supplementation alleviated LPS-induced increment in GFAP mRNA level and astrocyte density in the CA1 hippocampus of pups. To our knowledge, there is no MIA model study evaluating the effect of maternal zinc supplementation on the astrocyte density of adult offspring. However, in the fetal hippocampus at GD18, the previous study reported the protective influence of prenatal zinc treatment on LPS-induced astrogliosis (Chua et al., 2012).

Finally, this study has the following limitations. MIA animal model is profoundly considered a suitable tool to evaluate pathomechanism and develop new therapeutic agents for some of the most complex mental diseases, including schizophrenia (Reisinger et al., 2015). However, there is a stigma associated with this model, as this approach cannot represent all behavioral and pathological features of a particular neurodevelopmental disease. Additionally, the main focus of this study was to investigate hippocampal astrocyte changes in adult offspring. It should be taken into consideration that other glial cells, including microglia cells, have a role in producing inflammatory markers. Despite the abundant data for the cytokine disturbance in the brain of MIA offspring, literature has provided conflicting results about microglial activation in different brain regions. While some studies have documented an increase in microglial activation, others have demonstrated no significant alterations (Bergdolt & Dunaevsky, 2019; Solek et al., 2018). Therefore, the investigation of possible changes in astrocyte and microglia density in different hippocampal regions of MIA offspring is of utmost importance.

5. Conclusion

In conclusion, in the present study, we utilized the MIA animal model to examine the beneficial effect of maternal zinc supplementation in protection against LPS-induced hippocampal inflammation in adult offspring. Our findings showed that prenatal LPS exposure induced long-lasting alterations in the hippocampus of the resulting offspring, evidenced by the increased expression of NF-κB, TNF-α, IL-6, GFAP, and the astrocyte density in the CA1 area. In addition, our findings demonstrated that zinc supplementation during pregnancy mitigated the mentioned LPS-induced impairments. Therefore, considering a lack of comprehensive therapeutic strategy for schizophrenia, zinc supplementation during pregnancy might be an early treatment option to inhibit neurodevelopmental abnormalities induced by the maternal immune response to infectious agents.

The analyzed data are available from the corresponding author upon reasonable request.
**Ethical Considerations**

**Compliance with ethical guidelines**

All experimental procedures were based on the National Institutes of Health Guide for the care and use of laboratory animals. The Ethics Committee of Hormozgan University of Medical Sciences (HUMS) approved the research (IR.HUMS.REC.1397.276).

**Funding**

This study was supported by the Research Vice-chancellor of Hormozgan University of Medical Sciences (HUMS) (Grant No.: 970280).

**Authors’ contributions**

All authors equally contributed to preparing this article.

**Conflict of interest**

The authors declared no conflict of interest.

**Acknowledgments**

The authors would like to acknowledge the technical advice provided by Haniyeh Kazemi.

**References**

Adriano, F., Caltagirone, C., & Spalletta, G. (2012). Hippocampal volume reduction in first-episode and chronic schizophrenia: A review and meta-analysis. *The Neuroscientist: A Review Journal Bringing Neurobiology, Neurology and Psychiatry*, 18(2), 180-200. [DOI:10.1177/1073858410395147]

Alizadeh, F., Davoodian, N., Kazemi, H., Ghasemi-Kasman, M., & Shaerzadeh, F. (2020). Prenatal zinc supplementation attenuates lipopolysaccharide-induced behavioral impairments in maternal immune activation model. *Behavioural Brain Research*, 377, 112247. [DOI:10.1016/j.bbr.2019.112247]

Antoniades, M., Schoeler, T., Radua, J., Valli, I., Allen, P., Kempston, M. J., et al. (2018). Verbal learning and hippocampal dysfunction in schizophrenia: A meta-analysis. *Neuroscience & Biobehavioral Reviews*, 86, 166-175. [DOI:10.1016/j.neubiorev.2017.12.001]

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

Boksa, P. (2010). Effects of prenatal infection on brain development and behavior: A review of findings from animal models. *Brain, Behavior, and Immunity*, 24(6), 881-897. [DOI:10.1016/j.bbi.2010.03.005]

Brown, A. S., Begg, M. D., Gravenstein, S., Schaefer, C. A., Wyatt, R. J., Bresnahan, M., et al. (2004). Serologic evidence of prenatal influenza in the etiology of schizophrenia. *Archives of General Psychiatry*, 61(8), 774-780. [DOI:10.1001/archpsyc.61.8.774]

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., et al. (2009). The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry*, 55(4), 611–622. [DOI:10.1373/clinchem.2008.112797]

Cai, L., Chen, T., Yang, J., Zhou, K., Yan, X., Chen, W., et al. (2015). Serum trace element differences between Schizophrenia patients and controls in the Han Chinese population. *Scientific Reports*, 5, 15013. [DOI:10.1038/srep15013]

Cao, B., Yan, L., Ma, J., Jin, M., Park, C., Nozari, Y., et al. (2019). Comparison of serum essential trace metals between patients with schizophrenia and healthy controls. *Journal of Trace Elements in Medicine and Biology*. [DOI:10.1016/j.jtemb.2018.10.009]

Carey, L. C., Berbise, P. L., Coyle, P., Philcox, J. C., & Rofe, A. M. (2003). Zinc treatment prevents lipopolysaccharide-induced teratogenicity in mice. *Birth Defects Research, Part A, Clinical and Molecular Teratology*, 67(4), 240–245. [DOI:10.1002/bdra.10035]

Catts, V. S., Wong, J., Fillman, S. G., Fung, S. J., & Shannon Weickert, C. (2014). Increased expression of astrocyte markers in schizophrenia: Association with neuroinflammation. *The Australian and New Zealand Journal of Psychiatry*, 48(8), 722–734. [DOI:10.1177/0004867413501078]

Charan, J., & Kantharia, N. D. (2013). How to calculate sample size in animal studies? *Journal of Pharmacology & Pharmacotherapeutics*, 4(4), 303-306. [DOI:10.4103/0976-500X.119726]

Chasapis, C. T., Loutsidou, A. C., Spiroopoulos, C. A., & Stefanidou, M. E. (2012). Zinc and human health: An update. *Archives of Toxicology*, 86(4), 521-534. [DOI:10.1007/s00204-011-0775-1]

Chen, Y. H., Zhao, M., Chen, X., Zhang, Y., Wang, H., Huang, Y. Y., et al. (2012). Zinc supplementation during pregnancy protects against lipopolysaccharide-induced fetal growth restriction and demise through its anti-inflammatory effect. *Journal of Immunology (Baltimore, Md.: 1950)*, 189(1), 454–463. [DOI:10.4049/jimmunol.1105379]

Chiu, K., Lau, W. M., Lau, H. T., So, K. F., & Chang, R. C.-C. (2007). Micro-dissection of rat brain for RNA or protein extraction from specific brain region. *Journal of visualized Experiments*, 7, e269. [DOI:10.5971/jextr.269]

Chua, J. S., Cowley, C. J., Manavis, J., Rofe, A. M., & Coyle, P. (2012). Prenatal exposure to lipopolysaccharide results in neurodevelopmental damage that is ameliorated by zinc in mice. *Brain, Behavior, and Immunity*, 26(2), 326-336. [DOI:10.1016/j.bbi.2011.10.002]

Coyle, P., Tran, N., Fung, J. N., Summers, B. L., & Rofe, A. M. (2009). Maternal dietary zinc supplementation prevents aberrant behaviour in an object recognition task in mice offspring exposed to LPS in early pregnancy. *Behavioural Brain Research*, 197(1), 210-218. [DOI:10.1016/j.bbr.2008.08.022]

Funding

This study was supported by the Research Vice-chancellor of Hormozgan University of Medical Sciences (HUMS) (Grant No.: 970280).

Authors’ contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors would like to acknowledge the technical advice provided by Haniyeh Kazemi.

References

Adriano, F., Caltagirone, C., & Spalletta, G. (2012). Hippocampal volume reduction in first-episode and chronic schizophrenia: A review and meta-analysis. *The Neuroscientist: A Review Journal Bringing Neurobiology, Neurology and Psychiatry*, 18(2), 180-200. [DOI:10.1177/1073858410395147]

Alizadeh, F., Davoodian, N., Kazemi, H., Ghasemi-Kasman, M., & Shaerzadeh, F. (2020). Prenatal zinc supplementation attenuates lipopolysaccharide-induced behavioral impairments in maternal immune activation model. *Behavioural Brain Research*, 377, 112247. [DOI:10.1016/j.bbr.2019.112247]

Antoniades, M., Schoeler, T., Radua, J., Valli, I., Allen, P., Kempston, M. J., et al. (2018). Verbal learning and hippocampal dysfunction in schizophrenia: A meta-analysis. *Neuroscience & Biobehavioral Reviews*, 86, 166-175. [DOI:10.1016/j.neubiorev.2017.12.001]

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

Boksa, P. (2010). Effects of prenatal infection on brain development and behavior: A review of findings from animal models. *Brain, Behavior, and Immunity*, 24(6), 881-897. [DOI:10.1016/j.bbi.2010.03.005]
Amenorrhea in Wistar rats are dependent on sex. *Frontiers in Cellular Neuroscience*, 9, 489. [DOI:10.3389/fncel.2015.00489]

Ding, S., Hu, Y., Luo, B., Cai, Y., Hao, K., Yang, Y., et al. (2019). Age-related changes in neuroinflammation and prepulse inhibition in offspring of rats treated with Poly I:C in early gestation. *Behavioral and Brain Functions*, 15(1), 3.

Ewing, S. G., & Winter, C. (2013). The ventral portion of the CA1 region of the hippocampus and the prefrontal cortex as candidate regions for neuromodulation in schizophrenia. *Medical Hypotheses*, 86(6), 827-832.

Farina, C., Aloisi, F., & Meinl, E. (2007). Astrocytes are active players in cerebral innate immunity. *Trends in Immunology*, 28(3), 138-145. [DOI:10.1016/j.it.2007.01.005]

Fillman, S. G., Cloonan, N., Catts, V. S., Miller, L. C., Wong, J., Farina, C., Aloisi, F., & Meinl, E. (2007). Astrocytes are active players in cerebral innate immunity. *Trends in Immunology*, 28(3), 138-145. [DOI:10.1016/j.it.2007.01.005]

Farina, C., Aloisi, F., & Meinl, E. (2007). Astrocytes are active players in cerebral innate immunity. *Trends in Immunology*, 28(3), 138-145. [DOI:10.1016/j.it.2007.01.005]

Fillman, S. G., Cloonan, N., Catts, V. S., Miller, L. C., Wong, J., McCrossin, T., et al. (2013). Increased inflammatory markers identified in the dorsolateral prefrontal cortex of individuals with schizophrenia. *Molecular Psychiatry*, 18(2), 206-214. [DOI:10.1038/mp.2012.110]

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.

Giovanoli, S., Notter, T., Richetto, J., Labouesse, M. A., Vuillermondit, S., Riva, M. A., et al. (2015). Late prenatal immune activation causes hippocampal deficits in the absence of persistent inflammation across aging. *Journal of Neuroinflammation*, 12, 221. [DOI:10.1186/s12974-015-0437-y]

Hao, L. Y., Hao, X. Q., Li, S. H., & Li, X. H. (2010). Prenatal exposure to lipopolysaccharide results in cognitive deficits in age-increasing offspring rats. *Neuroscience*, 166(3), 573-570.

Heckers, S., & Konradi, C. (2002). Hippocampal neurons in schizophrenia. *Journal of Neuronal Transmission*, 109(5-6), 891-905. [DOI:10.1007/s007020020073]

Jarosz, M., Olbert, M., Wyszogrodzka, G., Mlyniec, K., & Li-Browski, W. (2017). Antioxidant and anti-inflammatory effects of zinc. Zinc-dependent NF-kB signaling. *Inflammopharmacology*, 25(1), 11-24.

Khandaker, G. M., Zimbron, J., Lewis, G., & Jones, P. B. (2013). Prenatal maternal infection, neurodevelopment and adult schizophrenia: A systematic review of population-based studies. *Psychological Medicine*, 43(2), 239-257.

Kirsten, T. B., Chaves-Kirsten, G. P., Bernardes, S., Scavone, C., Sarkis, J. E., Bernardi, M. M., et al. (2015). Lipopolysaccharide exposure induces maternal hypozincemia, and prenatal zinc treatment prevents autistic-like behaviors and disturbances in the striatal dopaminergic and mTOR systems of offspring. *PloS One*, 10(7), e0134565. [DOI:10.1371/journal.pone.0134565]

Lang, U. E., Puls, L., Muller, D. J., Strutz-Seebohm, N., & Gallinat, J. (2007). Molecular mechanisms of schizophrenia. *Cellular Physiology and Biochemistry*, 20(6), 687-702. [DOI:10.1159/00010430]

Lanz, T. A., Reinhart, V., Sheehan, M. J., Rizzo, S., Bove, S. E., James, L. C., et al. (2019). Postmortem transcriptional profiling reveals widespread increase in inflammation in schizophrenia: A comparison of prefrontal cortex, striatum, and hippocampus among matched tetrads of controls with subjects diagnosed with schizophrenia, bipolar or major depressive disorder. *Translational Psychiatry*, 9(1), 151. [DOI:10.1038/s41398-019-0492-8]

Lawrence, T. (2009). The nuclear factor NF-kB pathway in inflammation. *Cold Spring Harbor Perspectives in Biology*, 1(6), a001651.

Lieberman, J. A., Girgis, R. R., Brucato, G., Moore, H., Provenzano, F., Kegeles, L., et al. (2018). Hippocampal dysfunction in the pathophysiology of schizophrenia: A selective review and hypothesis for early detection and intervention. *Molecular Psychiatry*, 23(8), 1764-1772. [DOI:10.1038/mp.2017.249]

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

Mattei, D., Djodari-Irani, A., Hadar, R., Pez, A., de Cossio, L. F., Goetz, T., et al. (2014). Minocycline rescues decrease in neurogenesis, increase in microglia cytokines and deficits in sensorimotor gating in an animal model of schizophrenia. *Brain, Behavior, and Immunity*, 38, 175-184. [DOI:10.1016/j.bbi.2014.01.019]

Meyer, U., Feldon, J., & Yee, B. K. (2009). A review of the fetal brain cytokine imbalance hypothesis of schizophrenia. *Schizophrenia Bulletin*, 35(5), 959-972.

Meyer, U., Yee, B. K., & Feldon, J. (2007). The neurodevelopmental impact of prenatal infections at different times of pregnancy: The earlier the worse? *The Neuroscientist*, 13(4), 241-256. [DOI:10.1177/1073858406296401]

Moazed, A. A., Ghotbeddin, Z., & Parham, G. H. (2007). Comparison of the effects of dose-dependent zinc chloride on short-term and long-term memory in young male rats. *Pakistan Journal of Biological Sciences: PJBS*, 10(16), 2704-2708. [DOI:10.3923/pjbs.2007.2704.2708]

Mousaviyan, R., Davoodian, N., Alizadeh, F., Ghasemi-Kasman, M., Mousavi, S. A., Shaerzadeh, F., et al. (2021). Zinc supplementation during pregnancy alleviates lipopolysaccharide-induced gial activation and inflammatory markers expression in a rat model of maternal immune activation. *Biological Trace Element Research*, 199(11), 4139-4204.

Müller, N. (2018). Inflammation in schizophrenia: Pathogenetic aspects and therapeutic considerations. *Schizophrenia Bulletin*, 44(5), 973-982. [DOI:10.1093/schbul/sby024]

Patel, K. R., Cherian, J., Gohil, K., & Atkinson, D. (2014). Schizophrenia: Overview and treatment options. *P & T: A Peer-Reviewed Journal for Formulary Management*, 39(9), 638-645.

Potter, B. D., & Adlard, P. A. (2017). Zinc signal in brain diseases. *International Journal of Molecular Sciences*, 18(12), 2506. [DOI:10.3390/ijms18122506]

Potvin, S., Stip, E., Sepehry, A. A., Gendron, A., Bah, R., & Kouassi, E. (2008). Inflammatory cytokine alterations in schizophrenia: A systematic quantitative review. *Biological Psychiatry*, 63(8), 801-808. [DOI:10.1016/j.biopsych.2007.09.024]

Reisinger, S., Khan, D., Kong, E., Berger, A., Pollak, A., & Pollok, D. D. (2015). The poly (I:C)-induced maternal immune activation model in preclinical neuropsychiatric drug discovery. *Pharmacology & Therapeutics*, 149, 213-226. [DOI:10.1016/j.pharmthera.2015.01.001]

Ruiz, S., Birbaumer, N., & Sitaram, R. (2013). Abnormal neural connectivity in schizophrenia and fMRI-brain-computer in-
terface as a potential therapeutic approach. *Frontiers in Psychiatry*, 4, 17. [DOI:10.3389/fpsyg.2013.00017]

Samuelsson, A. M., Jennische, E., Hansson, H. A., & Holmäng, A. (2006). Prenatal exposure to interleukin-6 results in inflammatory neurodegeneration in hippocampus with NMDA/GABAA dysregulation and impaired spatial learning. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 290(5), R1345-R1356.

Solek, C. M., Farooqi, N., Verly, M., Lim, T. K., & Ruthazer, E. S. (2018). Maternal immune activation in neurodevelopmental disorders. *Developmental Dynamics*, 247(4), 588-619. [DOI:10.1002/dvdy.24612]

Sun, C., Fukushi, Y., Wang, Y., & Yamamoto, S. (2018). Astrocytes Protect Neurons in the Hippocampal CA3 Against Ischemia by Suppressing the Intracellular Ca2+ Overload. *Frontiers in Cellular Neuroscience*, 12, 280.

Tarisov, V. V., Svistunov, A. A., Chubarev, V. N., Sologova, S. S., Mukhortova, P., Levushkin, D., et al. (2020). Alterations of astrocytes in the context of schizophrenic dementia. *Frontiers in Pharmacology*, 10, 1612. [DOI:10.3389/fphar.2019.01612]

Trépanier, M. O., Hopperton, K. E., Mizrahi, R., Mechawar, N., & Bazinet, R. P. (2016). Postmortem evidence of cerebral inflammation in schizophrenia: A systematic review. *Molecular Psychiatry*, 21(8), 1009-1026. [DOI:10.1038/mp.2016.90]

van Erp, T. G., Hibar, D. P., Rasmussen, J. M., Glahn, D. C., Pearlson, G. D., Andreassen, O. A., et al. (2016). Subcortical brain volume abnormalities in 2028 individuals with schizophrenia and 2340 healthy controls via the ENIGMA consortium. *Molecular Psychiatry*, 21(4), 547-553. [DOI:10.1038/mp.2015.63]

Waterhouse, U., Roper, V. E., Brennan, K. A., & Ellenbroek, B. A. (2016). Nicotine ameliorates schizophrenia-like cognitive deficits induced by maternal LPS exposure: A study in rats. *Disease Models & Mechanisms*, 9(10), 1159-1167.

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 & Biological Psychiatry*, 57, 17–30.

Yolken, R. H., Dickerson, F. B., & Fuller Torrey, E. (2009). Toxoplasma and schizophrenia. *Parasite Immunology*, 31(11), 706-715.

Zhou, H. (2015). Region specific effects of maternal immune activation on offspring neuroimmune function. *Open Journal of Immunology*, 5(2), 51.
