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Loewen SM, Chavesa AM, Murray CJ, Traetta ME, Burns SE, H. Pekarik K, Tremblay M-È

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Review Article

The Outcomes of Maternal Immune Activation Induced with the Viral Mimetic Poly I:C on Microglia in Exposed Rodent Offspring

Sophia M. Loewen[a,b], Adriano M. Chaves[a,c], Colin J. Murray[a,b], Marianela E. Traetta[d,e], Sophia E. Burns[a,f], Keelin H. Pekarik [a,g], and Marie-Ève Tremblay[a,h,i,j,k,l]

[a] Division of Medical Sciences, University of Victoria, Victoria, BC, Canada

[b] Neuroscience Graduate Program, University of Victoria, Victoria, BC, Canada

[c] Department of Physiology and Pharmacology, Faculty of Medicine, Universidade Federal do Ceará, Fortaleza, CE, Brazil

[d] Instituto de Biología Celular y Neurociencia, CONICET, Universidad de Buenos Aires, Buenos Aires, Argentina

[e] Facultad de Farmacia y Bioquímica, Cátedra de Farmacología, Universidad de Buenos Aires, Buenos Aires, Argentina

[f] Department of Biology, University of Victoria, Victoria, BC, Canada

[g] Department of Neuroscience, University of Toronto, Toronto, ON, Canada

[h] Département de Médecine Moléculaire, Université Laval, Quebec City, QC, Canada

[i] Axe Neurosciences, Center de Recherche du CHU de Québec, Université Laval, Quebec City, QC, Canada

[j] Neurology and Neurosurgery Department, McGill University, Montréal, QC, Canada

[k] Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada

[l] Center for Advanced Materials and Related Technology (CAMTEC), University of Victoria, Victoria, BC, Canada

Short Title: Outcomes of Maternal Immune Activation on Microglia

Corresponding Author: Marie-Ève Tremblay

Full name: Marie-Ève Tremblay

Department: Division of the Medical Sciences

Institute/University/Hospital: University of Victoria

Street Name & Number: 3800 Finnerty Road

City, State, Postal code, Country: Victoria, BC, V8W 2Y2, Canada

Tel:250-721-8739

E-mail: evetremblay@uvic.ca

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Abstract

Maternal immune activation (MIA) can result from a variety of maternal inflammatory factors, including metabolic disorders, nutritional deficits, infections, and psychosocial stress. MIA has been consistently recognized as a major risk factor for neurodevelopmental disorders, and this association seems to be especially important for viral infections, as viral exposure during pregnancy was associated with a higher risk of developing neurodevelopmental disorders, such as schizophrenia. In MIA, the gestational parent's inflammatory response to an immune stimulus alters or interrupts fetal development, triggering neurodevelopmental consequences. As MIA can occur in any pregnancy it is important to understand the many factors at play that contribute to altered brain development in the offspring, especially considering recent global events such as the COVID-19 pandemic. The underlying mechanisms by which MIA results in deleterious outcomes are not yet clear, but due to the inflammatory response it initiates, it is becoming apparent that microglia are critically involved. Through investigation of MIA animal models, the role of microglia in this field is becoming more evident. Compelling evidence from animal models indicates that MIA can disrupt synaptic pruning, neuronal progenitor cell proliferation /differentiation, oligodendrogenesis and more. Microglia appear as an active player, assisting these neural-related functions during healthy development, but also mediating MIA-induced disturbances in these critical processes when neurodevelopment is challenged. The present review illustrates this complex web by reviewing recent literature, focusing on the outcomes of MIA resulting from viral mimetic poly I:C in rodents, to provide a clear description of how MIA impacts microglial functions and what this means for the offspring's neurodevelopment. Moreover, we discuss the possible implications of the COVID-19 pandemic on the neurodevelopment of the current and next generations in the frame of MIA models and propose some putative pharmacological and non-pharmacological approaches to prevent or attenuate MIA consequences.

Introduction

Maternal immune activation (MIA) is defined as any systemic inflammatory response taking place during pregnancy, which could, in turn, compromise brain function and behavior in the exposed offspring [1,2]. Emerging evidence based on epidemiological case-cohort and long-term prospective studies demonstrated that MIA, and consequently the exposure to inflammation during intrauterine development, leads to an increased risk for neurodevelopmental and neuropsychiatric disorders later in the offspring's life (e.g., autism spectrum disorder (ASD), schizophrenia (SCZ), and epilepsy) [3]. Preclinical studies in rodents and non-human primates, using environmental stressors, maternal diet, live microorganisms or related immunogens, were able to successfully mimic neurobehavioral alterations related to neurodevelopmental and neuropsychiatric disorders in animal models [4,5]. In this context, viral infections, including varicella, cytomegalovirus, mumps, and herpes simplex virus, can act as *in utero* inflammatory triggers [6–9]. Due to increased psychological and viral stressors in the modern world—especially with the COVID-19 pandemic—the need to better understand the association between MIA and potential detrimental outcomes in future generations is paramount.

Microglia—the resident immune cells of the brain—are an important link between immune stressors and brain health, acting as key players during development [10]. In this review, we will focus on viral infections as immune response triggers because of the drastic increase in risk for neurodevelopmental disorders after a viral infection during pregnancy and considering the relevance to current conditions associated with COVID-19. COVID-19, which is caused by SARS-CoV-2 viral infection, has potentially important, yet still largely undefined impacts on neurodevelopment [11–16]. Additionally, we will describe the current knowledge on the roles of microglia following MIA induced with the viral mimetic

polyriboinosinic-polyribocytidylic acid (poly I:C) in rodents. Therefore, this review aims to address the factors that trigger MIA focusing especially on viral pathogens, the use of poly I:C to model MIA in rodents, and the outcomes of this model on microglial functions in exposed rodent offspring. Furthermore, we will peer into the current implications and future directions of the field in the view of the COVID-19 pandemic.

2. Maternal Immune Activation by Viral Pathogens and Risk of Psychopathology

To introduce MIA, we can go back to the influenza epidemic in 1957 where vast numbers of individuals around the world contracted influenza while pregnant. Later, through meticulous observations of prenatal clinical records in Sweden, this viral infection during pregnancy was determined to significantly increase the likelihood of developing SCZ in the exposed offspring [17,18]. This link was shown not only in Sweden but also for other cohort-populations, including in Spain, the United States, Australia and Japan [19,20]. A similar association with SCZ was demonstrated for other viral infections, such as rubella [21]. Of note, other neurodevelopmental disorders, such as ASD and attention deficit hyperactivity disorder, follow this same trend, as their risk increases after exposure to *in utero* inflammation [11–14]. However, when using cohort studies to investigate MIA and the associated neurodevelopmental disorders, it is important to keep in mind that results from these studies can be hindered by confounding variables [8,9,22]. An excellent example of this is ASD, where it is important to acknowledge that the past 50 years have witnessed substantial changes to ASD diagnostic practices, as well as ASD awareness, resulting in increased diagnoses [23–25].

Having a better understanding into the pathophysiological mechanisms that drive increased risk of neurodevelopmental disorders after MIA is a critical step to propose better diagnostic, preventive and therapeutic strategies for these vulnerable populations. In this context, MIA is considered to act on a threshold basis; less severe infections may have no noticeable effect on the exposed offspring. However, subthreshold MIA can act as a predisposing factor, or a "tipping point" for the future emergence of various neurodevelopmental disorders [26–28]. This phenomenon is proposed as a "two-hit hypothesis", where an initial "hit", such as MIA, can increase the potential of a second "hit", such as psychosocial stress, drug exposure or infections occurring later in life, to induce the onset of severe neurodevelopmental disorders, like SCZ [29]. For example, inducing MIA in rodents using the viral mimetic poly I:C followed by exposure to a second stressor (e.g., short-term unpredictable stress) into adolescence resulted in behavioural abnormalities such as impairments in prepulse inhibition during adulthood in exposed offspring [30]. In this two-hit model, the tretracycline antibiotic minocycline, commonly used for its immunomodulatory actions, prevented the abnormal behavioral responses to the second stressor when administered in tandem during adolescence, indicating that 'priming' of the inflammatory response to subsequent stressors is tightly associated with these behavioural abnormalities [30]. However, despite this increased vulnerability to subsequent stressors, it is important to note that MIA alone can have significant effects on neurodevelopment, without the need for a second stressor later in life to trigger behavioral abnormalities [31,32]. In addition, recent evidence reported by Hayes et al., 2022 showed that inducing MIA with poly I:C in mice at embryonic day (E)9.5 did not cause an immune sensitization, but a lasting decrease of immune reactivity into adulthood [33]. This blunted immune response was accompanied by changes in nuclear chromatin accessibility and reduced transcription factor occupancy of the open chromatin in microglia, the immune cells of the brain which start colonizing the brain at E9.5, and altered neurotransmitter release in striatal dopaminergic medium spiny neurons [33].

While the severity of the infection can determine the neurodevelopmental outcome, an abundance of host-related factors are also of influence, including: genetic predispositions [27], timing of the inflammatory response [34], and offspring sex [35]. The influence of timing is notably related to the point of gestation, affecting different stages of neurodevelopment, as well as microglial brain colonization and functional maturation [34]. For example, a metanalysis of cohort and case-conroled studies conducted in humans showed an increased risk for ASD upeon exposure to viral infections during the second trimester. This is unlike SCZ, where the risk is higher with infection occurring in the first trimester [9,36].

The fact that MIA similarly results in an increased risk of neurodevelopmental disorders across different viral strains suggests the existence of a relationship between the antiviral-based innate immune response, characterized notably by increased levels of interferon (INF) type 1 and interleukins (IL) such as IL6, and psychopathology risk in the exposed offspring [37–39]. However, it is unknown if certain viral infections and/or certain host-specific factors (e.g., genetics) are correlated with specific developmental disorders (e.g. ASD, SCZ) [40,41]. However, it is well known that the activated innate immune response among the maternal-fetal axis induces neurobehavioral outcomes in animal models. As such, the activation of this axis serves as the underlying basis for modeling MIA in rodents and non-human primates, providing a useful approach to unravel the pathophysiological mechanisms of MIA and its effects during neurodevelopment [4,42]. In the next section, we will examine the insights provided by modelling of MIA, specifically using poly I:C, in rodents.

3. Maternal Immune Activation with Poly I:C: Insights from Rodent Models

Research on rodents and non-human primates has provided key insights into the downstream effects of MIA in the exposed offspring [43]. While this review focuses on studies performed in rodents, there is also a very strong non-human primate evidence in this field (refer to these reviews for more information [44–46]). Studies in rodents generally induce MIA with various inflammatory agents, including poly I:C, the bacterial endotoxin lipopolysaccharide (LPS), human influenza virus and others [43,47,48]. Poly I:C is a synthetic double-stranded (ds)RNA analog of retroviral genomic dsRNA. When administered to pregnant rodents, it mimics a viral infection *in utero* and therefore serves as a model to investigate the association between MIA and neurodevelopmental disorders such as ASD and SCZ [49–51].

In adult rodents, systemic administration of poly I:C causes peripheral and central inflammation resulting in fever and sickness-like behavior [52–54]. As defined by Dantzer et al., 2008 sickness behavior is a complex behavioral pattern commonly induced by infections and tissue injury that allows to conserve energy and enhance recovery against acute inflammation. This behavioral pattern is characterized by malaise, hyperalgesia, pyrexia, apathy, reduction of locomotor activity, reduction of reproductive performance, sleep disturbances and anxiety [55,56]. In this context, poly I:C mimics a viral infection through its binding to Toll-like receptor (TLR) 3 mainly found on macrophages and dendritic cells, but not restricted to these cells, in mammals [51,57]. Once bound, poly I:C causes a systemic increase in the production of pro-inflammatory cytokines by these cells, such as IFN-γ, IL-1β, IL-6, and tumor necrosis factor (TNF)α, thus mimicking the acute inflammatory state that would occur following a viral infection [52,54,58]. Antiviral innate immune responses are the body's front lines of defense against viral infections. Rapid and effective recognition of microbial infection or danger signals depends on pattern-recognition receptors (PRRs) that specifically recognize pathogen-associated molecular patterns (PAMPs)[59]. The most characterized PRRs are the TLRs, TLR3 mediating the transcriptional induction of type I interferons (IFNs), thereby collectively establishing an antiviral innate host response [51,57,60]. Studies have shown that unlike other TLR family members, TLR3 is the only RNA sensor that

is dependent on the Toll-interleukin-1 receptor (TIR)-domain-containing adaptor- inducing IFN-β (TRIF) and fundamentally involved in IFN-mediated antiviral responses [61].TLR3 main ligands are double-stranded RNAs (dsRNA), which are viral replication intermediates from different dsRNA viruses (e. g., rotavirus, respiratory syncytial virus, murine cytomegalovirus), and single-stranded RNA (ssRNA) virus (e. g., West Nile virus) [62,63]. Also, TLR3 extracellular domain can be activated by the synthetic dsRNA analog poly I:C, similarly inducing recruitment of the adaptor proteins involved in transcriptional activation of the TRIF pathway [63]. An essential role for TLR3 has been demonstrated in the antiviral responses against several viruses in humans, such as herpes simplex virus type 1, human immunodeficiency virus, rotavirus, influenza A virus and SARS-CoV-2 [64–66]. In mice, systemic injection of poly I:C activates the TLR-3 signaling cascade and induces a typical dose-response related sickness-like behavior, characterized by decreased locomotor activity, increased burrowing and decreased body weight, and mild hyperthermia. Along with this, poly I:C increases the serum and CNS levels of pro-inflammatory cytokines and type I IFNs (α and β) [52,54,58,67,68].

To model MIA in rodents, an intraperitoneal injection of poly I:C is commonly given to pregnant dams during the mid to late gestational stages (ranging from E ~ 9 to 20 in mice), which induces an acute inflammatory response in the maternal-placental-fetal axis [49,58]. The placenta is a transient organ that is fundamental for the bidirectional communication between gestational parent and fetus, which is commonly referred to as the 'maternal-placental-fetal axis' [42]. The placenta houses maternal immune cells, such as decidual macrophages and natural killer cells, which can respond to an inflammatory trigger by releasing proinflammatory factors and attracting maternal peripheral granulocytes and lymphocytes. This response impacts the placental-fetal barrier and reaches the fetal brain [69,70]. In this context, Monteiro et al., 2022 showed that a poly I:C challenge at E13.5 induced not only an acute pro-inflammatory response in gestational plasma, increasing IL-6, chemokine (C-X-C motif) ligand 1 (CXCL-1) and chemokine (C-C motif) ligand (CCL)-2/monocyte chemoattractant protein (MCP)-1 levels, but also decreased cell proliferation and increased cell death of placental cells in the junctional zone [71]. Additionally, poly I:C induced the expression of some placental efflux transporters in this placental zone, which are responsible for the transport of cholesterol, xenobiotics, and cytokines across the placental barrier [71]. Further, with poly I:C injection at E13.5 there was a decrease in the placental expression of some essential amino acid transporters, which are fundamental for fetal brain development [71]. Therefore, this evidence shows that poly I:C not only activates the parental innate immune system, but also disrupts the placental barrier, leading to an abnormal flux of soluble mediators, such as pro-inflammatory cytokines, that can reach and activate a fetal immune response.

As mentioned above, poly I:C induces an imbalance of pro- and anti-inflammatory cytokines in the maternal serum that can be transmitted to the fetus through a disrupted placental barrier [49,72,73], resulting in altered brain development and long-term behavioural outcomes. In this context, several authors have proposed that the overall extent and timing of the maternal cytokine-associated inflammatory response to infection is more relevant than the nature of the pathogen in determining outcomes on the exposed offspring [74–76]. Indeed, the exposure of pregnant mice to the IL-6 cytokine (administered at E12.5) was sufficient to induce multiple neurodevelopmental-related deficits in the adult offspring, such as sensorimotor gating and cognitive impairments [38,51].

Some of these cytokines (IL-6, IL-1α, IL-1β and TNF-α, for example) were shown in rodents and humans to enter the fetal blood-brain barrier [77]. Cytokines can also cross the placental barrier during gestation, while permeability of the placental barrier (to IL-6) was found to be highest in mid- compared to late-gestation in rats [78]. Furthermore, a study by Wu et al., 2017 showed that placental IL-6 is necessary to induce MIA with poly I:C at E12.5 in mice [79]. Additionally, infections, notably viral infections, can disrupt the placental barrier integrity, allowing for an abnormal flux of pro-inflammatory

mediators, and even pathogenic viral transmission, named vertical transmission [80,81]. Together, these findings indicate that the permeability for several cytokines can be altered in the context of MIA, thereby reaching the fetal circulation.

The timing of MIA challenge seems to be particularly relevant to determine the spectrum of behavioral and neurochemical phenotypes elicited in rodents. Considerable differences have been reported between prenatal poly I:C exposure during early/middle (E9-10) and late (E17-19) gestation in the offspring. Of note, early gestation poly I:C exposure in mice was associated with predominant SCZ-like symptoms in adulthood (12-16 weeks of postnatal life), such as deficits in prepulse inhibition and latent inhibition, and increased D2 dopamine receptors and tyrosine hydroxylase in the ventral striatum [82–84]. By contrast, late gestation MIA in mice induced phenotypes more consistent with affective/cognitive domain deficits in adult mice, such as impaired reversal learning and working memory deficits, and reduced NMDA receptor subunit, NR1, among the adult hippocampus [84,85]. These findings were partially replicated in adult rats, corroborating the predominant positive-like symptoms and dopaminergic alterations observed in the nucleus accumbens of animals exposed to poly I:C at early (E10) compared to late (E19) gestational age [86,87]. When translating neurodevelopmental timing between species, the first trimester of human gestation can be considered to represent up to E17 in mice and the second trimester up to postnatal day (P)5 in mice, while a human infant born at term is comparable to mice at approximately P10 [88]. This may vary depending on the process being studied, in particular it has been postulated that E11-13 cortical development corresponds to fetal development of the human brain gestational week 5-6 [89,90]. Together, this evidence suggests that the timing of MIA induced by viral-like stimuli determines the neurodevelopmental vulnerability associated with *in utero* infection and the emergence of traits related to specific neurodevelopmental disorders, such as ASD or SCZ, which are associated with alterations during distinct neurodevelopmental periods [34,91].

However, there is considerable variation in the protocols developed to induce MIA using poly I:C in rodents, which can account for the different neurobehavioral outcomes reported in the literature. In a recent systematic review and meta-analysis of rodent studies, the authors reported that poly I:C doses ranged from 0.25 mg/kg to 20 mg/kg, while the duration of the treatment varied from a single injection day to repeated treatment (up to three injection days) [35,92]. Additionally, the administration route can differ between intravenous, intraperitoneal and subcutaneous [49]. Furthermore, different mouse strains can result in differential response to poly I:C-induced MIA (changes found predominantly in behaviour but also in immune response) [93–95]. Therefore, despite poly I:C-induced MIA being an informative model to investigate the viral-like *in utero* infection and related neurodevelopmental outcomes in rodents, conclusions should be taken with caution considering the great intervariable differences observed between studies and protocols to induce MIA.

Another important aspect for MIA associated neurodevelopmental outcomes are sex differences and their strong interaction with other determining factors, such as the window of MIA exposure [96,97]. Of note, Nakamura et al., 2022 demonstrated that sex interacts with the MIA window of exposure to determine alterations of translationally relevant cognitive domains at postnatal day (P)70 in mice [35]. Early gestation MIA (induced by poly I:C) disrupted working memory in both adult males and females. Only females showed reduced perseverative behavior after early MIA (E9), while only males showed deficits in reversal learning after late MIA (E15). In both time windows and across sexes, MIA caused deficits in GABAergic markers, such as the homeobox protein Nkx2.1 and reelin expression, among the dorsal hippocampus of exposed adult offspring [35]. Furthermore, sex is also a determining factor for the long-term neurobehavioral alterations induced by a two-hit model combining neonatal poly I:C exposure and peripuberal stress [98,99]. In this model, female rats showed more pronounced deficits in prepulse inhibition of the startle reflex and hyperlocomotion in early adulthood (P60), while males

showed more deficits in social interaction at the same age. Both male and females exhibited similar working memory deficits in adulthood [98].

In the last decades, poly I:C induced MIA has been explored as a relevant strategy to model some translationally related psychopathology outcomes in rodents. The reduced biosafety risks conferred with poly I:C to the personnel and environment makes this strategy particularly convenient. Also, this model was proven to recapitulate distinctive developmental vulnerabilities associated with specific subsets of neurodevelopmental disorders, such as psychosis-related behavioral endophenotypes and ASD-related social and communication deficits [100,101]. However, that does not mean it is without limitations. Besides the already mentioned inter-variability reported in MIA protocols involving poly I:C, contrary to live pathogens (such as influenza), poly I:C results in an inherently less widespread immune response, differing from "natural" infections [102]. Therefore, the strategy of inducing MIA through "sterile inflammation" through viral-particle mimetics cannot fully recapitulate the actual immune response, recruiting both innate and adaptive branches, induced by live microorganisms such as influenza and other clinically relevant viruses [103,104]. Therefore, developing animal models with better translational validity capable of recapitulating fully the neuroimmune spectrum of MIA's long-term consequences remains a challenge in the field.

4. The Physiological Roles of Microglia in Neurodevelopment

Microglia perform a myriad of functions that are crucial during development and across the lifespan [43,102,105,106]. In mice, primitive hematopoiesis initiates in the yolk sac around E7.0, shortly after the onset of gastrulation, to generate erythrocytes and primitive macrophages [107,108]. The primitive macrophages spread into the embryo proper through the blood when the circulatory system becomes established (from E8.5 to E10) and they migrate into various tissues, including the brain around E9.5, giving rise to microglia [109,110]. Once microglia are seeded in the parenchyma, these cells proliferate and expand their population until the second week of postnatal life in rodents, and their density is maintained thereafter by low proliferation levels into adulthood at steady-state [105,110–114]. Microglia are long lived cells, meaning that unlike short-lived circulating monocytes which are replaced by newly produced cells, the overall microglial population ages with the brain as these cells undergo a slow but continuous turnover [115]. An implication is that an impaired functioning of microglia during development may be carried forward throughout the next stages of an individual's life [116].

Studies in rodents provide evidence that microglia in the embryonic brain initially localize to a few areas, including where newly forming blood vessels and developing neurons reside [105]. In these niches, microglia promote the sprouting and branching of budding blood vessels through direct contacts and release of soluble factors, such as vascular endothelial growth factor C and D, but also several wingless-related integration sites (Wnt) signaling components including Wnt5a and Wnt11 ligands, and Wnt receptors frizzled class receptor (Fzd)7, Fzd8 and low-density lipoprotein receptor-related protein (Lrp)5. These Wnt ligands usually suppress abnormal angiogenesis, allowing to shape vascular patterns during brain development [117–119]. Furthermore, there is a microglial state (termed capillary-associated microglia) which remains in close proximity to brain vessels and may play a role in vasodilation and blood flow modulation starting as early as P5 until 12 months of age in mice [120].

Microglia further localize to neurogenic regions like the subventricular zone (SVZ), especially in the early postnatal life (first two postnatal weeks in rodents) where they notably contribute to neurogenesis, oligodendrogenesis, myelination, and astrocyte fate-differentiation through neural precursor cells [121–125]. Another major role of microglia in the developing brain relates to neuronal survival and the

shaping/altering of neuronal circuits and synapses. Indeed, in the embryonic cerebral cortex of non-human primates and rats, microglia regulate the number of cortical neurons by phagocytosing neural precursor cells [126,127]. In rats, for instance, at E13 and E17, microglia immunolabeled for ionized calcium binding adaptor molecule (Iba)1—a binding protein specific to microglia and macrophages—were sparsely distributed throughout the cerebral cortex. However, at E19–20, during the peak of cortical layer 2 neurogenesis [128], Iba1 immunopositive microglia substantially increased their presence in the ventricular (VZ) and SVZ zones. These Iba1-positive cells within the mitotic VZ-SVZ showed predominant ameboid morphologies (greater than 95% of all Iba1-positive cells) and upregulated pro-inflammatory and phagocytic markers, such as iNOS, HLA-DR and CD11b. These Iba1-positive cells were also accompanied by reduced numbers of dividing precursor cells, suggesting their role in the thinning of these cellular zones at the end of rat embryonic development (E20) [126].

Furthermore, there is evidence showing that microglia play distinctive functions to support neurogenesis and neuronal survival during postnatal brain development [128,129]. For instance, Ueno et al., 2013 reported that in the mouse brain, ameboid microglia accumulate within the white matter, including the subcortical white matter, internal capsule and cerebral peduncle from P1–P3, while their numbers in these white matter regions peak at P7 [130]. Microglial modulation through minocycline or transient depletion through diphtheria toxin receptor (DTR) transgenic expression under the control of ITGAM (integrin alpha M or CD11b) promoter in mice during the P3–P4 period resulted in increased apoptotic neurons within the cortical layer. Also, this work identified microglia-secreted insulin growth factor (IGF)-1 as a trophic factor required to maintain neuronal survival during postnatal cortical development [130]. These findings were corroborated by *in vitro* studies showing that primary microglia from P1–P8 mice co-cultured with primary neural stem cells (NSCs) of the SVZ secrete factor(s) essential for postnatal neurogenesis [131] and differentiation into committed cell fates, such as astrocytes [132].

Microglia not only provide crucial trophic support (i.e., IGF-1) required for neuronal survival [133,134], but also dynamically respond to neuronal activity and engulf or “prune” synapses via different mechanisms including phagocytosis and trogocytosis [135–137]. These pruning actions of microglia play a significant role in determining the proper functioning of the brain, contributing to synaptic plasticity and the formation of appropriate neural circuits. The disruption of this key microglial physiological function can alter neurodevelopment, potentially contributing to various behavioural alterations (e.g., social interaction deficits, hyperlocomotion) associated with neurodevelopmental disorders like ASD and SCZ [138]. This is exemplified by the fractalkine receptor Cx3cr1 deficient mice. These mice show transiently reduced microglial density during the second and third postnatal weeks, accompanied by excessive weak and immature excitatory synapses in the hippocampus [135]. These findings were later extended by Zhan et al., 2014 reporting that Cx3cr1 deficient mice not only show deficient synaptic pruning, but also decreased functional connectivity between the prefrontal cortex and hippocampus, deficits in social interaction, and increased repetitive-behaviors that have been previously associated with ASD [139].

Oligodendrogenesis and myelin formation occur mainly during early postnatal days in mammals, during which microglia are also engaged in already mentioned functions, such as regulating neurogenesis and synaptic pruning, leading to hypothesize that similar mechanisms may contribute to microglia-oligodendrocyte interactions during the same developmental period [140]. In this context, Shigemoto-Mogami et al., 2014, demonstrated that minocycline treatment from P2 to P5 blocked the homeostatically secreted levels of some microglia-derived cytokines, such as IL-1β, IL-6, TNFα, and IFN-γ and inhibited oligodendrogenesis in the rat forebrain SVZ at P10 [125]. Also, it was reported that microglia-dependent pruning was important for normal myelin sheath formation [140]. Indeed, Nemes-Baran et al., 2020 further demonstrated that a population of ameboid microglia which migrates from

the VZ into the corpus callosum at P3–7 phagocytoses viable oligodendrocyte progenitor cells (OPCs) before the onset of myelination in mice [141]. Also, the engulfment of viable OPC by microglia does not take place during this period in Cx3cr1 deficient mice, increasing the number of immature oligodendrocytes and reducing axonal myelin thickness [141].

In the last years, sex was revealed as a major determining factor for the neurodevelopmental outcomes of early life adversity, including MIA, in both humans and animal models [96]. In this context, compelling evidence in humans advocate that males have increased vulnerability to early life adversity challenges, which are reflected by the male to female ratio for some neurodevelopmental disorders, such as ASD [142,143]. One of the possible underlying mechanisms for these sex-related differences is microglial sex-dependent responses [96,144]. A pioneering study by Schwarz et al., 2012 elegantly demonstrated in rats how sex influences microglial brain colonization, density, and morphology during early postnatal development into adulthood [145]. This study showed that male rats have higher microglial density, represented here by Iba1-positive cells, at P4 with predominantly ameboid morphologies, while females have a reduced microglial density in almost all examined brain regions (parietal cortex, CA1, CA3 and dentate gyrus of the hippocampus), except for the amygdala at the same age. Contrarily, at juvenile (P30) and adult (P60) stages, female rat microglia showed an increased density with a predominantly ameboid morphology. Also, at these ages, female rat microglia overexpressed several pro-inflammatory mediators (IL-1α, IL1f5, and IL1r1 mRNA expression and IL-1β protein), suggesting differences in microglial basal reactivity according to the sex and age [145]. These findings of microglial sex differences at steady state were further corroborated by studies examining the outcomes of immune challenges. Bacterial *Escherichia coli* infection in male rat pups (at P4) was shown to cause long-term changes in brain cytokine expression (increased IL-1β gene expression) in adulthood, while females did not exhibit the same vulnerability [146–148]. Also, the same challenge when performed in adolescent males (P30) did not cause the same long-term neuroimmune and behavioral changes [147]. Taken together, these microglial sex differences during development and later in life can provide a reasonable hypothesis for the strong sex bias in the prevalence and presentation of several neuropsychiatric disorders [96,145].

Altogether, the critical microglial contributions to brain angiogenesis, neural precursor cell survival, synaptogenesis and myelination in the developing brain across pre- and postnatal stages underscores the importance of microglial physiological functions for proper behavioral outputs into adolescence and adulthood [149–151]. Microglia respond very quickly to changes in their environment, notably according to sex and age/developmental period, conferring to immune challenges the ability to impair their physiological functions. MIA can thus have drastic impacts on microglial physiological functions during development and beyond, culminating into long-lasting disorders.

5. Microglial Functions Disturbed by Maternal Immune Activation

Given that microglia invade the developing brain around E9.5 in rodents and the majority of studies inject poly I:C after this time point, microglial presence in the brain at the time of MIA is unquestionable (Table 1; Figure1). This insult appears to affect microglia indirectly via MIA-induced systemic inflammation. As mentioned above, poly I:C is first recognized by TLR3 in maternal immune cells, then a viral-type response is mounted. Kwon and collaborators compared the administration of poly I:C *versus* resiquimod (ssRNA virus mimetic) to pregnant mice on E12.5 [152]. Contrary to poly I:C, resiquimod activates TLR7/8 [152]. This study showed that both viral mimetic compounds could engage the maternal immune system in a similar response characterized by increased maternal plasma levels of IL-6, TNFα, IL-10, C-C motif chemokine ligand (CCL)-2, CCL5, C-X-C motif chemokine ligand-10, and leukemia inhibitory factor after 4 hours [152]. However, the cytokine profile of the placenta depended

on the viral-like immune stressor: resiquimod increased *Tnfα*, *Ccl5*, *Ccl11* and *Cxcl1* mRNA levels while poly I:C induced *Il6*, *Il10*, and *Cxcl10* mRNA levels [152]. This emphasizes that even when viral infections can mount a similar immune response in the periphery, different viral stimuli can impact the local environment (e.g., the placenta) in different ways. In particular, placental IL6 signaling has been proved to be decisive in MIA (poly I:C, E12.5) outcomes [79]. Interestingly, Kwon and collaborators observed increased levels of cytokines in the fetal brain only with resiquimod after 4 hours with no observable changes for poly I:C. Since there are other reports that showed increased *Il-6* in the fetal brain after poly I:C injection at E12.5 as early as 3 hours post-injection [79] or later at E18 [153], this response seems to depend on the time of analysis. This last study [153] showed that MIA induced with poly I:C at E12.5 in mice similarly increased *Il6* in the maternal plasma and placenta, and later at E18 microglia from fetal brains showed an increase in *Il6* mRNA levels. Of note, the MIA-induced microglial expression of *Il1b* and *Tnf*α observed at E18 was dependent on the timing of poly I:C administration: *Tnf*α decreased when poly I:C was injected at E12, while *Il1b* increased when injected at E15 [153]. Interleukins, such as IL6 and IL17a, are known to have a critical role in linking MIA with poly I:C to behavioral outputs [38,154]. Furthermore, recent work showed that upregulation of maternal INF type I, as part of the maternal immune response due to poly I:C injection (E14), reduced fetal microglial proliferation and altered the mouse offspring's behavior [155], supporting the idea that microglia are responding to the maternal environment (Figure 1).

Microglial morphology was found to be relatively unchanged following MIA induced with poly I:C in mice, regardless of timing for induction (E12.5 and E15.5) and analysis (E18 and P10) [153]. Smolders et al, 2015 also investigated the impact of MIA with poly I:C at E11.5 or with double injection of poly I:C at E11.5 and E15.5 in mice, examining microglial density (CX3CR1-eGFP cells) and reactivity markers (Mac2/Galectin-3, inducible nitric oxide synthase and IL1β) by immunohistochemistry, finding no changes *versus* control animals at E11.5 or E17.5 [156]. In addition, a direct incubation for 24 hours with poly I:C did not alter the number of microglia positive for Mac-2, IL1β and iNOS *ex vivo* in mouse brain slices (E15.5) [156]. Although there were no significant changes found in microglial morphology, *Tmem119* expression and microglial motility were found altered in fetal and early postnatal brain following MIA in mice suggesting that microglial physiological functions could be altered early during development [152]. Ozaki et al. also found an increased microglial *Tmem119* mRNA expression in P10 microglia isolated by magnetic-activated cell sorting from offspring exposed to MIA with poly I:C at E15, highlighting differences in this homeostatic microglial marker [153]. Tmem119 is a microglial marker which has been found downregulated on reactive microglia in some pathological contexts [157]. Also, Ozaki et al. showed evidence of altered microglial motility following MIA with poly I:C, induced at E12 in mice, showing an increase in acute brain slices from E18 mice but a decrease in vivo, analyzed by two photon imaging, at P10 [153].

Mounting evidence showed that microglia transcriptional signature is deeply affected following MIA. Ben-Yehuda et al. determined that microglia from newborn mice (P0) following MIA (poly I:C, E15.5) showed reduced expression of genes related to proliferation regulated by IFN signaling [155]. Also, Hayes et al. demonstrated altered microglial gene expression in P4 mouse offspring exposed to MIA (poly I:C, E9.5), indicating a reduction in immune pathways related to INF signaling and phagocytosis [33]. Into adulthood, the offspring exposed to MIA showed no differences in microglial gene expression but after exposure to a second immune challenge (LPS), these microglia presented a blunted transcriptional immune response characterized by a reduced gene induction and suppression in

response to LPS compared to controls [33]. Consistently, microglia showed smaller lysosomal contents after a LPS challenge in adult offspring exposed to MIA suggesting reduced phagocytic activity [33]. This is in line with previous findings from Matcovitch-Natan et al., 2016 describing in mice a disrupted microglial transcriptional signature in newborns exposed to MIA (poly I:C, E12.5) which normalized into adulthood, unveiling a shift in microglial maturation [158]. Also, Ikezu et al. found alteration of microglial gene expression profiles early during development (E17 and P7) in mouse offspring exposed to MIA (poly I:C, E9.5) [159]. They also reported increased microglial hyper-ramification and contacts with glutamatergic presynaptic terminals, as well as immature dendritic spines in adult mice (P60) exposed to MIA [159]. Mattei et al., 2017 also found an altered microglial gene expression in the hippocampus of adult mice following MIA (poly I:C, E15) characterized by downregulation of pro-inflammatory and phagocytosis related genes (Fcgr1, Itgav, P2ry6, Sirpa, Siglece, Cx3cr1, Spi1, Irf8) [160]. This translated in a decreased phagocytic activity in hippocampal microglia isolated from adult mice (P60) exposed to MIA [160]. Yu and collaborators recently described microglial Gpr56 as a molecular target of MIA-induced neurodevelopmental disorder. Mice exposed to MIA (poly I:C, E12.5) showed a downregulation of microglial Gpr56 at E14.5 which was mediated by maternal IL17a . This downregulation increased Tnfα expression leading to PV+ interneuron deficits and autistic-like behavior in adult mice. Enriched genes in microglia from MIA animals were further associated with risks genes for SCZ and ASD supporting the link between compromised microglial function and neurodevelopmental disorders [33,159,161].

To understand microglial role in the alterations induced by MIA, studies have used a colony-stimulating factor 1 receptor (CSF1R) inhibitor to deplete microglia after MIA induction and allowed for microglial repopulation after withdrawal of the drug. Although this inhibitor can also affect peripheral immune cells [162] and leave unaffected some microglial states [163], it is an interesting tool to address microglia association to MIA outcomes. Using this strategy during gestation after MIA, Hayes et al. showed that microglial repopulation rescued microglial immune responses to LPS and excitatory neurotransmission in adult mice following MIA [33]. Ikezu et al. also applied the inhibitor at a postnatal stage (P21-P42) and observed a normalization of microglial hyper-ramification and aberrantly increased density of immature dendritic spines [159].

As microglia play critical roles in synaptic pruning, synapse maturation and plasticity [135], microglial dysfunction during development can have long-lasting effects on neuronal networks and behavior. Indeed, genetically engineered mice lacking Cx3cr1 or triggering receptor expressed on myeloid cells 2 (TREM2) display impaired synaptic pruning, as well as increased synaptic density and autistic-like behaviors [139,164]. Particularly interesting, Wegrzyn et al., 2021 demonstrated that microglia exposed to poly I:C *in vitro* became round-shaped (classified as ameboid morphology by the authors, suggesting increased activity) and their secretome was sufficient to alter perineuronal nets and increase synaptic activity of cultured hippocampal neurons [165].These studies allow us to move further down the path of unraveling how microglia may serve as a therapeutic target in the future.

Current Implications and Future Directions

Understanding how viral infections can impact offspring is especially of importance with the COVID-19 pandemic. Preliminary studies have found that contracting COVID-19 can elicit a diverse range of inflammatory responses in the body [166,167]. We hypothesize that the effects of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection on fetal brain development will be mediated through MIA, giving rise to both fetal brain and placental inflammation, as shown through multiple recent studies [168–170]. In more severe cases, COVID-19 induces a storm of proinflammatory

cytokines, including IL-2, IL-6, IL-10 and IL-1β, some of which were also identified as key players in MIA [166,167,170–172]. In a poly I:C mouse model (injections at E16 or P4), it was shown that cytokines IL-1β, IL-13, and chemokines were elevated in the fetal brain acutely after the immune challenge (6h and 24 hours later) and likely played a role in the negative effects on neurodevelopment during this time. A cohort study performed in Kuwait examined birth outcomes of infants born to people infected with SARS-CoV-2 during pregnancy. Most infants had typical outcomes, exhibiting no behavioural deficits; however, around 10% of them experienced neurodevelopmental and behavioural delays. Pregnant people infected by SARS-CoV-2 in the first or second trimesters of pregnancy had a greater likelihood of having offspring with developmental delays in communication, problem solving, personal-social and motor skills [173]. A SARS-CoV-2 infection during pregnancy, associated with immune imprinting (a previously acquired immune system response caused by a former exposure to a different strain of the antigen [174]), can increase cytokine levels which is one hypothesis for neurodevelopmental deficits in the offspring of infected pregnant people [170]. Similarly, Eldlow et al., 2022 reported an increased incidence of developmental disorders affecting motor function, expressive language, as well as speech and language in the offspring of mothers infected with SARS-CoV-2 during pregnancy [175]. However, in this cohort study, the association was found when maternal infection occurred during the third trimester [175]. As microglia are key players of inflammation, particularly producing these inflammatory cytokines, further research into the roles played by microglia in relation to SARS-CoV-2 infection *in utero* is warranted. With the recent and ongoing nature of COVID-19 pandemic, data is preliminary and studies currently lack long-term outcomes [176–179].

As previously discussed, MIA rodent models are often used to investigate the complex mechanisms of MIA, but they also allow to investigate how to attenuate or prevent MIA consequences in exposed offspring through relatively accessible means. Prevention, more than cure, has been intensively discussed in the field of neurodevelopmental disorders. The disorder's neuropathology usually starts during the critical periods of neurodevelopment, normally in gestational/perinatal life and adolescence, but once the disorder is fully established it is difficult to reverse the neurobehavioral patterns [180]. Therefore, in this context, MIA models provide a useful tool to investigate new pharmacological and non-pharmacological strategies, such as enriched environment [181] and specific nutritional interventions [99], to prevent or attenuate the neuroimmune consequences following these early life events [76].

In this context, Hashimoto 2020 suggest that a maternal diet high in anti-inflammatory nutrition (e.g., foods that contain sulforaphane – derived from glucoraphanin, such as broccoli) can be used to combat abnormal fetal brain development following maternal COVID-19 [177,182]. This is based on evidence from their previous studies that glucoraphanin consumption by the dams during pregnancy and by the offspring at weaning, helps prevent cognitive decline in adult mouse offspring exposed to MIA induced by poly I:C given for six consecutive days (E12 to E17) [183,184]. Furthermore, a study by Vuillermot et al., 2017 looked at the effect of vitamin D (400 ng/kg subcutaneous, E9) in a MIA mouse model (poly I:C , E9), which showed prevention of some autism-related phenotypes, such as stereotyped digging and impaired acquisition of fear memory, in adolescence (P30–40), however there was no reduction in the fetal pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) [185].Moreover, MIA rodent models have been used to assess the relevance of non-pharmacological strategies, leading to the discovery that environmental enrichment protects against the effects of MIA in offspring, likely due to improved maternal care as a result of the enhanced environment [181].

While further intensive investigation is required to unravel the mystery of microglia's relationship to MIA when induced with poly I:C, microglia clearly do play a role in determining the long-term consequences of this maternal challenge on the brain and behavior as highlighted in this review.

Analyzing only one piece of the picture can lead to the wrong conclusions. For instance, looking at morphology and not at changes in secreted mediators and functions or involving dynamic glia-glia and neuron-glia interactions, could lead to the wrong conclusions being drawn. When analyzing changes in microglia, it is best to compile a variety of measurements to better inform conclusions and paint a well-rounded picture[157]. Studying inter-cellular relationships in the lab can be difficult, but to create translationally relevant models, researchers need to consider how their factors of interest can simultaneously influence complex mechanisms [186]. In summary, this review compiled relevant information to provide brief insights into the mysterious worlds of MIA and microglia to help unravel their multidimensional interactions.

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Conflict of Interest Statement

The authors declare that this review was written without any commercial or financial relationships that could be construed as a potential conflict of interest.

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Figure 1. **Microglia at the crossroads of maternal immune activation and offspring future outcomes.** The administration of polyriboinosinic-polyribocytidylic acid (poly I:C) during gestation induces maternal immune activation (MIA). The gestational parent's immune cells recognize poly I:C by Toll-like receptor 3 (TLR3) resulting in an increased expression and secretion of inflammatory mediators such as interleukin (IL)-6 and interferon type (INF) 1. There is also a local inflammatory response mounted in the placenta characterized mainly by increased levels of *Il6*. This impacts the fetal brain, increasing the expression of *Il6* and reducing transmembrane protein (*Tmem)119* (marker for microglia) in fetal microglia. Even when no changes were found regarding microglial morphology, an increase in their motility was reported. Regarding gene expression, the reduction in G protein-coupled receptor 56 (*Gpr56*) proved to be key to determine future outcomes. Once born, the microglia from pups prenatally exposed to MIA showed a compromised transcriptional signature, a conserved morphology but a reduction in their motility. In adulthood, although microglial basal gene expression was found to be relatively unchanged, there are reports of increased process ramification, reduced response to lipopolysaccharide (LPS), decreased phagocytosis and increased neuronal contacts. (Other abbreviations: ASD, autism spectrum disorder; SCZ, schizophrenia)

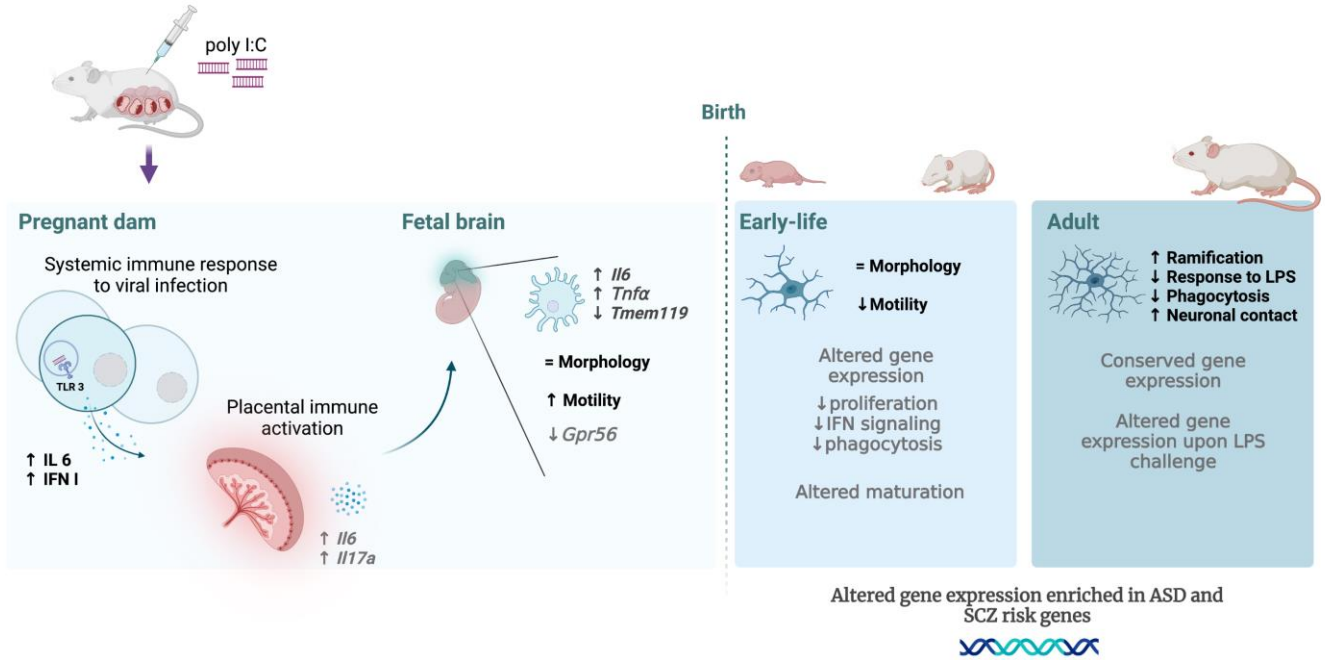

Table 1. Summary of the reviewed studies investigating changes of microglia in rodent offspring exposed to maternal immune activation (MIA) induced by the viral mimetic polyriboinosinic-polyribocytidylic acid (poly I:C).

Reference	Species/model	Sex of offspring	Age during injection	Poly I:C dose and route of administration	Age(s) when offspring analyzed	Main microglia findings in relation to changes in brain and behavior
Ben-Yehuda et al., 2020 [155]	C57BL/6J mice; Interferon α/β receptor 1 knockout (IFNARKO)-C57BL/6J mice; CX3CR1-GFP-C57BL/6J mice	Male	E14.5	5 mg/kg (i.v. injection)	E15.5 P90 P120	• MIA reduced microglial expression of genes related to proliferation and cell cycle • Maternal elevation of type-I interferons β resulted in schizophrenia-like behavior in the offspring • In a "two-hit" paradigm, offspring from mother with higher type-I interferons β showed increased sensitivity to postnatal stress
Giovanoli et al., 2016 [30]	C57BL/6J mice	Male	E9	1 mg/kg (i.v. injection)	Behavior: P70-P90 Immunohistochemistry (IHC): P41	• Tetracycline antibiotic MINO prevents some behavioral abnormalities (e.g., sensorimotor gating) normally associated with MIA • This treatment did not affect microglia density (measured by Iba1 positive cells) but did decrease CD68 positive cells (potentially a decrease in phagosomal activity) in the hippocampus and prefrontal cortex interleukin-1β expression
Hayes et al., 2022 [33]	C57BL/6J mice	Male variable	E9.5	10-20 mg/kg (i.p. injection); followed by LPS 4,500 endotoxin units/g (i.p. injection)	E18 P4 adult	• Second stimulus (LPS) indicates blunted microglia response in frontal cortex and especially the striatum of E18, P4 and adult mice • Poly I:C alone also blunts immune response in P4 mice • Repopulation of microglia ameliorated blunted response • Evidence for more robust blunting of microglia from males
Hui et al., 2020 [97]	C57BL/6 mice	Male and Female	E9.5	5 mg/kg (i.p. injection)	Behavior: P60-80 IHC: P80-90	• Increased microglial CD68 in the dentate gyrus of female offspring, suggesting enhanced phagolysosomal activity in MIA conditions • Increased density of dark microglia in males and behavioral impairments (e.g., anxiety and sensorimotor gating)
Ikezu et al. 2021 [159]	C57BL6/J mice	Male	E9.5	20 mg/kg (i.p. injection)	P60	• MIA alters microglial transcriptional signatures in the medial prefrontal cortex • Microglial depletion and repopulation normalize expression of neuritogenic molecules in microglia, as well as MIA-associated neurophysiological and behavioral changes
Kwon et al., 2021 [152]	C57BL/6J mice	Male and Female	E12.5	Subcutaneous injection of poly I:C (20 mg/kg) or resiquimod (2 mg/kg)	E12.5 (dams culled 4 hours after injection)	• The comparison of poly I:C and resiquimod determined that in maternal plasma there were increased levels of IL-6, TNFα, and chemokines • Only resiquimod caused increases in TNFα RNA and in the fetal brain; resiquimod elevated immune related cytokine and chemokine levels
Mattei et al., 2017 [160]	C57BL/6 mice	Male	E15	5 mg/kg (i.p. injection)	P60	• In adult offspring exposed to MIA had altered microglial transcriptome and phagocytic functions • Adult mice treated with minocycline showed complete reversal of the MIA-associated changes in microglia transcriptional, functional, and behavioral alterations
Ozaki et al., 2020 [153]	CX3CR1-EGFP- C57BL/6 mice	Male	E12 E15	10 mg/kg (i.p. injection)	E18 P10 P42	• MIA in mid-pregnancy (E15) increased IL-6 expression in embryonic microglia, however it did not cause any significant changes in morphology either at E18 or postnatally in cortical layers • MIA induced earlier (E12), caused alterations sustained through adolescence (P42) in the patterns of microglial process motility as well as in social and cognitive behavioral deficits
Smolders et al. 2015 [156]	CX3CR1-eGFP-C57BL6/J mice	Male and Female	E11.5 (for single injection) E11.5 and E15.5 (for double injection)	20 mg/kg (i.p. injection)	E11.5 E12.5 E17.5	• Microglial density and reactivity markers expression (antibodies against interleukin-1β, inducible nitric oxide synthase and Mac-2/Galectin-3) in the fetal cortex and hippocampus were not significantly different between the control and MIA groups
Zhang et al., 2019 [53]	Sprague-Dawley rats	Male and Female	E15	4 mg/kg (i.v.injection)	P2	• Increased density of microglia with amoeboid morphologies in supraventricular corpus callosum • Sex differences evident (e.g., differences in tangential and radial migration and morphology) • Reduced microglial density (decrease in IBA1 positive cells) in striatum, hippocampal fissure, CA1 and CA3 of hippocampus
Zhu et al., 2014 [151]	C57BL/6 mice	Male and Female	E9	20 mg/kg (i.p. injection)	P56-P62	• Poly I:C resulted in behavioral deficits (e.g., social deficits and prepulse inhibition deficits) that were all alleviated by minocycline • Increase microglial density (IBA1 positive cells)) that was inhibited with the addition of minocycline in the hippocampus, thalamus, and cerebral cortex • Microglia assume amoeboid morphologies in MIA exposed offspring

Abbreviation: i.v., intravenous; i.p., intraperitoneal; poly I:C, polyinosinic:polycytidylic acid; LPS, lipopolysaccharide, Iba1, ionized calcium binding adaptor molecule; IL, interlukin; CD11b, cluster of differentiation 11b (marker for dark microglia), CD68, cluster of differentiation 68 (marker for phagosomal activity in microglia), GABA, γ-aminobutyric acid; DG, dentate gyrus; TREM2, triggering receptor expressed on myeloid cells 2; IHC, immunohistochemistry; TNFα, tumor necrosis factor alpha.