

Controversies and prospects about microglia in maternal immune activation models for neurodevelopmental disorders.

Peer-reviewed author version

SMOLDERS, Silke; Notter, Tina; SMOLDERS, Sophie; RIGO, Jean-Michel & BRONE, Bert (2018) Controversies and prospects about microglia in maternal immune activation models for neurodevelopmental disorders.. In: BRAIN BEHAVIOR AND IMMUNITY, 73, p. 51-65.

DOI: 10.1016/j.bbi.2018.06.001

Handle: <http://hdl.handle.net/1942/28236>

**Controversies and prospects about microglia in maternal immune activation
models for neurodevelopmental disorders.**

Silke Smolders¹, Tina Notter², Sophie MT Smolders³, Jean-Michel Rigo⁴, Bert Brône⁴

1 BIOMED – U Hasselt, Hasselt, Belgium

Laboratory of Neuronal Differentiation, VIB Center for the Biology of Disease, Leuven and Center
for Human Genetics, KU Leuven Leuven, Belgium

2 Institute of Pharmacology and Toxicology, University of Zurich-Vetsuisse, Zurich, Switzerland

Neuroscience Center Zurich, University of Zurich and ETH Zurich, Zurich, Switzerland.

3 BIOMED – U Hasselt, Hasselt, Belgium

INSERM, UMR S 1130, Université Pierre et Marie Curie Paris, France; CNRS, UMR 8246,

Université Pierre et Marie Curie Paris, France; UM 119 NPS, Université Pierre et Marie Curie Paris,
France

4 BIOMED – U Hasselt, Hasselt, Belgium

Mailing addresses:

silke.smolders@kuleuven.vib.be, tina.notter@uzh.ch, sophie.smolders@uhasselt.be,

jeanmichel.rigo@uhasselt.be, bert.brone@uhasselt.be

**Individual, address, and telephone number to whom correspondence concerning manuscript should
be sent:**

Bert Brône

Martelarenlaan 42, 3500 Hasselt, Belgium

+32 11 26 92 37 bert.brone@uhasselt.be

Article type: Review

Declarations of interest: none

Abstract

Activation of the maternal immune system during pregnancy is a well-established risk factor for neuropsychiatric disease in the offspring, yet, the underlying mechanisms leading to altered brain function remain largely undefined. Microglia, the resident immune cells of the brain, are key to adequate development of the central nervous system (CNS), and are prime candidates to mediate maternal immune activation (MIA)-induced brain abnormalities. As such, the effects of MIA on the immunological phenotype of microglia has been widely investigated. However, contradicting results due to differences in read-out and methodological approaches impede final conclusions on MIA-induced microglial alterations. The aim of this review is to critically discuss the evidence for an activated microglial phenotype upon MIA.

Keywords: Maternal immune activation, Microglia, Neurodevelopmental disorders, Poly(I:C), LPS

1. Introduction

Maternal immune activation during pregnancy has been recognized to increase the risk for neurodevelopmental psychiatric disorders such as schizophrenia, Autism Spectrum Disorders (ASD) and bipolar disorder. Numerous epidemiological studies identified a number of inflammation-inducing agents to be associated with psychiatric illnesses including prenatal exposure to viruses such as rubella, herpes simplex, measles and polio (Torrey *et al.* 1988, Suvisaari *et al.* 1999, Brown *et al.* 2001, Buka *et al.* 2001), various bacterial pathogens (Sorensen *et al.* 2009), and protozoan parasite such as *Toxoplasma gondii* (Mortensen *et al.* 2007). Animal models of maternal immune activation (MIA) have further established causality by showing that immune activation during pregnancy can perturb processes of early neurodevelopment in the offspring and change developmental trajectories. These MIA models precipitate into long-term behavioral and neuronal deficits relevant for psychiatric illnesses such as schizophrenia, ASD and related disorders (as reviewed in a.o. (Boks 2010, Meyer 2014, Solek *et al.* 2017)).

In the MIA model, the maternal immune system can be stimulated by a variety of immune-activating agents including virus ((Fatemi *et al.* 1998) and others), lipopolysaccharide (LPS) ((Borrell *et al.* 2002) and others) and polyriboinosinic-polyribocytidilic acid (Poly(I:C)) ((Meyer *et al.* 2005) and others) resulting in viral, bacterial-like and viral-like immune responses respectively. Intriguingly, the structural and functional abnormalities in offspring exposed to MIA seem not to be dependent on the immune-activating agents per se but critically depend on the general maternal and fetal immune response (Smith *et al.* 2007, Choi *et al.* 2016). Specific cytokines including interleukin (IL) 6 and IL17a have been identified as key players in mediating the disruption of neurodevelopmental processes in the MIA models (Smith *et al.* 2007, Choi *et al.* 2016). Moreover, the importance of the maternal gut microbiota in the response to MIA has recently been elucidated by Kim *et al.* 2017, showing that treatment with the broad spectrum antibiotic vancomycin prior to MIA was able to prevent the emergence of behavioral abnormalities in the offspring (Kim *et al.* 2017). Despite this, however, the precise mechanisms through

1 which prenatal immune activation perturbs early brain development and leads to behavioral and
2 neuronal deficits remain largely unknown. In recent years, there has been an increasing interest in the
3 functional contribution of the neuro-immune system, and in particular aberrant microglia function, in
4 the pathophysiological processes underlying neurodevelopmental disorders such as schizophrenia and
5 ASD.

6 Microglia are the resident immune cells of the central nervous system (CNS). They are derived from
7 primitive yolk-sac macrophages that invade the brain during early development (Ginhoux *et al.* 2010).
8 In the adult brain, microglia actively scan the CNS parenchyma for potential changes in brain
9 homeostasis or harmful insults by protruding and retracting their processes (Nimmerjahn *et al.* 2005).
10 Once microglia sense changes in CNS integrity, they react accordingly. The classical response involves
11 a process that is often referred to as “microglia activation” and includes changes in the expression
12 profile of distinct markers of microglia, increased production and secretion of pro- and anti-
13 inflammatory cytokines and chemokines, and a shift in the morphological appearance from ramified
14 to a more amoeboid morphology (Ransohoff and Cardona 2010). This classical immune-related
15 activation refers to microglia responses towards harmful stimuli such as damage or invading
16 pathogens. Over the past decade, however, the functional role of microglia has been extended to non-
17 immunological functions including the regulation of neurogenesis, myelination and synaptic
18 remodeling (Bilimoria and Stevens 2015, Salter and Stevens 2017). As a consequence, the sustained
19 activity of microglial protrusions might also reflect a continuous contribution in the ongoing
20 remodeling of the brain instead of simply scanning the brain (Mosser *et al.* 2017). Microglia have thus
21 been attributed a key role in neurodevelopmental processes, including the establishment of proper
22 neuronal connectivity and brain homeostasis (as extensively reviewed in (Arnold and Betsholtz 2013,
23 Bilimoria and Stevens 2015, Wu *et al.* 2015, Frost and Schafer 2016, Reemst *et al.* 2016, Mosser *et al.*
24 2017, Paolicelli and Ferretti 2017)). Given these neuro-modulatory functions, abnormal microglia
25 activity may thus alter neuronal development and thereby contribute to the behavioral and neuronal
26 deficits observed in neurodevelopmental disorders.

Consequently, it has been proposed that prenatal immune activation could act directly on microglia and thereby alter their function in the developing brain, resulting in changing the developmental trajectories and ultimately leading to the behavioral and neuronal deficits detected in MIA models (Knuesel *et al.* 2014, Paolicelli and Ferretti 2017). In support of this hypothesis is the identification of signs of central inflammatory processes and microglia anomalies in post-mortem brains of a subset of schizophrenic and ASD patients (Vargas *et al.* 2005, Morgan *et al.* 2010, Trepanier *et al.* 2016, van Kesteren *et al.* 2017). Besides this, there have been numerous studies that investigated microglia phenotypes in the MIA model in order to associate microglia activity with brain and behavioral abnormalities (Arsenault *et al.* 2014, Bilbo *et al.* 2017, Paolicelli and Ferretti 2017). However, the reports are inconsistent with some detecting microglia with an altered phenotype (e.g. (Esslinger *et al.* 2016, Gumusoglu *et al.* 2017)) whereas others detect no changes in microglia biology in offspring prenatally exposed to MIA (e.g. (Giovannoli *et al.* 2015, Smolders *et al.* 2015)). It is one of the main aims of this review to thoroughly discuss the controversies in the field and critically revise possible factors that could contribute to the controversial findings regarding microglia alterations in the MIA model. Among these factors are those associated to the commonly used microglial activation read-outs (such as the age, sex and brain region analyzed) as well as MIA-model associated factors (such as the immune stimulant used and the age of MIA induction). We aim to conclude with suggestions/guidelines to improve the consistency in the field of MIA-microglia research.

2. Methods

The publications discussed in paragraph 3 were retrieved by searching the Title and/or Abstract field in pubmed using the following search terms: ‘maternal immune activation’, ‘prenatal immune activation’, ‘maternal immune stimulation’, ‘prenatal immune stimulation’, ‘maternal immune challenge’, ‘prenatal immune challenge’, ‘prenatal infection’, ‘prenatal viral infection’, ‘prenatal bacterial infection’, ‘maternal viral infection’, ‘maternal bacterial infection’, ‘maternal infection’ or

1 'maternal inflammation'. Every publication found by these search terms was opened and inspected for
2 analysis on microglia. Whilst the MIA model has been extended to pigs (Antonson *et al.* 2017) and
3 (non)-human primates (Careaga *et al.* 2017) we will only discuss and revise studies that systemically
4 administered immune agents such as LPS or Poly(I:C) or specific cytokines (excluding intrauterine
5 administration) in rodents including mice and rats. Although early postnatal injections (either through
6 direct infection of the neonate or through infection of the lactating dam) of Poly(I:C) have been used
7 to mimic late-gestation infection in humans (Ribeiro *et al.* 2013, Arad *et al.* 2017, Monte *et al.* 2017),
8 we will only consider studies inducing MIA at prenatal stages in order to include the effects of the
9 maternal immune system and contribution of the placenta.

11 **3. Microglial reaction to maternal infection**

12 The findings on MIA-induced alterations of microglia activity in rodents are divergent. There are
13 reports that describe alterations in the microglia activity state that point towards a pro-inflammatory
14 phenotype. These include increased microglia density (here defined as amount of cells/mm²) (e.g.
15 (Juckel *et al.* 2011, Manitz *et al.* 2013, Li *et al.* 2014, Zhu *et al.* 2014)), increased expression of microglia
16 "activation" markers (e.g. Major histocompatibility complex II (MHCII), Cluster of differentiation (CD)
17 68 or CD54 (Borrell *et al.* 2002, Van den Eynde *et al.* 2014, Esslinger *et al.* 2016)), and changes in the
18 morphological appearance (e.g. altered microglial branches (Juckel *et al.* 2011, Van den Eynde *et al.*
19 2014, Corradini *et al.* 2017)). On the other hand, there are multiple studies where no obvious changes
20 in microglia activity were detected (e.g. (Garay *et al.* 2013, Giovanoli *et al.* 2015, Giovanoli *et al.* 2016)).
21 Multiple reasons may underlie these contradictory results and should be considered when comparing
22 the outcome of different studies on "microglia activation".

23 Firstly, "microglial activation" can be assessed in several ways, some of which are more sensitive than
24 others. The most common methods used in MIA models include the estimation of the number/density

of microglia by means of stereology (e.g. (Mattei *et al.* 2014, Giovanoli *et al.* 2015, Hadar *et al.* 2016)), and the morphological appearance of microglia (e.g. (Giovanoli *et al.* 2015, Hsueh *et al.* 2017, Notter *et al.* 2017)). Another common approach to measure microglia activity is the assessment of the expression – usually by means of immunohistochemistry or biochemistry – of microglia activation markers (such as CD68 (Esslinger *et al.* 2016, Manitz *et al.* 2016, Notter *et al.* 2017) and Translocator protein (TSPO) (Mattei *et al.* 2017, Notter *et al.* 2017). To identify or isolate microglia from the brain, samples obtained from non-transgenic animals are stained with markers that are highly expressed by microglia (such as ionized calcium-binding adapter molecule 1 (Iba1), CD11b or CD68) or samples are obtained from transgenic animals where a reporter gene is expressed under the CX3C chemokine receptor 1 (CX3CR1) promotor. Although these molecular markers for microglia are often considered to be microglia-specific, they are also expressed by other members of the mononuclear phagocyte system such as monocytes and macrophages. Therefore, many groups have recently searched for a molecular marker that allows to distinguish between microglia and other immune cells in the brain. As such, the expression of the transcriptional repressor Spalt Like Transcription Factor 1 (Sall1) was recently shown to be largely restricted to microglia in the adult brain, although some cells in the liver, kidney and heart were also found to express this marker (Buttgereit *et al.* 2016). Similarly, Transmembrane protein 119 (Tmem119) was identified as a microglia-specific marker in the human and mouse CNS (Bennett *et al.* 2016). Although these markers will be of great value to distinguish microglia in the adult brain, their use in embryonic contexts should be further investigated.

Besides the methodology and readouts used to assess microglia activation state, the brain region investigated and time point/developmental stage when microglia are analyzed differ among the different studies, which makes direct comparisons merely impossible. In addition to these analytical issues, there are numerous other factors to be considered including the immune stimulant used, gestational window of immune activation, frequency of immune challenges, and route of administration that could all contribute to the observed inconsistencies in microglia phenotypes in the MIA model. Moreover, factors that are not directly related to the method of MIA-induction or microglia

activation assessment could influence the outcome of MIA studies, including housing conditions, food pellet composition and specific mouse strain (Babri *et al.* 2014, Li *et al.* 2015, Buschert *et al.* 2016, Weiser *et al.* 2016).

The vast majority of studies have analyzed MIA-induced alterations of microglial activity during postnatal stages while only a few studies have focused on microglial changes during prenatal developmental stages. In the first section, we focus on findings derived from offspring in postnatal stages subdivided according to the specific read-out used to assess microglia activation (number/density, morphology, expression of microglia activity dependent genes and proteins) in order to compare different MIA-microglia studies easier. However, as will be discussed later in this review, even by dividing studies in this manner, many other variables make direct comparison merely impossible. In the subsequent section, findings obtained during prenatal development are discussed.

3.1 Readout Postnatal Stage

Microglia Number/Density

Upon detection of an insult, microglia are known to migrate to the site of injury/infection and/or proliferate, a phenomenon called microgliosis (Streit *et al.* 1999). “Microglial activation” is thus often measured in relation to its number/density in specific brain regions of interest. Despite seemingly straightforward, the density of microglia can vary depending on the age and sex of the animal, as well as the brain region investigated (Schwarz *et al.* 2012) (as will be discussed in section 4). Moreover, studies differ with regard to the marker used to identify microglia (Iba1 or CD11b) and therefore, in the tables below, the specific markers are mentioned. In addition, the variety in techniques to determine microglia density (fluorescent vs chromogenic staining and software) might lead to variability in results between different studies.

A total of 21 studies were included in this review assessing microglia density in different brain regions of rat and mice offspring exposed to MIA (Table 1). Out of these, 19 studies measured microglia in

adult offspring (age > P60). A minority of these studies (only 5) reported an increased density of microglia in several brain regions while the vast majority (14 studies) did not detect a change in microglial density. Similar to the findings in adult brain, measurements in younger, adolescent offspring provides more evidence for unchanged microglial densities (4 studies) in comparison to increased density (only 2 studies).

These results suggest that MIA only sporadically induces microgliosis in certain brain regions. The biological function of increased numbers of microglia in specific brain regions of MIA exposed offspring and its consequences on brain formation and maturation has yet to be identified.

Table 1: Microglia number/density in postnatal MIA offspring

| Microglia Number/Density | | | | |
|----------------------------------------------------------|---------------------------------------------------|----------------------------------------------------|----------------|----------|
| Toxin/ Microglia marker/ Ref. | Species/ Dose/ Gestation Day | Age of Analysis | No Change | Increase |
| Poly(I:C) Iba1 (Juckel <i>et al.</i> 2011) | BALB/c mice/ 20mg/kg (i.p.)/ GD 9 | PND 30 | Cx | HPC, Str |
| Poly(I:C) CD11b (Hsiao <i>et al.</i> 2012) | C57BL6 mice/ 20mg/kg (i.p.) GD 12.5 | Adult | Whole brain | |
| Poly(I:C) Iba1 (Garay <i>et al.</i> 2013) | C57BL6 mice/ 20mg/kg (i.p.) GD 12.5 | PND 0, PND 7, PND 14, PND 30, PND 60 | FCx, CgCx, HPC | |

| | | | | |
|--------------------------------|----------------|--------------|-----------------|-------------|
| Poly(I:C) | BALB/c mice/ | PND 10 | Str, FaCx, HPC, | |
| Iba1 | 20mg/kg (i.p.) | | VCx, MCx | |
| (Manitz <i>et al.</i> 2013) | GD 9.5 | PND 30 | Str, MCx | FaCx, HPC |
| | | PND 100 | HPC, VCx, MCx | Str, FaCx |
| Poly(I:C) | C57BL6 mice/ | PND 85 | mPfcx, Str | |
| Iba1 | 5mg/kg (i.v.) | | | |
| (Willi <i>et al.</i> 2013) | GD 9 | | | |
| Poly(I:C) | C57BL6 mice/ | PND 2 | | HPC |
| Iba1 | 20mg/kg (i.p.) | PND 80 | HPC | |
| (Li <i>et al.</i> 2014) | GD 9.5 | | | |
| Poly(I:C) | C57BL6 mice/ | PND 62 | | Th, Cx, HPC |
| Iba1 | 20mg/kg (i.p.) | | | |
| (Zhu <i>et al.</i> 2014) | GD 9 | | | |
| Poly(I:C) | C57BL6 mice/ | PND 28, PND | HPC | |
| Iba1 | 5mg/kg (i.v.) | 140, PND 600 | | |
| (Giovanoli <i>et al.</i> 2015) | GD 17 | | | |
| Poly(I:C) | CD-1 mice/ | PND 172 | | |
| Iba1 | 20mg/kg (i.p.) | Standard | Cx, HPC | |
| (Buschert <i>et al.</i> 2016) | GD 9.5 | housing | | |
| | | Enriched | Cx, HPC | |
| | | housing | | |

| | | | | |
|--------------------------------|----------------------------------------------------------|----------------|--------------------|--------------|
| Poly(I:C) | C57BL6 mice/ | PND 35, PND 84 | HPC | |
| Iba1 | 5mg/kg (i.v.) | | | |
| (Giovanoli <i>et al.</i> 2016) | GD 9 | | | |
| Poly(I:C) | C57BL6 mice/ | PND 90 | Cx | |
| Iba1 | 2mg/kg (i.p.) | | | |
| (Corradini <i>et al.</i> 2017) | GD 9 | | | |
| Poly(I:C) | C57BL6 mice/ | PND 90 | mPfCx | |
| Iba1 | 5mg/kg (i.v.) | | | |
| (Notter <i>et al.</i> 2017) | GD 9 | | | |
| Poly(I:C) | C57BL6 mice/ | PND 80 | PfCx, Cb, HPC | |
| Iba1 | 5mg/kg (i.p.) | | | |
| (Hui <i>et al.</i> 2018) | GD 9.5 | | | |
| Poly(I:C) | Lewis rat/ | PND 9 | | Forebrain |
| Iba1 | 200 µg/kg | | | White matter |
| (Girard <i>et al.</i> 2010) | (i.p.)/every 12h starting from GD 17.5 until birth | | | |
| Poly(I:C) | Wistar rat/ | PND 128 | Str, Cb, HPC, PfCx | NAC |
| Iba1 | 4 mg/kg (i.v.) | | | |
| (Mattei <i>et al.</i> 2014) | GD 15.5 | | | |

| | | | | |
|-----------------------------|------------------|----------------|-------------------|----------|
| Poly(I:C) | Wistar-Hannover | PND 90 | FtCx, CC, HPC, | |
| CD11b/Iba1 | rat/ | | Th, Str, Pons | |
| (Missault <i>et al.</i> | 4 mg/kg (i.v.) | | | |
| 2014) | GD 15.5 | | | |
| Poly(I:C) | Sprague Dawley | PND 180 | Th, Cx, HPC, Str, | Pons, CC |
| CD11b | rat/ | | | |
| (Van den Eynde <i>et</i> | 4 mg/kg (i.v.) | | | |
| <i>al.</i> 2014) | GD 15.5 | | | |
| Poly(I:C) | Wistar rat/ | PND 130 | PfCx | NAC, HPC |
| Iba1 | 4 mg/kg (i.v.) | | | |
| (Hadar <i>et al.</i> 2016) | GD15.5 | | | |
| Poly(I:C) | Long-Evans rat/ | PND 7, PND 21, | MpCx, FaCx, | |
| Iba1 | 4 mg/kg (i.v.) | PND 35, PND 90 | PaCx, Am | |
| (Paylor <i>et al.</i> 2016) | GD 15.5 | | | |
| LPS | C57BL6 mice/ | PND 56 | Cx | |
| Iba1 | 25-50µ/kg (i.v.) | | | |
| (Hsueh <i>et al.</i> 2017) | GD 15 - 17 | | | |
| LPS | Sprague Dawley | PND 70 | HPC | |
| Iba1 | rat/ | | | |
| (Mouihate 2016) | 100 µg/kg (i.p.) | | | |
| | GD 15.5 | | | |

HPC: Hippocampus, NAc: Nucleus accumbens, Str: Striatum, Th: Thalamus, Cx: Cortex, CgCx: Cingulate Cortex, VCx: Visual Cortex, MCx: Motor cortex, FaCx: Frontal association Cortex, FCx: Frontal Cortex, FtCx: Frontotemporal Cortex, (m)Pfcx: (medial) Prefrontal Cortex, MpCx: Medial Prelimbic Cortex, PaCx: Primary Auditory Cortex, CC: Corpus Callosum, Cb: Cerebellum, Am: Amygdala

Microglia Morphology

A second parameter often used to assess “microglia activation” is the cell’s morphology. The fact that pathological insults can cause microglia to adopt a more amoeboid shape has led to the assumption that ramified microglia represent a “resting” state while amoeboid forms were interpreted as “activated” state (Hanisch and Kettenmann 2007). Single cell transcriptome studies in microglia, however, revealed that microglia activation states cannot be simply classified into “resting” and “active” states but rather represents a continuum at which ramified microglia represent an active form as well (Crotti and Ransohoff 2016). Hence, equating microglia morphology with microglia activation could therefore result in false negative results and misleading conclusions. Indeed, changes in microglia morphology represent an adequate response towards a specific insult but should not be taken as a general sign of microglia activation (Harry and Kraft 2012, Streit *et al.* 2014). On top of that, during brain development, the morphology of microglia is changing gradually from amoeboid to ramified and the age of the animals at which microglial morphology is studied might contribute to contradictory results. Conversely, the fact that microglia are ramified does not necessarily mean they are not responsive to an inflammatory stimulus on the transcriptional level: it was shown that 2-12 hours after a systemic inflammatory stimulus, transcriptional changes occurred in microglia while changes in morphology were only detected after 24-48 hours (Norden *et al.* 2016).

Nevertheless, multiple MIA studies have taken morphological measures to assess microglia activity (Table 2). Similar to the microglia density analyses, the assessment of microglial morphology in MIA has resulted in contradictory findings. The findings of these studies vary from decreased numbers of

processes (Juckel *et al.* 2011, Van den Eynde *et al.* 2014, Corradini *et al.* 2017, O'Loughlin *et al.* 2017, Hui *et al.* 2018) – indicative of the amoeboid state – to increased ramification (Gumusoglu *et al.* 2017). In comparison to these 6 studies, however, the 7 other studies presented in this review detected no changes in the microglia morphology of MIA exposed adult offspring (Garay *et al.* 2013, Missault *et al.* 2014, Giovanoli *et al.* 2015, Giovanoli *et al.* 2016, Mouihate 2016, Hsueh *et al.* 2017, Notter *et al.* 2017).

Table 2: Microglia morphology in postnatal MIA offspring

| Microglia Morphology | | | | |
|--------------------------------------------------------------------|---------------------------------------------------|------------------------------------------------------------------|------------------|----------------------------------------------------------------------------------|
| Toxin/ Microglia marker/ Ref. | Species/ Dose/ Gestation Day | Age of Analysis | Regions analyzed | Findings |
| Poly(I:C) Iba1 (Juckel <i>et al.</i> 2011) | BALB/c mice/ 20mg/kg (i.p.)/ GD 9 | PND 30 | HPC, Cx, Str | Decreased density and amount of branches and processes |
| Poly(I:C) Iba1 (Garay <i>et al.</i> 2013) | C57BL6 mice/ 20mg/kg (i.p.) GD 12.5 | PND 0, PND 7, PND 14, PND 30, PND 60 | FtCx, HPC, CgCx | No change in morphology |
| Poly(I:C) Iba1 (Giovanoli <i>et al.</i> 2015) | C57BL6 mice/ 5mg/kg (i.v.) GD 17.5 | PND 28, PND 140, PND 600 | HPC | No change in morphology (cell soma area and primary or secondary branches) |

| | | | | | |
|------------------------------------|-----------------|----------|-------------------|--|----------------------------------------------------|
| Poly(I:C) | C57BL6 mice/ | | | | |
| Iba1 | 5mg/kg (i.v.) | P35, P84 | HPC | | No change in morphology |
| (Giovanoli <i>et al.</i> 2016) | GD 9.5 | | | | (cell soma area and primary or secondary branches) |
| Poly(I:C) | C57BL6 mice/ | | | | Decreased microglial |
| Iba1 | 2mg/kg (i.p.) | PND 90 | Cx | | branches per cell |
| (Corradini <i>et al.</i> 2017) | GD 9 | | | | No change in length of microglia branches |
| Poly(I:C) | C57BL6 mice/ | | | | No change in cell soma area |
| Iba1 | 5mg/kg (i.v.) | PND 90 | mPFCx | | or primary branches of |
| (Notter <i>et al.</i> 2017) | GD 9.5 | | | | Iba1+-positive cells |
| Poly(I:C) | C57BL6 mice/ | PND 80 | | | Reduced arborization and |
| Iba1 | 5mg/kg (i.p.) | | HPC | | increased process area |
| (Hui <i>et al.</i> 2018) | GD 9.5 | | | | |
| Poly(I:C) | Wistar-Hannover | | | | |
| CD11b/Iba1 | rat/ | PND 90 | FtCx, HPC, Str, | | No change in morphology |
| (Missault <i>et al.</i> 2014) | 4 mg/kg (i.v.) | | CC, Pons, Th | | |
| | GD 15.5 | | | | |
| Poly(I:C) | Sprague Dawley | | | | |
| CD11b | rat/ | PND 180 | Cx, HPC, Str, CC, | | Reduced arborization |
| (Van den Eynde <i>et al.</i> 2014) | 4 mg/kg (i.v.) | | Pons, Th | | |
| | GD 15.5 | | | | |

| | | | | | |
|----------------------------|----------------------|---------|-----|--|-----------------------------|
| LPS | C57BL6 mice/ | | | | |
| Iba1 | 25-50µg/kg (i.v.) | PND 56 | Cx | | No change in morphology |
| (Hsueh <i>et al.</i> 2017) | GD 15 - 17 | | | | |
| LPS | C57BL6 mice/ | | | | Increased percentage of |
| Iba1 | 50µg/kg (i.p.) | PND 7, | | | amoeboid microglia and |
| (O'Loughlin <i>et al.</i> | GD 12 | PND 14, | Am | | decreased percentage of |
| 2017) | | PND 40 | | | ramified microglia at PND 7 |
| | | | | | and PND 40 |
| LPS | Sprague Dawley | | | | |
| Iba1 | rat/ | | | | |
| (Mouihate 2016) | 100 µg/kg (i.p.) | PND 70 | HPC | | No change in morphology |
| | GD 15.5 | | | | |
| IL6 | CD-1 Mice/ 100ng | | | | |
| Iba1 | (i.p.)/GD 12.5-birth | | | | |
| (Gumusoglu <i>et al.</i> | 3x/day | PND 84 | Cx | | More highly ramified |
| 2017) | | | | | microglia |

1

2 **HPC: Hippocampus, Str: Striatum, Th: Thalamus, Cx: Cortex, CgCx: Cingulate Cortex, FtCx:**

3 **Frontotemporal Cortex, (m)Pfcx: (medial) Prefrontal Cortex, CC: Corpus Callosum, Am: Amygdala**

4

5 *Expression of microglia activity dependent genes and proteins*

6 The analysis of a single aspect in the continuum of microglia activation states is insufficient to capture

7 subtle changes in microglia reactivity, especially in the MIA model, where subtle changes in microglia

activity have been detected using highly sensitive approaches including microglia-specific mRNA sequencing (Matcovitch-Natan *et al.* 2016). In order to capture subtle changes, one has to either apply microglia-specific protein or mRNA expression approaches or apply several complementary approaches. A widely used approach is to complement microglia density and morphological analyses with assessing the expression of microglial surface markers as well as cytokine and chemokine levels. Amongst the most widely used microglia markers are: CD68 (a lysosomal-associated membrane glycoprotein known to be upregulated in pro-inflammatory activity states of microglia), CD11b (subunit of the complement receptor 3 complex), MHCII, CX3CR1, CD200R (cell surface receptor of CD200, transmits inhibitory signals to microglia), and Iba1 as well as various different pro- (IL1 β , IL6, Tumor necrosis factor α (TNF α)) and anti-inflammatory (IL-5, IL10) cytokine and chemokine levels.

Again, the findings in the expression pattern of microglia markers in MIA models are diverse (Table 3). In addition, not all studies have implemented all complementary methods described before which precludes the determination of a microglia phenotype to some extent. For example, one study reported a decreased immunoreactivity of Iba1 in the dentate gyrus of MIA offspring with no changes in cell density (Mattei *et al.* 2014). In contrast, another study found an increased immunoreactivity of Iba1 in the dentate gyrus upon MIA (Mattei *et al.* 2017). In the latter study, the interpretation of what Iba1 expression changes signify when presented isolated from other microglia analyses can be difficult. For example, an observed increase in Iba1 could signify increased expression of Iba1 by microglia, increased density of microglia, increased microglia soma size (indicative for pro-inflammatory state) or an increase in ramification. Hence, the combination of other measures such as Iba1 expression with microglia density (Mattei *et al.* 2014) can aid the interpretation of the obtained results. In this example, decreased Iba1 expression together with no changes in density could be an indicator for either decreased expression of Iba1, or altered morphology with less ramification and smaller soma size. Findings from other microglia marker expression studies have yielded similarly contradicting results. In summary, while 8 out of 13 studies found no change in the expression of CD68, CD11, or Iba1, 7

studies did find changes in the expression (either increased or decreased) of these same markers and others (MHCII, CD124, CX3CR1, etc.)(Table 3).

Two recent studies measured changes in the expression of the mitochondrial TSPO in the brains of MIA offspring, similar to *in vivo* PET imaging studies in humans (Mattei *et al.* 2017, Notter *et al.* 2017). Both studies find opposite results, with increased TSPO in one study and decreased TSPO in the other. The reason for this discrepancy could be the difference in brain region (prefrontal cortex vs hippocampus) or the difference in the MIA protocol (i.v. vs i.p. administration of Poly(I:C) at GD9 or GD17). As discussed by the authors and recently reviewed in Notter *et al.* 2018, the use of TSPO to assess the activation status of microglia is debatable as it is also expressed in other CNS cell types, including astrocytes and endothelial cells (Notter *et al.* 2017, Tronel *et al.* 2017, Notter *et al.* 2018). The increase in TSPO could thus also signify processes other than inflammation, such as oxidative stress or cell activity per se.

Table 3: Microglia surface marker expression in postnatal MIA offspring

| Microglia Surface Marker Expression | | | | |
|------------------------------------------|------------------------------------------------|--------------------|------------------|---------------------------------------|
| Toxin/ Ref. | Species/ Dose/ Gestation Day | Age of Analysis | Regions analyzed | Findings |
| Poly(I:C) (Krstic <i>et al.</i> 2012) | C57Bl6 mice/ 5mg/kg (i.v.)/ GD 17.5 | PND 540 | HPC | No change in CD68 immunoreactivity |
| Poly(I:C) (Pineda <i>et al.</i> 2013) | C57Bl6 mice/ 2.5mg/kg (i.p.)/ GD 12 - 16 | PND 40 | HPC | No change in CD11b expression |

| | | | | |
|--------------------------------|---------------------------|--------------------|-------------|-----------------------------------------------------------------------------------------------------------------|
| Poly(I:C) | C57Bl6 mice/ | | | |
| (Willi <i>et al.</i> 2013) | 5mg/kg (i.v.)/ GD 9.5 | PND 85 | mPFC, Str | No change in amount of cells expressing CD68 |
| Poly(I:C) | BALB/c mice/ | | | No change in amount of cells expressing CD40, CD80, CD68, MHCII, CD200R, CD11c, CCR7; |
| (Esslinger <i>et al.</i> 2016) | 20mg/kg (i.p.)/ GD 9.5 | PND 30 | | Decreased amount of cells expressing CD124, CD206, CX3CR1; |
| | | | Whole brain | Increased amount of cells expressing CD54, CCR2 |
| | | PND 100 | | No change in amount of cells expressing CD40, CD54, CD80, CD86, MHCII, CD124, CD206, CD200R, CD11c, CCR7, CCR2; |
| | | | | Decreased amount of cells expressing CX3CR1 |
| Poly(I:C) | C57BL6 mice/ | | | |
| (Giovanoli <i>et al.</i> 2016) | 5mg/kg (i.v.)/ GD 9.5 | PND 35, PND 84 | HPC | No change in amount of cells expressing CD68 |
| Poly(I:C) | BALB/c mice/ | PND 10, PND 30, | Whole brain | Decreased CD11b and CD45 No change in Iba1 |

| | | | | |
|------------------------------------|---------------------------------|---------|----------------------------|-----------------------------------------------|
| (Manitz <i>et al.</i> 2016) | 20mg/kg (i.9.)/ GD 9 | PND 100 | | |
| Poly(I:C) | C57BL6 mice/ | | | Increased Iba1 reactivity |
| (Mattei <i>et al.</i> 2017) | 5mg/kg (i.p.)/ GD 15.5 | PND 120 | HPC | Increased TSPO binding potential |
| Poly(I:C) | C57BL6 mice/ | | | |
| (Corradini <i>et al.</i> 2017) | 2mg/kg (i.p.)/ GD 9 | PND 90 | Cx | No change in amount of cells expressing CD11b |
| Poly(I:C) | C57BL6 mice/ | | | No change in CD68 optical density |
| (Notter <i>et al.</i> 2017) | 5mg/kg (i.v.)/ GD 9 | PND 90 | mPFC, HPC | Decreased TSPO density |
| Poly(I:C) | Wistar Rat/ | | | |
| (Mattei <i>et al.</i> 2014) | 4mg/kg (i.v.)/ GD 15.5 | PND 128 | HPC, Cb | Decreased Iba1 reactivity |
| Poly(I:C) | Sprague Dawley | | | |
| (Van den Eynde <i>et al.</i> 2014) | Rat/ 4mg/kg (i.v.)/ GD 15.5 | PND 180 | Cx, HPC, Str, CC, Pons, Th | Increased CD11b or CD68 reactivity |
| LPS | C57BL6 mice/ | | | |
| (Hsueh <i>et al.</i> 2017) | 25-50µg/kg (i.v.) GD 15 - 17 | PND 56 | Cx | No change in Iba1 optical density |
| LPS | Wister rat/ | | | |
| (Borrell <i>et al.</i> 2002) | 1mg/kg (s.c.)/ | P100 | Internal capsule | Increased MHCII immunoreactivity |

HPC: Hippocampus, Str: Striatum, Th: Thalamus, Cx: Cortex, (m)PfCx: (medial) Prefrontal Cortex, CC: Corpus Callosum, Cb: Cerebellum

Besides microglia surface markers, a number of studies have assessed the expression of pro- and anti-inflammatory cytokines in MIA offspring (Table 4). Although not specific for microglia, several studies determined cytokine levels in brain homogenates in an attempt to give an idea about the inflammatory status of the brain. However, cytokines are not only produced by microglia, but also by other parenchymal cell types (such as astrocytes and neurons) and even by cells in the meningeal space. Moreover, many cytokines (such as IL1 β , IL6 and TNF α) not only play a role in inflammation but also in modulation of synaptic transmission and plasticity (as reviewed in (Vezzani and Viviani 2015)). Cytokine levels can be measured either on the mRNA or on the protein level but differences in transcriptional and translational dynamics might create additional variation between studies. In total, 8 studies determined cytokine levels in brain homogenates and findings are divergent. Yet, this might also be explained by dynamic changes in several brain cytokines in a region- and age-specific manner of early postnatal to adult MIA offspring (PND 0 – 60) (Garay *et al.* 2013). To our knowledge, only two studies measured cytokine levels (on the mRNA level) in isolated microglia from adult MIA offspring. Strikingly, although the protocols used to induce MIA and the age and brain region (hippocampus) analyzed are the same, Mattei *et al.* 2014 showed increased levels of IL1 β mRNA while Hadar *et al.* 2016 found no change in IL1 β mRNA levels (Mattei *et al.* 2014, Hadar *et al.* 2016).

Table 4: Microglia cytokine expression in postnatal MIA offspring

Cytokine mRNA/protein Expression

| Toxin/ Ref. | Species/ Dose/ Gestation Day | Age of Analysis | Regions analyzed | No change | Increase |
|-------------------------------------------------------|-------------------------------------------|--------------------------------------------------|---------------------|--------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Poly(I:C) (Mattei <i>et al.</i> 2014) | Wistar Rat/ 4mg/kg (i.v.)/ GD 15.5 | PND 128 | HPC, Cb | TNF α TNF α , IL1 β | IL1 β |
| Poly(I:C) (Hadar <i>et al.</i> 2016) | Wistar Rat/ 4mg/kg (i.v.)/ GD 15.5 | PND 130 | HPC, mPFC | TNF α , IL1 β , IL6 | |
| Poly(I:C) (Krstic <i>et al.</i> 2012) | C57Bl6 mice/ 5mg/kg (i.v.)/ GD 17.5 | PND 540 | Cx, HPC | IL1 α , IL6, TNF α | IL1 α , IL6 |
| Poly(I:C) (Garay <i>et al.</i> 2013) | C57Bl6 mice/ 2mg/kg (i.p.)/ GD 12.5 | PND 0, PND 7, PND 14, PND 30, PND 60 | HPC, FCx, CgCx | TNF α | IL1 α , IL1 β , IL6, IL10, IFN γ , IL2, IL3, IL4, IL5, IL9, IL12(p40) IL12(p70), IL13, IL17, G-CSF, GM-CSF, eotaxin, KC, MCP-1, MIP1 α , MIP1 β , RANTES (increased/decreased) |
| Poly(I:C) (Giovanoli <i>et al.</i> 2015) | C57Bl6 mice/ 5mg/kg (i.v.) GD 17.5 | PND 28, PND | HPC | IL1 β , IL4, IL6, TNF α | |

| | | | | | |
|--------------------------|----------------------|---------|-------------|----------------------------|-----------------------------------|
| | | 140, | | | |
| | | PND 600 | | | |
| Poly(I:C) | C57BL6 mice/ | PND 40, | | IL4, IL6, TNF α , | |
| (Giovanoli <i>et al.</i> | 5mg/kg (i.v.) | | HPC | IL1 β | |
| 2016) | GD 9 | PND 90 | | IL4, IL6, TNF α | IL1 β |
| Poly(I:C) | C57BL6 mice/ | PND 90 | mPFCx | | |
| (Notter <i>et al.</i> | 5mg/kg (i.v.) | | | IL1 β , IL5, IL6 | IL10, TNF α , IFN γ |
| 2017) | GD 9 | | | | |
| Poly(I:C) | C57BL6 mice/ | PND 120 | HPC | | |
| (Mattei <i>et al.</i> | 5mg/kg (i.p.) | | | IL1 β , TNF α | IL6 |
| 2017) | GD 15.5 | | | | |
| Poly(I:C) | Sprague Dawley | PND 1 | Whole brain | NGF, BDNF | TNF α (decrease) |
| (Gilmore <i>et al.</i> | rat/ 10mg/kg | PND 7 | FCx | NGF, BDNF, | |
| 2005) | (i.p.) GD16 | | | TNF α | |
| LPS | Sprague Dawley | PND 7 | FCx | | TNF α (decrease) |
| (Gilmore <i>et al.</i> | rat/ 100 μ g/kg | | | | |
| 2005) | (i.p.) GD14 - 16 | | | | |
| LPS | C57BL6 mice/ | PND 7 | | | IL1 β |
| (O'Loughlin <i>et</i> | 50 μ g/kg (i.p.) | PND 14 | Am | IL1 β , IL6, IL10 | |
| <i>al.</i> 2017) | GD 12 | PND 40 | | and/or TNF α | |

HPC: Hippocampus, Cx: Cortex, CgCx: Cingulate Cortex, FCx: Frontal Cortex, (m)PFCx: (medial) Prefrontal Cortex, Cb: Cerebellum, Am: Amygdala. The double line separates studies performed in isolated microglia (above) from those in brain homogenate (below).

By categorizing different studies according to their method for assessing microglia activation, we aimed to ease the comparison. However, contradictory results were found between different studies, which could, at least in part, be explained by the large variety in protocols used to induce MIA. However, even when comparing studies using comparable protocols for MIA induction, the results remain contradicting. For example, using an i.v. injection of Poly(I:C) (4mg/kg) at GD 15.5 in rats, 3 studies described increased microglial densities in several brain regions (Mattei *et al.* 2014, Van den Eynde *et al.* 2014, Hadar *et al.* 2016) while the same protocol did not induce changes in microglial density in 2 other studies (Missault *et al.* 2014, Paylor *et al.* 2016). Similarly, the same protocol induced no morphological changes at PND 90 in one study (Missault *et al.* 2014), while reduced arborization was observed in PND 180 offspring in another study (Van den Eynde *et al.* 2014). Despite applying the same MIA paradigm, these studies differ with regard to the age of the offspring when microglia were assessed, pointing towards a possible involvement of natural or pathological changes in cell density along the course of development

Taken together, when analyzing the effect of MIA on microglia in adult MIA-offspring, considerable controversy exists when only one parameter for microglia activation, i.e. density, morphology or expression of specific markers or cytokines, is assessed, even when comparing studies using the same or highly similar MIA induction protocols. In addition, the methods to detect ‘microglia activation’ described above are limited in their sensitivity and detect only obvious changes in microglia activation, and should therefore ideally be combined with more sensitive approaches, allowing the definition of distinct and very refined activity states. Although we conclude that morphology as a single readout is not sufficient to detect microglial activation, it is possible that subtle morphological changes are not detected with the standard microscopic analysis and in this aspect advanced and high resolution

1 imaging such as electron microscopy might shed some more light. Moreover, using direct RNA
2 sequencing, mRNA sequencing, gene and microRNA array, quantitative proteomic analysis and
3 chromatin accessibility analysis, several groups have defined the unique molecular signature and
4 'sensome' of microglia and their regulatory dynamics during brain development (Hickman *et al.* 2013,
5 Butovsky *et al.* 2014, Matcovitch-Natan *et al.* 2016, Hanamsagar *et al.* 2017, Thion *et al.* 2018)).
6 Interestingly, using these techniques, Mattei *et al.* 2017 and Matcovitch-Natan *et al.* 2016 showed a
7 MIA-induced deregulation of genes involved in microglial development, inflammation and
8 phagocytosis accompanied by increased pro-inflammatory signaling and decreased phagocytic activity
9 in microglia of MIA offspring (Maticovitch-Natan *et al.* 2016, Mattei *et al.* 2017), arguing in favor of
10 combining several methods to more thoroughly define the effect of MIA on microglia.

11 **3.2 Readout at embryonic age**

12 Activation of adult microglia due to an embryonic inflammatory insult – as described in the section
13 above – assumes some kind of activation memory. Much more strait forward is the hypothesis that
14 embryonic microglia are activated at the time of maternal immune activation and thereby influence
15 early neurodevelopment. The detection of increased mRNA levels of inflammatory cytokines in the
16 embryonic brain within a few hours upon MIA (Meyer *et al.* 2006) strengthened the hypothesis that
17 MIA could alter microglia activation states during prenatal development. Recently, the expression of
18 sensome genes was shown already in the progenitor phase of microglia (yolk sac macrophages –
19 GD10.5) (Thion *et al.* 2018), lending even more support to the notion that microglia are already able
20 to respond to (pathological) changes in the environment in early stages of development. In order to
21 test this, the microglia activation phenotype has been studied during embryonic periods (Table 5). As
22 for the findings in postnatal offspring, the results on embryonic microglia activation are divergent. For
23 example, out of 6 studies that measured microglial density in embryonic MIA offspring, 3 studies
24 measured no change while the other 3 studies detected increased microglial densities. Whilst other
25 studies did not measure alterations in microglia density or morphology upon MIA, they measured

1 altered gene and protein expression of inflammatory mediators in embryonic brain or microglia
2 (Cunningham *et al.* 2013, Pratt *et al.* 2013, Pont-Lezica *et al.* 2014, O'Loughlin *et al.* 2017, Schaafsma
3 *et al.* 2017). Similar to studies measuring cytokine levels in the brain of postnatal MIA-offspring, also
4 several studies analyzing embryonic brains measured cytokines in brain homogenates rather than in
5 isolated microglia. In this review, to exclude the possible contribution of maternally derived cytokines,
6 we consider only studies measuring cytokines at least 48 hours after the immune challenge. As such,
7 time-dependent changes in the levels of IL10, TNF α and IL1 β were detected (O'Loughlin *et al.* 2017).
8 To measure changes in microglia specifically, Pont-Lezica *et al.* 2014 used genome-wide gene
9 expression profiling of isolated embryonic microglia and found a differential gene expression of 3906
10 genes upon maternal inflammation (Pont-Lezica *et al.* 2014). They found that several immune genes
11 such as IL1 β , C-C motif Chemokine ligand 4 (CCL4) and Nuclear factor (NF)-kB were upregulated while
12 microglial genes involved in neuritogenesis such as Semaphorin (Sema)3c and Versican (Vcan) were
13 downregulated (Pont-Lezica *et al.* 2014). Cunningham *et al.* 2013 performed immunohistochemical
14 analyses on embryonic brain slices from rats exposed to LPS against markers indicative for different
15 inflammatory activation states including inducible nitric oxide synthase (iNOS) and IL1 β (pro-
16 inflammatory state) or arginase-1 and IL-1RA (anti-inflammatory state) respectively. While the amount
17 of microglia that expressed arginase-1 was not changed, the proportion of microglia expressing iNOS,
18 IL1 β or IL-1Ra was increased upon LPS administration (Cunningham *et al.* 2013). Similarly, repeated
19 prenatal administration of LPS in mice increased mRNA levels for IL1 β , IL6 and TNF α in isolated
20 microglia 3 hours after the last LPS injection (Schaafsma *et al.* 2017). Furthermore, Pratt *et al.* 2013
21 assessed both mRNA and protein levels of inflammatory mediators in isolated microglia from MIA-
22 exposed embryonic brains. They found increased levels of IL6 mRNA whereas mRNA levels for IL1 β ,
23 TNF α and IL10 were unchanged. On the protein level, they found increased expression of IL1 α , IL4 and
24 IL9 but not IL1 β , IL6, IL10 or TNF α (among others) (Pratt *et al.* 2013). The fact that mRNA levels for IL6
25 were increased while protein levels for this cytokine remained unchanged could be explained by
26 differences in transcriptional and translational dynamics, as mentioned before. In contrast to these

findings, no change in microglial protein levels of inflammatory markers including iNOS, IL1 β or Mac2 was detected in the hippocampus and cortex of embryos at different developmental stages upon Poly(I:C) exposure at GD 11.5 (Smolders *et al.* 2015). Also repeated immune stimulation (at GD 11.5 and GD 15.5) did not affect embryonic microglia at GD 17.5 (Smolders *et al.* 2015).

Table 5: Microglia alterations in embryonic MIA offspring

| Microglia alterations in embryonic MIA offspring | | | | | |
|--------------------------------------------------|--------------------------------------------|--------------------|---------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------|
| Toxin/ Ref. | Species/ Dose/ Gestation Day | Age of Analysis | Regions analyzed | No change | Increase |
| Poly(I:C) (Pratt <i>et al.</i> 2013) | C57BL6 mice/ 20mg/kg (i.9.)/ GD 12.5 | GD 16.5 | Whole brain | mRNA | mRNA |
| | | | | IL1 β , IL10, TNF α | IL6 |
| | | | | Protein | Protein |
| | | | | IL1 β , IL2, IL3, IL5, IL6, IL7, IL10, IL12p40, IL12p70, IL13, IL15, IL17, IFN γ , TNF α , ILF, G- CSF, VEGF, ... | IL1 α , IL4, IL9, GM-CSF, M-CSF |
| | | | | Density | |

| | | | | | |
|----------------------------|-------------------------|----------|-------------------|-------------------------|---------------------------------|
| Poly(I:C) | C57BL6 mice/ | GD 11.5, | Cx, HPC | Density | |
| (Smolders <i>et al.</i> | 20mg/kg (i.p.)/ | GD 12.5, | | IL1 β , iNOS, | |
| 2015) | 11.5 and/or 15.5 | GD 17.5 | | Mac2 | |
| LPS | C57BL6 mice/ | PND 0 | HPC | | Density |
| (Roumier <i>et al.</i> | 120 μ g/kg (i.p.)/ | | | | |
| 2008) | GD 15.5 | | | | |
| LPS | CD-1 mice/ 8 μ g/kg | GD 18 | Forebrain | | % Iba+ cells |
| (Le Belle <i>et al.</i> | (i.p.)/ GD 9.5 | | | | |
| 2014) | | | | | |
| LPS | C57BL6 mice/ | GD 17.5 | HPC, Fimbria, CC, | Morphology | 3906 |
| (Pont-Lezica <i>et al.</i> | 0.12 μ g/kg (i.p.)/ | | CgCx | | differentially |
| 2014) | GD 15.5 | | | | expressed |
| | | | | | genes |
| LPS | C57BL6 mice/ | GD 18.5 | Cx | | Density and |
| (Tronnes <i>et al.</i> | 140 μ g/kg (i.p.)/ | | | | morphology |
| 2016) | GD 17.5 | | | | (larger cell body |
| | | | | | and fewer and |
| | | | | | shorter |
| | | | | | processes) |
| LPS | C57BL6 mice/ | GD 17.5 | Whole brain | | mRNA |
| (Schaafsma <i>et al.</i> | 50 μ g/kg (i.p.)/ | GD | | | IL6, IL1 β , TNF α |
| 2017) | 15.5 – 17.5 | | | | |
| LPS | C57BL6 mice/ | GD 16 | | IL1 β , IL6, IL10 | TNF α , IL10 |
| | 50 μ g/kg (i.p.) | GD 18 | Whole brain | and/or TNF α | TNF α |

| | | | | | |
|-----------------------------------------------|------------------------------------------------------|---------|----|-----------------|--------------------------------------|
| (O'Loughlin <i>et al.</i> 2017) | GD 12 | PND 0 | | | IL6 |
| LPS (Cunningham <i>et al.</i> 2013) | Rat/ 100µg/kg (i.p.)/ GD 15.5 – 16.5 | GD 19.5 | Cx | Morphology | iNOS, IL1β, IL1Ra |
| IL6 (Gumusoglu <i>et al.</i> 2017) | CD-1 mice/ 100ng (i.p.)/ GD 12.5 – 14.5 3x/day | GD 14.5 | Cx | Overall density | More multivacuolated microglia |

1

2 **HPC: Hippocampus, Cx: Cortex, CgCx: Cingulate Cortex, CC: Corpus Callosum**

3 During rodent neurodevelopment, the brain environment substantially changes every day and
4 microglia are likely to adapt accordingly and even contribute to these changes (Cunningham *et al.*
5 2013, Matcovitch-Natan *et al.* 2016, Hanamsagar and Bilbo 2017). As a consequence, potential MIA-
6 induced alterations in microglia functions could be transient and its detection highly dependent on
7 when and where microglia phenotypes are assessed. This could in part explain some of the
8 controversial findings in microglia phenotypes described. For example Tronnes *et al.* 2016 found an
9 increased microglia density and altered microglia morphology in the somatosensory cortex 24 hours
10 after MIA at GD 17.5 (Tronnes *et al.* 2016), whilst no evidence of altered microglia density was detected
11 in the fetal cortex and hippocampus 24 hours after maternal injection of Poly(I:C) at GD 11.5 (Smolders
12 *et al.* 2015). Again direct comparison between these two studies is problematic due to the difference
13 in MIA paradigm and time of MIA induction. Nevertheless, others detected no changes in fetal
14 microglia density or morphology 48-72 hours post-MIA induction (Cunningham *et al.* 2013, Pratt *et al.*
15 2013, Pont-Lezica *et al.* 2014, Smolders *et al.* 2015) whilst one study described an increase in the
16 percentage of Iba1 positive cells 9 days post-LPS exposure (Le Belle *et al.* 2014). A similar controversy

1 arises when microglial protein expression is assessed. For example, while Cunningham *et al.* 2013
2 found increased protein expression of iNOS and IL1 β , 72 hours after maternal LPS administration at
3 GD 15.5 and GD 16.5, Smolders *et al.* 2015 found no change in the expression of these proteins 5, 24,
4 48 or 144 hours after maternal Poly(I:C) exposure at GD 11.5 or after a subsequent exposure at GD
5 15.5 (Cunningham *et al.* 2013, Smolders *et al.* 2015).

6 Taken together, clear conclusions cannot be drawn from the current data available on embryonic
7 microglia phenotypes after MIA, firstly due to the omnipresent variety in the protocols applied (a
8 similar phenomenon is seen in the studies assessing postnatal microglia phenotypes) and secondly due
9 to the dynamic changes of microglia phenotypes during normal early development, which necessitates
10 highly similar protocols of MIA induction and embryonic time points of analyses. However, as
11 suggested by the study of Mattei *et al.* 2017, it can be concluded that embryonic microglia are able to
12 sense and respond to MIA, causing long-term changes in their transcriptomic signature. This might
13 indicate that the early embryonic insult deviates the phenotypical track of microglia, which in turn
14 could also affect the development of other brain cells. Therefore, the characterization of MIA-induced
15 changes in embryonic microglia at different developmental stages is crucial in order to understand
16 how microglia could alter normal developmental trajectories that precipitate into the neuronal and
17 behavioral deficits detected in MIA.

19 **4. Age, sex and brain region might affect microglial phenotype upon MIA**

20 In the previous section, we discussed the influence of the specific read-out to assess microglia
21 activation (i.e. density, morphology, cytokine production and marker expression) on the precise
22 outcome of the study. However, even when considering the same read-out, factors that are indirectly
23 related to the analytical method will contribute to variability between studies. These factors include
24 physiological constraints that alter the microglial phenotype such as age, sex and brain region.

4.1 Brain region-specific changes in microglia of MIA offspring

In the normal brain, several microglial properties appear to be highly dependent on the specific brain region, including density, morphology, membrane properties and transcriptional profiles, possibly reflecting a different set of tasks in different brain regions (Schwarz *et al.* 2012, Yang *et al.* 2013, Grabert *et al.* 2016, De Biase *et al.* 2017). Moreover, a physiological function of microglia that received much attention in recent years, i.e. synaptic refinement, also appears to be region-dependent. In the hippocampus, elimination of synapses is dependent on CX3CR1 as mice lacking this receptor displayed increased hippocampal dendritic spine density accompanied by synaptic characteristics reminiscent of an immature connectivity and weak synaptic transmission (Paolicelli *et al.* 2011, Zhan *et al.* 2014). On the other hand, synaptic remodeling in the visual system was shown to be dependent on the complement system (in the lateral geniculate nucleus) and on P2Y12 (in the visual cortex) (Schafer *et al.* 2012, Sipe *et al.* 2016, Schechter *et al.* 2017). Hence, it should be of no surprise that environmental insults could affect microglia differently in different brain regions. In the context of MIA, several studies found changes in microglial density in one region while other regions were comparable to control mice upon Poly(I:C)-induced MIA (Juckel *et al.* 2011, Manitz *et al.* 2013, Mattei *et al.* 2014, Van den Eynde *et al.* 2014, Hadar *et al.* 2016, Hui *et al.* 2018).

4.2 Age of MIA offspring

Although schizophrenia and ASD share a subset of risk factors and symptoms (such as social interaction and communication deficits), they differ greatly in the age of disease onset. While ASD is usually diagnosed during childhood, schizophrenia typically emerges around adolescence. Similarly, the emergence of behavioral deficits appears to be age-dependent in the MIA model, which is effectively used to study the etiology of both ASD and schizophrenia. While deficits in prepulse inhibition, latent inhibition and amphetamine-induced activity are usually observed in adult MIA offspring (e.g. (Piontkewitz *et al.* 2011, Hadar *et al.* 2015, Fujita *et al.* 2016, Han *et al.* 2016)) (working) memory and

1 social interaction deficits can readily be detected in pubescence (e.g. (Malkova *et al.* 2012, Fujita *et al.*
2 2016, Han *et al.* 2016)). Moreover, on the neuroanatomical level, decreases in hippocampal volume
3 could be observed in pubescent MIA offspring while accelerated cortical thinning was only detectable
4 in adulthood (Piontkewitz *et al.* 2011, Crum *et al.* 2017).

5 In the normal brain, microglial properties such as density and morphology change substantially over
6 time (e.g. (Schwarz *et al.* 2012, Swinnen *et al.* 2013)). Also the transcriptional profile of microglia is
7 highly dependent on age, possibly allowing microglia to perform specific functions at specific stages
8 (Grabert *et al.* 2016, Matcovitch-Natan *et al.* 2016, Thion *et al.* 2018). Finally, it was recently shown
9 that the influence of the microbiome (as will be discussed later on in this review) on the microglial
10 transcriptome is different according to age (with smaller influence in early development) (Thion *et al.*
11 2018)).

12 Consequently, effects of MIA on microglia might also be dependent on the age at which analysis was
13 performed. Increased microglial densities were found in the hippocampus of Poly(I:C)-induced MIA
14 offspring between PND 2 and 30, which were no longer present in adulthood (Manitz *et al.* 2013, Li *et*
15 *al.* 2014) while increased striatal microglia density was only observed in PND 100 offspring (Manitz *et*
16 *al.* 2013). Furthermore, Esslinger *et al.* 2016 described changes in the expression of several microglial
17 surface markers in whole-brain microglia from 30-day-old Poly(I:C)-MIA offspring, a phenotype that
18 was lost when the offspring reached PND 100. A possible explanation for the loss of this phenotype
19 could be the fact that microglia function changes during development – from progenitor pool
20 maintenance in early brain development to synaptic remodeling during adolescence and adult brain
21 homeostasis – and that the changes observed in microglia are related to the age-specific function of
22 microglia at that specific point in time (Mosser *et al.* 2017).

23 **4.3 Sex of MIA offspring**

24 Neurodevelopmental disorders such as schizophrenia and ASD have a strong gender-bias, with a higher
25 prevalence in males than in females (Abel *et al.* 2010, Mukherjee 2017). Although only a handful of

1 studies directly compared the effects of MIA on behavior in both male and female offspring, most find
2 a more severe behavioral deficit in males compared to females (Taylor *et al.* 2012, Naviaux *et al.* 2013,
3 Xuan and Hampson 2014, Meehan *et al.* 2017, Hui *et al.* 2018). With regard to MIA studies investigating
4 the effect on microglia, about one third of studies did not report on whether they analyzed males or
5 females (e.g. (Roumier *et al.* 2008, Hsiao *et al.* 2012, Cunningham *et al.* 2013, Pratt *et al.* 2013)) and
6 two thirds of studies used either males or a mix of males and females (not analyzed separately) (e.g.
7 (Van den Eynde *et al.* 2014, Giovanoli *et al.* 2015, Hadar *et al.* 2016, Paylor *et al.* 2016)). Yet, several
8 recent reports indicate substantial differences between microglial properties of both sexes, including
9 microglia density and morphology, phagocytic activity and migration (Schwarz *et al.* 2012, Nelson *et*
10 *al.* 2017, Weinhard *et al.* 2017, Yanguas-Casas *et al.* 2018). Also on the transcriptional level, differences
11 have been described between male and female microglia. While the microglial transcriptome is similar
12 during development, sexual dimorphism increases in adulthood (with female microglia in a more
13 immune-activated state) (Hanamsagar *et al.* 2017, Thion *et al.* 2018). Moreover, the effects of
14 postnatal immune challenge or of pre- and postnatal microbiome composition on the microglial
15 transcriptome was dependent on sex (Hanamsagar *et al.* 2017, Thion *et al.* 2018). Hence, MIA might
16 differentially affect male versus female microglia and thus result in divergent behavioral outcomes.

17 To our knowledge, only 3 studies directly compared the effects of MIA on microglia between male and
18 female offspring. As such, Manitz *et al.* 2016 and Esslinger *et al.* 2016 showed sex-specific alterations
19 in the expression of several proteins associated with microglial activation, including CD45, CD54,
20 CD124 and CD206 ((Esslinger *et al.* 2016, Manitz *et al.* 2016)) in whole-brain lysates of Poly(I:C)-MIA
21 offspring. Hui *et al.* 2018 recently also showed more pronounced effects of MIA (induced by Poly(I:C)
22 on microglial distribution and arborization in the hippocampus of male offspring compared to females
23 (Hui *et al.* 2018). Of interest, the studies described in this section all used Poly(I:C) to induce maternal
24 inflammation when comparing the effects of MIA on microglia in different brain regions, between
25 sexes or at different ages. Besides O'Loughlin *et al.* 2017, who identified microglial morphological

changes at 3 different ages, no other studies that used LPS investigated the influence of the aforementioned factors.

Failure to report on the sex of the offspring examined is mostly prevalent in studies analyzing embryonic microglia, as sex determination by eye is not possible at this age. However, as done by Hsueh *et al.* 2017, genotyping of embryonic samples for the presence of the sex-specific SRY gene can readily distinguish between males and females at embryonic stages (Hsueh *et al.* 2017). Hence, the fact that microglia are measured at embryonic ages should not be a limitation to investigate gender differences.

5. MIA-model associated factors can influence the study outcome

As discussed in the previous section, different studies used different methodologies to determine microglia activation upon MIA, which could have led to the contradicting results on the effect of MIA on microglia. However, another notable difference between studies is the exact method used to induce MIA. Both Poly(I:C) and LPS are administered at several doses and at different gestational ages, all of which are factors that could contribute to the variation in results that have been found by different studies. Moreover, lab specific factors such as housing conditions and food composition are likely to play an important role in the diversity between studies. In the following sections, we will discuss the possible influence of these factors on the outcome of studies determining microglia activation.

5.1 Poly(I:C) vs LPS

The two most commonly used agents to induce MIA in rodents are LPS and Poly(I:C). MIA studies determining microglial alterations at postnatal or adult ages mostly used Poly(I:C) and only a few induced MIA with LPS. Regardless of which immune stimulant was used however, several studies suggest microglia “activation” in the adult MIA offspring while just as many studies find no evidence for changes in microglia. In contrast, studies assessing microglia “activation” at embryonic stages

1 mostly used LPS to induce MIA with the exception of Pratt *et al.* 2013 and Smolders *et al.* 2015, who
2 used Poly(I:C). Both Poly(I:C) and LPS elicit their effects via the toll like receptor (TLR) family, where
3 Poly(I:C) signals via the TLR3 and LPS via TLR4. Unlike TLR4, which is located at the cellular membrane,
4 TLR3 is localized to endosomal membranes. Although activation of both TRL3 and TLR4 seems to result
5 in similar, but not equal, intracellular and cytokine responses, the precise set of immune mediators
6 released by both immune activating agents has, to our knowledge, not been described. However, it
7 seems likely that the different immune stimulants elicit the production and secretion of different
8 subsets of cytokines (Reisinger *et al.* 2015, Bilbo *et al.* 2017). Indeed, specific cytokines have been
9 attributed to play a critical role in both the Poly(I:C)-based and LPS-based MIA model. In 2007, Smith
10 *et al.* showed that systemic injection of recombinant IL6 was able to evoke similar behavioral deficits
11 as Poly(I:C) and that simultaneous injection of IL6 blocking antibody could prevent the MIA-induced
12 behavioral changes (Smith *et al.* 2007). Complementary to this, Wu *et al.* 2017 showed that deletion
13 of the IL6 receptor specifically in the placenta could prevent MIA-induced deficits in the offspring (Wu
14 *et al.* 2017). Furthermore, it was shown that IL17a acts downstream of IL6 as a systemic injection of
15 IL17a-antibody before Poly(I:C)-induced MIA was able to prevent altered ultrasonic vocalizations in
16 MIA offspring (Choi *et al.* 2016). In addition to schizophrenia and ASD related symptoms, Pineda *et al.*
17 2013 also showed the importance of specific cytokines for epilepsy, a well-known co-morbidity in ASD
18 patients. While the presence of only IL6 in maternal blood was sufficient to induce social impairment
19 in the offspring, a combination of IL6 and IL1 β was required to increase susceptibility to seizures
20 (Pineda *et al.* 2013). In contrast to IL6, blocking IL1 β upon Poly(I:C) administration did not improve
21 social deficits (Pineda *et al.* 2013). Although the previous studies described the importance of IL6 and
22 IL17a in mediating the effects of Poly(I:C)-induced MIA on the offspring, IL1 was shown to be crucial
23 for LPS-mediated effects (Girard *et al.* 2010) as co-administration of an IL1 receptor antagonist could
24 alleviate LPS-induced placental damage, maintain microglia density and protect against motor
25 dysfunction in the offspring.

Beside the classical activation of TLR4, LPS was recently also shown to induce activation of transient receptor potential (TRP) channels in a TLR4-independent manner in airway epithelial cells and nociceptive sensory neurons (Meseguer *et al.* 2014, Alpizar *et al.* 2017). Activation of TRP channels in airway epithelial cells was speculated to be caused by mechanical perturbations induced by LPS in the plasma membrane and induced the production of bactericidal nitric oxide (Alpizar *et al.* 2017). Whether LPS also activates TRP channels in microglia is not known but, intriguingly, increased numbers of microglia expressing iNOS and IL1 β were detected when acute embryonic brain slices were challenged with LPS whereas exposure to Poly(I:C) and recombinant IL6 failed to do the same (Smolders *et al.* 2015). These results raise the question whether LPS might have more potent effects both on microglia and other cells due to its additional activation of TRP channels.

In addition to specific cytokines induced by either Poly(I:C) or LPS, a different intensity of the immune response (either by administration of a higher dose or by repeated exposure) provoked by both immune stimulants could explain the differential effects of these 2 agents. Indeed, dose-response studies by Shi *et al.* 2003 and Meyer *et al.* 2005 showed that increasing doses of Poly(I:C) correlated with increased levels of cytokines in maternal blood and with increased severity of behavioral deficits in the offspring while increasing doses of LPS were able to induce abortion (Hsueh *et al.* 2017). As different batches of LPS and Poly(I:C) can differ in the intensity of immune response they induce, dose-response studies are crucial and need to be performed. Moreover, the synthetic Poly(I:C) comes in different forms, low and high molecular weight or with added sodium salt, depending on the company, which could influence the precise dose. Hence, a clear statement on which Poly(I:C) was used and the exact dose should be included in every publication. Finally, a comparative study between LPS and Poly(I:C) addressing both classical immune activation and developmental functions of embryonic microglia would be of great value.

5.2 Timing of MIA induction

Besides the immune activating agent used to induce MIA, the embryonic age at which MIA is induced varies greatly between studies, ranging from early to mid (GD 9, GD 11, GD 12) and late gestation (GD 17). Intriguingly, studies comparing the behavioral deficits in offspring exposed to MIA during different gestational periods have shown that certain, but not all, behavioral phenotypes show a gestational dependency ((Meyer *et al.* 2006) and others). The precise mechanisms underlying this dependency remain to be fully identified (as discussed in detail in (Meyer *et al.* 2007)). The stage and ongoing processes of fetal development, as well as placental development have been suggested to play an important role. Besides this, microglial developmental stages could be perturbed differently depending on when MIA is induced, which could result in different perturbations of neurodevelopmental processes. Indeed recent findings of whole genome expression analyses of microglia isolated at different embryonic ages could identify distinct developmental stages of microglia (GD 10.5 - 14.5, GD 14.5 – PND 9 and PND 28) (Matcovitch-Natan *et al.* 2016). This could explain the findings described by Lai *et al.* 2013 where they detected an age-specific and activator-dependent activation profile of isolated, cultured microglia (Lai *et al.* 2013, Matcovitch-Natan *et al.* 2016). In line with this, Hanamsagar *et al.* 2017 identified a specific developmental maturation and immune reactivity profile of microglia in both mice and humans, which was shown to be gender- and age-dependent (Hanamsagar *et al.* 2017). Hence, it seems plausible that microglia could be differentially perturbed depending on when MIA was induced which leads to differential changes in brain developmental processes and thus subtle changes in behavioral deficits later in life.

In addition to the immediate effects of MIA on microglia functions and early neurodevelopment, prenatal immune stimulation might prime embryonic microglia resulting in an exacerbation of their response to stimulants later in life (for example stress during childhood or puberty) and as such cause a deregulation in critical processes of brain maturation (Eggen *et al.* 2013, Knuesel *et al.* 2014, Slusarczyk *et al.* 2015, Giovanoli *et al.* 2016). Such priming effects were shown by Krstic *et al.* 2012 where an increased CD68 reactivity and changes in microglial morphology was observed in aged offspring that were exposed to two Poly(I:C) immune challenges, once during prenatal development

(GD 17) and once during adulthood (Krstic *et al.* 2012). Giovanoli *et al.* 2013 and 2016 described increased expression of CD68 and CD11b together with an enlargement of the microglial cell soma in adolescent MIA offspring that was exposed to unpredictable stress during postnatal development (Giovanoli *et al.* 2013, Giovanoli *et al.* 2016). Intriguingly, they further showed that minocycline treatment – a broad-spectrum tetracycline antibiotic that was shown to inhibit microglia activation – during postnatal stress prevented the offspring to develop behavioral deficits (Giovanoli *et al.* 2016), pointing towards the importance of proper microglia function during postnatal periods for brain maturation, neuronal function and behavior.

5.3 Housing, food, microbiome, strain and species effect on MIA outcome

As briefly touched upon in the introduction, several lab-specific factors that are not directly related to the protocol used to induce MIA in rodents might influence the response to MIA and contribute to the variation in study outcome we observe.

The two rodent species that are by far mostly used in the lab are mice and rats. However, it was recently shown by Lam *et al.* 2017 that the microglial response to inflammatory stimuli is different between these species (Lam *et al.* 2017). Even within a rodent species, different **strains** are being used to study the effect of MIA on microglia. A handful of studies reported a different vulnerability of inbred mouse strains, with different genetic background, to the effects of MIA (Schwartzter *et al.* 2013, Babri *et al.* 2014, Morais *et al.* 2018). Alterations in ultrasonic vocalizations, social interaction and repetitive behavior were more pronounced in BTBR mice compared to C57BL6 mice (Schwartzter *et al.* 2013). Similarly, maternal inflammation in NMRI or NIH Swiss mice induced more pronounced anxiety and depression-like behavior compared to C57BL6 mice (Babri *et al.* 2014, Morais *et al.* 2018).

Interestingly, Morais *et al.* 2017 showed that the increased vulnerability of NIH Swiss mice to effects of MIA on the offspring was accompanied by an increased gut permeability in adulthood, a defect that has previously been associated with **microbiome** dysregulation (Hsiao *et al.* 2013, Morais *et al.* 2018). Accumulating evidence suggests an important effect of microbiome composition, both from the

1 mother and the offspring, on the development and homeostasis of microglia and the behavioral
2 outcome of MIA. Adult germ-free mice or mice with limited microbiota complexity displayed global
3 defects in microglia leading to an impaired innate immune response (Frick *et al.* 2013, Matcovitch-
4 Natan *et al.* 2016). Also in prenatal stages, absence of maternal gut microbiota resulted in dysregulated
5 microglia transcriptomic profiles, altered microglial colonization of the embryonic brain (i.e. increased
6 microglial density and excessive ramification) and increased chromatin accessibility (Thion *et al.* 2018).

7 The importance of microbiome composition in the context of MIA has also recently been addressed.
8 Eradication of the vancomycin-susceptible segmented filamentous bacteria (SFB) prior to MIA
9 induction prevented the emergence of behavioral deficits in the offspring (Kim *et al.* 2017).
10 Additionally, maternal infection in C57BL6 mice obtained from Taconic Biosciences (which contain
11 intestinal SFB) induced behavioral abnormalities in the offspring while the same protocol applied to
12 C57BL6 mice from Jackson Laboratories (which are devoid of intestinal SFB) failed to elicit a behavioral
13 phenotype (Kim *et al.* 2017). Finally, MIA was shown to induce a dysregulation of the offspring's
14 microbiome which in turn was associated with behavioral symptom severity (Hsiao *et al.* 2013).
15 Treatment of MIA offspring with microbiota partially prevented these gut alterations along with
16 stereotypical and anxiety-like behavior and deficits in sensorimotor gating (Hsiao *et al.* 2013).

17 Another variable between labs is the **composition of the food** provided to rodents. Nevertheless, the
18 composition of different food pellets can differ dramatically and influence the outcome of MIA studies.
19 One important aspect of rodent food pellets is the amount of anti-inflammatory dietary omega 3
20 polyunsaturated fatty acids (PUFA) relative to the amount of pro-inflammatory omega 6 PUFAs. The
21 importance of dietary omega 3 PUFA for brain development has been documented in rodents:
22 deficiency during development in rodents leads to deficits in cognition, vision and a wide array of
23 behaviors (Fedorova *et al.* 2009, Brenna 2011, Weiser *et al.* 2015) while feeding mice with diets high
24 in omega 6 PUFA during gestation produces autistic-like features in offspring (Jones *et al.* 2013). In the
25 context of MIA, Li *et al.* 2015 and Weiser *et al.* 2016 showed that omega 3 PUFA-enriched or

docosahexaenoic acid-supplemented diet could prevent the onset of behavioral deficits in MIA offspring (Li *et al.* 2015, Weiser *et al.* 2016). Given the anti-inflammatory properties of omega 3 PUFAs, it seems plausible that the preventive effects could partially be explained by reduced microglial activation.

Finally, **housing conditions** can influence the effect of MIA on offspring's microglial and behavioral phenotype. In the study of Buschert *et al.* 2016, MIA offspring was either housed in standard conditions or in an environmentally enriched cage. Although no typical MIA-induced behavioral changes were observed, prenatal MIA reduced overall exploration (in the sociability test) in the environmentally enriched group, while it failed to do so in the standard housed animals ~~as exploration~~. Similarly, a decrease in cortical microglial density upon MIA (which is in sharp contrast to the studies discussed in this review describing either unchanged or increased density) could only be observed in mice housed in an enriched cage, while both NaCl and Poly(I:C)-exposed mice housed in a standard cage displayed decreased microglial density compared to the environmental enrichment group. The increase in microglial density, which is often interpreted as a sign of inflammation, upon housing in an enriched environment seems to contradict the findings of an anti-inflammatory effect of an enriched environment in disease contexts (including Alzheimer and Depression) (Williamson *et al.* 2012, Chabry *et al.* 2015, Xu *et al.* 2016). However, as shown by Ziv *et al.* 2006 and speculated by Buschert *et al.* 2016, these microglia might be in a neuroprotective rather than an inflammatory state (Ziv *et al.* 2006, Buschert *et al.* 2016). Taken together, these findings suggest that standard housing conditions can mask behavioral deficits and possibly microglial alterations induced by MIA.

In conclusion, we want to stress that authors should mention as much methodological details as possible in the materials and methods section concerning strain, housing, food etc., as they all have been shown to be relevant for the exact outcome of MIA studies and as a consequence possibly for the microglial response to MIA.

6. Alterations in microglia beyond dysregulated immune functions

Over the last decade there has been a substantial shift in our understanding of the functional role of microglia with regard to their non-immunological functions. Microglia are the first glial cells present in the developing brain and have been attributed to play a role in neurogenesis, angiogenesis, myelination, synaptogenesis and synaptic pruning (Bilimoria and Stevens 2015, Reemst *et al.* 2016). Although these functions are considered “non-immune” functions, some of them still rely on immune components, e.g. synaptic pruning has been shown to depend on the complement system (Hong *et al.* 2016). The fact that microglia originate from primitive macrophages and exert classical immune functions in response to insults of the CNS in the adult brain has led to the assumption that embryonic microglia exert similar roles in the developing brain. However, although equipped with immune related machinery, their functional contribution during development was found to exceed that of classical immune functions.

In support of this, microglia depletion during the first two weeks after birth was shown to result in long-lasting behavioral deficits (VanRyzin *et al.* 2016). Furthermore, transcriptomic studies showed that microglia are able to adapt according to regional cues, which translates in a regional and age-dependent density, morphology, surface molecule expression and transcriptome profile of microglia (Hart *et al.* 2012, Schwarz *et al.* 2012, Butovsky *et al.* 2014, Grabert *et al.* 2016, De Biase *et al.* 2017, Mosser *et al.* 2017). In fact, microglia might adjust their functional phenotype as a response to developmentally changing factors in the neuro-environment to be able to perform their specific developmental task (Mosser *et al.* 2017). Hence, although studies investigating classical activation of microglia upon MIA are highly valuable, it might be more appropriate to look at gene profiles involved in the neurodevelopmental function of microglia, combined with assessing the related microglial functions during brain development. Indeed, recent studies point to MIA-induced changes in the microglial epigenome (Kaminska *et al.* 2016, Nardone and Elliott 2016) and transcriptome concerning genes involved in the developmental functions of microglia, such as genes involved in synaptogenesis

1 and neuronal network formation (Pont-Lezica *et al.* 2014, Squarzoni *et al.* 2014, Matcovitch-Natan *et*
2 *al.* 2016, Mattei *et al.* 2017). Matcovitch-Natan *et al.* described that microglia from mice subjected to
3 MIA and analyzed from GD 14 to early postnatal weeks were transcriptionally shifted toward a more
4 advanced developmental stage (Maticovitch-Natan *et al.* 2016). This implies that MIA renders microglia
5 more “mature”, which is in accordance with a recent microglial transcriptomics study suggesting an
6 accelerated development of these cells in autism patients (Hanamsagar *et al.* 2017).

7 In support of altered neurodevelopmental microglial functions upon MIA, several reports describe
8 increased phagocytosis of neuronal progenitor cells (Cunningham *et al.* 2013), a decrease in the
9 outgrowth of dopaminergic axons (Squarzoni *et al.* 2014) and defasciculation of dorsal callosal axons
10 (Pont-Lezica *et al.* 2014) in the embryonic brain of MIA offspring. In the latter case, mice lacking DNAX-
11 Activation Protein 12 (DAP12), a transmembrane protein that together with Triggering receptor
12 expressed on myeloid cells 2 (TREM2) forms an immunoreceptor signaling complex and is associated
13 with Nasu-Hakola disease, displayed similar deficits as observed after MIA. Moreover, MIA led to
14 transcriptional alterations in microglia with regard to genes involved in growth of neurites,
15 underscoring a disturbed developmental function of microglia after MIA (Pont-Lezica *et al.* 2014). Until
16 now, conclusive experimental evidence for a direct, causative role of microglia in neurodevelopmental
17 disorders after MIA is lacking. However, a recent study by Mattei *et al.* 2017 showed that MIA-induced
18 changes in the microglial transcriptome and phagocytic function were accompanied by behavioral
19 abnormalities (Mattei *et al.* 2017). Most interestingly, treatment of adult MIA offspring with
20 minocycline rescued the transcriptional, functional and behavioral deficits. This study is among the
21 first to point in the direction of dysfunctioning microglia as the culprits in inducing
22 neurodevelopmental disorders upon MIA. Furthermore, disturbance of microglia-related proteins in
23 humans such as Colony stimulating factor 1 receptor (CSF1r), TREM2, Methyl CpG binding protein 2
24 (MeCP2), CD33, Interferon regulatory factor 8 (Irf8), P2x7r, DAP12 and Complement component 4 (C4)
25 are associated with an increased likelihood to develop a plethora of neurological diseases, including
26 schizophrenia and possibly autism ((Sekar *et al.* 2016) and reviewed in (Frost and Schafer 2016)). These

data suggest that disturbance of specific functions of microglia during neurodevelopment correlate with disease outcome.

Taken together, microglia display specific and constantly changing transcriptional signatures throughout development and these profiles appear to be altered upon MIA. Although conclusive evidence to pinpoint microglial alterations as the cause for neurodevelopmental disorders is missing, accumulating evidence strongly suggests at least a partially shared causative role for microglia in MIA-induced cognitive and behavioral deficits.

7. Conclusion

In the search for evidence of altered microglia function as mediators for the neurological deficits caused by MIA, a lack of consistency is encountered. Several factors might explain these contradictory results and thereby add complexity to the comparison of different MIA studies with regard to microglial alterations. In this review, we addressed this issue of variability in the field and here we suggest guidelines that might result in convergence on findings with regard to the effects of MIA on microglia. On one hand, the exact protocol used to induce MIA in rodents differs substantially between studies, using different immune stimulants (mostly LPS or Poly(I:C)) at different doses and administration routes and administered at various gestational time points. These factors will result in a different environment to which the embryo is exposed and might differentially affect microglia. First, we want to urge researchers to fully state all experimental details (such as animal strain, housing conditions, food, sex, etc.) when reporting on the outcome of MIA studies. Secondly, we suggest to perform dose-response studies of the immune stimulant used before the start of the study, in order to better control the inflammatory environment that is created. Although behavioral testing would be the most ideal read-out for these dose-response studies, measurements of cytokines such as IL6 or IL17a in maternal serum might provide the minimal amount of information on the extent of maternal inflammation.

1 Finally, care should be taken to use the least invasive and stressful administration route, as stress could
2 be an additional confounding factor for the outcome of the experiments.

3 On the other hand, substantial variability could be caused by the precise readout to assess “microglia
4 activation” with respect to their immunological function, such as microglia density, morphology or
5 immune marker expression, but also by the time point and brain region where microglia were
6 analyzed. While many studies used only one of these read-outs to analyze the effect of MIA on
7 microglia, we suggest to use a combination of several complementary methods to better define the
8 precise changes observed in microglia. Moreover, although these relatively crude measures can aid
9 our understanding of the role of microglia in MIA models to some extent, they might not be suitable
10 to detect less obvious changes in microglia function related to developmental processes. Therefore,
11 we believe future research on immune disturbance of neurodevelopment will benefit from a holistic
12 approach in which developmental functions of microglia are studied using a combinatorial approach –
13 i.e. using readouts at transcriptional, protein and functional level – to study both intrinsic microglial
14 characteristics and the neuron-microglia interaction.

16 **Acknowledgements**

17 The authors thank the Research Foundation - Flanders (FWO) for funding the MIA-related research
18 (project nr G.OA05.13N).

20 **References**

- 21 Abel, K. M., *et al.* (2010). "Sex differences in schizophrenia." Int Rev Psychiatry **22**(5): 417-428.
- 22 Alpizar, Y. A., *et al.* (2017). "TRPV4 activation triggers protective responses to bacterial
23 lipopolysaccharides in airway epithelial cells." Nat Commun **8**(1): 1059.
- 24 Antonson, A. M., *et al.* (2017). "Maternal viral infection during pregnancy elicits anti-social behavior
25 in neonatal piglet offspring independent of postnatal microglial cell activation." Brain Behav Immun
26 **59**: 300-312.

1 Arad, M., *et al.* (2017). "Immune activation in lactating dams alters sucklings' brain cytokines and
2 produces non-overlapping behavioral deficits in adult female and male offspring: A novel
3 neurodevelopmental model of sex-specific psychopathology." Brain Behav Immun **63**: 35-49.

4 Arnold, T. and C. Betsholtz (2013). "Correction: The importance of microglia in the development of
5 the vasculature in the central nervous system." Vasc Cell **5**(1): 12.

6 Arsenault, D., *et al.* (2014). "The different effects of LPS and poly I:C prenatal immune challenges on
7 the behavior, development and inflammatory responses in pregnant mice and their offspring." Brain
8 Behav Immun **38**: 77-90.

9 Babri, S., *et al.* (2014). "Strain-dependent effects of prenatal maternal immune activation on anxiety-
10 and depression-like behaviors in offspring." Brain Behav Immun **37**: 164-176.

11 Bennett, M. L., *et al.* (2016). "New tools for studying microglia in the mouse and human CNS." Proc
12 Natl Acad Sci U S A **113**(12): E1738-1746.

13 Bilbo, S. D., *et al.* (2017). "Beyond infection - Maternal immune activation by environmental factors,
14 microglial development, and relevance for autism spectrum disorders." Exp Neurol.

15 Bilimoria, P. M. and B. Stevens (2015). "Microglia function during brain development: New insights
16 from animal models." Brain Res **1617**: 7-17.

17 Boksa, P. (2010). "Effects of prenatal infection on brain development and behavior: a review of
18 findings from animal models." Brain Behav Immun **24**(6): 881-897.

19 Borrell, J., *et al.* (2002). "Prenatal immune challenge disrupts sensorimotor gating in adult rats.
20 Implications for the etiopathogenesis of schizophrenia." Neuropsychopharmacology **26**(2): 204-215.

21 Brenna, J. T. (2011). "Animal studies of the functional consequences of suboptimal polyunsaturated
22 fatty acid status during pregnancy, lactation and early post-natal life." Matern Child Nutr **7 Suppl 2**:
23 59-79.

24 Brown, A. S., *et al.* (2001). "A.E. Bennett Research Award. Prenatal rubella, premorbid abnormalities,
25 and adult schizophrenia." Biol Psychiatry **49**(6): 473-486.

26 Buka, S. L., *et al.* (2001). "Maternal infections and subsequent psychosis among offspring." Arch Gen
27 Psychiatry **58**(11): 1032-1037.

28 Buschert, J., *et al.* (2016). "Prenatal immune activation in mice blocks the effects of environmental
29 enrichment on exploratory behavior and microglia density." Prog Neuropsychopharmacol Biol
30 Psychiatry **67**: 10-20.

31 Butovsky, O., *et al.* (2014). "Identification of a unique TGF-beta-dependent molecular and functional
32 signature in microglia." Nat Neurosci **17**(1): 131-143.

33 Buttgereit, A., *et al.* (2016). "Sall1 is a transcriptional regulator defining microglia identity and
34 function." Nat Immunol **17**(12): 1397-1406.

35 Careaga, M., *et al.* (2017). "Maternal Immune Activation and Autism Spectrum Disorder: From
36 Rodents to Nonhuman and Human Primates." Biol Psychiatry **81**(5): 391-401.

- 1 Chabry, J., *et al.* (2015). "Enriched environment decreases microglia and brain macrophages
2 inflammatory phenotypes through adiponectin-dependent mechanisms: Relevance to depressive-like
3 behavior." Brain Behav Immun **50**: 275-287.
- 4 Choi, G. B., *et al.* (2016). "The maternal interleukin-17a pathway in mice promotes autism-like
5 phenotypes in offspring." Science **351**(6276): 933-939.
- 6 Corradini, I., *et al.* (2017). "Maternal Immune Activation Delays Excitatory-to-Inhibitory Gamma-
7 Aminobutyric Acid Switch in Offspring." Biol Psychiatry.
- 8 Crotti, A. and R. M. Ransohoff (2016). "Microglial Physiology and Pathophysiology: Insights from
9 Genome-wide Transcriptional Profiling." Immunity **44**(3): 505-515.
- 10 Crum, W. R., *et al.* (2017). "Evolution of structural abnormalities in the rat brain following in utero
11 exposure to maternal immune activation: A longitudinal in vivo MRI study." Brain Behav Immun **63**:
12 50-59.
- 13 Cunningham, C. L., *et al.* (2013). "Microglia regulate the number of neural precursor cells in the
14 developing cerebral cortex." J Neurosci **33**(10): 4216-4233.
- 15 De Biase, L. M., *et al.* (2017). "Local Cues Establish and Maintain Region-Specific Phenotypes of Basal
16 Ganglia Microglia." Neuron.
- 17 De Biase, L. M., *et al.* (2017). "Local Cues Establish and Maintain Region-Specific Phenotypes of Basal
18 Ganglia Microglia." Neuron **95**(2): 341-356 e346.
- 19 Eggen, B. J., *et al.* (2013). "Microglial phenotype and adaptation." J Neuroimmune Pharmacol **8**(4):
20 807-823.
- 21 Esslinger, M., *et al.* (2016). "Schizophrenia associated sensory gating deficits develop after adolescent
22 microglia activation." Brain Behav Immun **58**: 99-106.
- 23 Fatemi, S. H., *et al.* (1998). "Differential expression of synaptosome-associated protein 25 kDa [SNAP-
24 25] in hippocampi of neonatal mice following exposure to human influenza virus in utero." Brain Res
25 **800**(1): 1-9.
- 26 Fedorova, I., *et al.* (2009). "Deficit in prepulse inhibition in mice caused by dietary n-3 fatty acid
27 deficiency." Behav Neurosci **123**(6): 1218-1225.
- 28 Frick, L. R., *et al.* (2013). "Microglial dysregulation in psychiatric disease." Clin Dev Immunol **2013**:
29 608654.
- 30 Frost, J. L. and D. P. Schafer (2016). "Microglia: Architects of the Developing Nervous System." Trends
31 Cell Biol **26**(8): 587-597.
- 32 Fujita, Y., *et al.* (2016). "Supplementation with D-serine prevents the onset of cognitive deficits in
33 adult offspring after maternal immune activation." Sci Rep **6**: 37261.
- 34 Garay, P. A., *et al.* (2013). "Maternal immune activation causes age- and region-specific changes in
35 brain cytokines in offspring throughout development." Brain Behav Immun **31**: 54-68.

- 1 Gilmore, J. H., *et al.* (2005). "Maternal poly I:C exposure during pregnancy regulates TNF alpha, BDNF,
2 and NGF expression in neonatal brain and the maternal-fetal unit of the rat." J Neuroimmunol **159**(1-
3 2): 106-112.
- 4 Ginhoux, F., *et al.* (2010). "Fate mapping analysis reveals that adult microglia derive from primitive
5 macrophages." Science **330**(6005): 841-845.
- 6 Giovanoli, S., *et al.* (2016). "Preventive effects of minocycline in a neurodevelopmental two-hit model
7 with relevance to schizophrenia." Transl Psychiatry **6**: e772.
- 8 Giovanoli, S., *et al.* (2013). "Stress in puberty unmasks latent neuropathological consequences of
9 prenatal immune activation in mice." Science **339**(6123): 1095-1099.
- 10 Giovanoli, S., *et al.* (2015). "Late prenatal immune activation causes hippocampal deficits in the
11 absence of persistent inflammation across aging." J Neuroinflammation **12**: 221.
- 12 Giovanoli, S., *et al.* (2016). "Prenatal immune activation causes hippocampal synaptic deficits in the
13 absence of overt microglia anomalies." Brain Behav Immun **55**: 25-38.
- 14 Girard, S., *et al.* (2010). "IL-1 receptor antagonist protects against placental and neurodevelopmental
15 defects induced by maternal inflammation." J Immunol **184**(7): 3997-4005.
- 16 Grabert, K., *et al.* (2016). "Microglial brain region-dependent diversity and selective regional
17 sensitivities to aging." Nat Neurosci **19**(3): 504-516.
- 18 Gumusoglu, S. B., *et al.* (2017). "The role of IL-6 in neurodevelopment after prenatal stress." Brain
19 Behav Immun.
- 20 Hadar, R., *et al.* (2016). "Deep brain stimulation during early adolescence prevents microglial
21 alterations in a model of maternal immune activation." Brain Behav Immun.
- 22 Hadar, R., *et al.* (2015). "Using a maternal immune stimulation model of schizophrenia to study
23 behavioral and neurobiological alterations over the developmental course." Schizophr Res **166**(1-3):
24 238-247.
- 25 Han, M., *et al.* (2016). "Intake of 7,8-Dihydroxyflavone During Juvenile and Adolescent Stages
26 Prevents Onset of Psychosis in Adult Offspring After Maternal Immune Activation." Sci Rep **6**: 36087.
- 27 Hanamsagar, R., *et al.* (2017). "Generation of a microglial developmental index in mice and in
28 humans reveals a sex difference in maturation and immune reactivity." Glia **65**(9): 1504-1520.
- 29 Hanamsagar, R., *et al.* (2017). "Generation of a microglial developmental index in mice and in
30 humans reveals a sex difference in maturation and immune reactivity." Glia.
- 31 Hanamsagar, R. and S. D. Bilbo (2017). "Environment matters: microglia function and dysfunction in a
32 changing world." Curr Opin Neurobiol **47**: 146-155.
- 33 Hanisch, U. K. and H. Kettenmann (2007). "Microglia: active sensor and versatile effector cells in the
34 normal and pathologic brain." Nat Neurosci **10**(11): 1387-1394.
- 35 Harry, G. J. and A. D. Kraft (2012). "Microglia in the developing brain: A potential target with lifetime
36 effects." Neurotoxicology **33**(2): 191-206.

1 Hart, A. D., *et al.* (2012). "Age related changes in microglial phenotype vary between CNS regions:
2 grey versus white matter differences." Brain Behav Immun **26**(5): 754-765.

3 Hickman, S. E., *et al.* (2013). "The microglial sensome revealed by direct RNA sequencing." Nat
4 Neurosci **16**(12): 1896-1905.

5 Hong, S., *et al.* (2016). "New insights on the role of microglia in synaptic pruning in health and
6 disease." Curr Opin Neurobiol **36**: 128-134.

7 Hsiao, E. Y., *et al.* (2012). "Modeling an autism risk factor in mice leads to permanent immune
8 dysregulation." Proc Natl Acad Sci U S A **109**(31): 12776-12781.

9 Hsiao, E. Y., *et al.* (2013). "Microbiota modulate behavioral and physiological abnormalities
10 associated with neurodevelopmental disorders." Cell **155**(7): 1451-1463.

11 Hsueh, P. T., *et al.* (2017). "Expression of cerebral serotonin related to anxiety-like behaviors in
12 C57BL/6 offspring induced by repeated subcutaneous prenatal exposure to low-dose
13 lipopolysaccharide." PLoS One **12**(6): e0179970.

14 Hui, C. W., *et al.* (2018). "Prenatal Immune Challenge in Mice Leads to Partly Sex-Dependent
15 Behavioral, Microglial, and Molecular Abnormalities Associated with Schizophrenia." Front Mol
16 Neurosci **11**: 13.

17 Jones, K. L., *et al.* (2013). "Maternal diet rich in omega-6 polyunsaturated fatty acids during gestation
18 and lactation produces autistic-like sociability deficits in adult offspring." Behav Brain Res **238**: 193-
19 199.

20 Juckel, G., *et al.* (2011). "Microglial activation in a neuroinflammatory animal model of
21 schizophrenia--a pilot study." Schizophr Res **131**(1-3): 96-100.

22 Kaminska, B., *et al.* (2016). "Signal transduction and epigenetic mechanisms in the control of
23 microglia activation during neuroinflammation." Biochim Biophys Acta **1862**(3): 339-351.

24 Kim, S., *et al.* (2017). "Maternal gut bacteria promote neurodevelopmental abnormalities in mouse
25 offspring." Nature **549**(7673): 528-532.

26 Knuesel, I., *et al.* (2014). "Maternal immune activation and abnormal brain development across CNS
27 disorders." Nat Rev Neurol **10**(11): 643-660.

28 Krstic, D., *et al.* (2012). "Systemic immune challenges trigger and drive Alzheimer-like
29 neuropathology in mice." J Neuroinflammation **9**: 151.

30 Lai, A. Y., *et al.* (2013). "Distinct activation profiles in microglia of different ages: a systematic study in
31 isolated embryonic to aged microglial cultures." Neuroscience **254**: 185-195.

32 Lam, D., *et al.* (2017). "Responses of rat and mouse primary microglia to pro- and anti-inflammatory
33 stimuli: molecular profiles, K(+) channels and migration." J Neuroinflammation **14**(1): 166.

34 Le Belle, J. E., *et al.* (2014). "Maternal inflammation contributes to brain overgrowth and autism-
35 associated behaviors through altered redox signaling in stem and progenitor cells." Stem Cell Reports
36 **3**(5): 725-734.

1 Li, Q., *et al.* (2015). "Dietary supplementation with n-3 fatty acids from weaning limits brain
2 biochemistry and behavioural changes elicited by prenatal exposure to maternal inflammation in the
3 mouse model." Transl Psychiatry **5**: e641.

4 Li, W. Y., *et al.* (2014). "Prenatal infection affects the neuronal architecture and cognitive function in
5 adult mice." Dev Neurosci **36**(5): 359-370.

6 Malkova, N. V., *et al.* (2012). "Maternal immune activation yields offspring displaying mouse versions
7 of the three core symptoms of autism." Brain Behav Immun **26**(4): 607-616.

8 Manitz, M. P., *et al.* (2013). "The role of microglia during life span in neuropsychiatric disease--an
9 animal study." Schizophr Res **143**(1): 221-222.

10 Manitz, M. P., *et al.* (2016). "Flow cytometric characterization of microglia in the offspring of PolyI:C
11 treated mice." Brain Res **1636**: 172-182.

12 Matcovitch-Natan, O., *et al.* (2016). "Microglia development follows a stepwise program to regulate
13 brain homeostasis." Science **353**(6301): aad8670.

14 Mattei, D., *et al.* (2014). "Minocycline rescues decrease in neurogenesis, increase in microglia
15 cytokines and deficits in sensorimotor gating in an animal model of schizophrenia." Brain Behav
16 Immun **38**: 175-184.

17 Mattei, D., *et al.* (2017). "Maternal immune activation results in complex microglial transcriptome
18 signature in the adult offspring that is reversed by minocycline treatment." Transl Psychiatry **7**(5):
19 e1120.

20 Meehan, C., *et al.* (2017). "Effects of immune activation during early or late gestation on
21 schizophrenia-related behaviour in adult rat offspring." Brain Behav Immun **63**: 8-20.

22 Meseguer, V., *et al.* (2014). "TRPA1 channels mediate acute neurogenic inflammation and pain
23 produced by bacterial endotoxins." Nat Commun **5**: 3125.

24 Meyer, U. (2014). "Prenatal poly(i:C) exposure and other developmental immune activation models
25 in rodent systems." Biol Psychiatry **75**(4): 307-315.

26 Meyer, U., *et al.* (2005). "Towards an immuno-precipitated neurodevelopmental animal model of
27 schizophrenia." Neurosci Biobehav Rev **29**(6): 913-947.

28 Meyer, U., *et al.* (2006). "The time of prenatal immune challenge determines the specificity of
29 inflammation-mediated brain and behavioral pathology." J Neurosci **26**(18): 4752-4762.

30 Meyer, U., *et al.* (2007). "The neurodevelopmental impact of prenatal infections at different times of
31 pregnancy: the earlier the worse?" Neuroscientist **13**(3): 241-256.

32 Missault, S., *et al.* (2014). "The risk for behavioural deficits is determined by the maternal immune
33 response to prenatal immune challenge in a neurodevelopmental model." Brain Behav Immun **42**:
34 138-146.

35 Monte, A. S., *et al.* (2017). "Two-hit model of schizophrenia induced by neonatal immune activation
36 and peripubertal stress in rats: Study of sex differences and brain oxidative alterations." Behav Brain
37 Res **331**: 30-37.

1 Morais, L. H., *et al.* (2018). "Strain differences in the susceptibility to the gut-brain axis and
2 neurobehavioural alterations induced by maternal immune activation in mice." Behav Pharmacol.

3 Morgan, J. T., *et al.* (2010). "Microglial activation and increased microglial density observed in the
4 dorsolateral prefrontal cortex in autism." Biol Psychiatry **68**(4): 368-376.

5 Mortensen, P. B., *et al.* (2007). "Early infections of *Toxoplasma gondii* and the later development of
6 schizophrenia." Schizophr Bull **33**(3): 741-744.

7 Mosser, C. A., *et al.* (2017). "Microglia in CNS development: Shaping the brain for the future." Prog
8 Neurobiol **149-150**: 1-20.

9 Mosser, C. A., *et al.* (2017). "Microglia in CNS development: Shaping the brain for the future." Prog
10 Neurobiol.

11 Mouihate, A. (2016). "Prenatal Activation of Toll-Like Receptor-4 Dampens Adult Hippocampal
12 Neurogenesis in An IL-6 Dependent Manner." Front Cell Neurosci **10**: 173.

13 Mukherjee, S. B. (2017). "Autism Spectrum Disorders - Diagnosis and Management." Indian J Pediatr
14 **84**(4): 307-314.

15 Nardone, S. and E. Elliott (2016). "The Interaction between the Immune System and Epigenetics in
16 the Etiology of Autism Spectrum Disorders." Front Neurosci **10**: 329.

17 Naviaux, R. K., *et al.* (2013). "Antipurinergic therapy corrects the autism-like features in the poly(IC)
18 mouse model." PLoS One **8**(3): e57380.

19 Nelson, L. H., *et al.* (2017). "Sex differences in microglial phagocytosis in the neonatal hippocampus."
20 Brain Behav Immun **64**: 11-22.

21 Nimmerjahn, A., *et al.* (2005). "Resting microglial cells are highly dynamic surveillants of brain
22 parenchyma in vivo." Science **308**(5726): 1314-1318.

23 Norden, D. M., *et al.* (2016). "Sequential activation of microglia and astrocyte cytokine expression
24 precedes increased Iba-1 or GFAP immunoreactivity following systemic immune challenge." Glia
25 **64**(2): 300-316.

26 Notter, T., *et al.* (2017). "Translational evaluation of translocator protein as a marker of
27 neuroinflammation in schizophrenia." Mol Psychiatry.

28 Notter, T., *et al.* (2018). "Reconceptualization of translocator protein as a biomarker of
29 neuroinflammation in psychiatry." Mol Psychiatry **23**(1): 36-47.

30 O'Loughlin, E., *et al.* (2017). "Acute in utero exposure to lipopolysaccharide induces inflammation in
31 the pre- and postnatal brain and alters the glial cytoarchitecture in the developing amygdala." J
32 Neuroinflammation **14**(1): 212.

33 Paolicelli, R. C., *et al.* (2011). "Synaptic pruning by microglia is necessary for normal brain
34 development." Science **333**(6048): 1456-1458.

35 Paolicelli, R. C. and M. T. Ferretti (2017). "Function and Dysfunction of Microglia during Brain
36 Development: Consequences for Synapses and Neural Circuits." Front Synaptic Neurosci **9**: 9.

1 Paylor, J. W., *et al.* (2016). "Developmental disruption of perineuronal nets in the medial prefrontal
2 cortex after maternal immune activation." Sci Rep **6**: 37580.

3 Pineda, E., *et al.* (2013). "Maternal immune activation promotes hippocampal kindling
4 epileptogenesis in mice." Ann Neurol **74**(1): 11-19.

5 Piontkewitz, Y., *et al.* (2011). "Abnormal trajectories of neurodevelopment and behavior following in
6 utero insult in the rat." Biol Psychiatry **70**(9): 842-851.

7 Pont-Lezica, L., *et al.* (2014). "Microglia shape corpus callosum axon tract fasciculation: functional
8 impact of prenatal inflammation." Eur J Neurosci **39**(10): 1551-1557.

9 Pratt, L., *et al.* (2013). "Maternal inflammation promotes fetal microglial activation and increased
10 cholinergic expression in the fetal basal forebrain: role of interleukin-6." Pediatr Res **74**(4): 393-401.

11 Ransohoff, R. M. and A. E. Cardona (2010). "The myeloid cells of the central nervous system
12 parenchyma." Nature **468**(7321): 253-262.

13 Reemst, K., *et al.* (2016). "The Indispensable Roles of Microglia and Astrocytes during Brain
14 Development." Front Hum Neurosci **10**: 566.

15 Reisinger, S., *et al.* (2015). "The poly(I:C)-induced maternal immune activation model in preclinical
16 neuropsychiatric drug discovery." Pharmacol Ther **149**: 213-226.

17 Ribeiro, B. M., *et al.* (2013). "Evidences for a progressive microglial activation and increase in iNOS
18 expression in rats submitted to a neurodevelopmental model of schizophrenia: reversal by
19 clozapine." Schizophr Res **151**(1-3): 12-19.

20 Roumier, A., *et al.* (2008). "Prenatal activation of microglia induces delayed impairment of
21 glutamatergic synaptic function." PLoS One **3**(7): e2595.

22 Salter, M. W. and B. Stevens (2017). "Microglia emerge as central players in brain disease." Nat Med
23 **23**(9): 1018-1027.

24 Schaafsma, W., *et al.* (2017). "Maternal inflammation induces immune activation of fetal microglia
25 and leads to disrupted microglia immune responses, behavior, and learning performance in
26 adulthood." Neurobiol Dis **106**: 291-300.

27 Schafer, D. P., *et al.* (2012). "Microglia sculpt postnatal neural circuits in an activity and complement-
28 dependent manner." Neuron **74**(4): 691-705.

29 Schechter, R. W., *et al.* (2017). "Experience-Dependent Synaptic Plasticity in V1 Occurs without
30 Microglial CX3CR1." J Neurosci **37**(44): 10541-10553.

31 Schwartz, J. J., *et al.* (2013). "Maternal immune activation and strain specific interactions in the
32 development of autism-like behaviors in mice." Transl Psychiatry **3**: e240.

33 Schwarz, J. M., *et al.* (2012). "Sex differences in microglial colonization of the developing rat brain." J
34 Neurochem **120**(6): 948-963.

35 Sekar, A., *et al.* (2016). "Schizophrenia risk from complex variation of complement component 4."
36 Nature **530**(7589): 177-183.

- 1 Sipe, G. O., *et al.* (2016). "Microglial P2Y₁₂ is necessary for synaptic plasticity in mouse visual cortex."
2 Nat Commun **7**: 10905.
- 3 Slusarczyk, J., *et al.* (2015). "Prenatal stress is a vulnerability factor for altered morphology and
4 biological activity of microglia cells." Front Cell Neurosci **9**: 82.
- 5 Smith, S. E., *et al.* (2007). "Maternal immune activation alters fetal brain development through
6 interleukin-6." J Neurosci **27**(40): 10695-10702.
- 7 Smolders, S., *et al.* (2015). "Maternal immune activation evoked by polyinosinic:polycytidylic acid
8 does not evoke microglial cell activation in the embryo." Front Cell Neurosci **9**: 301.
- 9 Solek, C. M., *et al.* (2017). "Maternal immune activation in neurodevelopmental disorders." Dev Dyn.
- 10 Sorensen, H. J., *et al.* (2009). "Association between prenatal exposure to bacterial infection and risk
11 of schizophrenia." Schizophr Bull **35**(3): 631-637.
- 12 Squarzoni, P., *et al.* (2014). "Microglia modulate wiring of the embryonic forebrain." Cell Rep **8**(5):
13 1271-1279.
- 14 Streit, W. J., *et al.* (1999). "Reactive microgliosis." Prog Neurobiol **57**(6): 563-581.
- 15 Streit, W. J., *et al.* (2014). "Microglial pathology." Acta Neuropathol Commun **2**: 142.
- 16 Suvisaari, J., *et al.* (1999). "Association between prenatal exposure to poliovirus infection and adult
17 schizophrenia." Am J Psychiatry **156**(7): 1100-1102.
- 18 Swinnen, N., *et al.* (2013). "Complex invasion pattern of the cerebral cortex by microglial cells during
19 development of the mouse embryo." Glia **61**(2): 150-163.
- 20 Taylor, P. V., *et al.* (2012). "Sexually dimorphic effects of a prenatal immune challenge on social play
21 and vasopressin expression in juvenile rats." Biol Sex Differ **3**(1): 15.
- 22 Thion, M. S., *et al.* (2018). "Microbiome Influences Prenatal and Adult Microglia in a Sex-Specific
23 Manner." Cell **172**(3): 500-516 e516.
- 24 Torrey, E. F., *et al.* (1988). "Schizophrenic births and viral diseases in two states." Schizophr Res **1**(1):
25 73-77.
- 26 Trepanier, M. O., *et al.* (2016). "Postmortem evidence of cerebral inflammation in schizophrenia: a
27 systematic review." Mol Psychiatry **21**(8): 1009-1026.
- 28 Tronel, C., *et al.* (2017). "Molecular Targets for PET Imaging of Activated Microglia: The Current
29 Situation and Future Expectations." Int J Mol Sci **18**(4).
- 30 Tronnes, A. A., *et al.* (2016). "Effects of Lipopolysaccharide and Progesterone Exposures on
31 Embryonic Cerebral Cortex Development in Mice." Reprod Sci **23**(6): 771-778.
- 32 Van den Eynde, K., *et al.* (2014). "Hypolocomotive behaviour associated with increased microglia in a
33 prenatal immune activation model with relevance to schizophrenia." Behav Brain Res **258**: 179-186.
- 34 van Kesteren, C. F., *et al.* (2017). "Immune involvement in the pathogenesis of schizophrenia: a meta-
35 analysis on postmortem brain studies." Transl Psychiatry **7**(3): e1075.

1 VanRyzin, J. W., *et al.* (2016). "Temporary Depletion of Microglia during the Early Postnatal Period
2 Induces Lasting Sex-Dependent and Sex-Independent Effects on Behavior in Rats." eNeuro **3**(6).

3 Vargas, D. L., *et al.* (2005). "Neuroglial activation and neuroinflammation in the brain of patients with
4 autism." Ann Neurol **57**(1): 67-81.

5 Vezzani, A. and B. Viviani (2015). "Neuromodulatory properties of inflammatory cytokines and their
6 impact on neuronal excitability." Neuropharmacology **96**(Pt A): 70-82.

7 Weinhard, L., *et al.* (2017). "Sexual dimorphism of microglia and synapses during mouse postnatal
8 development." Dev Neurobiol.

9 Weiser, M. J., *et al.* (2016). "Dietary docosahexaenoic acid alleviates autistic-like behaviors resulting
10 from maternal immune activation in mice." Prostaglandins Leukot Essent Fatty Acids **106**: 27-37.

11 Weiser, M. J., *et al.* (2015). "Dietary DHA during development affects depression-like behaviors and
12 biomarkers that emerge after puberty in adolescent rats." J Lipid Res **56**(1): 151-166.

13 Willi, R., *et al.* (2013). "Altered GSK3beta signaling in an infection-based mouse model of
14 developmental neuropsychiatric disease." Neuropharmacology **73**: 56-65.

15 Williamson, L. L., *et al.* (2012). "Environmental enrichment alters glial antigen expression and
16 neuroimmune function in the adult rat hippocampus." Brain Behav Immun **26**(3): 500-510.

17 Wu, W. L., *et al.* (2017). "The placental interleukin-6 signaling controls fetal brain development and
18 behavior." Brain Behav Immun **62**: 11-23.

19 Wu, Y., *et al.* (2015). "Microglia: Dynamic Mediators of Synapse Development and Plasticity." Trends
20 Immunol **36**(10): 605-613.

21 Xu, H., *et al.* (2016). "Environmental Enrichment Potently Prevents Microglia-Mediated
22 Neuroinflammation by Human Amyloid beta-Protein Oligomers." J Neurosci **36**(35): 9041-9056.

23 Xuan, I. C. and D. R. Hampson (2014). "Gender-dependent effects of maternal immune activation on
24 the behavior of mouse offspring." PLoS One **9**(8): e104433.

25 Yang, T. T., *et al.* (2013). "Differential distribution and activation of microglia in the brain of male
26 C57BL/6J mice." Brain Struct Funct **218**(4): 1051-1060.

27 Yanguas-Casas, N., *et al.* (2018). "Sex differences in the phagocytic and migratory activity of microglia
28 and their impairment by palmitic acid." Glia **66**(3): 522-537.

29 Zhan, Y., *et al.* (2014). "Deficient neuron-microglia signaling results in impaired functional brain
30 connectivity and social behavior." Nat Neurosci **17**(3): 400-406.

31 Zhu, F., *et al.* (2014). "Minocycline alleviates behavioral deficits and inhibits microglial activation in
32 the offspring of pregnant mice after administration of polyriboinosinic-polyribocytidilic acid."
33 Psychiatry Res **219**(3): 680-686.

34 Ziv, Y., *et al.* (2006). "Immune cells contribute to the maintenance of neurogenesis and spatial
35 learning abilities in adulthood." Nat Neurosci **9**(2): 268-275.

36