CHAPTER

19

Prenatal-induced psychopathologies: All roads lead to microglia

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KEY POINTS

- The consequences of maternal exposures are more dependent on the timing than on the nature of the exposure itself, which can be pathogen-induced, stress-related or environmental.
- Fetal brain development requires the cooperation of the immune system in the central nervous system.
- Microglia play a critical role in shaping neuronal migration, synaptogenesis, dendritic pruning, and axonation.
- Adverse prenatal exposures activate maternal cytokine release and fetal microglia, which increases susceptibility to psychopathologies.

Introduction

The in utero environment is critical for the healthy brain development of the offspring. This unique environment enables interactions of genetic and environmental factors with the capability of protecting or predisposing future offspring to psychopathologies[1–5] and neurodevelopmental disorders.[5–8] Prenatal exposures can be separated into three broad categories: (1) immune overactivation, e.g., immune activation by invading pathogens such as bacteria or viruses, chronic immune activation due to maternal autoimmunity, dysbiosis in the mother, or by pharmaceutical drugs that modulate stress or immune function; (2) stressful psychological exposures, e.g., the threat of physical harm; (3) adverse lifestyle factors of the mother, e.g., obesity, diet, smoking, alcohol consumption, and environmental toxins. All of them can directly or indirectly alter the neurodevelopment and immune function of the developing fetus.

During pregnancy, there is tight control of pro- and antiinflammatory pathways and responsiveness of the hypothalamic-pituitary-adrenal (HPA) axis to ensure several physiological processes.[9] For instance, during the first trimester, the immune system shifts toward innate immunity with monocyte enrichment, creating a proinflammatory state to ensure proper uterine implantation.[10,11] By the second trimester, there is a shift toward an antiinflammatory state with an increased number of microglia in the M2 stage of repair compared with microglia in the M1 stage of actively dealing with pathogens. In addition, there is a low monocyte ratio, and a shift from proinflammatory Th1 to antiinflammatory Th2 immunity occurs, reducing concentrations of natural killer cells, TNF-alpha, and interferon-gamma, thus preventing maternal rejection of the fetus.[12] Toward parturition, an influx of immune cells enters the myometrium to reactivate inflammatory processes. This proinflammatory environment supports the uterine contraction, the delivery of the baby, and the placenta. In addition, other adverse exposures can activate pathways that further shift to an inflammatory status and make the mother vulnerable to future infection. For example, stressful exposure activates the HPA axis and increases corticotropin-releasing factor (CRF) and inflammatory cytokines.

Since pregnancy is a sequential process of pro- and antiinflammatory conditions, environmental exposures during gestation, as seen in maternal immune activation (MIA), e.g., by pathogens, psychological stress, or environmental insult, cause an exaggerated immune

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pathway response.[12] In accordance with the timing of the environmental exposure and the current immune stage, brain development (as exhibited by morphological changes or compensatory cell signaling) is impacted. The actual impact of the environmental exposure causes a domino-like effect in which other parallel developing systems, such as excitatory-inhibitory balance and HPA axis development, are impacted by proxy. Thus, one type of gestational exposure may increase the risk for several developmental processes in parallel.

Adverse exposures during gestation can impact neurodevelopmental processes, such as neurons or interneurons migration, synaptogenesis, axonation, and more. The earlier the exposure, the more dramatic the outcomes are. Alterations in early fundamental neurodevelopmental processes like migration, cortical organization, connectivity, dendritic density, and pruning can lead to circuitry instability, epilepsy, and changes in social behavior[13–16] On the other hand, during the second trimester of gestation, synaptogenesis and neuronal migration are active,[17] while microglial maturation is only underway,[18,19] and stress/adverse exposures can influence synaptic circuitry. Finally, cytokines, modulated by stressors, are known to shape central nervous system (CNS) development by influencing migration, neurogenesis, and gliogenesis.[20] Chronic inflammation, or adverse lifestyle exposures, can dysregulate their level and impair multiple neurodevelopmental processes.[6] For example, maternal immune overactivation has been associated with developmental changes in the white to gray matter ratio in the frontal limbic cortex[21] and changes in connectivity related to the interpretation of salience in toddlers.[22] Maternal stress has also been found to negatively impact the offspring's emotional reactivity and mood disorders in adulthood. Levels of maternal emotional stress correlate with infant cortisol levels in preschool-age children.[23] Infant cortisol levels from mothers exposed to stress are lower than controls.[24] Lower cortisol levels in childhood can predispose them to depression due to increased glucocorticoid sensitivity in adulthood via FKBP5.[25,26] This change in sensitivity is also partially sexually dimorphic. The connectivity between the amygdala and frontal cortex—a functional biomarker of altered emotional reactivity[27]—is stronger in daughters of mothers with higher cortisol levels than in sons with similar cortisol exposure.[21]

Immune dysregulation has been identified as a hallmark of psychiatric and neurodevelopmental disorders such as depression, anxiety, autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), and schizophrenia.[28,29] For instance, depression has a proinflammatory biological signature, including elevation of the interleukin-6 (IL-6) cytokine.[29] Selective serotonin reuptake inhibitors (SSRIs) used to treat depression have been shown to have antiinflammatory effects, including reducing systemic IL-6 levels[30] and specifically inhibiting the activation of microglia.[31] In addition, cytokine activation, specifically IL-6, IL-1ß, and TNF-α, has been shown to impact SSRI responsiveness.[32] About one-third of schizophrenia patients have generalized severe inflammation and dysregulation of markers from innate to adaptive immunity, cytokines, chemokines, microglial density,[33] and more. Children with ASD also exhibit disturbances related to immune system function, including elevated antibody levels, increased allergy prevalence, asthma, eczema, and other autoimmune disorders.[34] Microglial activation has also been linked to ASD and was shown to be increased in postmortem tissue[35] and PET studies.[36] The question remains if these inflammatory states cause the onset of psychiatric disease or if the onset in adulthood depends on a second exposure, with prenatal exposure having primed to the disorder. Alternatively, is the onset of a neurodevelopmental disorder due to an inflammatory gestational state or early postnatal insults that were exacerbated or primed by an inflammatory gestational state? These questions can only be answered with precise attention paid to the timing of the exposure, in context with the developmental stage, and the intensity, which is related to how broad the impact is.

In this chapter, we will discuss animal models of MIA, prenatal chronic variable stress (CVS) or maternal restraint stress (RS), prenatal consumption of a Western or high-fat diet (WD or HFD), and maternal exposure to diesel exhaust pollution (DEP). All are known to activate microglia and can cause behavioral changes in adult offspring.

Microglial mechanisms of disturbance in brain development

Microglia support neuronal homeostasis

Microglia are one of several glial cells that support brain homeostasis. Microglia are the main glial cells responsible for phagocytosis by which they protect the CNS from pathogens and neuronal debris and are part of the immune defense system. Healthy brain development requires the cooperation of the immune system in the CNS. During human fetal development, microglia already begin colonization of the CNS by 4.5 weeks of gestation and depend on normal vasculogenesis. By 18 weeks, microglia have the full ability of mature microglial cells to survey their environment.[19]

The following measures are used to quantify changes in microglial behavior or function: absolute microglial numbers, the density of microglia, surveillance behaviors (i.e., motility, velocity, and tip directionality), phagocytic activity, and changes in morphology (i.e., lengthening or retraction of processes and soma size).

The number, density, and activity of microglial cells depend on the results of their surveillance of the CNS. They can be in a "resting" state or ramified, represented by the morphological characteristics of arborization or extension of processes from the soma (sometimes also referred to as types). Upon activation in the presence of stress, inflammation, or injury, the microglia undergo morphological changes characterized by an enlarged cell body and reduced arborization or shortened processes. These physiological changes allow the microglia to be capable of expedited movement toward a site of injury to perform phagocytosis. During phagocytic processes, activated microglia may perturb neurogenesis and/or activate inflammasomes via oxidative stress during neurotoxic processes.[37]

Additionally, like peripheral macrophages, activated microglia can show different phenotypes designated as M1 and M2. In the M1 stage, the cells increase the production of proinflammatory cytokines such as IL-1β and TNFα. This stage is meant to eliminate the pathogen or clear away dying neurons. Alternatively, the M2 stage, subdivided into M2a, M2b, and M2c, is antiinflammatory and is focused on facilitating repair, e.g., collagen formation and recruitment of regulatory T cells.[38]

Microglia are also influenced by external proinflammatory signaling from the body via interaction with the blood-brain barrier (BBB). When proinflammatory markers from the bloodstream cross the BBB, they activate microglia inside the brain. Consequently, neuronal morphology is altered by cytokine-induced activation of the microglia. Activated microglia, in their original quest for pathogens or injury, react by inhibiting the initiation of synaptic pruning or by regulating plasticity. These effects are mediated by regulating CX3CR1 and P2Y12R, a purinergic receptor.[39] Disruption of this neuronal-dependent signaling creates deficits in remodeling circuits and increases synaptic density instead of the needed pruning for circuit stabilization. Furthermore, microglial activation itself can increase the permeability of the BBB via COX2,[40] allowing the further entrance of proinflammatory cytokines into the CNS, causing damage from within the brain and not from exogenous pathogens. Increased BBB permeability due to the breakdown of tight junctions and increased flux of excitatory ions has been associated with neurodegenerative disorders and epilepsy.[41]

Microglia affect synapse formation, pruning, and maturation

In humans, the density of microglia in the midbrain peaks by 20–26 weeks, coinciding with the start of synaptogenesis and the generation of other glial cells.[18] In mice, microglial numbers continue growing postnatally and reach their peak 4 days after birth (P4). Between P8 and P10, the spread of microglia has increased, peaking its activity of synapse elimination. In parallel, the synapses in cortical areas, such as the anterior cingulate cortex (ACC) and thalamocortical, increase in density and form organized, pseudolaminated layers between P6 and P15.[42] In both control mice and mice prenatally exposed to DEP and maternal stress, the male offspring of both groups showed microglial heterogeneity at P25, indicating that microglial heterogeneity is neither abnormal nor pathological but rather a physiological feature of development.[43]

Microglia constantly survey the environment and, as such, are highly motile, making contact with a different synapse for about 5 min, about once every hour.[14] This interaction contributes to the regulation and maturation of synapses. Indeed, the balance between new synapse formation and the pruning of weak synapses is based on signaling between astrocytes, neurons, and microglia. Environmental input in critical windows in the form of molecular signaling is conveyed to the microglia, leading to retracting synapses. Retraction of synapses is crucial for ensuring functional connectivity and occurs after an interaction between receptors and ligands, identifying whether the synapse is active and mature or inactive and immature. The motility of the microglia allows them to reach synapses on mature and immature neurons and prune them if needed depending on the detected ligand's receptor and their expression on the neuron itself.

For example, retraction of synapses involves interaction between paired immunoglobulin-like receptor B (PirB) on the microglial processes and interaction with the receptor MHC class I on the neuron. Elimination of weaker synapses also occurs via the complement cascade. Astrocyte secretion of cytokines such as IL-33 or classical complement members such as C1q or C3 initiates phagocytosis via activation of the microglial C3R or C1R receptor on the neuron in the immature synapse.[44] This mechanism is utilized in the development of sensory systems, such as the lateral geniculate nucleus (LGN) of the thalamus, a key component of the visual system, leading to the pruning of the weak synapses in favor of the synapses that strongly fire to the required input.[45] Disruption of C3 or C3R decreases phagocytosis, leading to excessive arborization instead of synaptic pruning and shifting the balance from mature to immature neurons.[46]

In addition, microglia influence functional synaptic maturation via the recruitment of the chemokine receptor CX3CR1 in microglia and neuronal postsynaptic AMPA circuits. Neuronal CX3CR1, a regulator of microglial quantity, is an environmental cue used in hippocampal synaptic maturation. Deletion of CX3CR1 leads to a reduction in pruning and an increase in immature synapses.[47]

Microglia affect neurotransmission/excitatory-inhibitory tone

When microglia are in a resting state, glutamate reuptake proceeds via glutamate transporters in astrocytes and oligodendrocytes, and oxidative stress is regulated by glutathione levels.[48] However, when microglia enter an activated state due to sensing proinflammatory cytokines such as TNF-α and IL-1β, they can also disrupt neurotransmitter release and shift the excitatory-inhibitory balance in the brain toward excitation. Activated microglia release ATP, which activates the P2Y receptor on astrocytes, leading to an increase in glutamate release. Excess glutamate activates presynaptic metabotropic glutamate receptors, and causes enhanced neuroexcitation, as well as increased release of quinolate and D-serine.[14] This, in turn, stimulates brain-derived neurotrophic factor (BDNF) release from the activated microglia, which interferes with chloride homeostasis and reduces GABAergic transmission.[14]

Furthermore, activated microglia can interfere with the astrocyte reuptake of glutamate and disrupt signaling between astrocytes and neurons due to the accumulation of glutamate.[49] Excitotoxicity disrupts the conversion of cystine to cysteine, which is crucial for the production of glutathione, leading to increased levels of oxidative stress. Neurons are especially sensitive to oxidative stress, creating a cascade of cellular damage and neuronal death.[33]

Sex differences in microglia

Psychiatric disorders such as ASD, schizophrenia, depression, and anxiety are more prevalent in one sex; immune-sex differences and differences in the brain may play a key role. Across development, there are differences between males and females in the number of activated microglia. In addition, microglial development trajectories have been found to differ between sexes.[42,50]

Studies in rodents found no difference in microglial number between the sexes at E17. Sex differences arise after the onset of a testosterone spike at E18. At P4, males have more microglia within several brain regions, such as the prefrontal cortex (PFC), hippocampus, and amygdala. Females have more activated microglia in the same limbic regions 1 month after birth. In addition, at P0, females had more activated microglia in their paraventricular nucleus of the hypothalamus (PVN), which remained higher than in males.[51] In males, an increased number of microglia are active in surveillance during the time of neurogenesis, neuronal migration, and neurite outgrowth.[17] Morphological studies also show that females present with larger dendritic arborization and greater spine density in the PFC, consistent with the increased presence of activated microglia.[52]

The reactivity of the microglia is also different between sexes in adulthood. For example, CVS in adulthood affects males and females differently and depends on the stress duration. A short CVS protocol did not cause microglial activation in females in the hippocampus. At the same time, long CVS activated microglia with shortened processes in males with accompanying shortened dendritic length in the hippocampus. This effect was reversed in the nucleus accumbens, where both the short and the long CVS protocols activated microglia in females. At the same time, males showed activated microglia only in the shortened protocol. In both conditions, increased dendritic length was observed in the male nucleus accumbens, but no changes were seen in females despite activated microglia.[53] In summary, microglial number and activity may contribute to the differences observed in psychiatric disorders between the sexes.

Prenatal models that induce microglial activation

MIA and microglial involvement

Human prenatal exposure to the influenza virus, especially during the first and second trimesters, increases the risk for schizophrenia and autism spectrum disorder (ASD).[43,54,55] ASD has also been linked to bacterial or parasite infection and elevated levels of maternal antibodies, a sign of maternal immune dysregulation, especially infections accompanied by fever or during the first trimester.[43]

There are several preclinical models for MIA. These models use exposure to immune activators during gestation. These mainly rely on different types of immune stimulation meant to increase maternal IL-6 levels. This may be achieved by IL-6 injection or by inducing immune pathways that activate IL-6, such as other cytokines or toll-like receptor agonists.[56] The most commonly used model involves mimicking a bacterial or viral infection. Bacterial infection is simulated by the systemic injection of lipopolysaccharide (LPS), a component of the outer cell wall of gram-negative bacteria. LPS exposure during gestation activates maternal toll-like receptor 4 (TLR4) and eventually induces a cascade of proinflammatory cytokines such as IL-6 in the mother. Creating mature IL-1β requires activation of TLR, an increase in TNF-α, and priming activation of the purinergic receptor P2X7, to create an NLRP3 inflammasome that releases caspase 1. Inflammasomes are also created when cells are exposed to reactive oxygen species or sense lysosomal damage.[57] In either case, the released IL-1β then activates microglia.

Polyinosinic:polycytidylic acid (Poly I:C) is a virus-like double-stranded RNA, similar to influenza, that stimulates toll-like receptor 3 (TLR3) and elevates levels of maternal IL-6. Increases in cytokine levels are also seen in the placenta after a single Poly I:C injection and impact brain development. While the virus RNA from the mother does not enter the fetal circulation or brain, circulating cytokines activate proinflammatory pathways in the fetal brain via BBB interaction with microglia.[58] Most rodent models of MIA expose the dams to LPS or Poly I:C once or twice in the second week of gestation (between E9 and E15). This period parallels the first trimester in humans and marks the onset of cortical GABAergic interneuron production in mice.[59] Additionally, some studies use a "double hit" model adding LPS exposure in adolescence to investigate the "priming effect" of early exposure on the outcomes of the later exposure. The endpoints involve microglial quantification or behavioral outcomes.

A metaanalysis of MIA with either LPS or Poly I:C examined outcomes after a single immune exposure (without accounting for the timing of the exposure). A single hit led to increases in microglial Iba-1 reactivity, a marker for increased microglial number and decreased dendritic density, a sign of over pruning of synapses in the hippocampus, and attenuated neurotransmission of both serotonin and GABA.[17] The offspring exhibited an increase in anxiety-like behaviors.

Early maternal exposure to immune activators can structurally impact the developing brain, predisposing it to neurodevelopmental disorders. When comparing early maternal exposure to Poly I:C[60] at E9 vs. E17, the earlier exposure showed more dramatic effects in adulthood. Exposure at E9 was accompanied by enlarged lateral ventricles and reduced prepulse inhibition (PPI). After Poly I:C injection at E9, reversal learning in a T-maze was impaired, and the number of entries into the center of the open field was reduced.[61] Reduction in PPI and reversal learning are indicative of altered sensorimotor gating known to be impaired in ASD and schizophrenia. While Meyer et al.[61] observed changes in anxiety-like behavior after E9 injection but not E17 injection, anxiety-like behavior has not been shown in most studies with MIA exposure at E9-12.[62] The harsher influence of early exposure was also manifested by the differential vulnerability of GABAergic interneuron progenitors. Their proliferation was reduced by maternal inflammation at E9.5 and increased by maternal inflammation at E16.5.[63]

Single maternal Poly I:C injections at E12-E15 induced a wide range of behavioral outcomes from ASD-like or schizophrenia-like phenotypes to depressive-like behaviors and cognitive deficits. Poly I:C injection at E12.5 elevated serum IL-17A when examined at E14.5. This elevation was abolished in mice lacking IL-6. Maternal IL-17A activation by Poly I:C injection leads to an ASD-like morphology and behavioral phenotype. Offspring display disorganized cortical lamination by E18.5. Behaviorally, changes in vocalization during maternal separation were observed in early life, as well as increased time engaged in marble-burying and fewer social approaches in adulthood. These behavioral outcomes are related to ASD, e.g., communication deficits, repetitive behaviors, and changes in the initiation of social contact with unfamiliar mice. These effects were reversed when IL-17A was blocked before the maternal Poly I:C injection.[64] A single administration of IL-6[65] at E12 also leads to changes in PPI, and latent inhibition is also associated with ASD and schizophrenia, as well as reductions in social behavior and increased anxiety-like behavior. The administration of IL-6 antibodies can reverse these behaviors.

Injection of Poly I:C on E14 leads to changes in schizophrenia-related cortical genes. Changes were already observed by P14 but continued into adulthood. The changes include upregulation of ErbB4 and the BDNF receptor TrkB in cortical areas, such as the PFC, ACC, and parietal and piriform cortices, and downregulation of neuregulin-1, which is involved in proliferation, differentiation, and neuronal survival. Additionally, neuregulin-1 is associated with deficits in executive function in ASD as well.[66]

Long-term behavioral and cognitive effects of a single Poly I:C challenge at E12.5 were reversed when an inhibitor of microglial activation, minocycline, was administered at P21. Minocycline injection mitigated behavioral effects in PPI, and open field rescued cognitive deficits by improving time to find the platform in the Morris water maze and rescued GABAergic interneuron number in the dentate gyrus, preventing the offspring from a schizophrenic-like phenotype. The proposed mechanism of minocycline action was via upregulation of arginase 1 (Arg1). Arg1 may play a role in mitigating the glutamate toxicity known to occur with increases in microglial activation and conditions like schizophrenia.[67] It is also important to point out that identified alterations in brain development were reversed with intervention in early childhood. This may imply that, if prenatal microglial activation persisted postnatally, then the minocycline impact postnatally allowed normal developmental processes to continue, despite the prenatal interruption.

Other changes, such as mood and cognitive deficits, emerge after exposure at earlier time points. At E12.5, Khan et al. (2014)[68] showed that a single injection of Poly I:C caused depressive-like behaviors such as a reduction in sucrose preference and cognitive deficits in adulthood, which can be attributed to a reduction in hippocampal neurogenesis.[69] Additionally, injection of Poly I:C at E15 still caused cognitive deficits in reversal learning, studied in schizophrenia-like behaviors, as well as decreases in novelty seeking in novel object exploration and novel context exploration.[70]

Interestingly, maternal injection of LPS at a later developmental stage, such as E15-E17,[71] changed serotonin synthesis and dopamine levels at E18 in female embryos and increased anxiety-like behavior during adolescence.[71] The expression of critical genes regulating neurotransmitters and transporters of the serotonin and dopamine systems, classically implicated in depression, was downregulated. In addition, a decrease in the cerebral serotonin level was observed at 5–8 weeks postnatally. In MIA models at E17.5, only adult males showed deficits in working memory in T-maze tasks, while adult females showed impairment in novel object recognition.[62] No changes were reported in juvenile mice of either sex after exposure.

As illustrated in Fig. 1, the dramatic morphological and behavioral effects of immune activators between E12 and E15 were consistent with the disruption in developmental processes of neurogenesis and axonation, as well as dendritic growth and pruning, and the prevention of glutamate toxicity, which occur during the middle gestation when the mother's immune system shifts into an antiinflammatory state to retain the fetus's viability. However, MIA at E9 has the most dramatic outcomes due to the impact of microglial activation on earlier developmental processes, such as cortical organization and ventricle size, possibly due to disruption of early neuronal migration. Later immune activation changes are consistent with synaptic refinement and synaptogenesis.

Not all the literature agrees on the significant impact of structural and behavioral changes after a single prenatal injection of Poly I:C or LPS or the impact of an additional "second hit."

Carlezon et al. (2019)[72] modeled a double-hit immune exposure in early and late trimesters using a mouse model with combined hits of Poly I:C at E12.5 and LPS on postnatal day nine and showed behavioral changes in offspring. With a single injection at E12.5, male offspring showed reduced social behavior, increased repetitive behaviors, and no difference in the social interaction test. However, with the additional "second hit," mice had higher levels of IL-6, IL-1β, and Iba-1. In male mice, the most pervasive effects following the "second hit" were reduced vocalization, less social interaction, more anxiety-like behavior, and increases in repetitive-like behaviors. The proinflammatory marker upregulation was also more pervasive in males' PFC, hippocampus, and thalamus. In this model, they also observed changes in the sleep/wake cycle and the induction of epileptiform discharges, as well as epilepsy symptoms, a common comorbidity in ASD.[73] Since Poly I:C and LPS are known to act through TLR3 and TLR4 signaling pathways, respectively, this "second hit" would also indicate a broader immune insult given in critical windows in development. Indeed, the timing of immune maternal hyperactivation matters: P9 is a time of rapid brain growth and when preterm babies are more at risk for microbial infections. Preterm birth is also a risk factor for ASD.[74,75]

Ozaki et al.[16] used the double-hit model with the second exposure in adulthood to tease out the impact of a single Poly I:C administration and the impact of its timing. Injections of Poly I:C were given during pregnancy either at E12 or E15. These exposures led to increased microglial surveillance behaviors such as velocity, similar to the effect of maternal injection of IL-6. Increases in IL-6 were observed in the mother's liver, placenta, and fetus. However, morphological changes were not observed in the fetus by a single injection alone, neither at E12 nor E15.

Only after E12 exposure but not following E15 exposure, microglia increased motility at E18 but had lower velocities than controls at P10. These cells had an increase in repetitive surveillance movement and tip directionality, confirming the impact of E12 exposure at early stages and thus the capability of future priming when exposed to a "second" hit at later time points. Indeed, by P42, the effect of the E12 exposure was only discernible after an additional LPS challenge. Microglia of mice exposed to both E12 Poly I:C and P42 LPS injections exhibited a shorter process and increased velocity. Behaviorally, both E12 with P42 LPS and E15 with LPS exhibited less time in the center of the open field and less social sniffing. However, only the E12 groups spent less time interacting with unfamiliar mice. The amount of velocity and tip directionality of the microglia were correlated with time spent in the center and sniffing behaviors in the earlier exposed mice.

Desbonnet et al. (2022)[76] also demonstrated that Poly I:C itself, despite elevation in maternal IL-6, is insufficient to induce the altered phenotype in adulthood. A behavioral phenotype was only observed when a second behavioral stressor in adulthood, in this case, social isolation, was introduced. In addition, when Poly I:C was injected at E15, and then the mice were subjected to juvenile stress, no additional phenotype was observed in adulthood due to the "second hit."[77] The discrepancy between studies describing effects on morphology, surveillance behaviors, and offspring behavior after a single injection and those that only showed a phenotype after a "second hit" may reflect differences in the used protocols, including doses and mice strain. For example, recent studies use low doses of immune activators to minimize survival bias since higher doses decrease maternal and pup survival.[78,79] There are differences between mice and rat strains and differences based on which immune activator, Poly I:C or LPS, is used, in combination or not, and in which order in addition to the timing of the injections.[79]

Sex differences in offspring MIA models

Sex differences in offspring with respect to MIA have not been fully studied. While timing is important for understanding the behavior outcomes in the offspring,

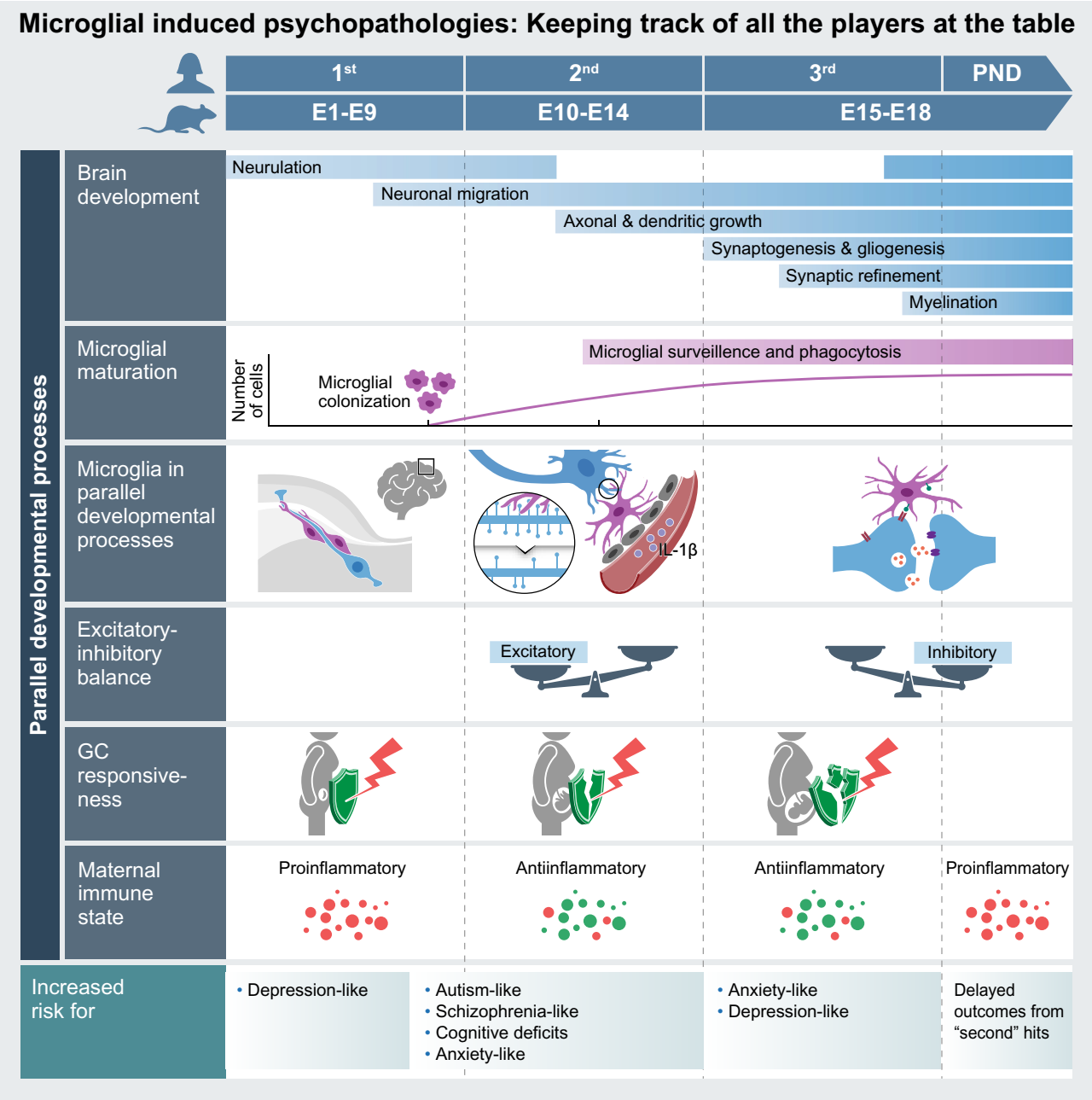

FIG. 1 Basal parallel processing in development schema. While normal brain development ensues, many immune activation, stress, lifestyle modifications, and environmental exposures can impact these developmental patterns, including the timing of microglial colonization and increase in microglia numbers. Depending on when the insult occurs, these processes (displayed vertically) in their individual developmental timelines (displayed horizontally) are impacted concurrently. Microglia after colonization and depending on the timing of insult with response to their surging numbers can modify their normal regulatory functions on the developing neurons and synapses when activated. Additionally, changes in excitation and inhibition in development can impact developmental trajectory by neurotoxicity. The interplay between the fetus and the mother regarding glucocorticoid sensitivity and immune state of the pregnancy mother can also affect the outcome of the offspring depending on when an insult is introduced. Other abbreviations include: glucorticoid (GC), embryonic day (E), postnatal day (PND).

sex-specific differences may also play a physiological role as microglia differ in basal numbers in brain-specific regions and the intensity of activation after exposure.

Prenatal RS at E12 combined with LPS in adulthood increases the microglial number in the CA1 region of the hippocampus in males.[80] On the other hand, ovariectomized females still show sex differences, although the impact of microglial sex differences that emerge after the testosterone spike at E18 was removed.[51] Females with ovariectomy still had higher postnatal microglial activation when exposed to the same/similar stress paradigm. The females showed Iba-1 immune-reactive staining, increases in IL-β, and proinflammatory signaling, as well as activated microglia with enlarged somas

and retracted processes in the hippocampus.[81] Increased IL-β may lead to enhanced HPA axis activity due to the interaction between cytokines and glucocorticoids. Specifically, IL-β exacerbates the release of mineralocorticoids over glucocorticoids in the hippocampus, leading to the phenotype observed in males.[82] The HPA axis is different in activation between sexes,[83] as is cytokine activation,[84] especially in psychiatric disorders,[85] which may influence hippocampal structure and function and be the basis of the microglial number vs. activation sex differences.

Additionally, different phenotypes were observed at different time points in different brain regions determined by sex. Two hours after maternal LPS injection at E12.5, proinflammatory cytokines, such as TNF-α and chemokine CXCL1, increased in the male placenta.[86] Another chemokine, CXCL10, known to initiate microglial activation, was also elevated in the female brain. In addition, the male cortex had more hypoxic tissue. In males, proliferating cells were predominantly in metaphase, impacting the number of radial glia, the brain's primary neural and glial progenitors. In adulthood, males made fewer approaches to social novelty, showed impairment in the Morris water maze, had rapid habituation to novel stimuli, and spent less time exploring social novelty.

In contrast, females were smaller in body size at birth and showed anxiety-like behavior in the open field in adulthood.[86] In addition, the sex of the offspring was shown to matter for other stressors. Maternal cold stress showed reduced oxytocin levels in the hypothalamus only in males and could be reversed with microglial depletion.[87] Prenatal injection of dexamethasone, given frequently in at-risk pregnancies, increased ramified microglia in males, whereas the number of activated microglial processes was reduced in females.[88]

In adulthood, females prenatally stressed with a "second hit" are more reactive to the second challenge. This is consistent with the higher glucocorticoids, cytokines, and more abundant activated microglia found in the female PVN from birth.[51] In addition, microglia can directly respond to circulating glucocorticoids since they express their receptor.[89] This sexual dimorphism may explain the number of microglia and accompanying higher prevalence of ASD-like and schizophrenia-like in males after maternal immune challenges and the microglial activation, affecting HPA activation and may accompany more anxiety-like behavior in females after later immune challenges. In addition, the increased microglial activation in females in the hippocampus and the basal elevation in the number of microglia in the PVN, two highly connected limbic areas, need to be further studied concerning mood disorder prevalence in females. Furthermore, sex differences in circulating hormones, CRF levels, and HPA axis activation may also influence the connections to other limbic areas.[90] Evidence for increased anxiety-like behaviors in females and a possible sex-dimorphic biological mechanism in PVN and limbic connections may explain the increased prevalence of mood disorders in women.

Psychological prenatal stress (CVS or RS)

Exposure to either psychological or physiological stressors activates the stress response. This includes activating the HPA axis and increasing hypothalamic corticotropin-releasing factor (CRF) secretion. Normally, CRF levels continuously rise during pregnancy and peak by late gestation.[9,91] However, CRF-binding protein (CRF-BP) keeps CRF from overactivating the downstream processes of the HPA axis, such as cortisol production. By the last trimester, CRF-BP levels decrease, allowing CRF in the circulation to mediate the secretion of ACTH and then increase circulating cortisol, a fundamental process for healthy fetus development. Fetal exposure to cortisol is further regulated by the expression of both placental and fetal 11 beta hydroxysteroid dehydrogenase type 2 (11b-HSD2, the enzyme that converts active cortisol to its inactive form), which is linked to emotional and cognitive disorders.[92] The elevated CRF levels make pregnancy a vulnerable period for the mother and the fetus, especially in the third trimester. Pregnant women are more vulnerable to mood disorders such as depression and anxiety, with a prevalence of 8%–13%. Prenatal stressors can also facilitate adverse pregnancy outcomes such as preterm birth, preeclampsia, gestational diabetes, and low birth weight.[9] The latter is associated with an increased risk of ASD, attention deficits, affective and anxiety disorders, and schizophrenia.

Psychological prenatal stress mainly includes models such as CVS or RS. CVS is usually applied using various mild stressors, including wet bedding, cage tilt, bright lights, loud sounds, cage changes, crowding, elevated platforms, and more.[93,94] The effect of psychological stressors depends on the time introduced, as well as the duration, intensity, and sex of the offspring. Exposure to such paradigms induces changes in the markers of HPA axis activation, such as increases in CRF or increases in circulating glucocorticoids. In rodents, the fetal glucocorticoid receptor is expressed already at embryonic day 10.5, while fetal glucocorticoid synthesis starts around embryonic day 14.[95] During the last trimester, the fetal brain is less shielded from the maternal glucocorticoids, and thus, the fetus is more susceptible to the downstream effects of glucocorticoid activation.[96] Maternal glucocorticoids, secreted following exposure to stress, can cross the placental barrier and induce epigenetic changes that modulate glucocorticoid sensitivity[91] and program the fetus to better sense danger on the outside.[97] Additionally, maternal stress lowers the placental

expression of 11 beta-HSD2 enzyme. This results in greater fetal exposure to active cortisol, further increasing the risk for proinflammatory processes in the fetus and psychiatric disorders.[91]

Mueller and Bale (2007)[93] and Bronson et al. (2014)[98] used an early gestational model of CVS between days E1 and E7. The adult male offspring, unlike the females, showed deficits in learning and memory tasks. In addition, males had depressive-like symptoms in the forced swim test and the sucrose preference test. Males also exhibited increased locomotor activity but no changes in sensorimotor gating or spatial learning. Since this CVS protocol was administered to mice at a stage of development before microglial colonization (occurring at E7.5 in mice), it may support the contribution of the microglia to the impaired performance in these tests as seen after MIA and microglial activation. Male mice also had altered levels of serotonin transporters in the CA1 and the CA3, and epigenetic changes in *Crf* and *Nr3c1*, the gene coding for the glucocorticoid receptor. Placentas from embryos extracted at E12.5 had increased placental proapoptotic factors, while proinflammatory markers were upregulated in male embryos only. When mice were exposed to prenatal CVS in the last week of gestation (between E14 and E21), spatial memory was impaired in males, consistently with cognitive deficits seen at the same time points in MIA models.

Prenatal stress administered midgestation (E10-E17) in the form of 2 h of daily RS led to an increase in Iba-1 expression, specifically in the male PFC, and reduced social interaction with novel mice. These alterations were accompanied by changes in serotonin metabolism and oxytocin receptor expression levels.[99] Importantly, the oxytocin receptor levels have been associated with social and behavioral impairments in ASD.[100,101]

Acute rather than chronic prenatal RS for 45 min did not increase Iba-1 immunoreactivity in a one-hit model. However, when an immune challenge of LPS was administered at 4 months, the double-exposed male offspring had higher Iba-1 immunoreactivity and increased numbers of activated microglia. This increased microglial activity with more processes was only seen when exposed to LPS in adulthood, suggesting a priming effect of prenatal stress when exposed to an adulthood immune challenge.[80]

Rat offspring of mothers exposed to CVS from E14 had increases in the microglial marker Iba-1 and immune markers, including CD40, in the hippocampus and PFC. C1q was also increased as part of the microglial-neuron pruning activation mechanism in adulthood. Those offspring exposed to prenatal stress also had higher levels of nitric oxide, in addition to increased levels of proinflammatory cytokines, as well as chemokines and chemokine receptors. The offspring also exhibited changes in neurotrophic factors, like BDNF and IGF-1.[102]

Female offspring exposed to prenatal RS during the third week of gestation, E14-E21, three times a day for 45 min showed increased latency to enter the center of the open field.[103] In addition, they had a reduction in benzodiazepine- and GABA-mediated receptor binding in the hippocampus and amygdala.[103,104] Grigoryan and Segal (2013) showed that a combination of RS, forced swim test, and platform exposure during days E14-E21 of gestation led to increased dendritic arborization, a sign of microglial activation, as well as an increase in GAD-positive neurons.[105] When prenatal RS overlapped between early and late gestations (begun at E7 and continued until E19), it mediated an increase in locomotor activity consistent with early gestation CVS of Mueller and Bale[93] and a reduced social interaction as seen in MIA after microglial activation. Changes in GABA circuitry can also be related to microglial activation and disruption of interneuron migration, which occurs during this later period of gestation and is responsible for changes in the excitatory-inhibitory balance due to microglial activation impacting glutamate accumulation.[14,49]

When pregnant mice were exposed to daily RS, the offspring had a reduction in intermediate progenitor cells by E13.5 and reduced neuronal production by E15.5. Interestingly, once the stressor had stopped and the offspring were born by P14, there was a recovery of neuronal production, but still a predisposition to depression- and anxiety-like behaviors in adulthood and high beta-diversity in the microbiome were observed in prenatally stressed offspring.[106]

A unique model mimicking maternal chronic stress used an inducible model of CRF release into the ventricles, specifically in the third trimester.[94,107] While immune and microglial markers were not measured in these studies, we assume microglial activation occurred due to the timing and type of prenatal exposure. PTSD-like behaviors were assessed based on a battery of behavioral testing related to anxiety-like behaviors.[108] In this study, male and female mice showed increased anxiety-like behaviors following the second stressor in adulthood. Males showed higher PTSD-like behaviors after exposure to traumatic stress and significant downregulation of glucocorticoid leucine zipper (GILZ), after maternal CRF overexpression in the third trimester alone. *GILZ* mRNA was uniquely downregulated after the third-trimester prenatal exposure and then even more dramatically reduced after traumatic stress in adulthood, especially in the subset that developed PTSD-like behaviors.[107] GILZ sits at a nexus between stress and immune pathway activation. It is activated by glucocorticoids and causes a proinflammatory downstream cascade.[109,110] Females prenatally exposed to CRF in the third trimester were more susceptible to eating disorders, specifically binge eating in adulthood with accompanying changes in hypothalamic POMC (measured after exposure to a

restricted eating protocol in adulthood). Interestingly, the ciliary neurotrophic factor is a known cytokine modulator of POMC in the arcuate nucleus of the hypothalamus, a region known for feeding behavior, suggesting a possible microglial interference in the hypothalamus of females exposed to maternal stress.[111]

Chronic environmental exposures: Focus on Western diet and air pollution

Chronic exposure to an unhealthy environment, such as an unbalanced diet or polluted air, is perhaps not referred to as a classical stressor per se but is not less harmful. These lasting environmental insults may be present long before conception. Both obesity and air pollution-associated pathologies are public health concerns with epidemic dimensions. Both conditions induce inflammation and affect the exposed individuals chronically.[112] A pregnant woman may be exposed to both, especially in the Western world. Therefore, these exposures during pregnancy may also affect the future generation. We will present data suggesting that these diverse environmental factors converge onto similar pathways, leading to microglial activation and affecting the offspring's developmental trajectory.

Importantly, paternal consumption of an unbalanced diet, or exposure to air pollution, may also impact the offspring's health through several mechanisms, including altered microRNA sperm profile[113,114] and sperm methylation profile, but these are beyond the scope of this chapter.

Air pollution

The influence of breathing polluted air goes far beyond the inhaling by the lungs and affects many body organs and systems.[115] Early pregnancy exposure to particulate matter (PM) was associated with increased methylation of placental 11b-HSD2 gene,[116] and maternal exposure to PM2.5, especially in the third trimester, is associated with higher odds of a child having ASD.[117]

Studies in mice prenatally exposed to diesel exhaust particles found that E18 embryos had a higher expression of TLR4, mainly in microglia. In addition, they had an increased cortical volume, which switches to a decreased volume by 1 month of age, specifically in males.[118] Adding a second challenge, namely, limited bedding from E14-E17 to the diesel exhaust particles exposure throughout gestation, activates the maternal immune system with elevated IL-6, IL-17A, TNF-α, and IL-12/IL-23p40,[119] a profile resembling MIA due to a viral infection. While at P8, offspring of both sexes weighed less and emitted more ultrasonic calls, several microglial-enriched genes were differentially expressed in the prefrontal cortex of both sexes, and only males had reduced expression of genes involved in synaptic structure and function. In the anterior cingulate cortex, males exhibited an overgrowth of thalamocortical synapses, which subsided with rapid synapse loss by P15, producing a net result of reduced connections. At P30, only males exhibited reduced social preference.[119] These studies may hint toward the underlying mechanisms for increased risk for ASD children of women that reside in the highest quartile of traffic-related air pollution during pregnancy.[120]

Unbalanced diet

One of the contributing factors to the obesity pandemic is the consumption of the Western diet (WD), namely palatable, calorie-dense, often ultraprocessed foods, and the reduced intake of healthy foods such as vegetables and legumes. The outcome is the consumption of foods with a proinflammatory index.[121] Chronic consumption of a Western diet mediates weight gain, pathological changes in energy metabolism, and immune system overactivation. Western diet consumption during pregnancy is associated with outcomes of ASD,[122] attention-deficit hyperactivity disorder (ADHD),[123] depression, and anxiety.[124] Additionally, severe maternal obesity strongly predicts increased neuropsychiatric problems in early childhood.[125] The outcome may be driven by dietary components, maternal obesity, or both.

Accumulating preclinical data show a central inflammatory state in the embryo due to unbalanced maternal diets such as Western, high-fat, or low-protein diets. Different dietary compositions and ingredient sources can lead to different outcomes.[126] For example, fat provides 40%–60% of the kilocalories in both high-fat and Western diets. However, the Western diet is also rich in sugar (sucrose or fructose), which adds harmful effects to the equation and, thus, more severely affects the offspring. Although exposure to an unbalanced diet is usually prolonged, it is important to consider that even a short exposure to an unbalanced diet during a distinct developmental phase may be sufficient to predispose the offspring to certain disorders. For example, postnatal overnutrition by maternal consumption of a high-fat diet during lactation predisposes offspring to metabolic disorders by impairing the projections from POMC to PVN preautonomic neurons.[127] In rodents, this finding is supported by other models of postnatal overnutrition, such as litter size adaptation.[128]

Mouse dams received a Western diet starting 5 weeks before conception and additional stress from E13 until the end of the lactation period. In the hippocampus of P21 offspring, there was an increase in microglial number, with higher microglial TLR9 expression and reduced neurogenesis.[129] In a similar setup, a higher number of hippocampal-activated microglia were seen with elevated IL-1β and TNF-α. In this case, the inflammatory

state was observed selectively in females, which showed a behavioral phenotype of an increased anxiety-like behavior and decreased sociability. It is unclear if the inflammatory state was present at birth or developed through lactation. Nevertheless, dietary intervention (switching to a regular diet) during lactation partially rescued this phenotype.[130] The option of dietary intervention during lactation is an easily translatable and efficacious intervention to offset some of the consequences of prenatal exposure.

Placental macrophages are intriguing mediators of the influence of HFD. In mice, there is a high correlation between fetal microglial activation and placental macrophages in both sexes. However, when tested ex vivo, male-originated macrophage and microglial cells have a higher TNF-α production in response to LPS.[131]

Notably, microglial activation in the offspring is not limited to a Western-style or a high-fat diet[132] and was also observed in rats born to females fed a low-protein diet.[133] These conditions can collectively be referred to as "suboptimal diet" due to maternal diet composition or large litter size. Food availability to the mother was not manipulated to prevent additional confounding stress.

Studies in pigs and monkeys also emphasize the importance of the maternal diet throughout gestation. In Yucatan minipig, sows consumed a Western diet from 4 weeks following fertilization until weaning. At 3 months of age, the piglets exposed to the maternal diet had more activated microglia, assessed by Iba-1 expression, in the prefrontal cortex but not in the hippocampus. However, hippocampal microglia had a decreased number of extensions, supporting an activated state.[134] Moreover, hippocampal neurogenesis was altered.[134] Also, in this case, it is unclear if the phenotype was present already at the prenatal stage or if it developed postnatally through lactation. Data from nonhuman primates demonstrated that maternal consumption of a Western diet before and during pregnancy leads to an inflammatory state in early third trimester embryos with activation of the fetus's hypothalamic microglia.[135] The immune phenotype included increased hypothalamic levels of IL-1β and IL-1 receptor type 1 and activated microglia markers, Iba-1.[135] The presence of active microglia in the fetal brain implies that the offspring is born with its brain already primed toward a proinflammatory response. In addition, prenatal exposure to the WD led to an altered serotonergic system with increased expression in rostral Raphe's TPH2 (the rate-limiting step in 5-HT synthesis) and 5-HT1AR (an inhibitory autoreceptor). At 4 months of age, female offspring (while on their mother's diet) exhibited increased anxiety-like behavior.[136] At 6 months of age, offspring had an increased idiosyncratic behavior and overall reduced social engagement. The offspring initiated less affiliative social behaviors and proximity to a peer without detectable sex differences.[137] The behavior was related to maternal adiposity and leptin alterations in the third trimester.[137] At 11 months, males and females reared on either Western or control diets, made fewer social approaches, and had increased anxiety-like behavior, further pointing to the importance of in utero programming effects.[138] At 13 months, CSF 5-HT levels were lower in monkeys exposed to a high-fat diet in utero.[136] The maternal diet affected *pomc* gene expression,[135] which may affect both energy balance and affective behavior due to its key roles in energy homeostasis and HPA function when spliced to alpha MSH and ACTH, respectively. Thus, the melanocortinergic system could be one of the mechanisms leading to the anxious phenotype.

The relationship between maternal adiposity, maternal diet, and offspring outcome is complex and may vary between brain regions. For example, when female monkeys consuming a Western diet for 4 years were reversed to consuming a control diet before pregnancy, the central proinflammatory phenotype and amygdala microglial activation were not observed in the offspring.[130] However, maternal diet and prepregnant adiposity but not maternal proinflammatory cytokines predicted offspring amygdala microglia count at 13 months.[112] This suggests that maternal diet and adiposity directly predict offspring amygdala microglial counts, whereas offspring peripheral inflammatory outcomes are influenced by the maternal inflammatory state due to adiposity.

In summary, maternal preconception obesity and an unbalanced maternal diet through pregnancy and lactation, regardless of the obese state, act as a prenatal/perinatal insult that influences the offspring's immune phenotype and behavior.[115]

Summary

In this chapter, we aim to examine the prenatal stress literature from a new perspective by putting the timing into proper context. The context, and not the stressor, is the underlying theme in this chapter: to investigate the context of parallel developmental processes at the time of stress exposure (see the schema, Fig. 1). One needs to consider the developmental stage, the immune state, the excitatory-inhibitory balance, and the sex-dependent trajectory over time on top of the stress intensity and duration. By looking at the stage in which these processes are at that specific moment of exposure, a pattern begins to emerge.

By examining multiple types of stressors, this chapter aims to see a common thread causing similar perturbations based on the context and not necessarily on the stressor. Maternal immune activation at early time points, E9-E14, has more dramatic effects on the brain's structural organization, changes in microglial

structure and function, and priming effects in adolescence and adulthood, either without or with another immune challenge, especially in males. Behaviorally, there are social novelty impairments and alterations in sensorimotor gating and latent inhibition in addition to anxiety-like behaviors suggesting altered synaptic structure affecting crucial sensory and motor signaling processing. This early MIA demonstrates the impact of the stage of development on the outcomes and neurodevelopmental trajectories of the offspring. As a result, early MIA may well serve as a model for ASD and schizophrenia. In addition, basal sex differences in microglia and responses to immune activation between sexes may also predispose to specific behavioral outcomes. Still, the effect of sex cannot be separated by the timing of the exposures from what is currently known.

Maternal exposure to psychological stress or environmental insults during gestation is not so different regarding the outcome. However, these stressors have not been evaluated side by side yet. Changes contributing to prenatal psychological stress can be explained once microglial activation is put into context. Changes in dendritic arborization result from stressors, specifically in the second part of gestation. The literature has barely scratched the surface understanding of a common mechanism contributing to these changes in dendritic arborization, but much evidence points to the microglia. In the context of both the microglia and the developmental stage in which the stressor occurs, a prediction about the outcome can be made. Social and behavioral tests and biological markers have just started to be examined as readouts in the context of prenatal psychological stressors. Importantly, the conclusions we can reach are limited to the capabilities of the tools we use. Classical methods used to measure mice behavior include short, controlled tests with limited social interaction that can be used together with new ethological methods for exploring complex social behaviors[139] and will increase the translational power of the models we discussed. It is plausible that the type of psychological stressor does matter, as well as the intensity of psychological stress. Robust changes induced by microglia would not be seen if microglial activation by cytokines did not occur in response to psychological stress. This is also similar if the dose of MIA was insufficient to cause cytokine activation. In contrast, maternal obesity alone is generally sufficiently robust to induce hyperactivity in offspring, a phenotype not ameliorated by dietary interventions.[126]

Similarly, lifestyle changes present a different challenge: timing is not specific but continuous and long term. The vulnerable periods at the end of the first trimester and early second trimester of male embryos may remain if the lifestyle exposure overlaps with this critical window or if there is a change in intensity such as degradation in diet quality in a certain developmental window or accumulation of environmental toxins. Sex-specific differences concerning these maternal lifestyle exposures also still need to be examined more in depth.

In modern-day life, we cannot avoid all possible stressful and immunological exposure during pregnancy, except perhaps for our dietary choices. Perinatal diet emerges as a critical modifiable variable that can influence the offspring's developmental trajectory and thus the behavioral and transcriptional response to physiological and psychological challenges.

Importantly, we need to acknowledge that birth is not a fixed starting point. Thus, we can try optimizing postnatal development to compensate for prenatal insults and support further brain development. In this context, we highlight the importance of the gut microbiota of the offspring as one of the primary postnatal mediators of microglial function. The mode of birth, the type of milk provided, the consequent food choices, and antibiotic administration may set the offspring's developmental trajectory. For example, it was elegantly shown that postnatal administration of *Lactobacillus reuteri*, or its metabolite BH4, rescues social deficits (assessed by a three-chamber test) in environmental, genetic, and idiopathic ASD models.[140]

The gut microbiome's role in shaping the offspring's immune function in general and, specifically, the microglial function can provide an intervention aiming to support a beneficial postnatal microglial function. Human data indicating modes of action that support healthy immune system maturation are accumulating. For example, administration of *Bifidobacterium infantis*, a strain that expresses all human-milk oligosaccharide genes,[141] daily probiotic treatment for three months,[142] or a single swab with a maternal vaginal gauze immediately after birth,[143] restores microbiota imbalance in Cesarean-born babies or ones who received antibiotics. Hopefully, these findings will be translated soon into concrete recommendations.

The evidence presented in this chapter shows that there are some burning questions to be addressed related to the timing of different prenatal exposures to bacteria, viruses, lifestyle, or stress and behavioral outcomes. In this context, the COVID-19 pandemic that the world has been facing since 2019 is a major concern. Even mild or asymptomatic COVID-19 during gestation influences the maternal-fetal interface and may tilt the equilibrium of decidual immune cells toward a proinflammatory state. Changes include attenuated antigen presentation and type I interferon signaling in decidual macrophages.[144] In fact, preliminary evidence suggests that maternal SARS-CoV-2 infection, especially during the third trimester, is associated with a 2.17 higher odds ratio for a neurodevelopmental disorder diagnosis in the first year.[145]

Like the brain connectome, we highlight the need for an "outcome-ome," a resource that integrates the knowledge of the various exposures and their consequences. The outcome-ome should include the prenatally exposed models we discussed here and other more ethological models such as maternal SARS-CoV-2 infection, maternal autoimmunity, and prenatal exposure to SSRIs, plastic, metals, pesticides, and other contaminants in the food supply.

Understanding the risks associated with timing of stress and other adverse exposures, sex, specific exposures, may help to prevent the negative impact on future generations.

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