STATE-OF-THE-ART REVIEW

Neurological consequences of neurovascular unit and brain vasculature damages: potential risks for pregnancy infections and COVID-19-babies

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Intragravidic and perinatal infections, acting through either direct viral effect or immune-mediated responses, are recognized causes of liability for neurodevelopmental disorders in the progeny. The large amounts of epidemiological data and the wealth of information deriving from animal models of gestational infections have contributed to delineate, in the last years, possible underpinning mechanisms for this phenomenon, including defects in neuronal migration, impaired spine and synaptic development, and altered activation of microglia. Recently, dysfunctions of the neurovascular unit and anomalies of the brain vasculature have unexpectedly emerged as potential causes at the origin of behavioral abnormalities and psychiatric disorders consequent to prenatal and perinatal infections. This review aims to discuss the up-to-date literature evidence pointing to the neurovascular unit and brain vasculature damages as the etiological mechanisms in neurodevelopmental syndromes. We focus on the inflammatory events consequent to intragravidic viral infections as well as on the direct viral effects as the potential primary triggers. These authors hope that a timely review of the literature will help to envision promising research directions, also relevant for the present and future COVID-19 longitudinal studies.

Introduction

Gestational stress can be produced by social and psychological adversities as well as by environmental factors, which primarily include prenatal infections. While the latter can directly cause a congenital fetal infection, all the above conditions pose a threat to the health of both the mother and the fetus through the action of immune factors. Long-term consequences of gestational stress on offspring's mental health have been studied for almost 80 years now. The hongerwinter, the Hollands famine winter that took place right after WWII, represents the first documented testimony of how maternal prenatal stress has an impact on the

occurrence of psychiatric disorders in the progeny [1,2]. Moreover, in the following years, several authors noted a link between the increased risk of neuropsychiatric disorders and winter-born babies, thus raising the attention on intragravidic infections as the underlying cause [3–5]. Nowadays, maternal influenza infection is one of the most well-replicated risk factors for schizophrenia—increasing the odds ratio by 3 [5]—and substantial epidemiological evidence exists to sustain a causal link for prenatal and perinatal infections to increase, later in life in the progeny, the susceptibility to neurodevelopmental disorders such as autism spectrum disorder (ASD) [6–8] and bipolar disorder [9,10], in addition to schizophrenia [11–13].

Recent studies have highlighted the activation of the immune system as a risk-shared mechanism in gestational stress-induced long-term newborn outcomes. Evidence span from findings of increased C-reactive protein, interleukin (IL)-6 plasma levels [14], and enhanced expression of various cytokines at the umbilical cord in the offspring of psychologically and socially stressed mothers [15] to the observations collected in translational animal models, collectively called maternal immune activation (MIA) models [16]. Together, these findings indicate that the physiological role of cytokines and immune factors in the government of brain development is likely to be disrupted upon stress and maternal immune responses. MIA models, mostly represented by rodents exposed to Toll-like receptors' agonists (mainly lipopolysaccharide —LPS and polyinosinic:polycytidylic acid—PolyI:C) during the gestational periods to mimic an inflammatory response to a pathological agent, are crucial to shed light on the underpinning molecular mechanisms which link the maternal–prenatal stress with the increased risk of psychiatric disorders in the progeny [17,18]. Of note, MIA has proved the non-necessity of congenital infections, of which there is limited evidence for influenza virus infections [19]. The latter are mostly due to the so-called 'TORCH' pathogens, a cluster of microbes that include Toxoplasma gondii, rubella virus, cytomegalovirus (CMV), and herpes simplex virus (HSV), known for their different ability to directly infect the fetus during gestation [20,21].

Potentially dangerous viruses have a distinctive spot in the world of microbes. They are high in number [22] and, differently from bacteria, have been associated with a twofold increased risk of adult diagnosis for nonaffective psychosis and schizophrenia [23]. In recent years, several viral outbreaks have affected large areas of the world. In < 20 years we have faced the coronavirus SARS-CoV (2002/2003)—named after the severe acute respiratory syndrome (SARS) that it

causes, the 'swine flu' caused by the influenza virus H1N1 (2009), the MERS-CoV (2012)—another coronavirus that prevalently spread in the Middle East area, the mosquito-borne Zika virus (ZIKV) (2015) and since December 2019, the worst global pandemic since 1918 'Spanish' influenza, caused again by another coronavirus: the SARS-coronavirus-2 (CoV-2).

Till now, 88 880 pregnant women have been found positive to COVID-19 just in the United States (CDC's data). At present, data are mostly available from third-trimester infections and there is a growing list of associated neonatal complications [24,25]. Major aspects, including the timing of the infection and the presence/absence of symptoms in the mothers-to-be, are being collected and will be evaluated. Still, marginal attention has been paid to the fetus as a patient during the pandemic and is too soon to understand the long-term effects of SARS-CoV-2 infection on progeny's mental and neurological disability. The average onset for schizophrenia coincides with adolescence-early twenties [26,27] while the diagnosis among children with ASD comes around 3.1 years of age [28]. In the context of gestational COVID-19 infections, if and how the virus can influence progeny neural system development will emerge in the next years.

Presently, we possess an extensive quantity of data on viral intragravidic infections and numerous MIA models. These data are being exploited to unveil the mechanisms which link the activation of the maternal immune system with the behavioral abnormalities in the offspring. These aspects, which are still not clarified, are of crucial relevance also for predicting the possible impact of the current pandemics in the neurodevelopmental trajectories of the next generation. Different processes have been brought into play to explain the effect of MIA on the offspring neurodevelopment, including (but not limited to) the altered expression of genes involved in neuronal migration [29] and the generation of an abnormal cortical phenotype [30], changes in the intrinsic excitability of neurons [18,31], impaired spine and synaptic development [32–34], and altered activation of microglia [35,36]. Notably, disturbances of the neurovascular unit (NVU) physiology and brain vasculature morphology have been identified as one of the leading causes of several neurological diseases, both in developing and aged brain. Moreover, multiple evidences indicate a key involvement of the vasculature in SARS-CoV-2 pathological manifestations [37]. The spike protein has recently been proved to alter the blood–brain barrier (BBB) function and integrity in an *in vitro* model with human brain microvascular endothelial cells (ECs) derived from fetal brains [38]. This review seeks to
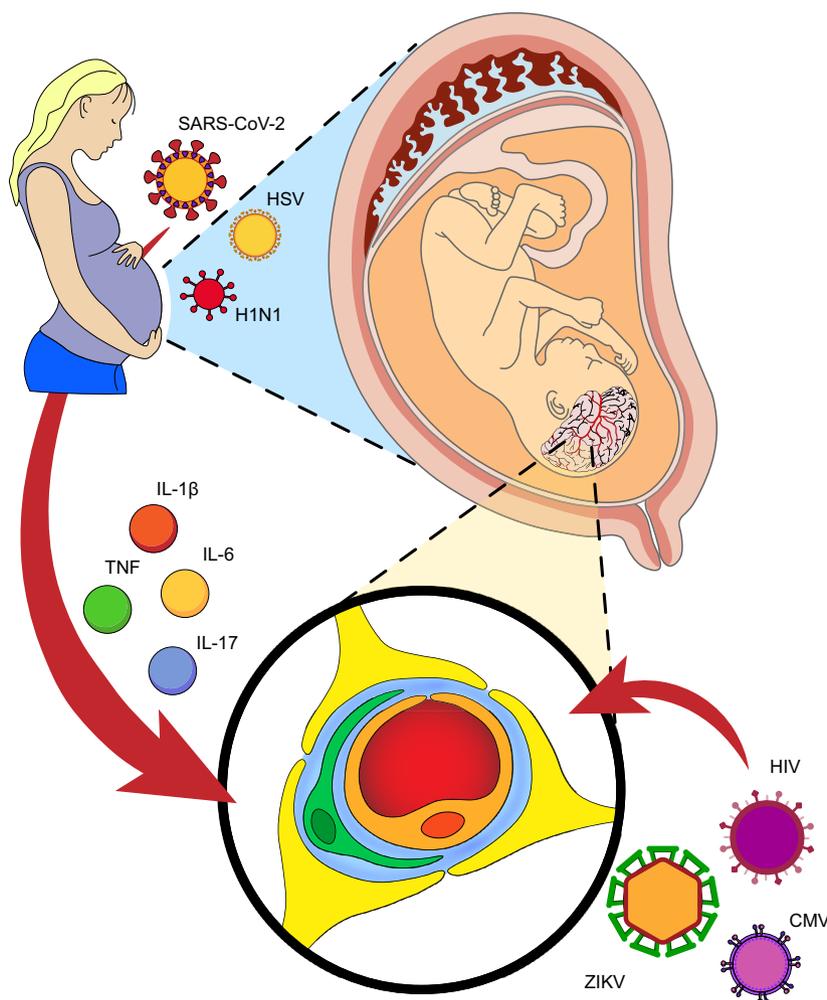
Fig. 1. Schematic representation of how viral infections affect the fetal NVU and the brain vasculature during pregnancy. Viruses can influence vessel development by both direct (i.e., congenital infection) and immune-mediated (i.e., inflammatory state) processes.

discuss state-of-art evidence indicating that NVU and brain vasculature damages may represent the etiological mechanism in neurodevelopmental syndromes. We specifically focus on inflammation, deriving from intragravidic viral infections, as well as on direct viral effects on the embryo, as potential primary triggers (Fig. 1). These authors hope that a timely review of the literature will help to envision research promising directions, also relevant for the present and future COVID-19 longitudinal studies.

Neurovascular unit dysfunctions result in pathological behavioral phenotypes

The NVU is a relatively recent concept that encloses highly dynamic and interactive cell types at the interface of brain parenchyma with the peripheral blood. Including both cellular and acellular components wrapped around the ECs that form the vasculature of the brain [39], the NVU controls the trafficking of blood-borne molecules and cells for homeostasis conservancy. The NVU starts to be formed early during brain development. Vascularization is then rapidly followed by the expression of tight junction proteins and transporters [40], allowing the brain protection from vasogenic edema, the passage of toxic substances, and microbe's invasion [41,42]. NVU development is a step-by-step process in which temporal and regional differences play a critical role in the functioning of the mature adult brain [43].

Many reviews have been published about NVU impairments and neurovascular anomalies in association with neurodevelopmental disorders [44–47]. BBB breakdown is considered an early marker for later cognitive dysfunctions [48]; further, neurovascular problems due to perinatal brain injuries—including inflammation, hypoxic-ischemia, or hemorrhagic events—are recognized as one of the leading causes of long-term mental disabilities [49–51]. Blood oxygen

level–dependent fMRI in ASD patients shows a reduced signal that correlates with symptoms severity, possibly indicating a faulty neurovascular coupling process [52]. Consistently, persistent angiogenesis (i.e., the growth of new vessels from the preexisting vasculature) has been recently observed in the brain of postmortem ASD patients [53], while several neurodevelopmental disorders, including autism [54], schizophrenia, depression [55], cerebral palsy [56], and bipolar disorder [57], have been epidemiologically associated with prenatal cerebral bleeding.

Here, we will analyze the direct impact of brain vasculature and NVU dysfunctions on behavioral disorders phenocopying, focusing on the lines of evidence that directly imply NVU/vasculature early damages as etiopathological in autism, schizophrenia, and depression (Fig. 2 and Table 1).

The case of pericytes

Pericytes, differently from astrocytes [58], are recruited by the ECs of the brain early during development, specifically by E10 in mice [59–61] and by the 8–10th week of gestation in humans [62–64]. Pericytes promote vascular structural stability and protect the brain from blood-borne elements and bleeding phenomena [59]. Anomalies involving pericytes biology have been proven to play key roles in the etiopathogenesis of different genetic diseases characterized by psychiatric comorbidities, including the Hereditary Hemorrhagic Telangiectasia [65,66], a vascular dysplasia condition affecting one in 5000 people worldwide, and the cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy [67,68], the most commonly inherited small-vessel disease. Furthermore, pericytes paucity has been recently found associated with intraventricular hemorrhage of the germinal matrix (GH-IVH), both in premature humans and in a rabbit premature pup model [69]. GH-IVH affects 12 000 premature babies every year just in the United States [70] and nearly 45% of all extremely premature infants [71], leading to seizures, mental retardation, cerebral palsy, and death [70,72]. Early treatments with angiogenesis inhibitors have been found to ameliorate pericytes coverage [69] and decrease bleeding incidence in a premature animal model [73].

Among pericytes paucity models, the Pdgfrβ$^{F7/F7}$ which bear homozygous F7 hypomorphic mutations of Pdgfrβ, and the Pdgfrβ$^{+/-}$, heterozygous null Pdgfrβ mice, are well-characterized models which show a leaky NVU [59,74,75]. In details, the Pdgfrβ$^{F7/F7}$ mice display a progressive functional deficit of the white matter, manifesting as impaired spatial working memory and reduced maximum velocity on the complex running test [74]. Only later in life (i.e., 36–48 weeks), Pdgfrβ$^{F7/F7}$ mice display hippocampus-dependent behavioral defects [74]. On the other hand, the Pdgfrβ$^{+/-}$ mice show a 20% loss in pericyte coverage by 1 month of age, which is sufficient to initiate progressive vascular damage [75]. By the age of 6–8 months, also *Pdgfrβ*$^{+/-}$ mice show hippocampal-dependent impairments in learning and spatial memory, although age-matched Pdgfrβ$^{F7/F7}$ mutants have worse performance [75]. The Pdgfrβ$^{F7/F7}$ and the Pdgfrβ$^{+/-}$ mice are considered good models of vascular age-dependent degenerative diseases resulting in neurodegeneration, memory, and learning impairments as Alzheimer's disease [76–78] and mild dementia [79]. Both Pdgfrβ$^{+/-}$ and Pdgfrβ$^{F7/F7}$ mice show a significant reduction in pericytes (i.e., 35–75%) in the neural tube already at E14.5 [80]. At present, to the best of our knowledge, no literature data describe the behavioral phenotype resulting from a pericyte loss restricted to the embryonic time window. Therefore, whether pericyte loss during fetal development contributes to the observed behavioral consequences is presently unknown. Recently, Cuervo *et al.* [81] developed a new tamoxifen-inducible Cre-model targeting pericyte, called PDGFRβ-P2A-CreER^{T2}, which will likely offer the opportunity for more detailed studies on this matter in the future. Of note, animal models of GH-IVH, as the collagenase-induced hemorrhage neonatal rat model, display a delay in motor abilities development and cognitive deficits [82].

Autism spectrum disorder

Recent studies have investigated the vascular contribution to ASD using autism mouse models (Fig. 2 and Table 1). This is the case for the 16p11.2 microdeletion [83], which approximately involves 2% of all ASD diagnosed cases [84] leading prevalently to large head size, intellectual disabilities, and language-related problems in humans [85]. The 16p11.2$^{df/+}$ mouse model shows alterations of the basal ganglia and synaptic defects beyond behavioral abnormalities that include stereotypic movements and hyperactivity [86]. Ouellette *et al.* [83] demonstrated that 16p11.2$^{df/+}$ adult males are affected by a form of endothelium-dependent neurovascular uncoupling. In particular, they evidenced in postnatal day 50 (P50) females and males, the occurrence of a reduced vessel relaxation in response to acetylcholine or adenosine, and a poor vessel constriction in response to nitric endothelial oxide synthase inhibition. Furthermore, isolated 16p11.2$^{df/+}$ ECs harvested at P14—but not at P50—exhibited a scant

Pdgfrβ[F7/F7] mice (Montagne *et al.* 2018)

Pdgfrβ[F7/F7] and Pdgfrβ^{+/−} mice (Bell *et al.* 2010)

Pericyte paucity

Astrocyte endfeet

Basal lamina

Pericyte

Hemorrhage

LPA and serum ICV
(Mirendil *et al.* 2015)

BCAA absence

LAT1

LUMEN

LAT1

Endothelial cell

Claudin-5 knockdown

AVV-shRNA-cldn5 mice
(Menard et al. 2017; Green *et al.* 2017)

Inducible claudin-5 knockdown (Green *et al.* 2017)

16p11.2[df/+] mouse model (Ouellette *et al.* 2020)

Cdh5-Cre[tg/+] and 16p11.2[flox/+] mouse model
(Ouellette *et al.* 2020)

Tie2-Cre; Slc7a5^{fl/fl} mouse model
(Tărlumgeanu *et al.* 2016)

Fig. 2. Research tools for the study of behavioral phenotypes consequent to the NVU dysfunctions. Pericyte paucity and reduced vascular coverage, hemorrhages, downregulation of the tight junction protein claudin-5, and lack of BCAAs into the brain parenchyma result in ASD- and schizophrenia-like behaviors, as well as depression and dementia.

Table 1. Animal models of NVU dysfunctions, resulting in behavioral phenotypes. A, anxiety-related; D, depression-related; LC, locomotor competence; LM, learning and memory; NS, not specified; S, schizophrenia-related; SL, sensory/locomotor.

Mouse model	Target	Age of treatment	Vascular phenotype	Age of assessment	Analyzed behavioral category	Behavioral profile	Sex	Reference
16p11.2$^{df/+}$	All cells	Constitutive	Neurovascular uncoupling	P14, P50	A, SL, LM, LC	ASD	M	[83]
Cdh5-Cre$^{tg/+}$; 16p11.2$^{flox/+}$	ECs	constitutive	↓cortex vascular density ↓cortex vascular branching	P0, P50	A, SL, LM, LC	ASD	Both	[83]
Tie2-Cre; Slc7a5$^{fl/fl}$	ECs	Constitutive	BCAA absence	E14.5, P2-65	A, LM, LC	ASD	both	[88]
LPA and serum ICV	none	E13.5	Prenatal cerebral hemorrhage	P70	S, A, LM, LC	Schizophrenia-like	F	[93]
inducible claudin-5 knockdown	ECs	P56-84	↓claudin-5	P56-84	S, A, LM, LC, D	Schizophrenia-like	NS	[97]
AVV-shRNA-cldn5	HP, PFC	P56-84	↓claudin-5	P56-84	S, A, LM, LC, D	Schizophrenia-like/Stress resistance	NS	[97]
AVV-shRNA-cldn5	HP, NAc	P56-70	↓claudin-5	P56-70	A, D, LC	Depression	M	[103]
Pdgfrβ$^{F7/F7}$	Pericyte	Constitutive	Pericyte paucity and low vessel coverage	P14-336	A, SL, LM, LC	Dementia	Both	[74]
Pdgfrβ$^{F7/F7}$ and Pdgfrβ$^{+/−}$	Pericyte	Constitutive	Pericyte paucity and low vessel coverage	P7-112	LM	Dementia	NS	[75]

capacity of network formation compared with WT. This phenomenon was recapitulated by ECs derived from human induced pluripotent stem cells obtained from donors carrying the 16p11.2 deletion. The authors excluded a contribution of smooth muscle cells by testing vasorelaxation in response to sodium nitroprusside and phenylephrine and by replicating most of their observations using an endothelial-specific 16p11.2 (7qF3) deletion mouse model [83]. The paper establishes a solid and direct link between neurovascular abnormalities and autistic phenotypes. Of note, many of the observed defects were sex- and age-specific. Another mutation, accountable for patients affected by ASD, but also found in cases of intellectual disability or epilepsy, is located in the gene branched-chain keto-acid dehydrogenase (BCKDK) [87]. The primary consequence of the mutation is the lack of branched-chain amino acids (BCAAs), for which the brain depends on supply from the periphery. Tărlungeanu *et al.* [88] studied a mouse model that mimics BCKDK absence by the deletion of Slc7a5, a gene responsible for the encoding of the large amino acid transporter 1, at its physiological site of expression: the BBB. The deletion of Slc7a5 from ECs, using a Tie2Cre line, led to neuronal activity imbalance and behavioral abnormalities in the mice, mostly revertible in adults by a 3-week long intracerebroventricular BCAAs administration. The observed behavior phenotypes spanned from

motor coordination and locomotion difficulties to more typical autistic tracts, including reduced explorative behavior, limited social interactions, decreased play activity, and reduced amount of isolation-induced ultrasonic vocalization [88]. Interestingly, BCAAs seem to be transported into the developing brain at higher levels than in the adult one [89,90]. Tărlungeanu *et al.* demonstrated that brain BCAA homeostatic concentrations—which are regulated at the BBB level—are critical for brain functioning and need to be taken into account in the context of ADS pathology.

Schizophrenia

Perinatal brain hemorrhages are a well-recognized risk factor for schizophrenia [91,92]. Still, very few authors have directly addressed the behavioral tracts resulting from developmental intracranial hemorrhages or increased permeability of serum elements into the brain (Fig. 2 and Table 1). Mirendil *et al.* [93] developed an animal model of prenatal hemorrhages by intraventricular injections of the bioactive phospholipid lysophosphatidic acid (LPA) in the fetus. LPA *in vivo* administration is known to induce bleedings and inflammation [94,95]. Fetuses intraventricularly treated with LPA or serum at E13.5 displayed schizophrenic-like behaviors in adulthood; in particular, the authors found a female-specific prepulse inhibition (PPI) deficit and

anxiety-related exploratory patterns [93]. Prenatal cerebral bleeding or diffusion of blood derivates (i.e., serum or LPA) are sufficient to induce schizophrenia symptoms in mice; if the observed sex-specific effect was due to the developmental stage chosen for the hemorrhagic hit, remains to be addressed.

Paracellular diffusion of serum proteins at the BBB is physiologically restrained by the means of occluding junctions, which seal the adjoining ECs. Claudin-5, encoded by the chromosomal region 22q11.21 is one of the most enriched tight junction proteins at the BBB and has been implicated in many neurological disorders, including depression and schizophrenia [44]. Deletions of the chromosomal region 22q11 confer the carrying individuals (i.e., ~ 1 in 4000) with a 30-fold increase risk for schizophrenia and other neuropsychiatric conditions [96]. Green *et al.* [97] generated an inducible ECs-specific claudin-5 knockdown mouse demonstrating that the prolonged suppression of claudin-5 at the BBB produces psychosis-like behaviors. Claudin-5 knocked-down animals showed a schizophrenia-like behavioral phenotype, consisting of altered spontaneous alternated Y-maze and T-maze performances, and reduced acoustic PPI response. The paper suggests the direct implication of claudin-5 anomalies in psychiatric disorder symptomatology, a concept strengthened by the fact that antipsychotic treatments (i.e., lithium, haloperidol, and chlorpromazine), among others, induce an increased expression of claudin-5 protein both *in vivo* and on EC cultures [97].

Depression

About 50 years are passed since the first vascular hypothesis on the origin of depression was formulated [98]. Today, multiple pieces of evidence indicate increased NVU permeability in both depressed patients [45,99] and animal models of depression [100–103] (Fig. 2 and Table 1). Menard *et al.* [103] recently proved that the sole downregulation of claudin-5 in the nucleus accumbens (NAc), which allows the passage of molecules till 800 Da through the BBB [104], is sufficient to induce depression-like behaviors in mice. The authors employed an inducible AVV-shRNA against claudin-5 to assess the behavioral phenotype resulting from the region-specific downregulation of the gene. Intra-NAc injections followed by doxycycline treatment effectively inhibited claudin-5 expression and resulted in stress-triggered depression-like behaviors. Specifically, mice subjected to a subthreshold micro-defeat routine showed anhedonia and significantly reduced their performances in the forced swim test [103]. Furthermore, mice displayed social avoidance,

compared with unstressed control and unstressed AAV-shRNA mice. Differently, mice subjected to the virus-mediated conditional knockdown of claudin-5 in the hippocampus—another brain region that showed claudin-5 downregulation following depression induced by chronic social defeat stress (CSDS)—still spent more time immobile in the forced swim test, but displayed no anhedonia [103]. These results suggest a region-specific effect of claudin-5 expression levels on behavior (Table 1). This concept was strengthened by the fact that CSDS-induced depression does not lead to a ubiquitous claudin-5 downregulation throughout the brain. In particular, the mice prefrontal cortex (PFC) does not show variations of claudin-5 expression upon CSDS [103]; on the same line, the AVV-shRNA mediated downregulation of claudin-5 in the PFC leads to impairments of recognition memory and working spatial memory but results in enhanced performances in the forced swim test [97]. These data indicate that the PFC is resilient to stress-induced NVU breakdown and that the experimental downregulation of claudin-5 in this region results in memory deficits accompanied by stress resistance. As a proof of concept for the relevance of claudin-5 homeostasis in brain physiology [44], only a chronic treatment with the antidepressant imipramine, known to reverse CSDS-induced depression-like behaviors [105], was able to normalize claudin-5 mRNA levels and behavioral phenotypes in the NAs-AVV-shRNA-cldn5 mice, while an acute administration of the same drug did not affect either [103].

Do viral infections cause neurovascular unit dysfunctions linked to neurodevelopmental diseases?

The above data support the concept that alterations of the NVU result in pathological behavioral phenotypes. Consistently, NVU defects are detectable in several neurodevelopmental diseases. In the next section, we will analyze the results of studies investigating the direct effects of the mother-to-embryo vertical viral infection and the indirect effect caused by inflammation consequent to prenatal infections.

Direct effects of congenital infections on the NVU and the brain vasculature

During pregnancy, viruses can reach and infect the fetus mainly through intrapartum or in utero (i.e., ascending or transplacental) pathways [21]. Both routes result in the vertical transmission of the

infection from the mother to the child, which poses an immediate threat to the newborn's immature immune system [106]. Of particular concern are in utero infections, which can occur at any time during the pregnancy and therefore interfere with the specific time schedule of development. Different viruses and TORCH infectious agents are known to target the fetal nervous system; some possess tropism for NVU components [107,108] and can induce prenatal brain vasculopathies and hemorrhages (Table 2) [109–111]. These complications constitute a common event in premature babies which can evolve in cerebral palsy, epilepsy, cognitive deficits, and/or behavioral difficulties in the adult [112–114]. Here, we will summarize recent findings of direct-viral-effects producing brain vascular anomalies and faulty NVU establishment, in the context of intragravidic infections.

Of interest is the case of a patient infected by CMV during the first 16 weeks of gestation, an important time window for brain vasculature development, who gave birth (at 21 weeks) to a child affected by cerebral hemorrhagic infarction [115]. This report is suggestive of a direct effect of CMV on the developing vasculature; in fact, even if the most frequent outcomes of CMV congenital infections are polymicrogyria, cortical dysplasia, and neuronal migration disorders [116,117], the occurrence of neonatal brain bleedings has been reported [118]. CMV can infect ECs of the brain [108] and induce in the fetus vasculitis that can evolve and manifest as thrombosis, coagulopathy, or hemorrhage even in the absence of thrombocytopenia

[111,119,120]. Vasculopathies are also a common complication associated with human immunodeficiency virus (HIV) infections [121], for which the NVU is believed to constitute the main brain route access. HIV, through a Tat-mediated downregulation of the BBB tight junctions, has been demonstrated to induce a loose NVU and an increase *in vitro* paracellular permeability [122,123]. Of particular interest, is the case report of hemorrhagic Moyamoya syndrome in a child with congenital HIV-1 infection [110], an uncommon manifestation in pediatric age, probably due to the HIV-mediated vascular damage. A higher amount of data is available on ZIKV congenital infections, which is known to have a huge impact on the cerebrovascular structure and functional development. Zika intragravidic infections can produce ischemic infarcts in human fetuses [124], encephalomalacia due to chronic infarction [125], and brain calcification in neonates [126,127]. All these outcomes are reproduced in the offspring of ZIKV-infected pregnant monkeys, adding to frequently occurring fetal loss, small brain size, hemorrhage, necrosis, vasculitis, and apoptosis of neuro-progenitor cells [128]. Moreover, offspring display more severe cerebrovascular signs if born from female monkeys challenged with viral particles at early (i.e., 6–7 weeks) relative to late gestation (i.e., 12–14 weeks) [128]. Similar anomalies are also detectable in the offspring of murine models of congenital Zika infection. For example, intra-amniotic administration of Zika particles at E15.5 leads to intracranial calcifications in the adult progeny [129], while the direct intracerebral inoculation of the virus at E14.5 induces enlargement of the brain's vessels accompanied by higher vascular density and increased permeability [130]. Furthermore, ZIKV has been shown to infect fetal brain ECs (IB4-positive vessels) in the study of Garcez *et al.*, where an E12.5-infected type 2 interferon-deficient pregnant mice model was used. In detail, at E15.5 the progeny of the infected mice showed a reduction in the vasculature percentage area and vessel branching, while by birth (i.e., at P2), pups showed only a significant decrease in branches length [131], a phenomenon possibly due to a virus-induced delayed vascular development.

Different from the above cases, we are still experiencing a lag in information about SARS-CoV-2. The huge global effort of clinicians and researchers in the last year provided a clear description of COVID-19 acute manifestations and cerebrovascular comorbidities, but our understanding of the long-COVID neurological implications is still in its infancy. Even more complex will be the analysis of possible neurological and psychiatric sequelae due to intragravidic SARS-

Table 2. Brain vascular outcomes in vertically transmitted infections.

Virus	Species	Vascular phenotype	References
CMV	Humans	Cerebral hemorrhagic infarction	[115]
	Humans	Brain bleeding	[118]
	Humans	Fetus vasculitis	[111,119,120]
HIV	Humans	Infant hemorrhagic Moyamoya Syndrome	[110]
Zika virus	Humans	Ischemic infarcts	[124]
	Humans	Encephalomalacia	[125]
	Humans	Brain calcification	[126,127]
	Monkey	Hemorrhage and vasculitis	[128]
	Monkey	Intracranial calcification	[129]
	Monkey	Increased vascular density and increased permeability	[130]
	Mice (type 2 interferon-deficient)	Delayed vascular development	[131]

CoV-2 infections in the progeny. Gestational COVID-19 infections could impact the developing brain, with possible consequences for behavioral impairment later in life, through several mechanisms. For example, IL-6, the major cytokine elevating during SARS-CoV-2 infections [132], has been shown to boost, during intragravidic infections, the excitatory synaptogenesis in the offspring, with consequences on brain connectivity [133]. Maternal T helper 17 cells have been implicated in cortical and behavioral abnormalities in MIA offspring [30]. Also, maternal infections have been found to reduce brain offspring's potassium-chloride co-transporter 2 expression, thus delaying the excitatory-to-inhibitory GABA switch [17,18]. Nevertheless, the NVU could directly play a role: We know that SARS-CoV-2 can directly affect the brain microvascular endothelium and thus force its way to the brain parenchyma [37]. MRI and autoptic studies showed endothelial activation, loss of cerebral vascular integrity, and multifocal hemorrhage in adult COVID-19-infected patients [134], while in children cases of Kawasaki-like disease vasculitis have been reported [135]. Furthermore, *in vitro* studies recently proved that the spike protein, and even just subunits of it, is sufficient to alter the BBB integrity [38]. Current data indicate that SARS-CoV-2 vertical transmission is relatively uncommon, occurring in about 3.2% of pregnant women who contracted COVID-19, while it is over 10% for ZIKV and roughly 30% for CMV [24,25,136]. Although it is too early to draw final conclusions, current reports on newborn outcomes show no increased risk of congenital anomalies [137]; also, cases of hypoxic-ischemic encephalopathy [138] and birth asphyxia [139] seem extremely rare. Nonetheless, the background knowledge about TORCH and other respiratory viruses suggests that SARS-CoV-2 might induce permanent neurological sequelae through damage of the NVU, so further studies are needed. Besides the possible direct effect of the virus on the NVU development and structural integrity, other elements need to be considered, including the proinflammatory state induced by the infection and the high rate of premature delivery (i.e., 12.9%) correlated with COVID-19 [137] which, by themselves, are known to be risk factors for NVU later malfunctions [49,140].

Inflammatory-mediated effects of gestational infections on the NVU and the brain vasculature

Tightly regulated changes in immune cell composition and cytokine expression at the maternal–fetal interface sustain and drive pregnancy. Longitudinal analysis of peripheral blood, decidua, and amniotic fluid demonstrated that the first trimester is characterized by the establishment of a mild proinflammatory environment and accumulation of immune cells at the decidua which allow trophoblast invasion, implantation, and placenta formation [141]. On the opposite, the second and third trimesters present a shift toward an anti-inflammatory phenotype [142–146] which facilitates fetal growth and immune tolerance [141]. Lastly, parturition display a proinflammatory bias: IL-6, tumor necrosis factor-alpha (TNF-α), and IL-1 increase at term, suggesting a role for these cytokines in normal labor [147]. Despite their relevant role in physiological pregnancy, inflammatory states can also be dangerous for the developing fetus as demonstrated by the harmful effects of late-pregnant serum on brain tissue, which promotes neuronal hyperexcitability and seizures, activating microglia cells, and promoting TNF-α production [148,149]. NVU formation and maturation during in utero development act therefore as a key brain shield to avoid these dangerous effects [150].

Upon maternal immune response to infection, acute or chronic disturbances can undermine these highly regulated immune adaptations and disrupt placental and NVU barriers ultimately affecting fetal development, pregnancy outcomes, and long-term progeny neurodevelopment (Table 3). Adinolfi was the first to propose that MIA during the course of infection may be harmful to the developing brain of the unborn infant [151]. Leviton further extended this hypothesis, suggesting that cytokines, such as TNF-α, may contribute to both preterm birth and periventricular white matter damage [152]. Nowadays, several maternal-derived cytokines have been identified as critical mediators of MIA on disease-related phenotypes in the offspring, including TNF-α, IL-1β, IL-6, and IL-17a [153]. Proinflammatory cytokines and their respective receptors are expressed in the brain and on the luminal side of cerebral ECs or NVU-associated cells, making the NVU a sensory organ for neuro-immune crosstalk [154–156].

Cytokines may have access to the fetal brain either by crossing the intact NVU [157–159] or by mediating NVU alteration and breakdown [160–162]. Activation of maternal immunity also presents a risk of developing autoantibodies, which have been implicated mainly in autism (i.e., maternal autoantibody-related autism) [163], and of local immune response activations in the fetal brain, which may lead to secondary cytokines production by microglia and astrocytes and, in turn, have cell damaging properties [154,164–168].

In the case of intragravidic infections, although the viruses are usually not transmitted to the fetus during

pregnancy thanks to effective multiple defense mechanisms specialized to protect the mother and the fetus [42,44], inflammatory responses in the placenta or infection-induced systemic changes in the pregnant mother might result in more severe or prolonged disease for the mother and long-term consequences for her newborn [43]. Viral infections modeled by the usage of PolyI:C (5 or 10 mg·kg^{-1}) intraperitoneal injections in pregnant mice (GD15.5) acutely elevate IL-6 concentration in the maternal plasma, concomitantly increasing fetal brain accumulation of P-glycoprotein (P-gp) substrates, an efflux transporter that protects the brain by limiting the transfer of substrates across the BBB [169]. The resulting BBB alteration and P-gp decreased activity may expose the

developing brain to xenobiotics and environmental toxins present in the maternal circulation.

Yan *et al.* [170] demonstrated an increased permeability of the NVU to albumin just 24 h after LPS 0.1 μg·kg^{-1} intravenous injection in 133-day gestation fetal sheep, suggesting that this is the result of prostaglandin production as indicated by cyclooxygenase (COX-2) expression in the fetal brain small vessels. Similarly, injections of LPS into the uterine artery of pregnant ewes resulted in extravasation of plasma albumin into the cerebellar parenchyma of the fetus [171], suggesting that both direct and maternal exposure to endotoxin inflammatory insults can compromise the fetal NVU tightness [170,171]. Dysregulation of NVU components was also demonstrated in a

Table 3. Brain vascular outcomes in MIA models. A, anxiety-related; E, embryonic day; GD, gestational day; LC, locomotor competence; LM, learning and memory; NA, not assessed; S, schizophrenia-related.

Inflammatory Agent	Dose	Target (species)	Age of treatment	Systemic features	Age of assessment	Vascular phenotype	Behavioral profile	References
PolyI:C	5 or 10 mg·kg^{-1}	Pregnant (mice)	GD14.5 ± 1	↑ IL-6 concentration in the maternal plasma after 4 h	+4/24 h	Accumulation of P-gp substrates	NA	[169]
LPS	0.1 μg·kg^{-1}	Fetus (sheep)	GD133±1	–	+24 h	↑ albumin permeability	NA	[170]
	30–60 μg	Uterine artery (sheep)	GD134-136	–	+72 h	↑ albumin permeability	LM	[171]
	100 ng·kg^{-1} over 24 h, followed by 250 ng·kg^{-1}/ 24 h for 96 h plus boluses of 1 μg LPS at 48, 72, and 96 h	Fetus (sheep)	GD103/104	–	+10 days	↓vessel density, ↓ pericyte and astrocyte microvascular coverage	NA	[172]
	0.2–10 mg·kg^{-1}	Postnatal (rats and opossum)	P0, P2, P4, P6, P8 and adult rats; P15,P20, P35, P50, P60 and adult opossum	↑ IL-1b and TNF-α plasma levels	P20 and adults	↑ permeability adulthood, preceded by claudin-5 altered distribution	S, A	[173–175]
	0.25 mg·kg^{-1}	pregnant (rat)	GD15	↑ cytokines and reactive oxygen species in the fetal brain	+6 h, 12 h and 24 h	↓ BBB and placental barrier integrities	LM, S, LC	[177]
IL-1β	0, 0.1, 0.5, 1 μg	Pregnant (mice)	E14-E17	–	+6 h	↓ placental ECs, red blood cells clumping	NA	[176]

model of prolonged in utero inflammation, exposing 103/104-day gestation fetal sheep (approximately similar to the preterm human brain between 28 and 32 weeks) to a low-dose LPS regimen [172]. This paradigm results in a significant decreased cortical and white matter microvascular density (measured by collagen type IV morphometric analysis) and reduced pericyte microvascular coverage (detected as desmin area overlapping with the vessel basal lamina) of the ovine fetal brain. Furthermore, the authors report increased astrogliosis and decreased astrocytes' end-foot coverage of the microvasculature in the white matter, suggesting an impaired gliovascular coupling. Prolonged in utero inflammation adversely affected multiple components of cortical and white matter cerebral NVU in the preterm ovine fetus, potentially predisposing to impaired brain development.

Stolp *et al.* were the first to demonstrate that changes in blood vessel tight junction distribution, caused by systemic inflammatory insults during early life, precede long-term alterations in both NVU permeability and behaviors. In particular, they conducted longitudinal studies in neonatal, adolescent, and adult rats exposed to intraperitoneal injections of $0.2 \text{ mg} \cdot \text{kg}^{-1}$ LPS during the early postnatal period [i.e., on postnatal day (P) 0, P2, P4, P6, and P8]. The systemic inflammatory response to LPS treatment—confirmed by increased IL-1β and TNF-α plasma levels—resulted in increased NVU permeability only in adulthood, preceded by claudin-5 altered distribution at an earlier time point [173,174]. Stolp *et al.* [173,175] demonstrated that NVU alterations due to inflammation during brain development can occur in several phases, each one leading to different behavioral modifications: at short-term, juvenile animals showed alteration in the PPI paradigm, while in the long-term, changes in NVU permeability (as shown by the sucrose permeability test) were correlated with anxiety-related behaviors (as shown by altered responses to the dark/light test).

Maternal immune activation can alter the permeability of the NVU and placental barriers [176] and so increase the circulation of inflammatory mediators to the fetal brain, such as cytokines and reactive oxygen species, ultimately leading to behavioral changes in offspring's adult life [177]. Altogether, animal experimental studies using models of MIA (Table 3), especially LPS and PolyI:C, suggest that maternal inflammatory response induced during the early postnatal period—hallmarked by excessive cytokine secretion and signal transduction, cellular immune activation, and recruitment—has been associated with altered NVU permeability, acting as mediator of

abnormal brain development, leading to long-term behavioral as well as neurochemical changes in adult offspring [178]. Brain injuries in the setting of systemic inflammatory processes can act as pathogenic mediator, not as a single hit in time [179], but holding for months or even for years and finally contributing to postinsult neuronal deficits as long-term ongoing process and lifelong disability, including cerebral palsy, seizure disorders, sensory impairment, and cognitive limitations.

Conclusions

Neurovascular interrelationship is essential for the proper functioning and development of the brain. Many animal models of NVU dysfunctions have been shown to be lethal, as for both the PDGFR-$\beta^{-/-}$ and the PDGF-B$^{-/-}$ mice, for which the loss of pericytes results in perinatal lethality caused by microaneurysms, mostly in the brain and kidneys [80,180] and the claudin-5-deficient mice, which exhibit a selective increased permeability for molecules under 800 Da [104]. Moreover, the cerebrovascular system serves as structural track and guide for the migration of various cell types. This is the case for oligodendrocyte precursors—which use vessels to migrate and finally engage neuronal axons to be enveloped with myelin sheaths [181]—and neurons—which rely on EC cues in order to find their final position [182]. Not only does the brain vasculature influence neuronal activity, but also the opposite occurs. Neural activity can indeed promote the formation of new vessels and ultimately modify vessel density and branching [183]. Furthermore, detrimental NVU events are consistently accompanied by microglia recruitment and enhanced release of cytokines, which in turn directly affect synapse function and cognitive processes [18,168,184]. For all these reasons, modifications of the NVU may have long-lasting effects on behavior, even resulting in psychiatric conditions. The undergoing SARS-CoV-2 pandemic stresses the need to build a solid net of clinical data and preclinical research evidence to understand not only the impact of intragravidic infections on the offspring's mental health, but also the underpinning mechanisms. Various registries, as the Pregnancy CoRonavIrus Outcomes RegIsTrY—a US study led by the University of California—or the International Registry of Coronavirus Exposure in Pregnancy—an international cohort—collect data on pregnancies and longitudinal birth outcomes, representing a valuable opportunity to collect information on possible NVU dysfunctions in the context of intragravidic infections. Therapies aimed at lowering intraventricular pressure,

distortion, free iron concentration, and cytokine levels in cases of posthemorrhagic brains have been already exploited. Recently, a 10-year follow-up study using the Drainage, Irrigation, and Fibrinolytic Therapy therapeutic approach on preterm babies presenting GH-IVH has shown improvements in child developmental profiles, cognitive ability, and global cerebral function, reducing the overall neurodevelopmental disability [185,186]. The future challenge will be to better understand the mechanisms through which the development of NVU and brain vasculature can shape our behavior, particularly when under the influence of inflammatory and viral hits.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

MR, EL, FM, and MM wrote and edited the paper. FM, MR, and EL prepared the figures and tables.

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