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1 **Review: Neuroinflammation in intrauterine growth restriction**

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1 **Abstract**

2 Disruption to the maternal environment during pregnancy from events such as hypoxia,

3 stress, toxins, inflammation, and reduced placental blood flow can affect fetal development.

4 Intrauterine growth restriction (IUGR) is commonly caused by chronic placental

5 insufficiency, interrupting supply of oxygen and nutrients to the fetus resulting in abnormal

6 fetal growth. IUGR is a major cause of perinatal morbidity and mortality, occurring in

7 approximately 5-10% of pregnancies. The fetal brain is particularly vulnerable in IUGR and

8 there is an increased risk of long-term neurological disorders including cerebral palsy,

9 epilepsy, learning difficulties, behavioural difficulties and psychiatric diagnoses. Few studies

10 have focused on how growth restriction interferes with normal brain development in the

11 IUGR neonate but recent studies in growth restricted animal models demonstrate increased

12 neuroinflammation. This review describes the role of neuroinflammation in the progression

13 of brain injury in growth restricted neonates. Identifying the mediators responsible for

14 alterations in brain development in the IUGR infant is key to prevention and treatment of

15 brain injury in these infants.

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1 **Introduction**

2   Intrauterine growth restriction (IUGR) is a major cause of perinatal morbidity and

3 mortality and occurs in approximately 5-10% of pregnancies [1, 2] with even higher rates

4 (21%) reported in the developing world [3]. IUGR is generally defined as a fetus that fails to

5 achieve appropriate growth potential due to genetic or environmental factors. It is

6 characterised by fetal weight dropping over time across growth percentiles; by birth most

7 IUGR infants weigh less than the 10th percentile for gestational age. Chronic placental

8 insufficiency is a common cause of IUGR. Placental insufficiency or utero-placental

9 dysfunction results in insufficient blood flow to the placenta during pregnancy and

10 inadequate supply of nutrients and oxygen to support normal growth of the fetus. Thus, the

11 fetus develops in a chronic hypoxic environment. Placental insufficiency can result in

12 changes in fetal metabolism, hormones, hematology, immunology and cardiovascular

13 function.

14   The adverse fetal environment can significantly affect the developing brain. In a

15 chronic hypoxic environment, fetal circulatory redistribution occurs; blood flow is selectively

16 redirected to the brain and away from other organs to maximise oxygen and nutrient supply.

17 This type of growth restriction is referred to as 'brain-sparing' or asymmetric IUGR because

18 the body is disproportionately smaller than the head. Asymmetrical IUGR is the most

19 common form of growth restriction affecting 70-80% of all IUGR infants with disruption to

20 fetal growth occurring mainly in the third trimester. Symmetric IUGR accounts for 20-25%

21 of all IUGR fetuses and is characterised by a global growth restriction throughout pregnancy.

22 Brain-sparing has been regarded as a protective mechanism in the IUGR fetus to protect and

23 promote brain development but recent evidence has challenged this idea (reviewed in [4]).

24 Several studies have demonstrated that asymmetric IUGR infants i.e. those with 'brain-

25 sparing', have worse neurodevelopmental outcomes than symmetric IUGR infants [5-10].

1 **Brain injury in IUGR**

2   The fetal brain is particularly vulnerable to the effects of IUGR [11]. Long-term

3 neurological disorders such as cerebral palsy (CP) and epilepsy, as well as learning and

4 attention difficulties, neurobehavioural disabilities, and other cognitive issues have been

5 attributed to restricted growth of the fetus [12-15]. A four- to six-fold increase in CP has been

6 shown in IUGR neonates [14] with others reporting up to a 30-fold increase [16]. The long-

7 term care of a child with compromised brain development is associated with emotional stress

8 for families and a direct cost on society. Currently there are limited treatments to prevent

9 neurological impairment in the IUGR neonate. Research is addressing IUGR health problems

10 from different angles; both the preventative aspect *in utero* as well as interventions from

11 birth. As many growth restricted fetuses may not be detected until after birth (especially in

12 the case of asymmetric IUGR) it is important to examine the vulnerable IUGR brain to best

13 determine treatment options to prevent long-term adverse neurological outcomes.

14 *Grey and white matter injury in IUGR*

15   Brain injury in the IUGR infant may be due to a combination of grey matter and white

16 matter disruption and disorganisation in the development of the brain. Clinical imaging

17 studies of preterm IUGR infants have demonstrated significant alterations in white and grey

18 matter volume and structure [17-19] including decreased cortical thickness, delayed cortical

19 development and altered brain connectivity [17-19] in comparison to non-IUGR preterm

20 infants. In IUGR infants cortical grey matter volume is 28% less than that of age equivalent

21 healthy term-born infants [17]. Reduced cerebral cortical grey matter volume in the term

22 IUGR neonate has been shown to correlate with attention disorders [17]. Furthermore, such

23 grey matter structural changes in the term IUGR infant that persist at 1 year of age have been

24 found to be associated with developmental disabilities [18, 20]. These alterations are also

1   evident in animal models of growth restriction with demonstrated neuronal and white matter

2   disruption [21-30]. Neuronal loss and disruption are observed in IUGR animal models

3   throughout many regions of the brain including the hippocampus [29, 31]. A decrease in

4   proliferation and differentiation of oligodendrocytes are also evident in many growth

5   restricted animal models [21, 24, 25, 27] with some demonstrating postnatal restoration of

6   myelin dependent on the severity of injury [25, 26, 30]. Miller *et al*., 2014 showed decreased

7   myelination with fragmentation and disorganisation of the white matter tracts in growth

8   restricted sheep [29]. They postulated these abnormal patterns may result in abnormal

9   neuronal activity and functionality in the IUGR brain. Even though characterisation of white

10   matter injury has been a major avenue of investigation in IUGR animal models, neuronal

11   disruption is also a critical neuropathological feature and brain injury in the IUGR neonate is

12   a combination of white and grey matter injury. As discussed above, grey matter injury is a

13   predominate neuropathological feature observed in human studies [17, 18, 20], therefore

14   further emphasis on mechanisms of neuronal injury in growth restricted animal models

15   studies are vital.

16

Mechanisms of neuronal injury

18   Few studies have focused on the detailed mechanisms of brain injury in the IUGR

19   neonate which is surprising given the high proportion of IUGR infants who exhibit adverse

20   long-term neurological outcomes [18, 19]. There is a considerable paucity of data from

21   human autopsy tissue of the pathology of the human IUGR brain. A classical study of six

22   term IUGR infants demonstrated a reduction in myelin lipids and DNA content (used as an

23   estimate of cell number) in cerebrum-brainstem and cerebellum fractions [32]. More recently

24   in nine IUGR fetuses a significant decrease in cell number in the developmental zones of the

1 cortex has been reported [33]. It is extremely challenging to acertain mechanisms of IUGR

2 injury from post-mortem human brain tissue. Difficulty in estimating the timing of an IUGR

3 insult as well as untangling variables of gestational age on brain dvelopment, insults such as

4 pregnancy hypertension and other factors confound intepretation from human IUGR autopsy

5 findings. Therefore animal models of IUGR are necessary to adequately explore mechanisms

6 of injury in the IUGR brain. It is likely that key normal developmental processes are affected

7 during the growth of the fetal brain and these may underlie the adverse neurodevelopmental

8 outcomes in the IUGR infant. Understanding the mechanisms behind grey matter and white

9 matter loss, and impairment in the IUGR infant is essential to identifying therapeutic targets

10 for intervention or prevention of brain injury. The mechanisms leading to neuronal injury in

11 the IUGR neonatal brain are complex and not well understood. Although the IUGR fetal

12 brain is often referred to as hypoxic-ischemic (HI) [34], the IUGR fetal brain is not generally

13 regarded as globally ischemic as blood flow is actually increased to many regions of the brain

14 [35-37]. However, the IUGR fetus is relatively hypoxic due to chronic placental oxygen

15 deprivation. The chronic IUGR insult leads to a reduction in oxygen delivery to the brain and

16 concomitant reduction in delivery of glucose and amino acids with potential effects on

17 immature neurons and neuroglia [34]. When cerebral oxygen is reduced, a cascade of cellular

18 and biochemical events occurs in the fetal brain causing cellular injury that can lead to cell

19 death [36]. Many of these events result in mitochondrial disruption and immediate or delayed

20 cell death [34]. The major putative mechanisms that may underpin the cellular death and

21 injury in IUGR brains are excitotoxicity, oxidative stress, necrotic and apoptotic degeneration

22 and neuroinflammation [34, 38].

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1   **Inflammation in the IUGR brain**

2   Recent studies in animal models of growth restriction have reported increased

3   numbers of activated microglia and astrogliosis, indicative of inflammatory responses in the

4   IUGR brain (Table 1) [22-27, 31, 39, 40]. Neuroinflammation encompasses a number of

5   processes including increased numbers of activated microglia, elevated production of

6   proinflammatory cytokines (particularly interleukin-1β (IL-1β) and tumour necrosis factor-α

7   (TNF-α)) [41-43], decreased production of anti-inflammatory cytokines [44], release of

8   chemokines [44-46], increased production of nitric oxide (NO) [47, 48], infiltration of

9   leukocytes [45] and astrogliosis [48-52]. However many previous IUGR studies have

10   examined only changes at one postnatal time point or changes of only select inflammatory

11   cytokines. The neuronal damage and loss which results from neuroinflammation is a dynamic

12   process and can continue for days or even weeks after a neonatal hypoxic insult [51, 53, 54].

13   It is important to focus future studies on the evolving impact of inflammation on neuronal

14   injury in the IUGR neonate. Whether the IUGR fetus adapts to this milder chronic HI event

15   such that the neuronal damage is not as severe is unclear. However, given the

16   neurodevelopmental disabilities prevalent in these children, these adaptations may be mild. A

17   thorough spatial and temporal examination of inflammation and neuronal injury in the IUGR

18   neonate is warranted.

19

20   **Proinflammatory cytokines**

21   Proinflammatory cytokines are shown to play a critical role in acute HI brain injury

22   and may cause and/or exacerbate brain damage to the fetal and neonatal brain. The on-going

23   presence of increased levels of proinflammatory cytokines contributes to white matter

24   damage as well as neuronal damage after acute neonatal HI [41, 48, 53, 55, 56]. In the

1 preterm infant, the occurrence of CP has been attributed, at least partially, to increased levels

2 of proinflammatory cytokines in the brain [57]. The long-term consequences on

3 neurodevelopment of the IUGR infant may be due to effects of neuroinflammation generated

4 by proinflammatory cytokines in the IUGR brain.

5 Proinflammatory cytokines, such as IL-1β, TNF-α, IL-6 and IL-8 are small, cell

6 signalling glycoproteins involved in communication between cells [58] and are secreted in

7 response to cellular injury. Proinflammatory cytokines are released by a variety of cells both

8 in the brain and also in the blood in response to hypoxic injury. As described below, activated

9 microglia are the major source of IL-1β and TNF-α in the central nervous system (CNS) [59-

10 61]. IL-6 is produced during astrogliosis where an abnormal increase in the number of

11 astrocytes occurs in the brain. IL-8's release into the cerebral spinal fluid after brain injury

12 has been shown to be associated with blood brain barrier (BBB) dysfunction [62].

13 Proinflammatory cytokines promote the progression of injury through complex interactive

14 networks, such as stimulating the synthesis of other cytokines and mediators of neuronal

15 injury including NO synthase, inducing leukocyte infiltration and the expression of adhesion

16 molecules, influencing glial gene expression and damaging oligodendrocytes [63]. Both

17 TNF-α and IL-1β can also activate matrix metalloproteinases (MMP) which leads to the

18 disruption of the immature BBB [64, 65]. Furthermore, altered BBB function after HI injury

19 facilitates entry of systemic proinflammatory cytokines into the brain of the fetus [66].

20 *Systemic proinflammatory cytokines in IUGR*

21 Recent human studies report the presence of systemic inflammation in IUGR neonates

22 [67, 68]. Severely growth restricted preterm neonates demonstrated significantly higher levels

23 of proinflammatory cytokines in the blood during the second postnatal week; however at

24 birth there was no evidence of this increase [67]. In umbilical cord serum from IUGR

25 neonates at birth, interferon-γ (INF-γ) levels have also been reported to be raised and may be

1 related to fetal growth restriction [69]. Furthermore, in mothers of IUGR infants, stimulation

2 of maternal peripheral blood by trophoblast antigens showed increased levels of

3 proinflammatory cytokines, IL-8, INF-γ and TNF-α [70] with decreased levels of the anti-

4 inflammatory cytokine IL-10 when compared with mothers of normally grown infants [70].

5 Whether the increases in systemic proinflammatory cytokine expression in these IUGR

6 infants are associated with adverse neurological outcome is not yet clear. However we can be

7 guided by a study on small for gestational age (SGA) newborns where blood concentrations

8 of inflammatory proteins were examined during the first two postnatal weeks and correlated

9 with mental development at two years of age [68]. Extremely preterm SGA newborns were at

10 increased risk of lower mental development scores. When these SGA infants presented with

11 systemic inflammation at two weeks of age, they were at an even greater risk of attaining a

12 lower mental development score at two years of age [68].

13 *Proinflammatory cytokines in the IUGR brain*

14 Overproduction of proinflammatory cytokines is proposed to be important in the

15 development of neonatal brain injury [61]. Yet, few studies have focused on its expression in

16 the IUGR brain. In an IUGR model of chronic hypoxia (chronic fetal hypoxemia; CHX),

17 inflammatory cytokines are found to be upregulated in the fetal brain [22]. TNF-α and IL-1β

18 are increased in response to CHX with the elevation in proinflammatory cytokines relative to

19 the severity of brain injury [22]. Similarly, in a rat model of growth restriction with

20 lipopolysaccharide treatment, a robust increase in cytokine macrophage chemoattractant

21 protein-1 (MCP-1) and cytokine induced neutrophil chemoattractant protein-1 (CINC-1) was

22 evident in the IUGR rats in comparison to controls [39]. Cytokine increases are not only

23 evident in the brain of IUGR animals; in a rat model of growth restriction an increase in the

24 proinflammatory cytokines IL-6, TNF-α and IL-1β have also been observed in both the

25 amniotic fluid and placentas [71]. The increased levels of these cytokines are evident as early

1   as 24 hours in the amniotic fluid and placenta after bilateral uterine artery ligation. These

2   studies confirm not only a systemic inflammatory response, but also a central inflammatory

3   response. As an increase in BBB permeability occurs in the IUGR neonate [40] it is uncertain

4   whether there is an infiltration of inflammatory mediators into the brain of the IUGR neonate

5   or whether inflammation is originating from the brain, and the BBB breakdown facilitates

6   brain derived inflammatory cells into the blood.

7

8   **Activated microglia and reactive astrocytes**

9   *Activated microglia*

10   Microglia are the first inflammatory cells that respond to hypoxic events in the

11   neonatal brain [72]. Microglial cells are resident macrophages in the brain and are present in

12   large numbers in the developing brain. Microglia are involved in cellular pruning during both

13   normal development and pathological conditions. Resting (ramified) microglia in the neonate

14   have multiple processes with a small cell body [73]. Microglial cells function to defend

15   against infections or toxic substances released from dying brain cells by scavenging and

16   engulfing unwanted pathogens and cellular debris [74]. Microglia become activated in

17   response to chemical signals from injured neurons where they increase in number and

18   migrate to sites of injury [45, 75-78]. Activated microglia are morphologically distinct from

19   resting microglia. When activated, their processes retract to develop a more rounded,

20   amoeboid appearance [73]. Activated microglia increase their tendency to bind lectins, up-

21   regulate immunological surface proteins, and release nitric oxide (NO) and proinflammatory

22   cytokines [79-81]. Activated microglia are largely responsible for the production of excessive

23   levels of the proinflammatory cytokines, IL-1β and TNF-α, that are toxic to neurons [45, 75,

24   78]. It is unclear whether the injured neurons initiate the activation of microglia, or the

1   activated microglia release factors which injure the neurons; regardless, the result is a cyclic

2   pro-inflammatory event.

3   *Reactive astrocytes*

4   Astrocytes, the most abundant glial cells in the brain, are involved in maintenance and

5   support of neurons as well as comprise a significant component of the BBB. In healthy neural

6   tissue, astrocytes play critical roles in energy provision, regulation of blood flow,

7   homeostasis of extracellular fluid, homeostasis of ions and transmitters, and regulation of

8   synapse function [82]. Astrocytes are critical in fetal development for providing scaffolding

9   for migrating neurons to form the layers and substructures of the brain [83]. Like microglia,

10   the morphology of astrocytes depends on the health of the tissue around them. In healthy

11   CNS, astrocytes exhibit large processes and a distinct star shape. Astrocytes become reactive

12   in response to signals released by injured neurons or activated microglia following an event

13   such as HI injury [84]. Reactive astrocytes undergo morphological changes where they divide

14   and become hypertrophic with short and thickened processes [84]. Reactive astrocytes release

15   various growth factors and cytokines (TNF-α and IL-1β) which exacerbate brain injury.

16   Reactive astrocytes are also known to physically block neuronal regeneration and therefore

17   inhibit functional recovery.

18   *Activated microglia and reactive astrocytes in IUGR brain*

19   The limited studies examining microglial and astrocytic response in the IUGR brain

20   have shown varying results. In a neonatal rat model, antenatal hypoxia-induced IUGR was

21   found to be associated with severe neuroinflammation and delayed myelination. Increased

22   microglial activation was apparent in the developing white matter at postnatal day 3 and 10 as

23   well as an increased density of astrocytes in the cingulate white matter of IUGR pups [27].

24   [27]. In contrast, a guinea pig model of chronic placental insufficiency (CPI) demonstrated no

1 difference between controls and IUGR guinea pigs in density or morphology of microglia in

2 the subventricular zone (SVZ) at 60 days of gestation [85]. In addition, no changes in the

3 density of astrocytes or evidence of reactive morphology of astrocytes in the SVZ were

4 apparent [85]. Yet the SVZ is a site where few activated glia reside. Furthermore neuronal

5 death was not observed in the SVZ but was evident in other regions of the brain [85],

6 therefore this region is likely less affected by inflammation in IUGR neonates. Further

7 regions of the brain warrant exploration at multiple postnatal time points.

8 However the majority of studies demonstrate a definitive astrocytic response in the

9 IUGR brain. A reduced number of mature cortical astrocytes was observed in an

10 experimental rabbit vascular IUGR model compared with controls [86]. This model of

11 asymmetric IUGR demonstrates that the brain is not 'spared' from cellular disruption. Not

12 only was there a disruption of astrocyte maturation in the cortical layer, but also a reduction

13 in cell size [86]. A rat model of placental insufficiency also reports reactive astrocytosis in

14 the corpus callosum and cingulum [23]. IUGR lambs show a loss of peri-vascular astrocyte

15 attachment. Astrocytes are also essential for the maintenance of the BBB [40] and disruption

16 to this cell type may mitigate the infiltration of systemic inflammatory mediators into the

17 brain.

18 A further study in rat pups with prenatal moderate and severe growth restriction

19 induced by unilateral ligation of the uterine artery reported increased activated microglia and

20 astrogliosis in the white matter [25]. A notable inflammatory response with concomitant

21 white matter injury in the severe growth restricted rat pup was apparent. Activated microglia

22 were significantly elevated 2 weeks after birth, at a critical time point when white matter

23 remodelling and neuronal pruning is occurring, which will have long-term consequences on

24 the developing brain [25].

1   Gender differences have also been reported in several IUGR studies. In a model of

2   uteroplacental insufficiency in the rat, region and gender specific changes in astrocytic

3   deficits were observed in the hippocampus [31]. A significant increase in the amount of

4   astrocytes in the dentate gyrus was apparent in males with a contrasting decrease in astrocytes

5   in the CA3 region. As IUGR males demonstrate worse behavioural deficits than females,

6   differences in astrocyte response to injury and subsequent inflammatory responses in the

7   brain may account for the higher rate of adverse outcomes in the IUGR male.

8

Conclusion

10   Chronic deprivation of oxygen and nutrients to the developing fetus through altered

11   placental function has dramatic consequences on fetal brain development. Activation of

12   inflammatory pathways both systemically and in the brain are thought to play a key role in

13   altered brain development and may contribute to the poor neurodevelopmental outcomes

14   associated with chronic placental insufficiency [67, 68]. Understanding how the IUGR brain

15   is damaged by examining where inflammation is occurring, when it is occurring and its

16   impact on various cell types and white matter will facilitate the development of appropriate

17   targeted therapies to improve neurodevelopmental outcomes.

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4

5 **Conflict of Interest statement**

6 The authors declare that they have no conflict of interest.

7

8 **Table 1. Glial and brain inflammatory responses in animal models of fetal growth**

9 **restriction.**

10 GD, gestational day; E, embryonic day; LPS, lipopolysaccharide; GR, growth restriction;

11 CPI, chronic placental insufficiency; P, postnatal day; BrSp, Brussel sprout; OL/Oligos,

12 oligodendrocyte; WM, white matter; NO, nitrous oxide; Mg, magnesium; IL-1β, interleukin-

13 1β; TNF-α, tumour necrosis factor-α; MCP-1, macrophage chemoattractant protein-1; CINC-

14 1, cytokine induced neutrophil chemoattractant protein-1

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Table 1. Glial and inflammatory responses in animal models of fetal growth restriction

IUGR Insult	Model	Insult	Examination	Brain region	Microglia	Astrocytes	Oligos	Neurons	Cytokines	Other
			Time Frame				Outcomes			
Pregnant rats exposed to hypoxic conditions and nitric oxide inhalation (Pham et al, 2015) [27]	Rat	E5-E19	P3, P10 and P21 (NO given 12-24 hrs prior to delivery until P5)	white matter	increased microglial activation		decreased proliferation and differentiation			Nitric oxide helps counteract hypoxia by decreasing cell death and microglial activation, increasing oligodendroglial proliferation and improving myelination.
Chronic placental insufficiency with broccoli sprout supplement (Black et al, 2015) [23]	Rat	GR E20; BrSp supplement given to dams E15-P14 (term is 23 days)	P3 - P21	hippocampus, corpus callosum and cingulum		astrogliosis		decreased hippocampus neural cells		BrSp alleviated the GR effects of diminished white matter, ventricular dilation, astrogliolis, and reduced hippocampal neural cells.
Bilateral uterine artery ligation and magnesium supplement (Roman et al, 2013) [71]	Rat	GD18	fluids taken post delivery	n/a					increase in cytokines	Mg supplements decreased effects of GR. No significant difference in maternal and fetal plasma. Significant increases in IL-6, IL-1β, TNF-α, and CCL2 (MCP-1) and CSCL1 levels 24 hours post-surgery in amniotic fluid and placenta.
Uteroplacental insufficiency (Fung et al, 2012) [31]	Rat	E19.5	at Birth P0	hippocampus		changes specific to gender	changes specific to gender	decreased locations specific to gender		GR induces neuronal, astrocytic and immature oligodendrocyte deficits in a region and sex specific manner. GR increased the amount of dentate gyrus astrocytes in males only. GR decreased the amount of CA3 astrocytes in males only. GR significantly increased amount of CA1 immature oligodendrocytes in females but decreased the amount in males.
Maternal uterine artery ligation and lipopolysaccharide (Campbell et al, 2012) [39]	Rat	GD14	P6	whole brain and entire periventricular white matter	no difference in numbers without LPS treatment	no astrogliosis without LPS treatment	no white matter impairment without LPS treatment		LPS elevated proinflammatory cytokines	GR rats with LPS treatment demonstrated enhanced brain damage with increased apoptosis, large ventricles and impaired myelination, activated microglia and astrogliosis.
Transient systemic hypoxia-ischemia/placental insufficiency (Mazur et al, 2010) [21]	Rat	GD18 (Third Trimester)	GD22 (P1)	frontal Lobe, coronal 'sections' (ventral hippocampal commissure-anterior temporal horn)			loss of oligodendrocytes	loss of neural cells		EPO administration rescued oligodendrocytes and neural cells.
Maternal unilateral uterine artery ligation (Olivier et al, 2007) [25]	Rat	E17	E21, Birth (P0), P3, P7, P10, P14, P21 and P60 (in adults)	white matter	microglial activation	astrogliosis	loss of oligodendrocytes - may be restored in P14 moderate GR pups			White matter lesions were smaller in pups with moderate GR and larger in pups with severe GR - rats with severe GR white matter damage persisted to adulthood.

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Model	Species	IUGR induction	Age examined	Brain region	Microglia	Astrocytes	Oligodendrocytes	Neurons	Cytokines	Outcome
Maternal unilateral uterine artery ligation (Olivier et al, 2005) [24]	Rat	E17	E21, Birth (P0), P3, P7, P10, P14, P21 and P60 (in adults)	white matter	increased microglia	astrogliosis	loss of oligodendrocytes			Defective myelination until adulthood.
Chronic placental insufficiency (Tolcos et al, 2015) [85]	Guinea Pig	GD30 (term is 67 days)	GD60-62	subventricular zone (SVZ)	no difference in density or morphology	no astrogliosis		increase in neuronal precursor cells		Blood vessel density increase with cell proliferation to counter effects of CPI.
Chronic placental insufficiency (Tolcos et al, 2011) [26]	Guinea Pig	GD30 (Mid gestation)	Day of birth (GD 67) 1 week or 8 weeks	cerebral Hemispheres/cerebellum/white matter	Increase in microglial density at GD60 in WM. No difference at 1 week of age	Increase in astrocyte density at GD60 in WM. No difference at 1 week of age	Oligods reduced in GR fetuses - post natal recovery			Delay in oligodendrocyte maturation and myelination in utero is transient. Myelin restored postnatally.
Chronic fetal hypoxemia (Guo et al, 2010) [22]	Guinea Pig	GD46-49 for 14 days	Days 64-65	hippocampus	microglial activation			loss of neuronal cells	increase in cytokines	Fetal adaptive response to chronic hypoxia is 'maladaptive'
Spontaneous growth restriction and unilateral uterine artery ligation (Tolcos et al, 2003) [87]	Guinea Pig	GD28-30 (term is 66-68 days)	P6	medulla		no difference between groups				Alterations in respiratory and thermoregulatory responses with greater effects seen following spontaneous GR rather than experimental GR.
Unilateral uterine artery ligation (Mallard et al, 2000) [28]	Guinea Pig	GD30	Day 4-7	hippocampus and cerebellum				decrease in amount of neurons		Reduced volume of cerebral WM.
Maternal unilateral uterine artery ligation (Tolcos and Rees 1997) [88]	Guinea Pig	GD28-30 (term is 66-68 days)	GD60-62	brainstem regions		proliferation of astrocytes		no change		Proliferation of astrocytes in the dorsal motor nucleus of the vagus, nucleus tractus solitarius and around blood vessels throughout the brainstem
Vascular IUGR (Bassan et al, 2010) [86]	Rabbit	GD25 (Third Trimester)	GD29 (term is 31 days)	cerebral hemispheres and posterior fossa		Decline in astrocyte index				Small fetuses, small body large head, asymmetric GR.
Chronic placental insufficiency (Castillo-Melendez et al, 2015) [40]	Lamb	GD105-110 (term is 145 days)	P1	white matter		astrocyte attachment lost				GR lambs demonstrate reduced vessel density within the WM. Attachment of astrocytes and pericytes to blood vessels is reduced in GR lambs, which may impact the integrity of the BBB.
Single umbilical artery ligation and melatonin treatment (Miller et al, 2014) [29]	Sheep	GD105-110	24 hours after birth	white and grey matter: forebrain, hippocampus, thalamus, cerebral cortex			white matter loss; hypomyelination	no consistent loss of neurons in cortex; neuronal degeneration in hippocampus		WM tracts were fragmented and disorganised in GR brains. Cortical organisation and neuronal morphology altered in some GR brains, especially within deeper layers of the cortex.

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