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## **Handbook of Animal Models in Neurological Disease**

**Editors:** Colin MARTIN, Vinood B PATEL, Victor R PREEDY

### **Prenatal Systemic Hypoxia-Ischemia: a Rat Model of NeuroDevelopmental Disorders related to Prematurity**

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**Abbreviated title:** MIUH reproduces the main symptoms of low birth weight (max 45 char)

World count: max = 7000 references included !

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#### **Abstract**

Premature birth represents about 11% of pregnancies overall and is increasing worldwide. The prevalence of brain damage and neurodevelopmental disorders (NDDs) related to prematurity is high and increasing. Etiological factors of perinatal brain injury in preterm children mainly involve exposure to systemic infection/inflammation, hypoxia/ischemia or hyperoxia, placental pathologies, foetal growth restriction or infections, maternal hypotension, hypoxemia, preeclampsia, diabetes, as

well as multiple birth, stress, air pollution or drug abuse. Several animal models have been developed to better understand the perinatal etiological factors and mechanisms underlying the emergence of NDDs. Among rat models of prenatal brain injury, mild intrauterine hypoperfusion (MIUH) at embryonic day 17 (E17), transient systemic hypoxia-ischemia (TSHI) at E18, reduced uterine perfusion pressure (RUPP) from E14 and permanent stenosis of uterine arteries at E17 using ameroid constrictors (AC) are gathered here as prenatal systemic hypoxia-ischemia (PSHI). Briefly, these models of PSHI induce intrauterine growth restriction, low birth weight, cardiovascular disease, metabolic syndrome, neuroinflammation, diffuse brain damage including white and gray matter injuries, astrogliosis, hyperexcitability related to homeostatic imbalance of intracellular chloride concentration, musculoskeletal pathologies, sensorimotor disturbances, impaired behavior and cognition, and deficits in executive functions related to maladaptive plasticity and network disorganization in the central nervous system (CNS). These preclinical models point out several mechanisms of action and are promising for developing new strategies of prevention, remediation and rehabilitation of the NDDs related to prematurity.

**Keywords:** (5-10) intrauterine growth restriction, mild intrauterine hypoperfusion, reduced uterine perfusion pressure, transient systemic hypoxia-ischemia, low birth weight, white matter injury, hyperactivity, neuroinflammation, maladaptive plasticity, hyperexcitability

#### **List of abbreviations :**

AC, ameroid constrictors; ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; EP, encephalopathy of prematurity; E17, embryonic day 17; GABA, gamma-aminobutyric acid; HI, hypoxia-ischemia; IUGR, intrauterine growth restriction; LBW, low birth weight; KCC2, potassium-chloride cotransporter 2; MIUH, mild intrauterine hypoperfusion; NKCC1, sodium-potassium-chloride cotransporter 1; NDDs, neurodevelopmental disorders; P10, postnatal day 10; PSHI, prenatal systemic hypoxia-ischemia; RUPP, reduced uterine perfusion pressure; TSHI, transient systemic hypoxia-ischemia; WMI, white matter injury.

#### **Introduction**

In the last decades, neurological disorders occurred in 25-30% of infants born preterm (< 37 weeks of gestation), while prematurity, low birth weight and intrauterine growth restriction (IUGR) have been increasing (Kurinczuk et al., 2010; Pierrat et al., 2017; Fleiss et al., 2020). The prevalence of the most common physical disorder in children, cerebral palsy (CP) was 5-8% of preterm children and higher in children born extremely preterm (< 28 weeks, (Anderson, 2014). CP involves a group of disorders

of movement, posture and motor functions including spasticity, as well as deficits in perception, cognition and learning, and comorbidities (Graham et al., 2016). More than 40% of children born preterm exhibit deficits in communication, sensorimotor and social skills, learning and problem solving (Pierrat et al., 2017) and up to 50% in very preterms (< 32 weeks) (Anderson, 2014). About 30% of preterm children were diagnosed with cognitive impairments including executive functions, 11% with attention deficit hyperactivity disorder (ADHD), 7-8% with autism spectrum disorder (ASD) and 10% with emotional disorders including anxiety (Heeren et al., 2017; Hirschberger et al., 2018). In addition, preterm birth occurs in 10.6% pregnancies in overall, ranging from 8.7% in Europe to 13.4% in North Africa, and is increasing worldwide (Vogel et al., 2018; Passera et al., 2021).

Encephalopathy of prematurity (EP) is a constellation of brain damage in preterm children that include loss of oligodendrocytes, diffuse white matter injury (WMI), axonal damage, premature subplate and neuronal loss, disorganized cortical maturation and gyrification (Volpe, 2009; Quezada et al., 2018; Fleiss et al., 2020). Postmortem brain tissues of preterm infants with WMI show reduced expression of ubiquitous chloride cotransporters NKCC1 and KCC2 (Robinson et al., 2010) that govern chloride homeostasis and cell excitability, thus perturbing the early switch of GABAergic synapses from excitatory to inhibitory, thereby disrupting cortical circuit formation and predisposing to epilepsy (Jantzie and Robinson, 2015; Ben-Ari, 2017).

Etiological factors related to perinatal brain injury in infants born preterm mainly include exposure to systemic infection/inflammation, hypoxia/ischemia or hyperoxia, placental pathologies, foetal growth restriction or infections, maternal hypotension, hypoxemia, preeclampsia, diabetes, as well as multiple birth, stress, air pollution or drug abuse (Hagberg et al., 2015; Fleiss et al., 2020; Hahad et al., 2020; Ophelders et al., 2020). To better understand the mechanisms underlying the emergence of NDDs and to improve strategies for their prevention or early remediation animal models are crucial for preclinical investigation. However, the development of the central nervous system (CNS) differs across species. For example, the rodent CNS at birth is immature in comparison to the human CNS at birth, the latter is approximately equivalent to that of rodents at the postnatal day 10 (P10) or even later depending on the cell type or brain structure or function (see (Jantzie and Robinson, 2015; Hamdy et al., 2020) for reviews). Neonatal and postnatal models of both hypoxia-ischemia (HI) including the well-known Rice-Vannucci model and inflammation replicate only postnatal insults without the maternal-placental-fetal components. Brain injury related to prematurity is much less studied and characterized in larger animals such as rabbits, piglets, sheeps or non human primates than in rodents (Jantzie and Robinson, 2015). In fact, this chapter deals with prenatal studies in rodents that recapitulate or explain the emergence of the encephalopathy of prematurity and neurodevelopmental disorders (NDDs), disabilities associated with brain functions.

## **Animal Models of PSHI**

To produce IUGR and HI in rat pups, we performed a stenosis of uterine arteries by first using ligation at embryonic day 17 (E17) (Olivier et al., 2005; Delcour et al., 2012a) and then metal-coated coils wrapped around intrauterine arteries at E17 (Ohshima et al., 2016). In the latter, the blood flow perfusion in the placenta and fetus was reduced about 15% to 20% a few hours after stenosis and then returned to pre-stenosis levels at E20 (Fig. 1) (Ohshima et al., 2016; Coq et al., 2018; Tsuji et al., 2018). Pups were delivered by spontaneous labor at E21-22 and those with weight < 5.5 g were considered growth retarded and part of the mild intrauterine hypoperfusion (MIUH) group (Olivier et al., 2005; Delcour et al., 2012a; Ohshima et al., 2016).

Comparable procedures to reduce intrauterine blood flow in pregnant rats involve transient systemic HI (TSHI: clamping uterine arteries for 60 min at E18) as a model of placental insufficiency (Robinson et al., 2005; Jantzie et al., 2013), reduced uterine perfusion pressure (RUPP: permanent stenosis of uterine arteries from E14) as a model of preeclampsia (Granger et al., 2006; Clayton et al., 2018; Coats et al., 2021) or permanent stenosis of uterine arteries at E17 using ameroid constrictors (AC) as a model of hypertensive disease of pregnancy (Kitase et al., 2020). We thus postulate that all these procedures correspond to a prenatal systemic HI (PSHI) that could help to better understand the underlying mechanisms of the early emergence of EP (Jantzie et al., 2016).

## **Main results after prenatal systemic hypoxia-ischemia**

### *- Body growth restriction, placental insufficiency and metabolic syndrome*

MIUH at E17 and RUPP at E14 resulted in hypotrophic pups at birth (Olivier et al., 2005; Delcour et al., 2011; Ohshima et al., 2016; Coats et al., 2021; Rains et al., 2021); whereas, TSHI at E18 did not (Mazur et al., 2010; Robinson et al., 2018). LBW persisted in 1 and 2 months-old MIUH rats according to sex (Delcour et al., 2011; Ohshima et al., 2016) but MIUH rats caught up BW at P90-110 (Delcour et al., 2011, 2012a). After MIUH at E19, adult LBW females weighted more (i.e., higher body fat) than age-matched control females; whereas, the blood pressure in adult LBW males was higher than in control males, suggestive of greater risk for developing metabolic syndrome in MIUH females while the risk for cardiovascular complications is higher in MIUH males (Ogawa et al., 2019).

RUPP at E14 did not alter placental protein expression (i.e. nutrient transport) but impaired the placental development that lead to fetal oxidative stress and energetic imbalance (Coats et al., 2021; da Silva et al., 2021), as shown by increased pro-oxidation markers and lipid peroxidation, and reduced anti-oxidative markers in plasma (Rains et al., 2021) and in the aorta and mesenteric arteries (da Silva et al., 2021) of young and adult offspring. Increased water content, brain edema and impaired myogenic tone in the middle cerebral arteries have been reported after RUPP (Ryan et al., 2011). In addition, RUPP has also been shown to reduce the surface area of uterine-placental

junctions and to increase uterine artery resistance, indicative of reduced placental perfusion and development at E20 (Coats et al., 2021). Thus, several studies using RUPP at E14 or PSHI demonstrated the development of metabolic syndrome in adult IUGR rats, corresponding to endothelial dysfunctions, and increased rates of cardiovascular diseases and mortality that has been observed in adults born preterm (da Silva et al., 2021). The hypoperfused placenta releases pro-inflammatory and anti-angiogenic factors that cause vascular dysfunction, high blood pressure, maternal organ damage and systemic inflammation (LaMarca et al., 2016).

- *Neuroinflammation and hyperexcitability*

Recent studies based on IUGR in both humans and rats show systematic perinatal neuroinflammation that encompasses several cascades and steps including systemic inflammation along with the production of circulating pro-inflammatory cytokines leading to the activation and infiltration of microglia in the brain, thereby inducing neuroinflammation cascades that results in NDDs, described briefly here and elsewhere (Hagberg et al., 2015; Jantzie and Robinson, 2015; Wixey et al., 2017; Fleiss et al., 2021). PSHI including MIUH at E17 led to caspase 3 activation in the corpus callosum, cerebral cortex including S1 and hippocampus but no increase in ED1 or CD11b positive cells in these areas (Robinson et al., 2005; Delcour et al., 2012a; Jia et al., 2020). Rats submitted to prenatal AC showed increased expression of markers for microglia M1 and M2 phenotypes in the cerebral cortex (Kitase et al., 2020). RUPP has been shown to induce brain-blood barrier permeability during pregnancy and in offspring, and increased expression of pro-inflammatory cytokines and microglial markers in the cerebral cortex and hippocampus of 2 month-old offspring (LaMarca et al., 2016; Clayton et al., 2018). One of the striking consequence of early neuroinflammation is the excess enzymatic activity of calpains in the plasma, subplate, cerebral cortex and hippocampus from P2 to P12 while calpastatin expression, which contributes to regulate calpain activity, was reduced after TSHI at E18 (Jantzie et al., 2014b, 2015a). Excess calpain activity cleaves the oligomeric or active form of KCC2 to inactive, monomeric form. KCC2 is a ubiquitous K-Cl cotransporter and the main chloride extrusion system of the CNS, and thus regulates chloride homeostasis and neuronal excitability. Thus, reduced KCC2 function increases the intracellular concentration of chloride, thereby maintaining or reversing (in pathological conditions) the effect of GABA from hyperpolarization to depolarization, leading to hyperexcitability (Blaesse et al., 2006; Gackière and Vinay, 2015; Brocard et al., 2016). MIUH at E17 reduces the expression levels of active KCC2 in the lumbar spinal cord at P8 and in adulthood, leading to spinal hyperexcitability (Coq et al., 2018). Hyperexcitability has been also evidenced in the sensorimotor cortex, thereby resulting in sensorimotor loop disorganization and motor disturbances including hyperreflexia and spasticity after MIUH (Coq et al., 2018, 2019), and in the hippocampus and layer IV cortex leading subsequently to behavioral and memory deficits after

TSHI (Jantzie et al., 2014a, 2015a, 2015b, 2016; Robinson et al., 2018, 2021). Postmortem cerebral samples from infants born preterm with WMI showed reduced expression of KCC2 (Robinson et al., 2010). Thus, early neuroinflammation leads to neuronal and oligodendrocyte precursor death, gliosis, epigenetic dysfunction, altered synaptogenesis and other neural dysfunctions and brain disorders (Fleiss et al., 2021).

- *White matter injury (WMI)*

MIUH at E17 led to myelination deficits in the corpus callosum and cingulum of rats from birth to P21 (Olivier et al., 2005, 2007) and hypomyelination/axonal markers began to reduce in fetal brains at E20 (Ohshima et al., 2016). TSHI at E18 led to postnatal reductions of the expression of MBP, production and survival of progenitors and precursors of oligodendrocytes and immature oligodendrocytes as well as reduced oligodendrocyte process extensions (Jantzie et al., 2013, 2015a). Hypomyelination and axonal degeneration persisted in the internal and external capsules, corpus callosum genu, and pontocerebellar tract, within the primary somatosensory (S1) cortex and in white matter zones below the cingulate and S1 cortices of adult MIUH rats (Fig. 2). No hypomyelination nor axonal degeneration was detected in white matter zones below the primary motor cortex (M1) or in the corticospinal tract. Degenerating axons were also detected in the hippocampal CA3 region, lateral and medial entorhinal cortices and fornix but not in the prefrontal cortex. Diffusion tensor imaging showed white matter injury and impaired axonal integrity (i.e. neurofilament expression, reduced fractional anisotropy and increased radial, axial and mean diffusivity) in the corpus callosum, internal capsule and corona radiata in adult TSHI rats (Robinson et al., 2018). Interestingly, the severity of hypomyelination in MIUH adult rats was correlated with the gradient of growth restriction at birth (Olivier et al., 2005, 2007; Delcour et al., 2012a).

- *Gray matter damage and astrogliosis*

The thickness of the cerebral cortex was reduced in MIUH rats (Delcour et al., 2012a; Ohshima et al., 2016). MIUH at E17 decreased the neuronal density in the S1 cortex, particularly inhibitory interneurons, and decreased the height of cortical gray matter in adult rats. In contrast, the M1 cortex showed no structural changes (Fig.2(Delcour et al., 2012a). MIUH decreased the neuronal density in the lateral and medial entorhinal cortices but not in the CA1, dentate gyrus of the hippocampus, cingulate and prefrontal cortices, in which the density of inhibitory interneurons was surprisingly increased (Delcour et al., 2012a, 2012b). Prenatal AC also induced reduced neuronal densities in both the cerebral cortex and hippocampus (Kitase et al., 2020). However, adult TSHI rats showed no changes of the diffusion MRI parameters in the sensory cortex including the barrel cortex, but diffusion abnormalities were present in the hippocampus, striatum and thalamus of TSHI rats

(Robinson et al., 2018). In addition, prenatal TSHI induced to premature loss of the subplate and cortical plate, leading to abnormalities in cortical differentiation and gyrification (Jantzie et al., 2015a). It is worth to note that MIUH at E17.5 appeared to reduce early neuronal proliferation and to increase neuronal apoptosis of fetal neurons and autophagy from E18 to P28 in the hippocampus concomitant to alterations of Wnt, Akt and PTEN signaling pathways (Yin et al., 2020, 2021). MIUH at E17 induced mRNA changes, indicative of incomplete axonal outgrowth and astrocyte dysfunction but little to no impairment of immature neurons and pre-oligodendrocytes in MIUH fetuses at E20 (Ohshima et al., 2016).

Adult MIUH rats showed no obvious tissue damage, such as cystic and necrotic lesions or inflammatory cell infiltration (Ohshima et al., 2016). MIUH at E17 produced massive astrogliosis in the cingulate and S1 cortices and white matter below, the dentate gyrus, fornix, lateral and medial entorhinal cortices, and CA1 and CA3 of the hippocampus, but not in the M1 and prefrontal cortices (Delcour et al., 2011, 2012a, 2012b) as well as an enlargement of the lateral ventricles of adult MIUH rats (Ohshima et al., 2016).

#### - *Musculoskeletal pathologies and sensorimotor disabilities*

While MIUH is systemic, we investigated the adult anatomy of lower limbs. Adult MIUH rats exhibited fewer myofibres overall, hypertrophy of the myofibres in the gastrocnemius and a greater variation in size of myofibers in the gastrocnemius (Fig. 2), rectus femoris and tibialis anterior (Delcour et al., 2011). They also exhibited changes in soleus muscle contractile properties toward a lower twitch tension and the persistence of neonatal myosin heavy chain isoform, showing abnormal maturation of muscle properties and phenotype (Coq et al., 2018). More satellite cells in the gastrocnemius after MIUH suggested a prior injury to that muscle and now a repair or regeneration process because the myofiber number was reduced (Delcour et al., 2011). We detected mild knee and ankle joint chondromalacia after MIUH that could result from an imbalance in muscle forces across joints (Delcour et al., 2011). Such mild changes in joints and muscles and minor gait abnormalities have been reported in patients with CP (Pierce et al., 2007) and suggest the possible presence of spasticity.

Negative geotaxis appeared to be impaired as the pivoting time in pups submitted to either MIUH or AC was longer than in age-matched controls (Ohshima et al., 2016; Kitase et al., 2020), showing delayed or impaired multisensory integration. Despite apparent normal posture and gait during automatic locomotion on treadmill, MIUH rats exhibited reduced length, amplitude and velocities of the foot during the stride phase when they were juvenile (P30) and young adults (P65). Only adult MIUH rats showed a significant overextension of the knee and ankle that was compensated by an overflexion of the hip (Fig. 2; (Delcour et al., 2011). TSHI at E18 also disturbed treadmill locomotion in



young adult rats, such as reduced durations of propel phase and swing, reduced stride length, increased stepping frequency and signs of ataxia for hind limbs, as well as reduced surface area of the hind paw (i.e., footprint), indicative of digitigrade locomotion or toe-walking. The forelimb was less affected than hind limb during locomotion (Robinson et al., 2018). Rats submitted to AC spent less time on the rotarod, compared to shams (Kitase et al., 2020). MIUH rats exhibited muscle weakness with their hind limbs but not with their forelimbs when tested with a reaction meter (Ohshima et al., 2016). Grip strength and perturbations of motor coordination have also been reported after RUPP at E14 (Rains et al., 2021). Acrobatic locomotion involving the M1 cortex has not yet been investigated to our knowledge.

We postulate that postural and locomotor disturbances may be related to hyperreflexia observed in early and adult MIUH rats (Coq et al., 2018), leading to muscle dyssynergies and cocontractions. In addition, juvenile MIUH rats exhibited lower variations in many kinematic measures than age-matched controls, whereas with maturation, young-adult MIUH rats showed higher variations, suggestive of maturation deficits of posture and locomotion after MIUH (Delcour et al., 2011). Increased variations of locomotor parameters has also been reported after TSHI (Robinson et al., 2018).

- *Impaired behavior, cognition and executive functions*

MIUH at E17 induced spontaneous locomotor hyperactivity in P15 pups and adult rats in openfield tests (Delcour et al., 2011, 2012b; Ohshima et al., 2016). Other authors using either RUPP or TSHI also showed hyperactivity in openfield as well as disinhibition since rats spent more time or ambulated more in the center of the openfield (Robinson et al., 2018; da Silva et al., 2021). MIUH at E17 induced deficits in short-term and long-term memory in object recognition memory tasks including novel object recognition, and disturbances in encoding spatial configurations and object features in adult rats (Fig. 2). Comparable results were found in novel object recognition with TSHI and RUPP rats (Rains et al., 2021; Robinson et al., 2021). Although MIUH rats exhibited no significant deficits in spatial learning, spatial working memory and reversal behavior in the Morris water maze tests (Delcour et al., 2012b), other studies based on TSHI at E18 showed mild but significant deficits in watermaze tests (Jia et al., 2020). Of interest, the more the MIUH rats were growth-retarded at birth, the more they exhibited hyperactivity, degraded performances in memory tasks, astrogliosis in the fornix and decreased neuronal density in the entorhinal cortex (Delcour et al., 2012b).

Regarding executive functions, TSHI at E18 induced increased immobility in forced swim test, indicative of depressive behavior (da Silva et al., 2021) and impaired reversal learning during a visual discrimination task, indicative of more perseveration and poor cognitive flexibility (Robinson et al., 2021). Impaired reversal learning has also been reported in the watermaze task (Jia et al., 2020; Yin

et al., 2020). In addition, the time spent sniffing, playing and grooming by TSHI rats from different litters at P30 was reduced, showing impaired social interactions (Robinson et al., 2018). Thus, rats that were submitted to PSHI exhibit hyperactivity, disinhibition, signs of depressive-like behavior, less cognitive flexibility and impaired social interactions, but attention and emotional reactions have not been investigated so far. Associated with hippocampal and prefrontal/cingular cortex damage, these NDDs are usually often encountered in children born preterm who display WMI, ADHD or CP ((Pascal et al., 2018), ref).

- *Maladaptive CNS plasticity and network disorganization*

The emergence of sensorimotor reflexes such as negative geotaxis after MIUH appears delayed or impaired. Early and enduring increased stretch reflex and the likely signs of spasticity and contractile dysfunction may be at the origin and persistence of the mild locomotor and coordination impairments found in adult PSHI rats. In addition, MIUH rats exhibited musculoskeletal pathologies. Then, we postulate that hyperreflexia, musculoskeletal pathologies and motor deficits interplay to maintain each disorder and produce abnormal proprioceptive feedback or reafference to the immature spinal network, and finally to the S1 cortex, leading thereby to disorganized sensorimotor circuitry from development to adulthood (Fig. 3;(Delcour et al., 2012a; Coq et al., 2019). Indeed, MIUH at E17 degraded the S1 neuronal properties and the organization of cortical somatotopic maps, devoted to represent the hind limb skin surfaces. This degradation was mainly characterized by a reduction in cortical map size, and enlarged and multiple receptive fields, leading to a topographic disorganization and a patchy representation in S1 that correspond to degraded abilities in tactile discrimination (Xerri et al., 2005), as observed in preterm children (Wingert et al., 2008). This functional disorganization of S1 appears related to neuroanatomical changes observed in the S1 cortex such as lower densities of neuron, especially inhibitory interneurons, the WMI below the S1 cortex, massive astrogliosis and inflammation (i.e., diffuse brain damage). Abnormal lamination of the parietal cortex, presumably due to premature disruption of the cortical subplate, has been associated with gait disturbances in adult rats exposed to TSHI at E18 (Jantzie et al., 2015a). In addition to abnormal reafference and CNS hyperexcitability, the WMI below the S1 cortex may have also contributed to the disorganization of the S1 maps and sensorimotor circuitry through asynchronous afferences, inappropriate spike timing-dependent plasticity and maladaptive plasticity, which we previously postulated (Coq et al., 2016, 2019). Although the absence of motor cortex changes and normal white matter in the corticospinal tractus, the early lumbar hyperreflexia, altered multisensory integraton and disorganized sensorimotor circuitry may also produce maladapted motor commands, which in turn may have induced, maintained or even aggravated the muscle

dyssynergies, locomotor disturbances and musculoskeletal histopathologies in the form of self-perpetuating cycles (Fig. 3).

Besides the early and enduring disorganization of the sensorimotor circuitry at multiple scales, PSHI appears to induce neuroinflammation, premature subplate and neuronal loss, WMI and axonal damage, astrogliosis, and disorganized lamination of the hippocampal complex and cingular/prefrontal cortex, as described above. Such a disorganization of the hippocampal-prefrontal circuitry has been also evidenced to induce several deficits in behavior, cognition and executive functions (Fig. 3) and may include dysfunctions of many pathways and the cerebellum involved in the processing and integration of multisensory information, which has been shown to be altered after PSHI. Therefore, PSHI that includes several models of placental insufficiency and early HI leading to IUGR and perinatal neuroinflammation appears to recapitulate the variety of symptoms observed in children born preterm who develop NDDs. Such a preclinical model also contributes to better understand the underlying mechanisms and to develop novel and innovative strategies of prevention and early remediation of these NDDs.

### **Advantages/disadvantages**

PSHI encompasses several models ranging from transient (i.e., MIUH and TSHI) to permanent (AC stenosis and RUPP) stenosis leading to graded IUGR from E14 to E18. In the overall, all these models share the same mechanisms underlying the emergence of NDDs, such as neuroinflammation, reduced expression of KCC2 leading to hyperexcitability from the spinal cord to the hippocampus and cerebral cortex, neuronal, WMI and astrogliosis.

Compared to other techniques of stenosis such as ligation, the use of microcoils in MIUH or clamps increased the survival of pups and reproducibility per dam. The use ameroid constrictors is much expensive than clamps or metal microcoils.

These models are easy-to-use and very promising for preclinical studies of innovative treatments such as anti-inflammatory drugs including mesenchymal stromal cells (Tanaka et al., 2018; Kitase et al., 2020; Mukai et al., 2021) and other drugs including erythropoietin (Jantzie et al., 2015a; Robinson et al., 2018) or melatonin (Chen et al., 2012; Jantzie et al., 2018), as well as novel strategies of early rehabilitation based on dynamic and funny interactions such as HABIT-ILE and Care Toy System in children (Sgandurra et al., 2017; Araneda et al., 2020).

### **Key facts ?**

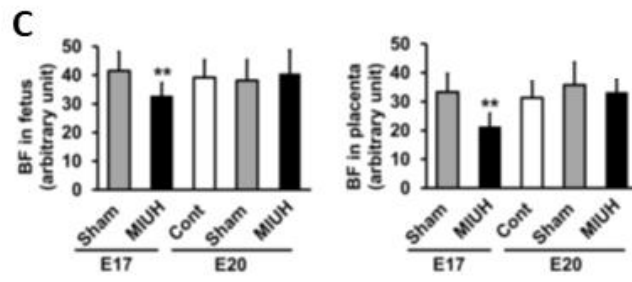
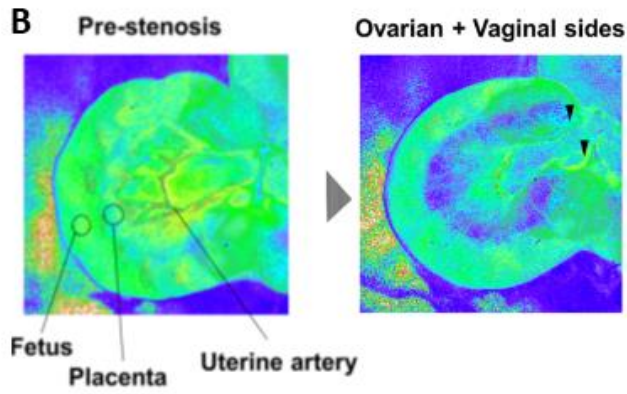
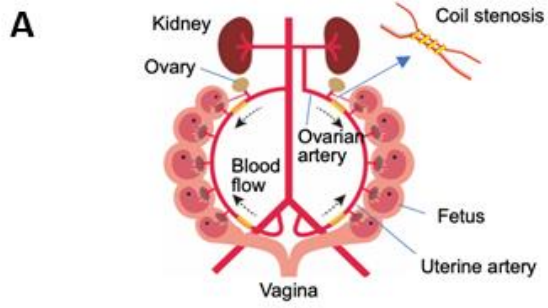
- These models induce IUGR, neuroinflammation, diffuse brain damage and the emergence of NDDs observed in children born preterm.

- The emergence of NDDs appears mainly due to maladaptive plasticity in relation to the interplay between periphery (altered sensorimotor functions and musculoskeletal pathologies) and CNS (diffuse brain damage).

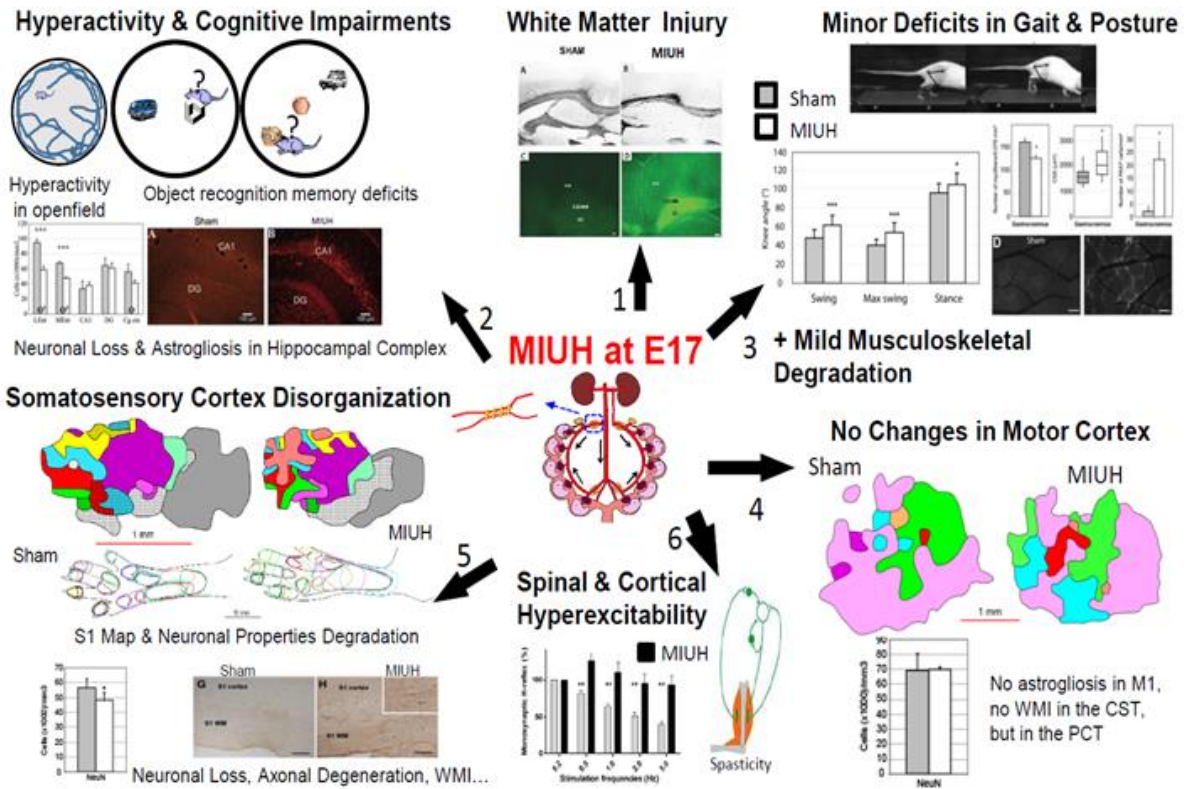
**Summary points (min of 5)**

- MIUH transiently reduces intrauterine blood perfusion and leads to neuroinflammation and IUGR observed at birth
- Hypotrophic pups exhibit diffuse brain damage
- PSHI leads to maladaptive CNS plasticity and the emergence of brain dysfunctions
- Hypotrophic rats display the main symptoms of the EP from early postnatal stages to adulthood
- PSHI appears to be a promising preclinical model for leads of prevention/remediation

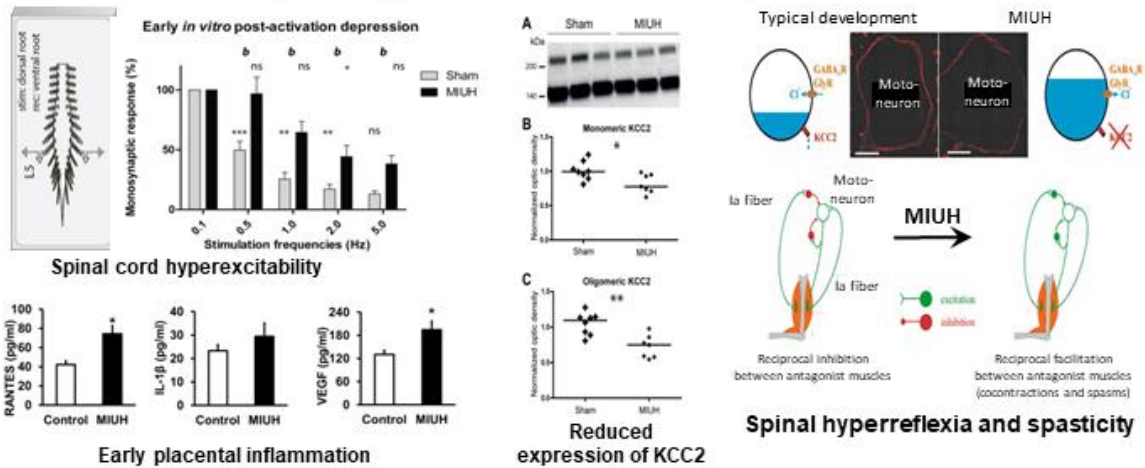
Figure 1



# Mild IntraUterine Hypoperfusion (MIUH): adult disorders



## MIUH: early emergence of neurodevelopmental disorders



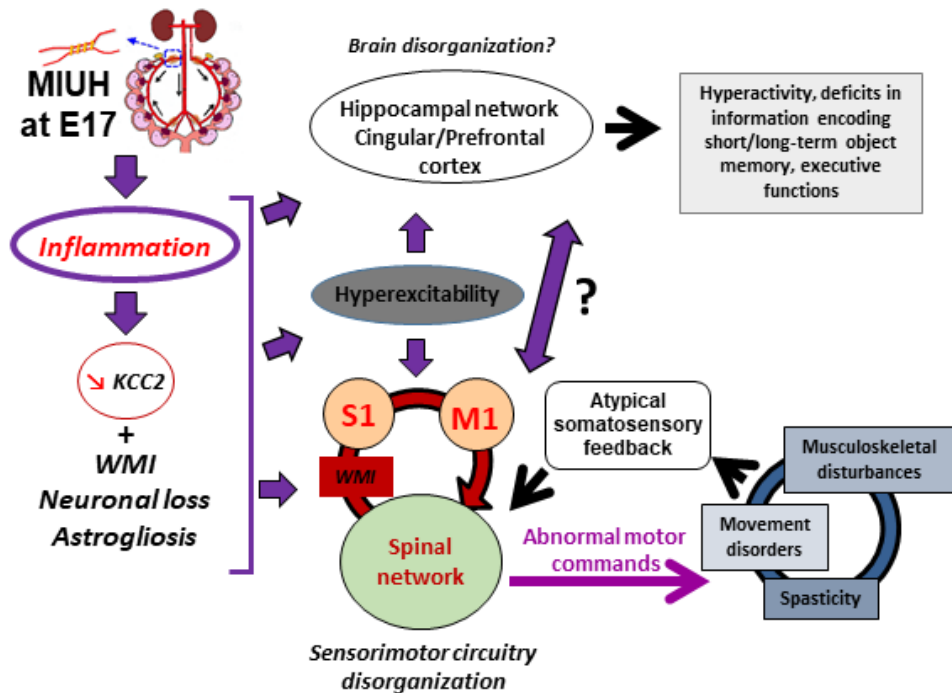


Figure 3

### Figure legends

Figure 1. Mild intrauterine hypoperfusion (MIUH) transiently reduces blood flow perfusion in placentas and fetuses. A. Schematic illustration of the uterine artery system with stenosis performed at embryonic day 17 (E17) using 4 metal microcoils wrapped around arteries. B. Representative images of changes in the uterine artery blood flow before and immediately after stenosis. Blue and purple zones indicates reduced perfusion at the opposite of yellow and red colours. C. Mean blood flow changes in each group immediately after stenosis (E17) and 3 days after (E20) in the fetuses and placentas. The blood flow reduced in both placentas (28%) and fetuses (16%) immediately after surgery and returned to control/sham pre-stenosis levels at E20. Sham, sham-surgery ; Cont, no-surgery control. B and C from Ohshima et al., 2016.

Figure 2. Schematic illustration of the diversity of neurodevelopmental disorders observed in our rat model of prematurity/low birth weight based on mild intrauterine hypoperfusion (MIUH). CST, corticospinal tract; E17, embryonic day 17; KCC2, potassium-chloride cotransporter 2; PCT, pontocerebellar tractus; WMI, white matter injury. From Delcour et al., 2011, 2012ab; Ohshima et al., 2016; Coq et al., 2018.

Figure 3. Putative mechanisms underlying the emergence of neurodevelopmental disorders observed after prenatal systemic hypoxia-ischemia (PSHI), which recapitulates mild intrauterine hypoperfusion (MIUH), transient systemic hypoxia-ischemia (TSHI), reduced uterine perfusion pressure (RUPP) and stenosis with ameroid constrictors (AC). See text. E17, embryonic day 17; KCC2, potassium-chloride cotransporter 2; MIUH, mild intrauterine hypoperfusion; M1, primary motor cortex; S1, primary somatosensory cortex; WMI, white matter injury.

## References

- Anderson PJ (2014) Neuropsychological outcomes of children born very preterm. *Semin Fetal Neonatal Med* 19:90–96.
- Araneda R, Sizonenko SV, Newman CJ, Dinomais M, Le Gal G, Nowak E, Guzzetta A, Riquelme I, Brochard S, Bleyenheuft Y, Early HABIT-ILE group (2020) Functional, neuroplastic and biomechanical changes induced by early Hand-Arm Bimanual Intensive Therapy Including Lower Extremities (e-HABIT-ILE) in pre-school children with unilateral cerebral palsy: study protocol of a randomized control trial. *BMC Neurol* 20:133.
- Ben-Ari Y (2017) NKCC1 Chloride Importer Antagonists Attenuate Many Neurological and Psychiatric Disorders. *Trends Neurosci* 40:536–554.
- Blaesse P, Guillemain I, Schindler J, Schweizer M, Delpire E, Khiroug L, Friauf E, Nothwang HG (2006) Oligomerization of KCC2 correlates with development of inhibitory neurotransmission. *J Neurosci Off J Soc Neurosci* 26:10407–10419.
- Brocard C, Plantier V, Boulenguez P, Liabeuf S, Bouhadfane M, Viallat-Lieutaud A, Vinay L, Brocard F (2016) Cleavage of Na<sup>+</sup> channels by calpain increases persistent Na<sup>+</sup> current and promotes spasticity after spinal cord injury. *Nat Med* 22:404–411.
- Chen Y-C, Tain Y-L, Sheen J-M, Huang L-T (2012) Melatonin utility in neonates and children. *J Formos Med Assoc Taiwan Yi Zhi* 111:57–66.
- Clayton AM, Shao Q, Paauw ND, Giambrone AB, Granger JP, Warrington JP (2018) Postpartum increases in cerebral edema and inflammation in response to placental ischemia during pregnancy. *Brain Behav Immun* 70:376–389.
- Coats LE, Bakrania BA, Bamrick-Fernandez DR, Ariatti AM, Rawls AZ, Ojeda NB, Alexander BT (2021) Soluble guanylate cyclase stimulation in late gestation does not mitigate asymmetric intrauterine growth restriction or cardiovascular risk induced by placental ischemia in the rat. *Am J Physiol Heart Circ Physiol* 320:H1923–H1934.
- Coq JO, Delcour M, Massicotte VS, Baud O, Barbe MF (2016) Prenatal ischemia deteriorates white matter, brain organization, and function: implications for prematurity and cerebral palsy. *Dev Med Child Neurol* 58:7–11.
- Coq JO, Delcour M, Ogawa Y, Peyronnet J, Castets F, Turle-Lorenzo N, Montel V, Bodineau L, Cardot P, Brocard C, Liabeuf S, Bastide B, Canu M-H, Tsuji M, Cayetanot F (2018) Mild Intrauterine Hypoperfusion Leads to Lumbar and Cortical Hyperexcitability, Spasticity, and Muscle Dysfunctions in Rats: Implications for Prematurity. *Front Neurol* 9:423.



- Coq J-O, Kochmann M, Lacerda DC, Khalki H, Delcour M, Toscano AE, Cayetanot F, Canu M-H, Barbe MF, Tsuji M (2019) From cerebral palsy to developmental coordination disorder: Development of preclinical rat models corresponding to recent epidemiological changes. *Ann Phys Rehabil Med*.
- da Silva TFG, de Bem GF, da Costa CA, Santos IB, Soares R de A, Ognibene DT, Rito-Costa F, Cavalheira MA, da Conceição SP, Ferraz MR, Resende AC (2021) Prenatal hypoxia predisposes vascular functional and structural changes associated with oxidative stress damage and depressive behavior in adult offspring male rats. *Physiol Behav* 230:113293.
- Delcour M, Olivier P, Chambon C, Pansiot J, Russier M, Liberge M, Xin D, Gestreau C, Alescio-Lautier B, Gressens P, Verney C, Barbe MF, Baud O, Coq JO (2012a) Neuroanatomical, Sensorimotor and Cognitive Deficits in Adult Rats with White Matter Injury following Prenatal Ischemia. *Brain Pathol* 22:1–16.
- Delcour M, Russier M, Amin M, Baud O, Paban V, Barbe MF, Coq JO (2012b) Impact of prenatal ischemia on behavior, cognitive abilities and neuroanatomy in adult rats with white matter damage. *Behav Brain Res* 232:233–244.
- Delcour M, Russier M, Xin D, Massicotte VS, Barbe MF, Coq JO (2011) Mild musculoskeletal and locomotor alterations in adult rats with white matter injury following prenatal ischemia. *Int J Dev Neurosci* 29:593–607.
- Fleiss B, Gressens P, Stolp HB (2020) Cortical Gray Matter Injury in Encephalopathy of Prematurity: Link to Neurodevelopmental Disorders. *Front Neurol* 11:575.
- Fleiss B, Van Steenwinckel J, Bokobza C, K Shearer I, Ross-Munro E, Gressens P (2021) Microglia-Mediated Neurodegeneration in Perinatal Brain Injuries. *Biomolecules* 11.
- Gackière F, Vinay L (2015) Contribution of the Potassium-Chloride Cotransporter KCC2 to the Strength of Inhibition in the Neonatal Rodent Spinal Cord In Vitro. *J Neurosci* 35:5307–5316.
- Graham HK, Rosenbaum P, Paneth N, Dan B, Lin J-P, Damiano DL, Becher JG, Gaebler-Spira D, Colver A, Reddihough DS, Crompton KE, Lieber RL (2016) Cerebral palsy. *Nat Rev Dis Primer* 2:15082.
- Granger JP, LaMarca BBD, Cockrell K, Sedeek M, Balzi C, Chandler D, Bennett W (2006) Reduced uterine perfusion pressure (RUPP) model for studying cardiovascular-renal dysfunction in response to placental ischemia. *Methods Mol Med* 122:383–392.
- Hagberg H, Mallard C, Ferriero DM, Vannucci SJ, Levison SW, Vexler ZS, Gressens P (2015) The role of inflammation in perinatal brain injury. *Nat Rev Neurol*.
- Hahad O, Lelieveld J, Birklein F, Lieb K, Daiber A, Münzel T (2020) Ambient Air Pollution Increases the Risk of Cerebrovascular and Neuropsychiatric Disorders through Induction of Inflammation and Oxidative Stress. *Int J Mol Sci* 21:E4306.
- Hamdy N, Eide S, Sun H-S, feng Z-P (2020) Animal models for neonatal brain injury induced by hypoxic ischemic conditions in rodents. *Exp Neurol*:113457.
- Heeren T, Joseph RM, Allred EN, O’Shea TM, Leviton A, Kuban KCK (2017) Cognitive functioning at the age of 10 years among children born extremely preterm: a latent profile approach. *Pediatr Res* 82:614–619.

- Hirschberger RG, Kuban KCK, O'Shea TM, Joseph RM, Heeren T, Douglass LM, Stafstrom CE, Jara H, Frazier JA, Hirtz D, Rollins JV, Paneth N, ELGAN Study Investigators (2018) Co-occurrence and Severity of Neurodevelopmental Burden (Cognitive Impairment, Cerebral Palsy, Autism Spectrum Disorder, and Epilepsy) at Age Ten Years in Children Born Extremely Preterm. *Pediatr Neurol* 79:45–52.
- Jantzie LL, Corbett CJ, Berglass J, Firl DJ, Flores J, Mannix R, Robinson S (2014a) Complex pattern of interaction between in utero hypoxia-ischemia and intra-amniotic inflammation disrupts brain development and motor function. *J Neuroinflammation* 11:131.
- Jantzie LL, Corbett CJ, Firl DJ, Robinson S (2015a) Postnatal Erythropoietin Mitigates Impaired Cerebral Cortical Development Following Subplate Loss from Prenatal Hypoxia–Ischemia. *Cereb Cortex* 25:2683–2695.
- Jantzie LL, Getsy PM, Denson JL, Firl DJ, Maxwell JR, Rogers DA, Wilson CG, Robinson S (2015b) Prenatal Hypoxia-Ischemia Induces Abnormalities in CA3 Microstructure, Potassium Chloride Co-Transporter 2 Expression and Inhibitory Tone. *Front Cell Neurosci* 9:347.
- Jantzie LL, Getsy PM, Firl DJ, Wilson CG, Miller RH, Robinson S (2014b) Erythropoietin Attenuates Loss of Potassium Chloride Co-Transporters Following Prenatal Brain Injury. *Mol Cell Neurosci* 61:152–162.
- Jantzie LL, Miller RH, Robinson S (2013) Erythropoietin signaling promotes oligodendrocyte development following prenatal systemic hypoxic-ischemic brain injury. *Pediatr Res* 74:658–667.
- Jantzie LL, Oppong AY, Conteh FS, Yellowhair TR, Kim J, Fink G, Wolin AR, Northington FJ, Robinson S (2018) Repetitive Neonatal Erythropoietin and Melatonin Combinatorial Treatment Provides Sustained Repair of Functional Deficits in a Rat Model of Cerebral Palsy. *Front Neurol* 9 Available at: <https://www.frontiersin.org/articles/10.3389/fneur.2018.00233/full> [Accessed April 24, 2018].
- Jantzie LL, Robinson S (2015) Preclinical Models of Encephalopathy of Prematurity. *Dev Neurosci* 37:277–288.
- Jantzie LL, Winer JL, Corbett CJ, Robinson S (2016) Erythropoietin Modulates Cerebral and Serum Degradation Products from Excess Calpain Activation following Prenatal Hypoxia-Ischemia. *Dev Neurosci* 38:15–26.
- Jia L, Wang J, Cao H, Zhang X, Rong W, Xu Z (2020) Activation of PGC-1 $\alpha$  and Mitochondrial Biogenesis Protects Against Prenatal Hypoxic–ischemic Brain Injury. *Neuroscience* 432:63–72.
- Kitase Y, Sato Y, Arai S, Onoda A, Ueda K, Go S, Mimatsu H, Jabary M, Suzuki T, Ito M, Saito A, Hirakawa A, Mukai T, Nagamura-Inoue T, Takahashi Y, Tsuji M, Hayakawa M (2020) Establishment of a Novel Fetal Growth Restriction Model and Development of a Stem-Cell Therapy Using Umbilical Cord-Derived Mesenchymal Stromal Cells. *Front Cell Neurosci* 14:212.
- Kurinczuk JJ, White-Koning M, Badawi N (2010) Epidemiology of neonatal encephalopathy and hypoxic-ischaemic encephalopathy. *Early Hum Dev* 86:329–338.

- LaMarca B, Amaral LM, Harmon AC, Cornelius DC, Faulkner JL, Cunningham MW (2016) Placental Ischemia and Resultant Phenotype in Animal Models of Preeclampsia. *Curr Hypertens Rep* 18:38.
- Mazur M, Miller RH, Robinson S (2010) Postnatal erythropoietin treatment mitigates neural cell loss after systemic prenatal hypoxic-ischemic injury: laboratory investigation. *J Neurosurg Pediatr* 6:206.
- Mukai T, Di Martino E, Tsuji S, Blomgren K, Nagamura-Inoue T, Ådén U (2021) Umbilical cord-derived mesenchymal stromal cells immunomodulate and restore actin dynamics and phagocytosis of LPS-activated microglia via PI3K/Akt/Rho GTPase pathway. *Cell Death Discov* 7:46.
- Ogawa Y, Coq J-O, Tsuji M (2019) Intrauterine Mild Hypoperfusion Model Rats Have a Risk of Developing Metabolic Syndrome. *J Dev Orig Health Dis* 10:S219.
- Ohshima M, Coq JO, Otani K, Hattori Y, Ogawa Y, Sato Y, Harada-Shiba M, Ihara M, Tsuji M (2016) Mild intrauterine hypoperfusion reproduces neurodevelopmental disorders observed in prematurity. *Sci Rep* 6:39377.
- Olivier P, Baud O, Bouslama M, Evrard P, Gressens P, Verney C (2007) Moderate growth restriction: deleterious and protective effects on white matter damage. *Neurobiol Dis* 26:253–263.
- Olivier P, Baud O, Evrard P, Gressens P, Verney C (2005) Prenatal ischemia and white matter damage in rats. *J Neuropathol Exp Neurol* 64:998–1006.
- Ophelders DRMG, Gussenhoven R, Klein L, Jellema RK, Westerlaken RJJ, Hütten MC, Vermeulen J, Wassink G, Gunn AJ, Wolfs TGAM (2020) Preterm Brain Injury, Antenatal Triggers, and Therapeutics: Timing Is Key. *Cells* 9:E1871.
- Pascal A, Govaert P, Oostra A, Naulaers G, Ortibus E, Van den Broeck C (2018) Neurodevelopmental outcome in very preterm and very-low-birthweight infants born over the past decade: a meta-analytic review. *Dev Med Child Neurol* 60:342–355.
- Passera S, Boccazzi M, Bokobza C, Faivre V, Mosca F, Van Steenwinckel J, Fumagalli M, Gressens P, Fleiss B (2021) Therapeutic potential of stem cells for preterm infant brain damage: Can we move from the heterogeneity of preclinical and clinical studies to established therapeutics? *Biochem Pharmacol* 186:114461.
- Pierce SR, Barbe MF, Barr AE, Shewokis PA, Lauer RT (2007) Co-contraction during passive movements of the knee joint in children with cerebral palsy. *Clin Biomech Bristol Avon* 22:1045–1048.
- Pierrat V, Marchand-Martin L, Arnaud C, Kaminski M, Resche-Rigon M, Lebeaux C, Bodeau-Livinec F, Morgan AS, Goffinet F, Marret S, Ancel P-Y, EPIPAGE-2 writing group (2017) Neurodevelopmental outcome at 2 years for preterm children born at 22 to 34 weeks' gestation in France in 2011: EPIPAGE-2 cohort study. *BMJ* 358:j3448.
- Quezada S, Castillo-Melendez M, Walker DW, Tolcos M (2018) Development Of The Cerebral Cortex And The Effect Of The Intrauterine Environment. *J Physiol O* Available at: <https://physoc.onlinelibrary.wiley.com/doi/abs/10.1113/JP277151> [Accessed October 20, 2018].

- Rains ME, Muncie CB, Pang Y, Fan L-W, Tien L-T, Ojeda NB (2021) Oxidative Stress and Neurodevelopmental Outcomes in Rat Offspring with Intrauterine Growth Restriction Induced by Reduced Uterine Perfusion. *Brain Sci* 11.
- Robinson S, Corbett CJ, Winer JL, Chan LAS, Maxwell JR, Anstine CV, Yellowhair TR, Andrews NA, Yang Y, Sillerud LO, Jantzie LL (2018) Neonatal erythropoietin mitigates impaired gait, social interaction and diffusion tensor imaging abnormalities in a rat model of prenatal brain injury. *Exp Neurol* 302:1–13.
- Robinson S, Mikolaenko I, Thompson I, Cohen ML, Goyal M (2010) Loss of cation-chloride cotransporter expression in preterm infants with white matter lesions: implications for the pathogenesis of epilepsy. *J Neuropathol Exp Neurol* 69:565–572.
- Robinson S, Petelenz K, Li Q, Cohen ML, DeChant A, Tabrizi N, Bucek M, Lust D, Miller RH (2005) Developmental changes induced by graded prenatal systemic hypoxic–ischemic insults in rats. *Neurobiol Dis* 18:568–581.
- Robinson S, Winer JL, Kitase Y, Brigman JL, Jantzie LL (2021) Neonatal administration of erythropoietin attenuates cognitive deficits in adult rats following placental insufficiency. *J Neurosci Res* Available at: <http://onlinelibrary.wiley.com/doi/abs/10.1002/jnr.24815> [Accessed March 10, 2021].
- Ryan MJ, Gilbert EL, Glover PH, George EM, Masterson CW, McLemore GR, LaMarca B, Granger JP, Drummond HA (2011) Placental ischemia impairs middle cerebral artery myogenic responses in the pregnant rat. *Hypertens Dallas Tex* 1979 58:1126–1131.
- Sgandurra G, Lorentzen J, Inguaggiato E, Bartalena L, Beani E, Cecchi F, Dario P, Giampietri M, Greisen G, Herskind A, Nielsen JB, Rossi G, Cioni G, On behalf of The CareToy Consortium (2017) A randomized clinical trial in preterm infants on the effects of a home-based early intervention with the “CareToy System” van Wouwe JP, ed. *PLOS ONE* 12:e0173521.
- Tanaka E, Ogawa Y, Mukai T, Sato Y, Hamazaki T, Nagamura-Inoue T, Harada-Shiba M, Shintaku H, Tsuji M (2018) Dose-Dependent Effect of Intravenous Administration of Human Umbilical Cord-Derived Mesenchymal Stem Cells in Neonatal Stroke Mice. *Front Neurol* 9 Available at: <https://www.frontiersin.org/articles/10.3389/fneur.2018.00133/full> [Accessed June 21, 2018].
- Tsuji M, Coq JO, Ogawa Y, Yamamoto Y, Ohshima M (2018) A Rat Model of Mild Intrauterine Hypoperfusion with Microcoil Stenosis. *J Vis Exp* Available at: <https://www.jove.com/video/56723/a-rat-model-of-mild-intrauterine-hypoperfusion-with-microcoil-stenosis> [Accessed January 18, 2018].
- Vogel JP, Chawanpaiboon S, Moller A-B, Watananirun K, Bonet M, Lumbiganon P (2018) The global epidemiology of preterm birth. *Best Pract Res Clin Obstet Gynaecol* 52:3–12.
- Volpe JJ (2009) Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. *Lancet Neurol* 8:110–124.
- Wingert JR, Burton H, Sinclair RJ, Brunstrom JE, Damiano DL (2008) Tactile sensory abilities in cerebral palsy: deficits in roughness and object discrimination. *Dev Med Child Neurol* 50:832–838.

Wixey JA, Chand KK, Colditz PB, Bjorkman ST (2017) Review: Neuroinflammation in intrauterine growth restriction. *Placenta* 54:117–124.

Xerri C, Bourgeon S, Coq JO (2005) Perceptual context-dependent remodeling of the forepaw map in the SI cortex of rats trained on tactile discrimination. *Behav Brain Res* 162:207–221.

Yin shaowei, Wang Y, Meng Y-L, Liu cai-X (2020) Effects of mild intrauterine hypoperfusion in the second trimester on memory and learning function in rat offspring. *Neural Regen Res* 15:2082–2088.

Yin S, Meng Y, Liu C, Wang Y (2021) MIUH Inhibits the Hippocampal Neuron Growth in Fetal Rat by Affecting the PTEN Pathway. *Neurochem Res* 46:2046–2055.