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# Cortical grey matter injury in Encephalopathy of Prematurity: Link to neurodevelopmental disorders

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## Abstract

Preterm born infants frequently suffer from an array of neurological damage, collectively termed Encephalopathy of Prematurity (EoP). They also have an increased risk of presenting with a neurodevelopmental disorder (e.g. autism spectrum disorder; attention deficit hyperactivity disorder) later in life. It is hypothesised that it is the grey matter injury to the cortex, in addition to white matter injury, injury to the cortex in EoP that is responsible for the altered behaviour and cognition in these individuals. However, the-although it is established that grey matter injury that occurs in infants following preterm birth that exact nature of these changes is still not fully elucidated. Here we will review the current state of knowledge in this field, amalgamating data from both clinical and preclinical studies. This will be placed in the context of normal processes of developmental biology and the known pathophysiology of neurodevelopmental disorders. Novel diagnostic and therapeutic tactics required integration of this information so that in the future we can combine mechanism based approaches with patient stratification to ensure the most efficacious and cost-effective clinical practice.

## Abbreviations:

ADHD - Attention Deficit Hyperactivity Disorder; ASD - Autism Spectrum Disorder; EEG - Electroencephalogram; EoP - Encephalopathy of Prematurity; GM - Grey Matter; FA - fractional anisotropy; IVH - Intraventricular Haemorrhage; MRI - Magnetic Resonance Imaging; NDD - Neurodevelopmental Disorder; PVL - Periventricular Leukomalacia; WM - White Matter

## Introduction

Preterm birth is defined as delivery before 37 completed weeks of gestation, and although the shorter the gestation the higher risk of mortality and morbidity, even the late preterm born infants are vulnerable to injury, including to the brain. The hallmarks of brain injury to the preterm born infant are: neuroinflammation, oligodendrocyte maturation arrest and hypomyelination, axonopathy, reduced fractional anisotropy and cortical volume determined by magnetic resonance imaging (MRI), and eventually, significant cognitive deficits (Volpe 2009). Collectively the brain damage associated with preterm birth is called ~~the~~ Encephalopathy of Prematurity (EoP).

As long-term cognitive and behavioural consequences of preterm birth are increasingly recognised, neuropathological studies have focused on grey matter (GM), in addition to the white. It was initially thought that cortical GM injury only occurred in preterm infants in cases of very severe injury. Increased understanding of cortical development and more detailed post-mortem studies revealed that this not the case (Kostovic *et al.* 1995, Marin-Padilla 1997). Over the past few years work increasingly indicates a widespread subtle neuronal injury in infants born preterm, that in some cases, (such as interneuron deficits,) may be independent of white matter (WM) injury (Panda *et al.* 2018). Further to this, swathes of clinical and preclinical studies indicate that the cortical GM injury found in preterm infants significantly contributes to their increased risk of neurodevelopmental disorder (NDD), such as autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD) and other learning and behavioural disorders.

The large and long running EPICURE (UK), EPIPAGE (France) and ELGAN (USA) studies have provided invaluable data on the incidence of neurological injury following very premature birth (e.g. Marlow *et al.* 2005, O'Shea *et al.* 2009, Pierrat *et al.* 2017). Together these epidemiological studies confirm that preterm infants have 25-30% incidence of neurological disorder, with as many as 40% of affected individuals having more than one diagnosable disorder (Marlow *et al.* 2005, O'Shea *et al.* 2009, Pierrat *et al.* 2017). In all studies, incidence of cerebral palsy was 5-8% of preterm children, consistent between 2, 6 and 10 years of age (Marlow *et al.* 2005, Johnson *et al.* 2010, Pierrat *et al.* 2017, Hirschberger *et al.* 2018). In addition, more than 40% of children at 2 years were below threshold on the for communication, motor, problem solving and social skills (Pierrat *et al.* 2017), 30% of children were diagnosed with cognitive impairment at 6 years of age (Marlow *et al.* 2005), while at school age (10-11 years), 7-8% of preterm born children were diagnosed with ASD, 11% with ADHD, and 10% with emotional disorders such as anxiety (Johnson *et al.* 2010, Hirschberger *et al.* 2018). Using latent profile analysis in school age preterm born children (10 years of age), 25% of children

were shown to have impaired executive functioning across a range of cognitive domains, while 41% of children fell into a 'low-normal' category, where impairment was related to reasoning and working memory (Heeren *et al.* 2017).

## Environmental contributors to EoP and mechanisms of injury

The maternal fetal membranes surrounding the amniotic cavity represent the boundaries of a sort of 'black box', inside which we struggle to know and understand the processes preceding preterm birth. This is due to technical difficulties in safely monitoring the biochemical processes ongoing in the uterine space. However, processes causing brain injury in the preterm born infants certainly begin before delivery, as indicated by a small study of brain functional connectivity in fetuses who went on to be born preterm (Thomason *et al.* 2017) and an increasingly number of studies showing predictive biomarkers in maternal blood months before preterm birth (Nunthapiwat *et al.* 2019, Souza *et al.* 2019, Leow *et al.* 2020). Numerous events and antenatal exposures have also been associated with preterm birth and EoP via epidemiological study and verified with preclinical studies. These include placental abruption or twin-twin transfusion, preeclampsia or placental insufficiency (potentially contributing to a hypoxic-ischemic like insult and/or intrauterine growth retardation), and, less commonly, complications linked to oligohydramnios and maternal substance abuse (Ananth and Vintzileos 2008). A predominant role of hypoxia in EoP with no other complications (such as those described above) is not supported by clinical data (Gilles *et al.* 2018). Chorioamnionitis, leading to a maternal-fetal inflammatory response, is a chief driver of the process of early parturition leading to preterm birth - demonstrated across clinical and preclinical studies (Kaga *et al.* 1996, Romero *et al.* 2001, Fidel *et al.* 2003). Maternal-foetal inflammatory response not only precipitates preterm birth, but a wealth of epidemiological and clinical studies have shown that although it is often clinically silent it is a major driver of EoP (Dammann and Leviton 1997; Lau *et al.* 2005) and its associated long-term neurological and behavioral/psychiatric deficits (see reviews, Hagberg *et al.* 2015, Kuban *et al.* 2015). While EoP can be initiated prenatally, there is evidence of continued disruption of the brain postnatally, that could be driven by a mixture of pre- and postnatal factors. For instance, Bouyssi-Kobar *et al.* (2016) show reduced brain growth trajectories in preterm born infants compared to age-matched *in utero* controls, associated with (antenatal) chorioamnionitis, as well as postnatal sepsis. Inflammatory drivers include pre- and post-natal events and conditions: chorioamnionitis, funisitis, early and late onset sepsis and necrotizing enterocolitis. Other, non-inflammatory, postnatal contributors to EoP may include hyperoxia (Wellmann *et al.* 2014), as the *ex utero* environment is relatively high in oxygen compared to the *in utero* environment (Manley *et al.* 2017) and reduced exposure to maternal hormones such as oestrogen and other neuroactive precursors (Shaw *et al.* 2019). While there has been little follow up of the oestrogen hypothesis clinically (Hunt *et al.* 2004), recent animal models have suggested a potential protective effect (Panda *et al.* 2018, Tibrewal *et al.* 2018, Klebe *et al.* 2019). That hyperoxia plays a role in EoP is also supported by animal studies (Vottier *et al.* 2011, Schmitz *et al.* 2012, Scheuer *et al.* 2015).

## Cellular mediators of brain injury

How specifically do these perinatal events lead to EoP? In the case of maternal-foetal inflammation, systemic inflammation drives changes in the brain after either crossing directly through the endothelial cells making up the blood-brain barrier or by stimulating, via receptors for cytokines such as interleukin-1 (IL-1), production of pro-inflammatory molecules by the

endothelial cells that are secreted into the brain parenchyma (Banks *et al.* 1995, Verma *et al.* 2006). It is currently unclear whether these immune mediators act directly on neurons, or have their actions via stimulation of glial cells such as microglia and astrocytes, though it is likely that both processes occur. Inflammation has been shown in the foetal brain to reduce neurogenesis in embryonic proliferative zones (Stolp *et al.* 2011) or to increase neurogenesis in the SVZ and dentate gyrus (Pang *et al.* 2016), which has been linked to microglia activity in some cases (reviewed in Sato 2015, Fan and Pang 2017, Prins *et al.* 2018). ~~How neuroinflammatory processes effect neuron is still being explored. In one example, neurons cultured from foetal mice after maternal administration of lipopolysaccharide four hours prior, demonstrated delayed/disrupted maturation *in vitro*, compared to controls, in the absence of microglia during their time *in vitro* (Burd *et al.* 2010).~~ The timing of the exposure of neurons to inflammatory stimuli is undoubtedly critical. The exact timing of events impacting EoP is not clear, though it is likely events occurring in the third trimester will be most influential. Although the vast amount of proliferation is complete in the third trimester there are specific cell types, interneurons of note, that are still being born and migrating in this period and which are increasingly demonstrated to be vulnerable in preterm born infants and animal models (Malik *et al.* 2013, Stolp *et al.* 2019). Susceptibility of processes such as neuronal arborisation and synapse formation may be even more important, given the intersect between injury events sensitising to EoP and the developmental timetable of the brain (discussed in more detail below).

Over the past decade the importance of microglial activation has been exhaustively demonstrated in human preterm born infant post-mortem brain samples and in models of perinatal brain injury (reviewed in Mallard *et al.* 2019, McNamara and Miron 2020). These studies have included experimental evidence that microglia are necessary for the evolution of injury in the developing brain (Dommergues *et al.* 2003, Van Steenwinckel *et al.* 2019) but conversely that microglia also play protective roles in perinatal brain injury (Faustino *et al.* 2011, Fernández-López *et al.* 2016). Microglia are also activated by other modulators of brain injury, such as hypoxia, or hypoxia-ischemia, as these events lead to cell injury and the release of damage associated messenger proteins (DAMPs) and/or the production of toxic metabolites that also activate microglia directly (Lafemina *et al.* 2006, Gao and Tsirka 2011). Early microglia establish territories in the developing brain from early in embryonic life day 40-42.5 in mouse, and are intimately involved in the processes of brain building ~~and especially synaptic pruning from embryonic day 14.5 to postnatal day 9, before undertaking immune surveillance and maintaining the health of the brain via the clearance of protein aggregates (from 6 weeks plus; Matecovitch-Natan *et al.* 2016).~~ Thus, microglia activation to an immune responsive state leads to EoP via a double hit, whereby there is production of toxic factors for neighbouring neural cells and loss of normal microglial functions to shape axonal connectivity and synaptic elimination/function. This has been well described in the WM (Pang *et al.* 2010, Favrais *et al.* 2011, Scafidi *et al.* 2014, Xie *et al.* 2016). There is substantially less information on the specific effects of activated microglia on the GM - including synapses and interneurons which is relevant to EoP. However, as increasing evidence shows that microglial dysfunction persists for weeks to years after insult (Ramlackhansingh *et al.* 2011, Loane *et al.* 2014, Mattei *et al.* 2017, Frost *et al.* 2019) the importance of this phenomena may become more apparent with further study. It is also plausible that the GM and WM are differentially vulnerable as microglia in these tissues have differing gene expression patterns in the basal state and after injury based on studies in adults (Lawson *et al.* 1990, Hart *et al.* 2012). However, nothing is known of this difference in a model relevant to EoP. As such, although we can speculate that

there may be specific soluble factors or regulators (microRNAs, cytokines etc) released (possible via vesicles) from GM microglia that influence GM development in ways that would offer therapeutic avenues (Verderio *et al.* 2012, Lener *et al.* 2015) evidence is still required.

Reactive astrogliosis is also observed in some forms of human perinatal WM injury (Back and Miller 2014, Shiw *et al.* 2017) and is associated with deleterious effects mediated by agents including hyaluronic acid (Preston *et al.* 2013), bone morphogenic protein (Wang *et al.* 2011), cyclooxygenase-2 induction and associated prostaglandin E2 production (Shiw *et al.* 2017), and endothelin-1 (Hammond *et al.* 2014) that can impair oligodendrocyte precursor cell maturation. ~~In the human WM, glial fibrillary protein (GFAP) positive astrocytes are present from gestational week 20 onwards, but rapid increases in cell number are reported from gestational week 28 onwards (Roessmann and Gambetti 1986). A similar phenomena was reported in the developing baboon, and the authors cautioned that this apparently increasing reactivity could be mistaken for an astroglial injury response (Dieni *et al.* 2004).~~ Clinical and experimental studies have shown a role for GFAP-positive astrocytes in WM injury in preterm born infants, but specifically in older preterm born infants (>32 weeks; Gelot *et al.* 2009, Verney *et al.* 2012) and during equivalent stages of rodent development (5 postnatal days plus; Tahraoui *et al.* 2001). In the GM, astrocytes increase in number with gestational age. ~~Compared to the WM, it must be noted that~~ GFAP positive cells represent a far smaller proportion of cells (less than 1%) ~~that also increase with increasing gestational age (Roessmann and Gambetti 1986), and response of the populations a whole to injury is under studied.~~ In preterm born infants, a small increase in the number of GM astrocytes was reported in a study of infants with cystic periventricular leukomalacia (cPVL; severe injury), but as the control group had a significantly lower gestational age this effect did not survive correcting for development (Pierson *et al.* 2007). However, studies in animals support the hypothesis that astrocytic dysfunction proceeds neuronal damage in at least some injury paradigms (Sullivan *et al.* 2010).

~~Of interest is that microglia and astrocytes can possibly have beneficial effects in the context of perinatal brain injury due to their abilities to produce a wide range of regenerative factors with the right input (Nobuta *et al.* 2012, Bylicky *et al.* 2018). How these protective functions can be enhanced and whether tuning these cells to help the WM and GM will require different approaches requires ongoing research.~~

## Developmental events susceptible to injury in the preterm brain

When assessing how pre- and postnatal factors contribute to EoP, it is necessary to consider what developmental events happen during the preterm period that may be affected by preterm birth. The prenatal period most associated with EoP (from 23-32 weeks), is characterised by the final stages of neurogenesis in the human telencephalon, neuronal migration, differentiation and maturation, and the very early stages of cortical myelination. Neurogenesis peaks very early in gestation (8-12 weeks), but continues both in the ventricular zone of the dorsal cortex and within the ganglionic eminences up to 29 weeks (Malik *et al.* 2013). The cortical plate forms around 11 weeks gestation (reviewed in Sidman and Rakic 1973) until shortly after the end of neurogenesis. Excitatory neurons primarily come from the progenitors in the ventricular zone of the dorsal cortex, and migrate radially to the cortical plate (reviewed in Sidman and Rakic 1973), while inhibitory cortical neurons derive from the ganglionic

eminences, and migrate tangentially to the cortical plate (reviewed in Marin 2013). Once neurons reach their final positions in the cortical plate, they start to mature, a process which includes extending dendritic arbours and forming synapses, detectable from 19-23 weeks gestation (Huttenlocher 1979). At the same time, radial projections of the neural progenitors are lost, and tangential extension of subcortical and cortico-cortical projections continues. These processes continue through the prenatal and postnatal period of brain development, with an extensive period of synaptic modulation and pruning throughout the first year of life. Local electrical activity and connectivity between neurons can be detected early, undergoing numerous changes over development, and doesn't appear to find a mature state until early adolescence (reviewed by Kilb *et al.* 2011). Details of these events and many of the mechanisms underlying them are reviewed extensively in Molnar *et al.* (2019) and Volpe (2019). On top of these microstructural changes, is a general increase in cortical thickness, and a semi-stereotypical pattern of cortical folding, with primary sulci forming from 16 to 19 weeks gestation, and secondary and tertiary sulci formation starting from 32 and 36 weeks respectively (Armstrong *et al.* 1995).

The real time development of the brain, including the increasing complexity in the cortical structure, can be detected with non-invasive imaging methods such as T1/T2 or diffusion-weighted MRI (Neil *et al.* 1998, McKinstry *et al.* 2002, Batalle *et al.* 2019; reviewed by Lodygensky *et al.* 2010) and these techniques have allowed the detection of delayed or impaired cortical development in preterm born infants. However, given the relatively low resolution, integrated nature of diffusion MRI signal, interpreting the specific structural changes in relation to neurodevelopmental processes is difficult. Techniques are in development to scale match MRI and histological data (e.g. Stolp *et al.* 2018), though changes in diffusion MRI are currently interpreted through comparison with standard histological measures. There is evidence that preterm birth can result in changes to all the processes of cortical development described above, including reduced progenitor proliferation, arborisation and myelination, as well as direct injury outcomes such as cell death. The rest of this review will discuss a number of these in detail, including potential mechanisms of injury, overlap with NDD and potential therapeutics.

## Macroscale alterations in cortical GM

Many elegant neuroimaging studies have begun to correlate both typical and pathological behaviour with specific brain areas or functional readouts. Based on these we know that many brain regions contribute to the diverse array of neurological disorders presented by preterm born children. In particular, it is increasingly clear the important role of cortical dysfunction underlying these cognitive disorders (but not so prominently in motor disorders). Rathbone *et al.* (2011), in their study of cortical growth (cerebral volume and cortical surface area) in the 20 weeks between birth and term equivalent age in preterm infants, showed that slow rates of cortical growth correlated directly with neurocognitive ability at 2 and 6 years of age. In particular, impaired cortical surface area growth correlated with poorer scores in numerous features of executive function, learning, memory and attention, as well as social ability. However, there was a clear lack of correlation between cortical growth and motor abilities (Rathbone *et al.* 2011). Numerous neuroimaging studies, using different post-imaging assessment methods, have shown reductions in cortical GM volumes in preterm infants, both in the preterm population as a whole ([Boardman \*et al.\*, 2006](#), Ajayi-Obe *et al.* 2000, Makropoulos *et al.* 2016), or specifically those with periventricular leukomalacia (PVL; Inder

*et al.* 1999, Zubiaurre-Elorza *et al.* 2011), and in very preterm born children ([assessed at 7 years](#); Zhang *et al.* 2015) and adolescence ([assessed at 15 years](#); Nosarti *et al.* 2002, Nosarti *et al.* 2008). Reductions in volume of the deep GM have also been reported (Ball *et al.* 2012, Bjuland *et al.* 2014); changes in thalamic volume were found to be a predictor of reduced cortical GM volume, and alterations in diffusivity within the thalamocortical networks (Ball *et al.* 2012) were found to correlate with cognitive performance at 2 years of age, though they only accounted for 11% of the variance (Ball *et al.* 2015).

Importantly, Bora *et al.* (2014) showed that very preterm infants had a 13% increased risk of inattention and hyperactivity behaviour at school age (4, 6 & 9 years), that correlated with decreased GM volumes, particularly within the prefrontal region. Increased anxiety like behaviour has also been associated with preterm born infants with reduced GM volume (Rogers *et al.* 2014). In a small study group, very preterm infants that went on to have a diagnosis of ASD were found to have increased incidence of cystic WM lesions, and reduced cerebellar volume, but not changes in GM volume (Ure *et al.* 2016). However, only 8 children in the cohort (4.7%) were diagnosed with ASD by the age of 7 years, so the study [may have been](#) underpowered for detecting more subtle changes in cortical GM.

In animal models, mimicking changes in ~~these exact~~ cortical volume parameters is difficult, due to differences in the relative GM and WM volumes in experimental species and the differences in the size/complexity of the individual regions relative to one another. Sheep are used in studies of perinatal brain injury, with advantages regarding physiological and neurological similarities to preterm humans including grey-white ratios (discussed in Jantzie and Robinson 2015). Dean *et al.* (2011), have studied ~~a sheep paradigm of~~ intra-amniotic LPS [in sheep, leading to inflammation significant enough a paradigm able](#) to cause cystic WM injury. This inflammatory exposure caused no obvious cell loss in the GM, but reduced cortical volume by approx. 18%. In further work in sheep models, Dean *et al.* (2013), also showed a reduction in cortical GM volume after *in utero* hypoxia-ischemia in which there was again no cortical cell loss. Interestingly, there was no early reduction in cortical volume (+7 days) but ~~these cortical reductions~~ became increasingly apparent with ~~greater~~ time after injury (starting at 2 weeks and at least up to 4 weeks; Dean *et al.* 2013). In a mouse model of maternal immune activation using poly:I:C, subtle decreases in GM volumes were observed throughout development (Crum *et al.* 2017), though changes in cortical volumes were not specifically reported.

In addition to alterations in GM volume, complex changes in cortical architecture have also been identified. For instance, Zhang *et al.* (2015) determined that there was a decrease in cortical surface area and the gyrification index of 7 year olds following very preterm birth compared to term born controls. Maps of cortical folding patterns in neonates suggest that preterm infants have fewer and shallower sulci than term equivalent controls (Engelhardt *et al.* 2015). Data suggested that the lower gyrification index and cortical surface area in preterm born neonates was likely to be due to a combination of altered *in utero* and postnatal growth, and was a finding independent of reduced total brain volume (Engelhardt *et al.* 2015, Kersbergen *et al.* 2016). Reduced cortical folding has also been associated with increased incidence of NDDs later in life (Kersbergen *et al.* 2016), matching, at least partly with data from specific disorder cohorts (Wolosin *et al.* 2009, Kohli *et al.* 2019, Kohli *et al.* 2019, Libero *et al.* 2019), though the data is not substantial here, as existing studies are small.



Collectively, this data points to the possibility that alterations in cortical folding are driven by numerous age-specific microstructural changes. The theories behind cortical folding are many, and currently no single one is sufficient to explain the biological underpinning to normal or abnormal cortical folding, and to date including processes such as the rate of neurogenesis, processes of tangential migration and neuronal arborisation (reviewed by Bayly *et al.* 2014, Striedter *et al.* 2015, Llinares-Benadero and Borrell 2019). Numerous aspects of the *in utero* environment and preterm injury have been associated with changes in cortical folding (reviewed by Quezada *et al.* 2018), though the mechanism by which this injury is produced is still unclear. Recent work by Garcia *et al.* (2018) has shown regional differences in cortical growth rate between post menstrual age 30 and term equivalent age (based on 2-4 MRI scans over this period in preterm born infants), that are disrupted in preterm born infants with gross injury such as intraventricular haemorrhage (IVH). Their work suggests that severe injury in preterm born infants may alter local cortical growth and subsequently cortical folding (Garcia *et al.* 2018), supporting the hypothesis that cortical folding may result from mechanical instability as the GM grows faster than underlying WM (Van Essen *et al.* 2019). Alternatively, recent compelling evidence also shows that the extracellular matrix is essential in normal cortical folding (Long *et al.* 2018), likely contributing to the mechanical tension within the brain. The link between these highly reductionist *ex vivo* studies and EoP is currently unclear, though they have suggested that hyaluronic acid can inhibit cortical folding (Long *et al.* 2018), and increases in hyaluronic acid have been found within the preterm brain (Buser *et al.* 2012).

## Grey matter neuropathology associated with EoP

There are few neuropathological studies of GM injury in preterm born infants, compared to the number of studies of WM injury. Complicating matters, due to the difficulties of defining appropriate controls, GM studies typically use evidence of WM injury as a starting point in the assessment of the GM rather than searching for independent patterns of injury. We have created **Table 1** that summarizes studies performed on human preterm born post-mortem tissue that have included GM analyses. From this we can generalise that in studies of infants with severe and contemporaneously uncommon WM injury (cystic PVL) there are reductions in neuronal number and increased neuronal cell death (where assessed; 6/6 studies). However, in studies of infants predominantly with diffuse WM injury, global reductions in cell number are less frequently reported (1/5 studies), but interneurons seem to be a vulnerable subpopulation (present in 3/3 studies ~~where assessed~~) and dysmaturation in cerebellar lamination are reported (1 study ~~where assessed~~). Interestingly, ~~for interneurons~~, there are complex subtle changes in interneurons in cases with non-cystic WM injury versus controls (Stolp *et al.* 2019), and ~~also changes~~ when comparing very preterm infants to less preterm infants (irrespective of WM injury; Panda *et al.* 2018). It is necessary to note that of the 12 studies identified, six of these were performed on archival tissue collected between 1993-2007 from the Department of Pathology at the Children's Hospital Boston. An additional observation study was not included in the table as the data were expanded upon in a later study (Haynes *et al.* 2003). It is not possible from the published details of the Boston groups work to determine whether cases in these studies have been reused. Thus, reports of cell death across regions in these studies may be interdependent due to case severity in this centre, and studies of other centres and in more contemporaneous cohorts are needed to determine the state of neuronal injury in preterm born infants more generally.

~~Understanding the presence and mechanisms of cell death are important for understanding injury and developing therapies. A recent thorough review was undertaken on cell death in preterm born infant and animal models (Truttmann *et al.* 2020). The authors build a rational case that the current use of cleaved caspase 3 and TUNEL underestimates the occurrence of cell death in preterm born infants. In support for this, in studies of diffuse WM injury relevant to contemporaneous infants exhaustive cell counts do show changes in cell number in the thalamus (Vontell *et al.* 2015). However, with the same approach in the same infants there was no cell loss in the cortex (Stolp *et al.* 2019) indicating that we must indeed look harder for cell loss, but that it may still be highly region and injury type specific.~~

## Animal models of GM pathology – Severe injury

Severe brain injury including cystic lesions and severe IVH occurs in very few preterm born infants (<5% cystic lesions, <5% IVH; Ancel *et al.* 2006). Historically the proportion of infants with these forms of injury was much higher. ~~Also,~~ it was also once considered that hypoxia/ischemia was the leading (possibly the predominant) cause of perinatal injury, including in preterm born infants. Because of these historical trends and (now updated) ideas, much of the data that we have on GM injury in EoP is from animal models of gross clastic lesion (30-80% hemispheric ablation). This initial wave of data suggested that of the cortical layers, the subplate was most susceptible to hypoxic-ischemic injury at preterm equivalent ages (McQuillen *et al.* 2003), possibly due to its relatively early birth and maturation. However, subsequent studies agree that the extent of cortical injury is dependent on the severity of the insult, and all lower cortical layers have the capacity for cell loss after severe HI (Okusa *et al.* 2014). In a study of partial uterine artery occlusion, modelling hypoxia-ischemia in the foetal sheep, immediate low level necrotic cell death was found throughout the deep and cortical GM, followed by extensive apoptosis in both the GM and WM at 3 hours post-injury (Goni-de-Cerio *et al.* 2007). Other studies of *in utero* hypoxia-ischemia in sheep have shown some increase in pyknotic cells and activated caspase 3 staining from 24 hours up to 4 weeks in the caudate nucleus and subplate (McClendon *et al.* 2014, McClendon *et al.* 2017), reduced NeuN and somatostatin positive neurons in the caudate and putamen (Ardalan *et al.* 2019), specific loss of glutamate decarboxylase interneurons (a marker of GABAergic neurons) and their perineuronal nets in the cortex (Fowke *et al.* 2018), along with reduced arborisation complexity and spine density in both the caudate, hippocampus and cortex (Dean *et al.* 2013, McClendon *et al.* 2014, McClendon *et al.* 2017, Ardalan *et al.* 2019, McClendon *et al.* 2019).

## Animal models of GM pathology – Moderate/mild injury

Improvements in antenatal and postnatal care, including the use of prenatal steroids and postnatal surfactants and improved respiratory support have collectively led to the decrease in severe brain injury so that now the vast majority of infants suffer from diffuse WM injury (Buser *et al.* 2012). This has inspired the revision / creation of animal models focused on modelling white matter dysmaturation. A number of these new(er) models providing insights into the role of contemporaneously relevant insults to GM injury are described. A landmark study in our understanding of the GM injury induced by preterm birth came from the team led by Sandra Rees (Dieni *et al.* 2004), wherein they delivered baboons preterm and held them in a NICU environment for two weeks. This important study isolated the roles of prematurity itself from exogenous/precipitating factors (such as chorioamnionitis and sepsis). WM injury and haemorrhage were most common in preterm baboons, but there were significant pockets

of necrosis in layer IV/V cortical neurons (4/16 cases) and in the head of the caudate (1/16 cases). One of the first attempts to nail down the cellular substrate of GM injury was the analysis of the effects of intrauterine hypoxia-ischemia on the fetal sheep (Dean et al. 2013). Although it can be debated whether hypoxia-ischemia is particularly relevant to the majority of cases of EoP (Gilles et al. 2018), this team used cutting edge combinations of high field MRI and detailed neuropathological assessments of cell number and structure to reveal novel insights into brain injury. The team found that overall reductions in GM volume were not precipitated by neuronal cell loss, but that there were frank changes in dendritic arborizations (length, number, intersections; Dean et al. 2013).

Sheep models of moderate inflammatory injury are also providing important information. Exposure to intra-amniotic inflammation prior to preterm birth, was used to reveal the evolution of *in utero* inflammatory brain injury (Gussenhoven et al. 2018). Interestingly, sheep were all exposed to inflammation on the same gestational day, but sub-groups were culled every 24 hours providing a detailed time course of events. Cleaved caspase-3 positive cells were increased in number in the hippocampus at 2, 4, 8, and 15 days following LPS exposure, and in the cortex (at 8 days only), along with increases in MAP2 staining in both GM regions. Strikingly, there was only moderate WM injury (few cleaved caspase-3 positive cells, microgliosis and mild demyelination) suggesting that neurons may be vulnerable to injury in the absence of [frankoverl](#) WM damage. Reduced cortical neurons were found in another study exposing the developing sheep to LPS (Stojanovska et al. 2018), seven days after a single LPS challenge. In these experiments there was no difference in either astrogliosis or microgliosis at the time point analysed compared with the previous sheep study (Gussenhoven et al. 2018) in which microgliosis was present, but astrogliosis was not.

Rodent models are by far the most common for studying potential neuropathology of EoP. In a rat model of inflammatory exposure (maternal LPS exposure at the end of gestation), significant postnatal reduction in brain and body weight were observed, and a small increase in cell death in the striatum and germinal matrix (Rousset et al. 2006). In a milder injury model of prolonged induction of systemic inflammation (using systemic IL-1 $\beta$  exposure; Favrais et al. 2011), there was no gross body weight change, no evidence of caspase-3 positive dying cells or alteration in the number of NeuN positive neurons in the neocortex. However, in this injury model there were numerous changes in gene expression for synaptic and neurotransmission related genes (Fleiss et al. 2015). In this same animal model, and similar to data reported in human cases, a specific alteration in interneuron number was identified in the neocortex (Stolp et al. 2019); a finding supported by a number of other early-life inflammatory exposure models (Canetta et al. 2016, Vasistha et al. 2019) and preterm birth models (Panda et al. 2018). It is likely that migration and differentiation of these cell populations is affected, though many studies show that injury reduces or repairs in adult mice following early-life inflammation (Stolp et al. 2019, Vasistha et al. 2019). The important advantage of rodent models is the potential for behavioural testing, where aspects of human clinical disease can be recapitulated. In the inflammatory injury models just described behavioural dysfunction has been reported, including reduced social interaction (Vasistha et al. 2019), short and long-term memory deficits (Favrais et al. 2011, Van Steenwinckel et al. 2019), attention-shifting deficits and anxiety-like behaviour (Canetta et al. 2016). These behaviours are commonly found in preterm infants, as described above, and in other NDDs, thus supporting the face validity of these models. This is further supported by an extensive body of work showing reduction in GABAergic interneurons, or expression of [parv](#)albumin (as distinct from a reduction in cell number) in

clinical ASD cases (Zikopoulos and Barbas 2013, Adorjan *et al.* 2017, Lunden *et al.* 2019), and genetic models of NDDs (Yekhelef *et al.* 2015, Lauber *et al.* 2018, Wiebe *et al.* 2019).

## EoP as a synaptopathy

A synaptopathy is a disease or disorder caused by dysfunction of synapses. This dysfunction can arise due to a mutation in a gene encoding a synaptic-related protein such as an ion channel, a neurotransmitter receptor, or a protein involved in neurotransmitter release; alternatively a synaptopathy may be due to structural deficits in extension of neuronal arbours and synaptic process. Whether EoP can be defined as synaptopathy requires further study, but we suggest that this is likely to be an important part of the neuropathology of this condition. The changes in EoP of gross grey matter volume changes, variations in growth rate and patterns of cortical folding discussed above all reflect a combination of microstructural deficits (Ball *et al.* 2013) and connectivity (Ball *et al.* 2013, Pandit *et al.* 2014, Ball *et al.* 2015) including delayed acquisition of the default mode network, as assessed by MRI techniques. Additionally, there is the fact that EoP predisposes to strikingly increased odds of a diagnosis of a NDD that are clearly recognised as synaptopathies such as: ASD, up to 17 fold increased rates (Limperopoulos *et al.* 2008, Johnson *et al.* 2010); attention deficit disorder, up to 2.5 fold increased rates (Delobel-Ayoub *et al.* 2009, Lindstrom *et al.* 2011); epilepsy, up to 5 fold increased rates (Hagberg *et al.* 1996, Crump *et al.* 2011); and decreases in IQ directly proportional to the severity of their preterm birth (Wood *et al.* 2005, Costeloe *et al.* 2012).

Considering the developmental events happening during the period of preterm birth, it may be expected that alterations should be found in patterns of neuronal migration, time frames and degrees of arborisation, axon extension and synapse formation. On this subject, the recent study by Petrenko *et al.* (2018), provides a number of important insights. In a highly reductionist model of selective cell neuronal apoptosis in layer 5 of the cortex, induced by diphtheria toxin (Petrenko *et al.* 2015), the authors showed a progressive loss of ~20% of neurons within the cortex over a 14 day period. While this degree of neuronal loss is unlikely to occur in EoP, the pre and post-apoptosis changes to the brain have interesting correlates for the injury observed in EoP. Specifically, there was an associated increasing presence of astro- and microgliosis, retraction of dendritic arbours in dying neurons (days 3 - 5) and increased arborisation (branch number and length) in the surviving neurons (day 14; Petrenko *et al.* 2018). Alterations in dendritic arborisation have been found in the GM in a number of experimental studies, many of which have already been referred to [above](#) (e.g. Dean *et al.* 2013, Gussenhoven *et al.* 2018). Additionally, a model of intrauterine growth restriction in pig, initiated at 100 days of pregnancy, and assessed 22 days later showed a loss of MAP2 staining in the parietal cortex and hippocampus, that was interpreted as disrupted somatodendritic neurites (Kalanjati *et al.* 2017). Intrauterine growth restriction is an important contributor to poor perinatal outcomes, particularly in preterm born infants (see Fleiss *et al.* 2019). Reduced dendritic branching and spine immaturity have also been reported in the CA1 region of hippocampus in a model of preterm birth in rabbit kits (Klebe *et al.* 2019), and in the granular layer of the dentate gyrus in a maternal inflammatory activation (using i.p. PolyIC exposure) model in mice (Li *et al.* 2014). These assessments are harder to perform in neuropathology on clinical samples, though reduced dendritic complexity (branch number and length) have been described for somatostatin and neuropeptide Y positive neurons in the subcortical WM of preterm born infants with WM injury (Stolp *et al.* 2019). Dendritic arborisation, and relatedly, synapse formation (something also disrupted in these models; Dean *et al.* 2011, Balakrishnan

*et al.* 2013, Stolp *et al.* 2019), are essential developmental events for ensuring appropriate connectivity in the brain, and disruption in these processes have been implicated in a number of functional disorders of the brain (discussed below).

~~A clear example of~~ The vulnerability of synapse structure in preterm born infants is clearly shown in our study that revealed a relationship between brain injury in preterm born infants and single nucleotide polymorphisms (SNP) variants in the gene for the post-synaptic protein 95 (PSD-95, *DLG4*; Krishnan *et al.* 2017). ~~This~~ Our work focused on a novel role for PSD-95 expressed specifically by microglia in early development in EoP, but the patient SNP data also suggests a wider vulnerability of synapse structure in preterm born infants.

While evidence of EoP as a synaptopathy inevitable comes from neuropathological studies, our best capacity to clinical recognise disease, stratify patients for treatment and monitor progress, comes from neuroimaging. When relating *in vivo* imaging to pathology, the study by Petrenko *et al.* (2018) suggested that a) neurons loss could be detected by decrease in N-acetylaspartate and N-acetylaspartylglutamate, and astrogliosis with reduced Glutamate/Glycine ratio using magnetic resonance spectroscopy within 3 days of injury; b) diffusion MRI could also detect microstructural injury within 3 days of cell death induction, starting with increased water diffusivity (mean diffusivity) and extending to reduced fractional anisotropy (FA) due to altered dendritic arrangement. Ball *et al.* (2013) has shown a developmental decrease in FA in the cortex over the preterm period, using diffusion MRI, with preterm born infants lagging behind term born infants in this maturational process, i.e. with a higher FA, at term equivalent age. Modelling by Dean *et al.* (2013) supports the idea that this increased FA value is due to delays in the normal dendritic arborisation of the cortical neurons over this period. Vinall *et al.* (2013), studied variation in diffusion MRI values between two scans in a cohort of very preterm infants. Their work showed that increased FA in the cortical GM at scan two was independently associated with reduced gestational age, birth weight and slow weight gain. In addition, changes in FA were related to the second and third eigenvector direction, rather than the primary eigenvector direction. Collectively, these data imply that delays in cortical maturation were most likely driven by delays in neuronal process formation, or cell loss, and that cortical maturation was associated primarily with the phase of neonatal growth (Vinall *et al.* 2013). Structural connectivity studies, typically based on the integrity of WM tracts using diffusion MRI, have shown a topographically dependent timetable of connectivity developing brain, that is impaired in the preterm brain (Pannek *et al.* 2013, Batalle *et al.* 2017), and that is altered in nature over time, but persist in some form to adulthood (Karolis *et al.* 2016). While these measures are not directly assessing cortical GM injury, it is likely that an interplay between WM and GM development occurs, and that altered connectivity maps will reflect changes in GM development. These structural alterations are also likely to have functional consequences that reflect both local and global connectivity.

## Interplay of structural and functional deficits in EoP

Altered structural and functional connectivity can be identified in the brains of preterm infants at term equivalent age, using combined diffusion and functional MRI (Arichi *et al.* 2014). Aside from studies testing passive function, including touch and auditory stimulation, the majority of functional MRI studies in preterm infants have ~~been conducted to look at~~ investigated resting states. Collectively these resting state studies suggest that there is modular organisation of the connectivity of the preterm brain, as is seen in the mature brain, but that integration

between networks is altered (Ball *et al.* 2016, Gozdas *et al.* 2018, Bouyssi-Kobar *et al.* 2019). In these studies, there is evidence for disruption in both cortico-cortical and cortico-subcortical networks (Ball *et al.* 2016, Bouyssi-Kobar *et al.* 2019), and reduced connectivity between areas associated with motor function, cognition, language and executive function (Gozdas *et al.* 2018).

Electroencephalogram (EEG) is a clinical tool that has been shown to have some potential to monitor and predict severity and outcome of EoP. EEG waveforms are immature in the preterm brain, but appear to have some characteristic changes that can be used as a biomarker, including seizures, EEG suppression and mechanical delta brush activity (Ranasinghe *et al.* 2015, Song *et al.* 2015, Pavlidis *et al.* 2017, Whitehead *et al.* 2017). The rate of spontaneous activity transients on EEG in preterm born infants with or without GM-IVH, measured ~~in preterm born infants~~ over the first 48 hours of life, was associated with cortical GM volume growth, increased gyrification index and increased FA in WM tracts (Tataranno *et al.* 2018). Additional studies of the association between early EEG and cortical growth have revealed very specific band frequency relationships and with spontaneous activity transients (SATs; Benders *et al.*, 2015). As we begin to understand the biological drivers of these events it will provide further information of the structure function relationship of the EEG recordings. Whitehead *et al.* (2020), using EEG ~~in preterm born infants with or without GM-IVH~~, showed that gross injury initially disrupts signal recruitment from cortical circuits in preterms. Signal recruitment appears to eventually be reinstated following injury but remains different from individuals without gross injury. Importantly, EEG abnormalities assessed shortly after birth (a week to a month after birth) were able to predict both developmental delay and cerebral palsy at 18-24 months (Hayashi-Kurahashi *et al.* 2012, Reynolds *et al.* 2014).

In animal models fMRI has not been used, but EEG has been used extensively in sheep models of *in utero* hypoxic ischemic injury, (more closely modelling hypoxic ischemic encephalopathy), and show reduced maturation of the EEG signal over time, seizure susceptibility and microscale epileptiform events in the latent phase (up to 7 hours post injury) prior to seizure onset that correlates with cell death (Abbasi *et al.* 2018, van den Heuij *et al.* 2019). Following intrauterine inflammatory exposure in foetal sheep, changes in developmental patterns in alpha, beta power (reduced) and delta power (increased) have also been reported (Keogh *et al.* 2012). However, while there is widespread evidence of altered EEG parameters in both clinical and preclinical studies, it is not clear how well these changes related to the neuropathology, and how predictive they are for outcome. This work is only just beginning in clinical populations (e.g. Hayashi-Kurahashi *et al.* 2012, Reynolds *et al.* 2014, Whitehead *et al.* 2020), but in pre-clinical studies a number of studies have found a disconnect between EGGEEG results and activity and arousal (Plomgaard *et al.* 2019) or neuropathology (van de Looij *et al.* 2014, Galinsky *et al.* 2017). However, it should be noted that the pathology examined in the study by Galinsky *et al.* (2017) was largely focused on WM, rather than GM, features, and therefore may provide a limited understanding the pathological correlates of EEG. van den Heuij *et al.* (2019), for instance, have reported improved EEG findings together with reduced cortical and deep grey matter damage following intrauterine artery occlusion in the foetal sheep. In rodent models, EEG studies are less common, due to the size of the postnatal brain. Using *ex vivo* multi-electrode arrays, Mordel *et al.* (2016) showed that inflammation and hypoxia, alone or together, increased the excitability of cortical neurons, in a glutamate receptor dependent manner. Interestingly, this research group has also shown that inflammation-induced alterations in cortical neuron spontaneous burst activity

subsequently results in an increase in apoptosis in the same cell population (Nimmervoll *et al.* 2013). The work of Mordel *et al.* (2016) suggests that altered electrical activity in the cortex occurs only in the first few weeks after injury, and recovers in adulthood. However, long-term alterations in spontaneous and mini inhibitory postsynaptic currents, a more subtle measure of neuronal activity, was found specifically for parvalbumin-positive interneurons following foetal exposure to inflammation (Canetta *et al.* 2016). Electrophysiological studies in the preterm sheep have shown altered excitability in subplate neurons (McClendon *et al.* 2017), as well as reductions in intrinsic excitability, altered polarisation dynamics, and reduced long-term synaptic plasticity in the hippocampus following hypoxia-ischemia and hypoxia alone (McClendon *et al.* 2019).

The relationship with structure and function is complex, and needs to be understood better [at a \(sub\)cellular level](#) in the context of EoP. However, the study of Zaslavsky *et al.* (2019) in iPSCs from ASD patients, shows increased dendritic arborisation and synaptic connectivity associated with a significant increase in sESPC frequency, supporting suggestions that altered [neuronal](#) morphology does change cellular function (rather than being compensated for in the function of the cellular communications pathway). This link between structure and function, the capacity for one to affect the other and the plasticity for recovery, is a particularly important point to consider when exploring new therapeutic targets, and optimal periods of treatment, for EoP and NDDs. This concept has recently been supported in a study of genetically encoded epilepsy, where timely treatment with Bumetanide altered long-term neuronal activity and network formation (Marguet *et al.* 2015).

## Potential therapies for EoP - Grey matter targets

The most obvious change in the GM of preterm born infants are reductions in volumes on MRI, changes that persist with increasingly age. These gross changes are likely mediated by limited but significant cell death, changes in sub-classes of interneurons and, across neuronal classes, reductions in arborisation and/or synaptic number. There are no therapies designed to target GM injury in the preterm specifically. Given that there are striking similarities between the GM changes in EoP and NDDs it would seem appropriate to consider if any therapeutic candidates from the NDD field might have efficacy in EoP. Current pharmacological strategies for the treatment of ADHD focus on normalising, but not repairing, disturbances in synaptic transmission and activity (Mattingly *et al.* 2017) and the same is the case for the various forms of epilepsy (Goldenberg 2010, De Crescenzo *et al.* 2017). For ASD, therapy focuses on treating the symptoms of the disorder, such as risperidone to reduce irritability via antagonism of central type 2 serotonergic (5-HT<sub>2</sub>) receptors and central dopamine D<sub>2</sub> receptors (Heylen and Gelders 1992). There are no therapies for ASD to treat the underlying deficits in social abilities. Other NDDs such as intellectual disability, and learning disorders (dyslexia and dyscalculia) together with ASD and ADHD are successfully treated with behavioural interventions. It is believed that these therapies do rewire the brain (Stavropoulos 2017), but whether they are capable of repair is not at all established.

A recent review of the drugs under investigation review for ASD highlighted that potential therapies fall into several clear classes - GABA/Glutamate modulators, neuropeptides, immunologics, and dietary supplementation (Hong and Erickson 2019). The only therapies whose specific underlying premise is to permanently alter the structure of the brain are

immunological therapies. Although neurotransmitter modulators given at the optimal stage of development may normalise aspects of structural and functional development, something that needs to be considered in future research. That immunological therapies might enable repair is based on the underlying idea that in the brains of people with ASD there is a persistent immunological dysfunction that itself is the cause of the core social deficits. As such, removing this dysfunction allows the brain to return to a normal structural and functional state. A very similar process of persisting and damaging inflammation is hypothesised to occur in the brain after perinatal brain injury (Fleiss and Gressens 2012) that evidence begins to accrue that in this context it is also a valid therapeutic target (Han *et al.* 2016, Mattei *et al.* 2017).

Another exploratory area of understanding and treating ASD and other NDDs is the gut-microbiome-brain axis (Iannone *et al.*, 2020, Hill-Yardin *et al.*, 2017). Gene mutations associated with autism pathogenesis impair brain and gut function and contribute to core and comorbid symptoms reported in autism (Katayama *et al.*, 2016; Hosie *et al.*, 2019, Lasheras *et al.*, 2020). The gut and brain share cellular structures, molecular pathways and processes that likely cause shared vulnerability to processes leading to autism (Hill-Yardin *et al.*, 2017). For instance, gut and brain synaptic structure and function are similarly vulnerable to disturbances in structural proteins such as neuroligins, postsynaptic density proteins and Shanks (Krishnan *et al.*, 2017, Saur *et al.*, 2019, Burrows *et al.*, 2015; Hosie *et al.*, 2018; Matta *et al.*, 2019; Lee *et al.*, 2020). An inexorable production of gut microbe-derived neuroactive metabolites influences gastrointestinal function, and these also traverse the BBB to exert potent effects on the brain (Hsiao *et al.*, 2013; Sharon *et al.*, 2019; Sgritta *et al.*, 2018). Importantly, microbiome-mediated gut and brain crosstalk even alters early brain development (Erny *et al.*, 2015; Matcovitch-Natan *et al.*, 2016) via dysbiosis that impairs the function of the brain's chief 'building manager' and resident immune cells - microglia. Microbe-derived metabolites also regulate the function of the BBB itself (Hoyle *et al.*, 2018) demonstrating the integral nature of the microbiome-gut-brain axis in brain health. As such, research investigating factors modulating the gut-microbiome-axis in NDDs may uncover novel mechanisms for treatment (Swiderski *et al.*, 2019; Nithianantharajah *et al.*, 2017).

Considering the options from the classes of drugs already being tested in models of EoP, we find that despite many compounds being tested (with mixed results) most have not considered outcomes in the GM. There are some notable exceptions, such as MgSO<sub>4</sub> pre-treatment in a rat model of preterm HI (modelling antenatal treatment in at risk mothers) significantly reduced tissue loss in the hippocampus and striatum, and was associated with reduced neurological injury score (Koning *et al.* 2018). MgSO<sub>4</sub> has also been tested in a sheep model of perinatal asphyxia, reporting reduced seizure burden, but worse WM outcomes and no GM neuropathology (Galinsky *et al.* 2017). Clinically, MgSO<sub>4</sub> has a number to treat of 54 (Doyle *et al.* 2007), though due to the nature of pre-treatment of at risk individuals, the exact efficacy is difficult to determine; a Cochrane review of four trials of antenatal treatment of at risk women showed no significant effect on mortality or neurological outcome (Doyle *et al.* 2007). Erythropoietin in this environment has not been shown to be protective for qualitative WM or GM injury when administered as 3 doses of 25µg/kg within the first 2 days of birth in preterm infants (Leuchter *et al.* 2014). This is despite positive GM outcomes in rodent (van de Looij *et al.* 2014, Robinson *et al.* 2018) and sheep models (Wassink *et al.* 2017). Robinson *et al.* (2018) showed that 2000U/kg erythropoietin (~17µg/kg), administered postnatally following intrauterine occlusion, was beneficial for both WM and GM, ameliorating behavioural deficits in gait and social interaction, and fractional anisotropy changes in the WM, hippocampus and



striatum. In their study of perinatal injury, hypoxia-ischemia in the postnatal day 3 rat, (van de Looij et al. (2014) showed that erythropoietin improved somatosensory evoked potentials and diffusion parameters in the WM, when measured with MRI, not didn't prevent cortical tissue loss. Wassink et al. (2017) assessed neuronal number and cell death in the caudate, showing a significant improvement with erythropoietin (5000IU loading dose, followed by 832IU/hour) in the preterm sheep, as well as reduced seizure burden. More positive data on [erythropoietin EPO](#) has been found for WM injury (reviewed in Fleiss and Gressens 2019, Volpe 2019), supporting the numerous on-going clinical trials for this drug, however it is clear that additional therapeutic agents need to be tested for GM efficacy.

## Links between EoP and neurodevelopmental diseases

It has been unequivocally established that preterm born infants have increased rates of diagnosis for NDDs, including ASD, ADHD and generalised learning disorders (Marlow et al. 2005, Johnson et al. 2010, Sucksdorff *et al.* 2015, Pierrat et al. 2017, Hirschberger et al. 2018). It is also clear that in the brains of people who suffered from EoP and those with NDDs (and from their matched preclinical models) ~~that~~ there are a striking number of shared pathomechanisms. In this section we will highlight key phenotypic, macrostructural, genetic, cellular and sub-cellular processes shared with EoP and in cases of NDD. We will focus on the GM, but we wish to highlight that for the WM these links between EoP and NDD are more established - such as shared deficits in corpus callosum structure in people after EoP and those with ASD and ADHD (Indredavik *et al.* 2005).

Recent work has assessed in detail the specific characteristics of behavioural disturbances in people born preterm with NDD compared with people born at term with a NDD (see reviews (Bokobza *et al.* 2019, Fitzallen *et al.* 2020). In general, in preterm versus term NDD the phenotypic presentations are similar. However, there are important differences. For instance, in people born at term there is a higher rate of ADHD in males compared with females, but this sex differences in not observed in people with ADHD that were born preterm (Elgen *et al.* 2002). For ASD, a greater proportion of preterm (versus term born) males reported comorbidities (sleep apnoea, seizure disorders, and ADHD) and people born preterm (particularly females) were more often non-verbal (Bowers *et al.* 2015). Another recent small study of children with ASD demonstrated that compared to term children, the preterm children had higher quality peer relationships and socioemotional reciprocity but poorer nonverbal behaviours that regulate social communication (Chen *et al.* 2019). None of the current literature has indicated a problem with diagnosing those born preterm using the current diagnostic criteria. However, we speculate that just as autism has been expanded and refined into a complex spectrum of disorders that in the future ASD phenotypes specific to preterm born infants may be defined.

With increased MRI analyses of the GM in individuals with EoP, we begin to see a clear pattern of similarities in changes in brain structure in people with ASD and those born preterm - including that there are shared changes in the orbitofrontal regions, the amygdala, the basal ganglia, the hippocampus and the cerebellum (reviewed in Batalle *et al.* 2017, Bokobza et al. 2019). There is also a parallel with the altered cortical growth in preterm born infants and equivalent findings in ASD and ADHD patients. In MRI studies of ASD and ADHD, decreased GM volume has been associated with both conditions (McAlonan *et al.* 2005, Batty *et al.* 2010, Greimel *et al.* 2013, Griffiths *et al.* 2016, Sato *et al.* 2017). In ASD, decreases have particularly

been found in areas related to social behaviour networks (McAlonan et al. 2005, Greimel et al. 2013, Sato et al. 2017), while regions associated with inhibitory control (Batty et al. 2010) were changed in ADHD. In both cases, it is clear that patterns of GM deficits alter through the disease course (Nakao *et al.* 2011, Greimel et al. 2013). Changes in the volume of GM in preterm infants/children/adolescents have been found in many of these regions (e.g. Zubiaurre-Elorza (Zubiaurre-Elorza et al. 2011, Lawrence *et al.* 2014, Zhang et al. 2015), but are typically more widespread. Variation between studies have, of course, been reported, with not all studies finding cortical GM volume changes, or associating them with neurological outcome. However, these are in the minority, and it has been suggested that these may be due to difficulties in accurately recognising the grey-white matter boundary [in the developing brain](#) (Keunen *et al.* 2016). Interestingly, in addition to this overlap in affected brain areas in both EoP and NDDs, MRI studies are also showing alterations in cortical networks (e.g. Ecker *et al.* 2013, Griffiths et al. 2016) in ASD and ADHD that warrant further exploration, and may come from as similar anatomical basis as in the EoP studies.

A newer avenue with which to link EoP and NDD are genetic studies such as genome wide association studies (GWAS), copy number variant (CNV) studies, single nucleotide polymorphisms (SNP) and haplotype studies, and these are revealing common risk factors. For instance, we have recently uncovered that a SNP in the gene for PSD95 is associated with poorer outcome for preterm born infants (Krishnan *et al.* 2017), mentioned above, as genetic variation in polymorphisms for PSD95 is a known risk factor for ASD (Coley and Gao 2018). Common genetic variants and methylation patterns have been revealed in focused studies of people with ASD with and without prior history of preterm birth (Sajdel-Sulkowska *et al.* 2019). Changes uncovered by these targeted studies include tyrosine-protein kinase Met (*MET*), Neuregulin 3 (*NRG3*), and serotonin transporter (*SLC6A4*). A great deal can also be learnt from comparing findings from studies of NDDs and studies of prematurity and EoP. For NDDs, there are numerous genes associated with synapse formation identified from GWAS studies including Shanks, Neuroregulin, Neurexin and Contactins (reviewed by Guang *et al.* 2018, Rylaarsdam and Gumez-Gamboa 2019). Many of these genes also associate with preterm birth or outcomes after preterm birth. Of note, neuregulin is found associated with infant outcome, with polymorphisms increasing mRNA levels in patients associated with better outcomes in babies born preterm (Hoffmann *et al.* 2010).

A key vulnerable neuronal subpopulation in EoP is interneurons, although it is still unclear which populations are the most vulnerable at which time point and in what regions based on the human and preclinical studies (Panda et al. 2018, Stolp et al. 2019). Research into neuropathology in NDD, via post-mortem studies and animal models of NDD also conclusively illustrates changes in interneurons (Lunden et al. 2019). Quite strikingly, in a synaptic protein knockout model of ASD (PTEN KO), interneuron transplantation rescues social behaviour deficits (Southwell *et al.* 2020). This study also questions the established idea that interneuron deficits associate with NDDs due to negative effects on inhibitory circuit activation (Pizzarelli and Cherubini 2011), as although interneuron transplantation rescued the behavioural phenotype there were no improvements in circuit function.

No discussion of the similarities between NDD and EoP could be complete with highlighting the shared common pathological process of neuroinflammation, which has at its core the aberrant activation of microglia. Indeed, across NDD and EoP models and humans studies evidence shows that microglial activities are altered (thoroughly reviewed in Bilbo *et al.* 2012,

Hagberg *et al.* 2015, Petrelli *et al.* 2016, Vezzani *et al.* 2019). ~~This microglial dysfunction and the concept of EoP and NDDs as synaptopathies aligns clearly.~~ A chief function of microglia during development, but also throughout life, is regulation of connectivity via refinement of synaptic number (Paolicelli *et al.* 2011, Zhan *et al.* 2014, Mallya *et al.* 2019; and reviewed in Schafer *et al.* 2012, Bilimoria and Stevens 2015, Tay *et al.* 2017). ~~The similar synaptic profiles of EoP and NDD is discussed further below.~~ Based on all the evidence for the role of microglia and the presence of inflammation (both systemic and central) in EoP and NDD it is clear that microglia (and their effects on synapses and neurogenesis) are an important starting point in understanding grey matter pathology across NDDs and EoP and also a shared target for neurorepair.

We outlined above the reasons that EoP can be considered a synaptopathy - including genetic associations between injury severity and synaptic genes, connectivity deficits, and preclinical studies show synaptic immaturity, plus arborisation deficits. These characteristics are also common among NDDs, and NDDs are clearly characterized as synaptopathies (Won *et al.* 2013, Ebrahimi-Fakhari and Sahin 2015, Lepeta *et al.* 2016, Luo *et al.* 2018). For example, about half of the genes identified as candidate genes in people with ASD code synaptic proteins (Delorme *et al.* 2013). Additionally, animal models of abnormal synaptic pruning induced by abnormal microglial function (Bokobza *et al.* 2019), or via genetic perturbation of synaptic structure (Giovedì *et al.* 2014), have cognitive and behavioural deficits reminiscent of NDDs. Thus, perhaps it is the collective change in these functional units of the neuron that give rise to the shared gross volumetric changes and pervasive behavioural problems in people with NDDs and due to EoP. Though it should also be said that a great many children and adults who were born preterm and who had EoP have typical neurodevelopmental profiles; potentially and interaction of genetics and environmental challenges in these case, leads to structurally resilient synapses in these case. There is clearly need for is a better understanding of the vulnerabilities leading to NDDs and negative consequences after EoP.

## Conclusions

Imaging and neuropathological studies indicate changes in GM are a subtle but substantial contributor to EoP. The full nature of this injury is probably only just being discovered, and would benefit from more longitudinal MRI studies, with closer integration of both patient genetics data and neuropathology where possible. Given the link between GM injury and long-term cognitive and behavioural disorders, it is important to therapeutically target this injury, distinct from the WM injury aspects of EoP. In particular, while preterm birth and EoP increases risk of NDD in later life, the current evidence suggests that preterm born infants may make up a specific subset of cases in these disorder spectrums and could benefit from a distinct treatment paradigm. In terms of what this therapeutic paradigm might look like, it is likely that a combination of ameliorating (e.g. anti-inflammatory or growth supporting) agents and restorative agents (e.g. drugs facilitating normal structural-functional development) will be required. If these treatments are delivered at optimal periods of brain development it may be possible to limit the need for life-long symptom controlling medication. In this regard, it is necessary to focus more research on the synaptopathy aspects of EoP. Current research in this area is only the tip of the iceberg, particularly lacking in clinical studies, and increased understanding of the injury mechanisms and plasticity during the postnatal period may identify new therapeutic targets. Our great hope is actually that this proposed work becomes redundant. We hope that our highly skilled and motivated counterparts working on prediction

and prevention of preterm birth have major breakthroughs. However, pragmatically, even major breakthroughs will take decades to make it across high and middle/low economic settings means that millions more babies are going to need us to better understand the GM and its changes after EoP.

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## References

- Abbasi, H., P. P. Drury, C. A. Lear, A. J. Gunn, J. O. Davidson, L. Bennet and C. P. Unsworth (2018). "EEG sharp waves are a biomarker of striatal neuronal survival after hypoxia-ischemia in preterm fetal sheep." *Sci Rep* **8**(1): 16312.
- Adorjan, I., B. Ahmed, V. Feher, M. Torso, K. Krug, M. Esiri, S. A. Chance and F. G. Szele (2017). "Calretinin interneuron density in the caudate nucleus is lower in autism spectrum disorder." *Brain* **140**(7): 2028-2040.
- Ajayi-Obe, M., N. Saeed, F. M. Cowan, M. A. Rutherford and A. D. Edwards (2000). "Reduced development of cerebral cortex in extremely preterm infants." *Lancet* **356**(9236): 1162-1163.
- Ananth, C. V. and A. M. Vintzileos (2008). "Medically indicated preterm birth: recognizing the importance of the problem." *Clin Perinatol* **35**(1): 53-67, viii.
- Ancel, P. Y., F. Livinec, B. Larroque, S. Marret, C. Arnaud, V. Pierrat, M. Dehan, S. N'Guyen, B. Escande, A. Burguet, G. Thiriez, J. C. Picaud, M. Andre, G. Breart and M. Kaminski (2006). "Cerebral palsy among very preterm children in relation to gestational age and neonatal ultrasound abnormalities: the EPIPAGE cohort study." *Pediatrics* **117**(3): 828-835.
- Andiman, S. E., R. L. Haynes, F. L. Trachtenberg, S. S. Billiards, R. D. Folkerth, J. J. Volpe and H. C. Kinney (2010). "The cerebral cortex overlying periventricular leukomalacia: analysis of pyramidal neurons." *Brain Pathol* **20**(4): 803-814.
- Ardalan, M., P. Svedin, A. A. Baburamani, V. G. Supramaniam, J. Ek, H. Hagberg and C. Mallard (2019). "Dysmaturation of Somatostatin Interneurons Following Umbilical Cord Occlusion in Preterm Fetal Sheep." *Front Physiol* **10**: 563.
- Arichi, T., S. J. Counsell, A. G. Allievi, A. T. Chew, M. Martinez-Biarge, V. Mondì, N. Tusor, N. Merchant, E. Burdet, F. M. Cowan and A. D. Edwards (2014). "The effects of hemorrhagic parenchymal infarction on the establishment of sensori-motor structural and functional connectivity in early infancy." *Neuroradiology* **56**(11): 985-994.
- Armstrong, E., A. Schleicher, H. Omran, M. Curtis and K. Zilles (1995). "The ontogeny of human gyrification." *Cereb Cortex* **5**(1): 56-63.
- Back, S. A. and S. P. Miller (2014). "Brain injury in premature neonates: A primary cerebral dysmaturation disorder?" *Ann Neurol* **75**(4): 469-486.
- Balakrishnan, B., H. Dai, J. Janisse, R. Romero and S. Kannan (2013). "Maternal endotoxin exposure results in abnormal neuronal architecture in the newborn rabbit." *Dev Neurosci* **35**(5): 396-405.
- Ball, G., P. Aljabar, T. Arichi, N. Tusor, D. Cox, N. Merchant, P. Nongena, J. V. Hajnal, A. D. Edwards and S. J. Counsell (2016). "Machine-learning to characterise neonatal functional connectivity in the preterm brain." *Neuroimage* **124**(Pt A): 267-275.
- Ball, G., J. P. Boardman, P. Aljabar, A. Pandit, T. Arichi, N. Merchant, D. Rueckert, A. D. Edwards and S. J. Counsell (2013). "The influence of preterm birth on the developing thalamocortical connectome." *Cortex* **49**(6): 1711-1721.
- Ball, G., J. P. Boardman, D. Rueckert, P. Aljabar, T. Arichi, N. Merchant, I. S. Gousias, A. D. Edwards and S. J. Counsell (2012). "The effect of preterm birth on thalamic and cortical development." *Cereb Cortex* **22**(5): 1016-1024.
- Ball, G., L. Pazderova, A. Chew, N. Tusor, N. Merchant, T. Arichi, J. M. Allsop, F. M. Cowan, A. D. Edwards and S. J. Counsell (2015). "Thalamocortical Connectivity Predicts Cognition in Children Born Preterm." *Cereb Cortex* **25**(11): 4310-4318.
- Ball, G., L. Srinivasan, P. Aljabar, S. J. Counsell, G. Durighel, J. V. Hajnal, M. A. Rutherford and A. D. Edwards (2013). "Development of cortical microstructure in the preterm human brain." *Proc Natl Acad Sci U S A* **110**(23): 9541-9546.
- Banks, W. A., A. J. Kastin and R. D. Broadwell (1995). "Passage of cytokines across the blood-brain barrier." *Neuroimmunomodulation* **2**(4): 241-248.
- Batalle, D., A. D. Edwards and J. O'Muircheartaigh (2017). "Annual Research Review: Not just a small adult brain: understanding later neurodevelopment through imaging the neonatal brain." *J Child Psychol Psychiatry*.

- Batalle, D., E. J. Hughes, H. Zhang, J. D. Tournier, N. Tusor, P. Aljabar, L. Wali, D. C. Alexander, J. V. Hajnal, C. Nosarti, A. D. Edwards and S. J. Counsell (2017). "Early development of structural networks and the impact of prematurity on brain connectivity." Neuroimage **149**: 379-392.
- Batalle, D., J. O'Muircheartaigh, A. Makropoulos, C. J. Kelly, R. Dimitrova, E. J. Hughes, J. V. Hajnal, H. Zhang, D. C. Alexander, A. D. Edwards and S. J. Counsell (2019). "Different patterns of cortical maturation before and after 38 weeks gestational age demonstrated by diffusion MRI in vivo." Neuroimage **185**: 764-775.
- Batty, M. J., E. B. Liddle, A. Pitiot, R. Toro, M. J. Groom, G. Scerif, M. Liotti, P. F. Liddle, T. Paus and C. Hollis (2010). "Cortical gray matter in attention-deficit/hyperactivity disorder: a structural magnetic resonance imaging study." J Am Acad Child Adolesc Psychiatry **49**(3): 229-238.
- Bayly, P. V., L. A. Taber and C. D. Kroenke (2014). "Mechanical forces in cerebral cortical folding: a review of measurements and models." J Mech Behav Biomed Mater **29**: 568-581.
- Benders MJ, Palmu K, Menache C, Borradori-Tolsa C, Lazeyras F, Sizonenko S, Dubois J, Vanhatalo S, Huppi PS (2015) Early Brain Activity Relates to Subsequent Brain Growth in Premature Infants. Cereb Cortex 25:3014-3024 10.1093/cercor/bhu097
- Bilbo, S. D., S. H. Smith and J. M. Schwarz (2012). "A lifespan approach to neuroinflammatory and cognitive disorders: a critical role for glia." J Neuroimmune Pharmacol **7**(1): 24-41.
- Bilimoria, P. M. and B. Stevens (2015). "Microglia function during brain development: New insights from animal models." Brain Res **1617**: 7-17.
- Bjuland, K. J., L. M. Rimol, G. C. Lohaugen and J. Skranes (2014). "Brain volumes and cognitive function in very-low-birth-weight (VLBW) young adults." Eur J Paediatr Neurol **18**(5): 578-590.
- Boardman JP, Counsell SJ, Rueckert D, Kapellou O, Bhatia KK, Aljabar P, Hajnal J, Allsop JM, Rutherford MA, Edwards AD (2006) Abnormal deep grey matter development following preterm birth detected using deformation-based morphometry. Neuroimage 32:70-78 10.1016/j.neuroimage.2006.03.029
- Bokobza, C., J. Van Steenwinckel, S. Mani, V. Mezger, B. Fleiss and P. Gressens (2019). "Neuroinflammation in preterm babies and autism spectrum disorders." Pediatr Res **85**(2): 155-165.
- Bora, S., V. E. Pritchard, Z. Chen, T. E. Inder and L. J. Woodward (2014). "Neonatal cerebral morphometry and later risk of persistent inattention/hyperactivity in children born very preterm." J Child Psychol Psychiatry **55**(7): 828-838.
- Bouyssi-Kobar, M., J. De Asis-Cruz, J. Murnick, T. Chang and C. Limperopoulos (2019). "Altered Functional Brain Network Integration, Segregation, and Modularity in Infants Born Very Preterm at Term-Equivalent Age." J Pediatr **213**: 13-21 e11.
- Bouyssi-Kobar, M., A. J. du Plessis, R. McCarter, M. Brossard-Racine, J. Murnick, L. Tinkleman, R. L. Robertson and C. Limperopoulos (2016). "Third Trimester Brain Growth in Preterm Infants Compared With In Utero Healthy Fetuses." Pediatrics **138**(5).
- Bowers, K., L. K. Wink, A. Pottenger, C. J. McDougale and C. Erickson (2015). "Phenotypic differences in individuals with autism spectrum disorder born preterm and at term gestation." Autism **19**(6): 758-763.
- Burd, I., A. I. Bentz, J. Chai, J. Gonzalez, H. Monnerie, P. D. Le Roux, A. S. Cohen, M. Yudkoff and M. A. Elovitz (2010). "Inflammation-induced preterm birth alters neuronal morphology in the mouse fetal brain." J Neurosci Res 88(9): 1872-1881.
- Burrows EL, Laskaris L, Koyama L, Churilov L, Bornstein JC, Hill-Yardin EL, Hannan AJ (2015) A neuroligin-3 mutation implicated in autism causes abnormal aggression and increases repetitive behavior in mice. Molecular autism 6:62 10.1186/s13229-015-0055-7
- Buser, J. R., J. Maire, A. Riddle, X. Gong, T. Nguyen, K. Nelson, N. L. Luo, J. Ren, J. Struve, L. S. Sherman, S. P. Miller, V. Chau, G. Henderson, P. Ballabh, M. R. Grafe and S. A. Back (2012). "Arrested preoligodendrocyte maturation contributes to myelination failure in premature infants." Ann Neurol **71**(1): 93-109.

- Bylicky, M. A., G. P. Mueller and R. M. Day (2018). "Mechanisms of Endogenous Neuroprotective Effects of Astrocytes in Brain Injury." Oxid Med Cell Longev **2018**: 6501031.
- Canetta, S., S. Bolkan, N. Padilla-Coreano, L. J. Song, R. Sahn, N. L. Harrison, J. A. Gordon, A. Brown and C. Kellendonk (2016). "Maternal immune activation leads to selective functional deficits in offspring parvalbumin interneurons." Mol Psychiatry **21**(7): 956-968.
- Chen, L. W., S. T. Wang, L. W. Wang, Y. C. Kao, C. L. Chu, C. C. Wu, Y. T. Hsieh, C. H. Chiang and C. C. Huang (2019). "Behavioral characteristics of autism spectrum disorder in very preterm birth children." Mol Autism **10**: 32.
- Coley, A. A. and W. J. Gao (2018). "PSD95: A synaptic protein implicated in schizophrenia or autism?" Prog Neuropsychopharmacol Biol Psychiatry **82**: 187-194.
- Costeloe, K. L., E. M. Hennessy, S. Haider, F. Stacey, N. Marlow and E. S. Draper (2012). "Short term outcomes after extreme preterm birth in England: comparison of two birth cohorts in 1995 and 2006 (the EPICure studies)." BMJ **345**: e7976.
- Crum, W. R., S. J. Sawiak, W. Chege, J. D. Cooper, S. C. R. Williams and A. C. Vernon (2017). "Evolution of structural abnormalities in the rat brain following in utero exposure to maternal immune activation: A longitudinal in vivo MRI study." Brain Behav Immun **63**: 50-59.
- Crump, C., K. Sundquist, M. A. Winkleby and J. Sundquist (2011). "Preterm birth and risk of epilepsy in Swedish adults." Neurology **77**(14): 1376-1382.
- Dammann, O. and A. Leviton (1997). "Maternal intrauterine infection, cytokines, and brain damage in the preterm newborn." Pediatr Res **42**(1): 1-8.
- De Crescenzo, F., S. Cortese, N. Adamo and L. Janiri (2017). "Pharmacological and non-pharmacological treatment of adults with ADHD: a meta-review." Evidence Based Mental Health **20**(1): 4.
- Dean, J. M., E. McClendon, K. Hansen, A. Azimi-Zonooz, K. Chen, A. Riddle, X. Gong, E. Sharifnia, M. Hagen, T. Ahmad, L. A. Leigland, A. R. Hohimer, C. D. Kroenke and S. A. Back (2013). "Prenatal cerebral ischemia disrupts MRI-defined cortical microstructure through disturbances in neuronal arborization." Sci Transl Med **5**(168): 168ra167.
- Dean, J. M., Y. van de Looij, S. V. Sizonenko, G. A. Lodygensky, F. Lazeyras, H. Bolouri, I. Kjellmer, P. S. Huppi, H. Hagberg and C. Mallard (2011). "Delayed cortical impairment following lipopolysaccharide exposure in preterm fetal sheep." Annals of Neurology **70**(5): 846-856.
- Delobel-Ayoub, M., C. Arnaud, M. White-Koning, C. Casper, V. Pierrat, M. Garel, A. Burguet, J. C. Roze, J. Matis, J. C. Picaud, M. Kaminski, B. Larroque and E. S. Group (2009). "Behavioral problems and cognitive performance at 5 years of age after very preterm birth: the EPIPAGE Study." Pediatrics **123**(6): 1485-1492.
- Delorme, R., E. Ey, R. Toro, M. Leboyer, C. Gillberg and T. Bourgeron (2013). "Progress toward treatments for synaptic defects in autism." Nat Med **19**(6): 685-694.
- ~~Dieni, S., T. Inder, B. Yoder, T. Briscoe, E. Camm, G. Egan, D. Denton and S. Rees (2004). "The pattern of cerebral injury in a primate model of preterm birth and neonatal intensive care." J Neuropathol Exp Neurol **63**(12): 1297-1309.~~
- Dieni, S., T. Inder, B. Yoder, T. Briscoe, E. Camm, G. Egan, D. Denton and S. Rees (2004). "The Pattern of Cerebral Injury in a Primate Model of Preterm Birth and Neonatal Intensive Care." Journal of Neuropathology & Experimental Neurology **63**(12): 1297-1309.
- Dommergues, M. A., F. Plaisant, C. Verney and P. Gressens (2003). "Early microglial activation following neonatal excitotoxic brain damage in mice: a potential target for neuroprotection." Neuroscience **121**(3): 619-628.
- Doyle, L. W., C. A. Crowther, P. Middleton and S. Marret (2007). "Magnesium sulphate for women at risk of preterm birth for neuroprotection of the fetus." Cochrane Database Syst Rev(3): CD004661.
- Ebrahimi-Fakhari, D. and M. Sahin (2015). "Autism and the synapse: emerging mechanisms and mechanism-based therapies." Curr Opin Neurol **28**(2): 91-102.
- Ecker, C., L. Ronan, Y. Feng, E. Daly, C. Murphy, C. E. Ginestet, M. Brammer, P. C. Fletcher, E. T. Bullmore, J. Suckling, S. Baron-Cohen, S. Williams, E. Loth, M. A. Consortium and D.

- G. Murphy (2013). "Intrinsic gray-matter connectivity of the brain in adults with autism spectrum disorder." Proc Natl Acad Sci U S A **110**(32): 13222-13227.
- Elgen, I., K. Sommerfelt and T. Markestad (2002). "Population based, controlled study of behavioural problems and psychiatric disorders in low birthweight children at 11 years of age." Arch Dis Child Fetal Neonatal Ed **87**(2): F128-132.
- [Erny D, Hrabe de Angelis AL, Jaitin D, Wieghofer P, Staszewski O, David E, Keren-Shaul H, Mahlakoiv T, Jakobshagen K, Buch T, Schwierzeck V, Utermohlen O, Chun E, Garrett WS, McCoy KD, Diefenbach A, Staeheli P, Stecher B, Amit I, Prinz M \(2015\) Host microbiota constantly control maturation and function of microglia in the CNS. Nat Neurosci 18:965-977 10.1038/nn.4030](#)
- Engelhardt, E., T. E. Inder, D. Alexopoulos, D. L. Dierker, J. Hill, D. Van Essen and J. J. Neil (2015). "Regional impairments of cortical folding in premature infants." Ann Neurol **77**(1): 154-162.
- Fan, L.-W. and Y. Pang (2017). "Dysregulation of neurogenesis by neuroinflammation: key differences in neurodevelopmental and neurological disorders." Neural regeneration research **12**(3): 366-371.
- Faustino, J. V., X. Wang, C. E. Johnson, A. Klibanov, N. Derugin, M. F. Wendland and Z. S. Vexler (2011). "Microglial cells contribute to endogenous brain defenses after acute neonatal focal stroke." J Neurosci **31**(36): 12992-13001.
- Favrais, G., Y. van de Looij, B. Fleiss, N. Ramanantsoa, P. Bonnin, G. Stoltenburg-Didinger, A. Lacaud, E. Saliba, O. Dammann, J. Gallego, S. Sizonenko, H. Hagberg, V. Lelievre and P. Gressens (2011). "Systemic inflammation disrupts the developmental program of white matter." Ann Neurol **70**(4): 550-565.
- Fernández-López, D., J. Faustino, A. L. Klibanov, N. Derugin, E. Blanchard, F. Simon, S. L. Leib and Z. S. Vexler (2016). "Microglial Cells Prevent Hemorrhage in Neonatal Focal Arterial Stroke." The Journal of Neuroscience **36**(10): 2881.
- Fidel, P., F. Ghezzi, R. Romero, T. Chaiworapongsa, J. Espinoza, J. Cutright, N. Wolf and R. Gomez (2003). "The effect of antibiotic therapy on intrauterine infection-induced preterm parturition in rabbits." The Journal of Maternal-Fetal & Neonatal Medicine **14**(1): 57-64.
- Fitzallen, G. C., H. G. Taylor and S. Bora (2020). "What do we know about the preterm behavioural phenotype? A narrative review." Frontiers in Psychiatry [doi.org/10.3389/fpsy.2020.00154](https://doi.org/10.3389/fpsy.2020.00154).
- Fleiss, B. and P. Gressens (2012). "Tertiary mechanisms of brain damage: a new hope for treatment of cerebral palsy?" Lancet Neuro **11**(6): 556-566.
- Fleiss, B. and P. Gressens (2019). "Neuroprotection of the preterm brain." Handb Clin Neurol **162**: 315-328.
- Fleiss, B., C. J. Tann, V. Degos, S. Sigaut, J. Van Steenwinckel, A.-L. Schang, A. Kichev, N. J. Robertson, C. Mallard, H. Hagberg and P. Gressens (2015). "Inflammation-induced sensitization of the brain in term infants." Developmental Medicine & Child Neurology **57**(S3): 17-28.
- Fleiss, B., F. Wong, F. Brownfoot, I. K. Shearer, O. Baud, D. W. Walker, P. Gressens and M. Tolcos (2019). "Knowledge Gaps and Emerging Research Areas in Intrauterine Growth Restriction-Associated Brain Injury." Front Endocrinol (Lausanne) **10**: 188.
- Fowke, T. M., R. Galinsky, J. O. Davidson, G. Wassink, R. N. Karunasinghe, J. D. Prasad, L. Bennet, A. J. Gunn and J. M. Dean (2018). "Loss of interneurons and disruption of perineuronal nets in the cerebral cortex following hypoxia-ischaemia in near-term fetal sheep." Sci Rep **8**(1): 17686.
- Frost, P. S., F. Barros-Aragao, R. T. da Silva, A. Venancio, I. Matias, E. S. N. M. Lyra, G. C. Kincheski, P. M. Pimentel-Coelho, F. G. De Felice, F. C. A. Gomes, S. T. Ferreira, C. P. Figueiredo and J. R. Clarke (2019). "Neonatal infection leads to increased susceptibility to Abeta oligomer-induced brain inflammation, synapse loss and cognitive impairment in mice." Cell Death Dis **10**(4): 323.
- Galinsky, R., V. Draghi, G. Wassink, J. O. Davidson, P. P. Drury, C. A. Lear, A. J. Gunn and L. Bennet (2017). "Magnesium sulfate reduces EEG activity but is not neuroprotective after asphyxia in preterm fetal sheep." J Cereb Blood Flow Metab **37**(4): 1362-1373.



- Gao, Z. and S. E. Tsirka (2011). "Animal Models of MS Reveal Multiple Roles of Microglia in Disease Pathogenesis." Neurol Res Int **2011**: 383087.
- Garcia, K. E., E. C. Robinson, D. Alexopoulos, D. L. Dierker, M. F. Glasser, T. S. Coalson, C. M. Ortinau, D. Rueckert, L. A. Taber, D. C. Van Essen, C. E. Rogers, C. D. Smyser and P. V. Bayly (2018). "Dynamic patterns of cortical expansion during folding of the preterm human brain." Proc Natl Acad Sci U S A **115**(12): 3156-3161.
- Gelot, A., S. Villapol, T. Billette de Villemeur, S. Renolleau and C. Charriaut-Marlangue (2009). "Astrocytic demise in the developing rat and human brain after hypoxic-ischemic damage." Dev Neurosci **31**(5): 459-470.
- Gilles, F., P. Gressens, O. Dammann and A. Leviton (2018). "Hypoxia-ischemia is not an antecedent of most preterm brain damage: the illusion of validity." Dev Med Child Neurol **60**(2): 120-125.
- Giovedì, S., A. Corradi, A. Fassio and F. Benfenati (2014). "Involvement of Synaptic Genes in the Pathogenesis of Autism Spectrum Disorders: The Case of Synapsins." Frontiers in Pediatrics **2**(94).
- Goldenberg, M. M. (2010). "Overview of drugs used for epilepsy and seizures: etiology, diagnosis, and treatment." P t **35**(7): 392-415.
- Goni-de-Cerio, F., A. Alvarez, A. Caballero, V. E. Mielgo, F. J. Alvarez, M. C. Rey-Santano, E. Gastiasoro, A. Valls-i-Soler, J. Bilbao and E. Hilario (2007). "Early cell death in the brain of fetal preterm lambs after hypoxic-ischemic injury." Brain Res **1151**: 161-171.
- Gozdas, E., N. A. Parikh, S. L. Merhar, J. A. Tkach, L. He and S. K. Holland (2018). "Altered functional network connectivity in preterm infants: antecedents of cognitive and motor impairments?" Brain Struct Funct **223**(8): 3665-3680.
- Greimel, E., B. Nehrkorn, M. Schulte-Ruther, G. R. Fink, T. Nickl-Jockschat, B. Herpertz-Dahlmann, K. Konrad and S. B. Eickhoff (2013). "Changes in grey matter development in autism spectrum disorder." Brain Struct Funct **218**(4): 929-942.
- Griffiths, K. R., S. M. Grieve, M. R. Kohn, S. Clarke, L. M. Williams and M. S. Korgaonkar (2016). "Altered gray matter organization in children and adolescents with ADHD: a structural covariance connectome study." Transl Psychiatry **6**(11): e947.
- Guang, S., N. Pang, X. Deng, L. Yang, F. He, L. Wu, C. Chen, F. Yin and J. Peng (2018). "Synaptopathology Involved in Autism Spectrum Disorder." Frontiers in cellular neuroscience **12**: 470-470.
- Gussenhoven, R., R. J. J. Westerlaken, D. Ophelders, A. H. Jobe, M. W. Kemp, S. G. Kallapur, L. J. Zimmermann, P. T. Sangild, S. Pankratova, P. Gressens, B. W. Kramer, B. Fleiss and T. Wolfs (2018). "Chorioamnionitis, neuroinflammation, and injury: timing is key in the preterm ovine fetus." J Neuroinflammation **15**(1): 113.
- Hagberg, B., G. Hagberg, I. Olow and L. von Wendt (1996). "The changing panorama of cerebral palsy in Sweden. VII. Prevalence and origin in the birth year period 1987-90." Acta Paediatr **85**(8): 954-960.
- Hagberg, H., C. Mallard, D. M. Ferriero, S. J. Vannucci, S. W. Levison, Z. S. Vexler and P. Gressens (2015). "The role of inflammation in perinatal brain injury." Nat Rev Neurol **11**(4): 192-208.
- Haldipur, P., U. Bharti, C. Alberti, C. Sarkar, G. Gulati, S. Iyengar, P. Gressens and S. Mani (2011). "Preterm delivery disrupts the developmental program of the cerebellum." PloS one **6**(8): e23449-e23449.
- Hammond, Timothy R., A. Gadea, J. Dupree, C. Kerninon, B. Nait-Oumesmar, A. Aguirre and V. Gallo (2014). "Astrocyte-Derived Endothelin-1 Inhibits Remyelination through Notch Activation." Neuron **81**(3): 588-602.
- Han, M., J. C. Zhang, W. Yao, C. Yang, T. Ishima, Q. Ren, M. Ma, C. Dong, X. F. Huang and K. Hashimoto (2016). "Intake of 7,8-Dihydroxyflavone During Juvenile and Adolescent Stages Prevents Onset of Psychosis in Adult Offspring After Maternal Immune Activation." Sci Rep **6**: 36087.
- Hart, A. D., A. Wyttenbach, V. H. Perry and J. L. Teeling (2012). "Age related changes in microglial phenotype vary between CNS regions: grey versus white matter differences." Brain Behav Immun **26**(5): 754-765.

- Hayashi-Kurahashi, N., H. Kidokoro, T. Kubota, K. Maruyama, Y. Kato, T. Kato, J. Natsume, F. Hayakawa, K. Watanabe and A. Okumura (2012). "EEG for predicting early neurodevelopment in preterm infants: an observational cohort study." *Pediatrics* **130**(4): e891-897.
- Haynes, R. L., S. S. Billiards, N. S. Borenstein, J. J. Volpe and H. C. Kinney (2008). "Diffuse axonal injury in periventricular leukomalacia as determined by apoptotic marker fractin." *Pediatr Res* **63**(6): 656-661.
- Haynes, R. L., R. D. Folkerth, R. J. Keefe, I. Sung, L. I. Swzeda, P. A. Rosenberg, J. J. Volpe and H. C. Kinney (2003). "Nitrosative and oxidative injury to premyelinating oligodendrocytes in periventricular leukomalacia." *J Neuropathol Exp Neurol* **62**(5): 441-450.
- Haynes, R. L. and K. van Leyen (2013). "12/15-lipoxygenase expression is increased in oligodendrocytes and microglia of periventricular leukomalacia." *Dev Neurosci* **35**(2-3): 140-154.
- Heeren, T., R. M. Joseph, E. N. Allred, T. M. O'Shea, A. Leviton and K. C. K. Kuban (2017). "Cognitive functioning at the age of 10 years among children born extremely preterm: a latent profile approach." *Pediatr Res* **82**(4): 614-619.
- Heylen, S. L. and Y. G. Gelders (1992). "Risperidone, a new antipsychotic with serotonin 5-HT<sub>2</sub> and dopamine D<sub>2</sub> antagonistic properties." *Clin Neuropharmacol* **15 Suppl 1 Pt A**: 180A-181A.
- [Hill-Yardin EL, McKeown SJ, Novarino G, Grabrucker AM \(2017\) Extracerebral Dysfunction in Animal Models of Autism Spectrum Disorder. Adv Anat Embryol Cell Biol 224:159-187 10.1007/978-3-319-52498-6\\_9](#)
- Hirschberger, R. G., K. C. K. Kuban, T. M. O'Shea, R. M. Joseph, T. Heeren, L. M. Douglass, C. E. Stafstrom, H. Jara, J. A. Frazier, D. Hirtz, J. V. Rollins, N. Paneth and E. S. 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.
- Hoffmann, I., W. Bueter, K. Zscheppang, M. J. Brinkhaus, A. Liese, S. Riemke, T. Dork, O. Dammann and C. E. Dammann (2010). "Neuregulin-1, the fetal endothelium, and brain damage in preterm newborns." *Brain Behav Immun* **24**(5): 784-791.
- Hong, M. P. and C. A. Erickson (2019). "Investigational drugs in early-stage clinical trials for autism spectrum disorder." *Expert Opinion on Investigational Drugs* **28**(8): 709-718.
- [Hosie, S., Malone, D. T., Liu, S., Glass, M., Adlard, P. A., Hannan, A. J., & Hill-Yardin, E. L. \(2018\). Altered amygdala excitation and CB1 receptor modulation of aggressive behavior in the neuroligin-3R451C mouse model of autism. Frontiers in cellular neuroscience, 12, 234.](#)
- [Hosie S, Ellis M, Swaminathan M, Ramalhosa F, Seger GO, Balasuriya GK, Gillberg C, Rastam M, Churilov L, McKeown SJ, Yalcinkaya N, Urvil P, Savidge T, Bell CA, Bodin O, Wood J, Franks AE, Bornstein JC, Hill-Yardin EL \(2019\) Gastrointestinal dysfunction in patients and mice expressing the autism-associated R451C mutation in neuroligin-3. Autism Res 12:1043-1056 10.1002/aur.2127](#)
- Hunt, R., P. G. Davis and T. Inder (2004). "Replacement of estrogens and progestins to prevent morbidity and mortality in preterm infants." *Cochrane Database Syst Rev*(4): CD003848.
- Huttenlocher, P. R. (1979). "Synaptic density in human frontal cortex - developmental changes and effects of aging." *Brain Res* **163**(2): 195-205.
- [Iannone LF, Gomez-Equilaz M, Citaro R, Russo E \(2020\). The potential role of interventions impacting on gut-microbiota in epilepsy. Expert Rev Clin Pharmacol:1-13 10.1080/17512433.2020.1759414.](#)
- Inder, T. E., P. S. Huppi, S. Warfield, R. Kikinis, G. P. Zientara, P. D. Barnes, F. Jolesz and J. J. Volpe (1999). "Periventricular white matter injury in the premature infant is followed by reduced cerebral cortical gray matter volume at term." *Ann Neurol* **46**(5): 755-760.

- Indredavik, M. S., J. S. Skranes, T. Vik, S. Heyerdahl, P. Romundstad, G. E. Myhr and A. M. Brubakk (2005). "Low-birth-weight adolescents: psychiatric symptoms and cerebral MRI abnormalities." *Pediatr Neurol* **33**(4): 259-266.
- Jantzie, L. L. and S. Robinson (2015). "Preclinical Models of Encephalopathy of Prematurity." *Dev Neurosci* **37**(4-5): 277-288.
- Johnson, S., C. Hollis, P. Kochhar, E. Hennessy, D. Wolke and N. Marlow (2010). "Autism spectrum disorders in extremely preterm children." *J Pediatr* **156**(4): 525-531 e522.
- Johnson, S., C. Hollis, P. Kochhar, E. Hennessy, D. Wolke and N. Marlow (2010). "Psychiatric disorders in extremely preterm children: longitudinal finding at age 11 years in the EPICure study." *J Am Acad Child Adolesc Psychiatry* **49**(5): 453-463 e451.
- Kaga, N., Y. Katsuki, M. Obata and Y. Shibutani (1996). "Repeated administration of low-dose lipopolysaccharide induces preterm delivery in mice: A model for human preterm parturition and for assessment of the therapeutic ability of drugs against preterm delivery." *American Journal of Obstetrics and Gynecology* **174**(2): 754-759.
- Kalanjati, V. P., J. A. Wixey, S. M. Miller, P. B. Colditz and S. T. Bjorkman (2017). "GABAA receptor expression and white matter disruption in intrauterine growth restricted piglets." *Int J Dev Neurosci* **59**: 1-9.
- Karolis, V. R., S. Froud-Walsh, P. J. Brittain, J. Kroll, G. Ball, A. D. Edwards, F. Dell'Acqua, S. C. Williams, R. M. Murray and C. Nosarti (2016). "Reinforcement of the Brain's Rich-Club Architecture Following Early Neurodevelopmental Disruption Caused by Very Preterm Birth." *Cereb Cortex* **26**(3): 1322-1335.
- Keogh, M. J., L. Bennet, P. P. Drury, L. C. Booth, S. Mathai, A. S. Naylor, M. Fraser and A. J. Gunn (2012). "Subclinical exposure to low-dose endotoxin impairs EEG maturation in preterm fetal sheep." *Am J Physiol Regul Integr Comp Physiol* **303**(3): R270-278.
- Kersbergen, K. J., A. Makropoulos, P. Aljabar, F. Groenendaal, L. S. de Vries, S. J. Counsell and M. J. Benders (2016). "Longitudinal Regional Brain Development and Clinical Risk Factors in Extremely Preterm Infants." *J Pediatr* **178**: 93-100 e106.
- Keunen, K., I. Isgum, B. J. van Kooij, P. Anbeek, I. C. van Haastert, C. Koopman-Elseboom, P. C. Fieret-van Stam, R. A. Nievelstein, M. A. Viergever, L. S. de Vries, F. Groenendaal and M. J. Benders (2016). "Brain Volumes at Term-Equivalent Age in Preterm Infants: Imaging Biomarkers for Neurodevelopmental Outcome through Early School Age." *J Pediatr* **172**: 88-95.
- Kilb, W., S. Kirischuk and H. J. Luhmann (2011). "Electrical activity patterns and the functional maturation of the neocortex." *Eur J Neurosci* **34**(10): 1677-1686.
- Kinney, H. C., R. L. Haynes, G. Xu, S. E. Andiman, R. D. Folkerth, L. A. Sleeper and J. J. Volpe (2012). "Neuron deficit in the white matter and subplate in periventricular leukomalacia." *Ann Neurol* **71**(3): 397-406.
- Klebe, D., M. Tibrewal, D. R. Sharma, R. Vanaparthi, S. Krishna, M. Varghese, B. Cheng, P. R. Mouton, J. Veliskova, K. Dobrenis, P. R. Hof and P. Ballabh (2019). "Reduced Hippocampal Dendrite Branching, Spine Density and Neurocognitive Function in Premature Rabbits, and Reversal with Estrogen or TrkB Agonist Treatment." *Cereb Cortex* **29**(12): 4932-4947.
- Kohli, J. S., M. K. Kinnear, C. H. Fong, I. Fishman, R. A. Carper and R. A. Muller (2019). "Local Cortical Gyrfication is Increased in Children With Autism Spectrum Disorders, but Decreases Rapidly in Adolescents." *Cereb Cortex* **29**(6): 2412-2423.
- Kohli, J. S., M. K. Kinnear, I. A. Martindale, R. A. Carper and R. A. Muller (2019). "Regionally decreased gyrfication in middle-aged adults with autism spectrum disorders." *Neurology* **93**(20): e1900-e1905.
- Koning, G., E. Lyngfelt, P. Svedin, A. L. Leverin, M. Jinnai, P. Gressens, C. Thornton, X. Wang, C. Mallard and H. Hagberg (2018). "Magnesium sulphate induces preconditioning in preterm rodent models of cerebral hypoxia-ischemia." *Int J Dev Neurosci* **70**: 56-66.
- Kostovic, I., M. Judas, Z. Petanjek and G. Simic (1995). "Ontogenesis of goal-directed behavior: anatomic-functional considerations." *Int J Psychophysiol* **19**(2): 85-102.
- Krishnan, M. L., J. Van Steenwinckel, A.-L. Schang, J. Yan, J. Arnadottir, T. Le Charpentier, Z. Csaba, P. Dournaud, S. Cipriani, C. Auvynet, L. Titomanlio, J. Pansiot, G. Ball, J. P.

- Boardman, A. J. Walley, A. Saxena, G. Mirza, B. Fleiss, A. D. Edwards, E. Petretto and P. Gressens (2017). "Integrative genomics of microglia implicates DLG4 (PSD95) in the white matter development of preterm infants." *Nature Communications* **8**(1): 428.
- Kuban, K. C., T. M. O'Shea, E. N. Allred, R. N. Fichorova, T. Heeren, N. Paneth, D. Hirtz, O. Dammann, A. Leviton and E. S. Investigators (2015). "The breadth and type of systemic inflammation and the risk of adverse neurological outcomes in extremely low gestation newborns." *Pediatr Neurol* **52**(1): 42-48.
- Lafemina, M. J., R. A. Sheldon and D. M. Ferriero (2006). "Acute hypoxia-ischemia results in hydrogen peroxide accumulation in neonatal but not adult mouse brain." *Pediatr Res* **59**(5): 680-683.
- [Lasheras I, Seral P, Latorre E, Barroso E, Gracia-Garcia P, Santabarbara J \(2020\) Microbiota and gut-brain axis dysfunction in autism spectrum disorder: Evidence for functional gastrointestinal disorders. Asian J Psychiatr 47:101874 10.1016/j.ajp.2019.101874.](#)
- Lau, J., F. Magee, Z. Qiu, J. Hoube, P. Von Dadelszen and S. K. Lee (2005). "Chorioamnionitis with a fetal inflammatory response is associated with higher neonatal mortality, morbidity, and resource use than chorioamnionitis displaying a maternal inflammatory response only." *Am J Obstet Gynecol* **193**(3 Pt 1): 708-713.
- Lauber, E., F. Filice and B. Schwaller (2018). "Dysregulation of Parvalbumin Expression in the Cntnap2-/- Mouse Model of Autism Spectrum Disorder." *Front Mol Neurosci* **11**: 262.
- Lawrence, E. J., S. Froudast-Walsh, R. Neilan, K. W. Nam, V. Giampietro, P. McGuire, R. M. Murray and C. Nosarti (2014). "Motor fMRI and cortical grey matter volume in adults born very preterm." *Dev Cogn Neurosci* **10**: 1-9.
- Lawson, L. J., V. H. Perry, P. Dri and S. Gordon (1990). "Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain." *Neuroscience* **39**(1): 151-170.
- [Lee, CYQ; Franks AE, Hill-Yardin EL. The microbiota-gut-brain axis in mouse models of neurodevelopmental disorders. Brain, behavior, and immunity, in press, 2020.](#)
- [Leembruggen AJL, Balasuriya GK, Zhang J, Schokman S, Swiderski K, Bornstein JC, Nithianantharajah J, Hill-Yardin EL \(2019\) Colonic dilation and altered ex vivo gastrointestinal motility in the neuroligin-3 knockout mouse. Autism Res 10.1002/aur.2109.](#)
- Lener, T., M. Gimona, L. Aigner, V. Borger, E. Buzas, G. Camussi, N. Chaput, D. Chatterjee, F. A. Court, H. A. Del Portillo, L. O'Driscoll, S. Fais, J. M. Falcon-Perez, U. Felderhoff-Mueser, L. Fraile, Y. S. Ghosh, A. Gorgens, R. C. Gupta, A. Hendrix, D. M. Hermann, A. F. Hill, F. Hochberg, P. A. Horn, D. de Kleijn, L. Kordelas, B. W. Kramer, E. M. Kramer-Albers, S. Laner-Plamberger, S. Laitinen, T. Leonardi, M. J. Lorenowicz, S. K. Lim, J. Lotvall, C. A. Maguire, A. Marcilla, I. Nazarenko, T. Ochiya, T. Patel, S. Pedersen, G. Pocsfalvi, S. Pluchino, P. Quesenberry, I. G. Reischl, F. J. Rivera, R. Sanzenbacher, K. Schallmoser, I. Slaper-Cortenbach, D. Strunk, T. Tonn, P. Vader, B. W. van Balkom, M. Wauben, S. E. Andaloussi, C. Thery, E. Rohde and B. Giebel (2015). "Applying extracellular vesicles based therapeutics in clinical trials - an ISEV position paper." *J Extracell Vesicles* **4**: 30087.
- Leow, S. M., M. K. W. Di Quinzio, Z. L. Ng, C. Grant, T. Amitay, Y. Wei, M. Hod, P. M. Sheehan, S. P. Brennecke, N. Arbel and H. M. Georgiou (2020). "Preterm birth prediction in asymptomatic women at mid-gestation using a panel of novel protein biomarkers: the Prediction of PreTerm Labor (PPeTaL) study." *American Journal of Obstetrics & Gynecology MFM*: 100084.
- Lepeta, K., M. V. Lourenco, B. C. Schweitzer, P. V. Martino Adami, P. Banerjee, S. Catuara-Solarz, M. de La Fuente Revenga, A. M. Guillem, M. Haidar, O. M. Ijomone, B. Nadorp, L. Qi, N. D. Perera, L. K. Refsgaard, K. M. Reid, M. Sabbar, A. Sahoo, N. Schaefer, R. K. Sheehan, A. Suska, R. Verma, C. Vicidomini, D. Wright, X.-D. Zhang and C. Seidenbecher (2016). "Synaptopathies: synaptic dysfunction in neurological disorders - A review from students to students." *Journal of neurochemistry* **138**(6): 785-805.
- Leuchter, R. H., L. Gui, A. Poncet, C. Hagmann, G. A. Lodygensky, E. Martin, B. Koller, A. Darque, H. U. Bucher and P. S. Huppi (2014). "Association between early administration of high-dose erythropoietin in preterm infants and brain MRI abnormality at term-equivalent age." *JAMA* **312**(8): 817-824.

- Li, W. Y., Y. C. Chang, L. J. H. Lee and L. J. Lee (2014). "Prenatal Infection Affects the Neuronal Architecture and Cognitive Function in Adult Mice." Developmental Neuroscience **36**(5): 359-370.
- Libero, L. E., M. Schaer, D. D. Li, D. G. Amaral and C. W. Nordahl (2019). "A Longitudinal Study of Local Gyrification Index in Young Boys With Autism Spectrum Disorder." Cereb Cortex **29**(6): 2575-2587.
- Ligam, P., R. L. Haynes, R. D. Folkerth, L. Liu, M. Yang, J. J. Volpe and H. C. Kinney (2009). "Thalamic damage in periventricular leukomalacia: novel pathologic observations relevant to cognitive deficits in survivors of prematurity." Pediatr Res **65**(5): 524-529.
- Limperopoulos, C., H. Bassan, N. R. Sullivan, J. S. Soul, R. L. Robertson, Jr., M. Moore, S. A. Ringer, J. J. Volpe and A. J. du Plessis (2008). "Positive screening for autism in ex-preterm infants: prevalence and risk factors." Pediatrics **121**(4): 758-765.
- Lindstrom, K., F. Lindblad and A. Hjern (2011). "Preterm birth and attention-deficit/hyperactivity disorder in schoolchildren." Pediatrics **127**(5): 858-865.
- Llinares-Benadero, C. and V. Borrell (2019). "Deconstructing cortical folding: genetic, cellular and mechanical determinants." Nat Rev Neurosci **20**(3): 161-176.
- Loane, D. J., A. Kumar, B. A. Stoica, R. Cabatbat and A. I. Faden (2014). "Progressive neurodegeneration after experimental brain trauma: association with chronic microglial activation." J Neuropathol Exp Neurol **73**(1): 14-29.
- Lodygensky, G. A., L. Vasung, S. V. Sizonenko and P. S. Huppi (2010). "Neuroimaging of cortical development and brain connectivity in human newborns and animal models." J Anat **217**(4): 418-428.
- Long, K. R., B. Newland, M. Florio, N. Kalebic, B. Langen, A. Kolterer, P. Wimberger and W. B. Huttner (2018). "Extracellular Matrix Components HAPLN1, Lumican, and Collagen I Cause Hyaluronic Acid-Dependent Folding of the Developing Human Neocortex." Neuron **99**(4): 702-719 e706.
- Lunden, J. W., M. Durens, A. W. Phillips and M. W. Nestor (2019). "Cortical interneuron function in autism spectrum condition." Pediatric Research **85**(2): 146-154.
- Luo, J., R. H. Norris, S. L. Gordon and J. Nithianantharajah (2018). "Neurodevelopmental synaptopathies: Insights from behaviour in rodent models of synapse gene mutations." Progress in neuro-psychopharmacology & biological psychiatry **84**(Pt B): 424-439.
- Makropoulos, A., P. Aljabar, R. Wright, B. Huning, N. Merchant, T. Arichi, N. Tusor, J. V. Hajnal, A. D. Edwards, S. J. Counsell and D. Rueckert (2016). "Regional growth and atlas of the developing human brain." Neuroimage **125**: 456-478.
- Malik, S., G. Vinukonda, L. R. Vose, D. Diamond, B. B. Bhimavarapu, F. Hu, M. T. Zia, R. Hevner, N. Zecevic and P. Ballabh (2013). "Neurogenesis continues in the third trimester of pregnancy and is suppressed by premature birth." J Neurosci **33**(2): 411-423.
- Mallard, C., M. E. Tremblay and Z. S. Vexler (2019). "Microglia and Neonatal Brain Injury." Neuroscience **405**: 68-76.
- Mallya, A. P., H. D. Wang, H. N. R. Lee and A. Y. Deutch (2019). "Microglial Pruning of Synapses in the Prefrontal Cortex During Adolescence." Cereb Cortex **29**(4): 1634-1643.
- Manley, B. J., L. S. Owen, S. B. Hooper, S. E. Jacobs, J. L. Y. Cheong, L. W. Doyle and P. G. Davis (2017). "Towards evidence-based resuscitation of the newborn infant." Lancet **389**(10079): 1639-1648.
- Marguet, S. L., V. T. Le-Schulte, A. Merseburg, A. Neu, R. Eichler, I. Jakovcevski, A. Ivanov, I. L. Hanganu-Opatz, C. Bernard, F. Morellini and D. Isbrandt (2015). "Treatment during a vulnerable developmental period rescues a genetic epilepsy." Nat Med **21**(12): 1436-1444.
- Marin-Padilla, M. (1997). "Developmental neuropathology and impact of perinatal brain damage. II: white matter lesions of the neocortex." J Neuropathol Exp Neurol **56**(3): 219-235.
- Marin, O. (2013). "Cellular and molecular mechanisms controlling the migration of neocortical interneurons." Eur J Neurosci **38**(1): 2019-2029.
- Marlow, N., D. Wolke, M. A. Bracewell, M. Samara and E. P. S. Group (2005). "Neurologic and developmental disability at six years of age after extremely preterm birth." N Engl J Med **352**(1): 9-19.

- Matcovitch-Natan, O., D. R. Winter, A. Giladi, S. Vargas Aguilar, A. Spinrad, S. Sarrazin, H. Ben-Yehuda, E. David, F. Zelada González, P. Perrin, H. Keren-Shaul, M. Gury, D. Lara-Astaiso, C. A. Thaiss, M. Cohen, K. Bahar Halpern, K. Baruch, A. Deczkowska, E. Lorenzo-Vivas, S. Itzkovitz, E. Elinav, M. H. Sieweke, M. Schwartz and I. Amit (2016). "Microglia development follows a stepwise program to regulate brain homeostasis." Science **353**(6301): aad8670.
- [Matta, S. M., Hill-Yardin, E. L., & Crack, P. J. \(2019\). The influence of neuroinflammation in Autism Spectrum Disorder. Brain, behavior, and immunity.](#)
- Mattei, D., A. Ivanov, C. Ferrai, P. Jordan, D. Guneykaya, A. Buonfiglioli, W. Schaafsma, P. Przanowski, W. Deuther-Conrad, P. Brust, S. Hesse, M. Patt, O. Sabri, T. L. Ross, B. J. L. Eggen, E. Boddeke, B. Kaminska, D. Beule, A. Pombo, H. Kettenmann and S. A. Wolf (2017). "Maternal immune activation results in complex microglial transcriptome signature in the adult offspring that is reversed by minocycline treatment." Transl Psychiatry **7**(5): e1120.
- Mattingly, G. W., J. Wilson and A. L. Rostain (2017). "A clinician's guide to ADHD treatment options." Postgrad Med **129**(7): 657-666.
- McAlonan, G. M., V. Cheung, C. Cheung, J. Suckling, G. Y. Lam, K. S. Tai, L. Yip, D. G. Murphy and S. E. Chua (2005). "Mapping the brain in autism. A voxel-based MRI study of volumetric differences and intercorrelations in autism." Brain **128**(Pt 2): 268-276.
- McClendon, E., K. Chen, X. Gong, E. Sharifnia, M. Hagen, V. Cai, D. C. Shaver, A. Riddle, J. M. Dean, A. J. Gunn, C. Mohr, J. S. Kaplan, D. J. Rossi, C. D. Kroenke, A. R. Hohimer and S. A. Back (2014). "Prenatal cerebral ischemia triggers dysmaturation of caudate projection neurons." Ann Neurol **75**(4): 508-524.
- McClendon, E., D. C. Shaver, K. Degener-O'Brien, X. Gong, T. Nguyen, A. Hoerder-Suabedissen, Z. Molnar, C. Mohr, B. D. Richardson, D. J. Rossi and S. A. Back (2017). "Transient Hypoxemia Chronically Disrupts Maturation of Preterm Fetal Ovine Subplate Neuron Arborization and Activity." J Neurosci **37**(49): 11912-11929.
- McClendon, E., K. Wang, K. Degener-O'Brien, M. W. Hagen, X. Gong, T. Nguyen, W. W. Wu, J. Maylie and S. A. Back (2019). "Transient Hypoxemia Disrupts Anatomical and Functional Maturation of Preterm Fetal Ovine CA1 Pyramidal Neurons." J Neurosci **39**(40): 7853-7871.
- McKinstry, R. C., A. Mathur, J. H. Miller, A. Ozcan, A. Z. Snyder, G. L. Schefft, C. R. Almlı, S. I. Shiran, T. E. Conturo and J. J. Neil (2002). "Radial organization of developing preterm human cerebral cortex revealed by non-invasive water diffusion anisotropy MRI." Cereb Cortex **12**(12): 1237-1243.
- McNamara, N. B. and V. E. Miron (2020). "Microglia in developing white matter and perinatal brain injury." Neuroscience Letters **714**: 134539.
- McQuillen, P. S., R. A. Sheldon, C. J. Shatz and D. M. Ferriero (2003). "Selective vulnerability of subplate neurons after early neonatal hypoxia-ischemia." J Neurosci **23**(8): 3308-3315.
- Molnar, Z., G. J. Clowry, N. Sestan, A. Alzu'bi, T. Bakken, R. F. Hevner, P. S. Huppi, I. Kostovic, P. Rakic, E. S. Anton, D. Edwards, P. Garcez, A. Hoerder-Suabedissen and A. Kriegstein (2019). "New insights into the development of the human cerebral cortex." J Anat **235**(3): 432-451.
- Mordel, J., A. Sheikh, S. Tsohataridis, P. O. Kanold, C. M. Zehendner and H. J. Luhmann (2016). "Mild systemic inflammation and moderate hypoxia transiently alter neuronal excitability in mouse somatosensory cortex." Neurobiol Dis **88**: 29-43.
- Nakao, T., J. Radua, K. Rubia and D. Mataix-Cols (2011). "Gray matter volume abnormalities in ADHD: voxel-based meta-analysis exploring the effects of age and stimulant medication." Am J Psychiatry **168**(11): 1154-1163.
- Neil, J. J., S. I. Shiran, R. C. McKinstry, G. L. Schefft, A. Z. Snyder, C. R. Almlı, E. Akbudak, J. A. Aronovitz, J. P. Miller, B. C. Lee and T. E. Conturo (1998). "Normal brain in human newborns: apparent diffusion coefficient and diffusion anisotropy measured by using diffusion tensor MR imaging." Radiology **209**(1): 57-66.
- Nimmervoll, B., R. White, J. W. Yang, S. An, C. Henn, J. J. Sun and H. J. Luhmann (2013). "LPS-induced microglial secretion of TNF $\alpha$  increases activity-dependent neuronal apoptosis in the neonatal cerebral cortex." Cereb Cortex **23**(7): 1742-1755.

[Nithianantharajah, J., Balasuriya, G. K., Franks, A. E., & Hill-Yardin, E. L. \(2017\). Using animal models to study the role of the gut–brain axis in autism. \*Current developmental disorders reports\*, 4\(2\), 28-36.](#)

- Nobuta, H., C. A. Ghiani, P. M. Paez, V. Spreuer, H. Dong, R. A. Korsak, A. Manukyan, J. Li, H. V. Vinters, E. J. Huang, D. H. Rowitch, M. V. Sofroniew, A. T. Campagnoni, J. de Vellis and J. A. Waschek (2012). "STAT3-mediated astrogliosis protects myelin development in neonatal brain injury." *Ann Neurol* **72**(5): 750-765.
- Nosarti, C., M. H. Al-Asady, S. Frangou, A. L. Stewart, L. Rifkin and R. M. Murray (2002). "Adolescents who were born very preterm have decreased brain volumes." *Brain* **125**(Pt 7): 1616-1623.
- Nosarti, C., E. Giouroukou, E. Healy, L. Rifkin, M. Walshe, A. Reichenberg, X. Chitnis, S. C. Williams and R. M. Murray (2008). "Grey and white matter distribution in very preterm adolescents mediates neurodevelopmental outcome." *Brain* **131**(Pt 1): 205-217.
- Nunthapiwat, S., R. Sekararithi, C. Wanapirak, S. Sirichotiyakul, F. Tongprasert, K. Srisupundit, S. Luewan and T. Tongsong (2019). "Second Trimester Serum Biomarker Screen for Fetal Aneuploidies as a Predictor of Preterm Delivery: A Population-Based Study." *Gynecol Obstet Invest* **84**(4): 326-333.
- O'Shea, T. M., E. N. Allred, O. Dammann, D. Hirtz, K. C. Kuban, N. Paneth and A. Leviton (2009). "The ELGAN study of the brain and related disorders in extremely low gestational age newborns." *Early Hum Dev* **85**(11): 719-725.
- Okusa, C., F. Oeschger, V. Ginet, W. Z. Wang, A. Hoerder-Suabedissen, T. Matsuyama, A. C. Truttmann and Z. Molnar (2014). "Subplate in a rat model of preterm hypoxia-ischemia." *Ann Clin Transl Neurol* **1**(9): 679-691.
- Panda, S., P. Dohare, S. Jain, N. Parikh, P. Singla, R. Mehdizadeh, D. W. Klebe, G. M. Kleinman, B. Cheng and P. Ballabh (2018). "Estrogen Treatment Reverses Prematurity-Induced Disruption in Cortical Interneuron Population." *J Neurosci* **38**(34): 7378-7391.
- Pandit, A. S., E. Robinson, P. Aljabar, G. Ball, I. S. Gousias, Z. Wang, J. V. Hajnal, D. Rueckert, S. J. Counsell, G. Montana and A. D. Edwards (2014). "Whole-brain mapping of structural connectivity in infants reveals altered connection strength associated with growth and preterm birth." *Cereb Cortex* **24**(9): 2324-2333.
- Pang, Y., L. Campbell, B. Zheng, L. Fan, Z. Cai and P. Rhodes (2010). "Lipopolysaccharide-activated microglia induce death of oligodendrocyte progenitor cells and impede their development." *Neuroscience* **166**(2): 464-475.
- Pang, Y., X. Dai, A. Roller, K. Carter, I. Paul, A. J. Bhatt, R. C. Lin and L. W. Fan (2016). "Early Postnatal Lipopolysaccharide Exposure Leads to Enhanced Neurogenesis and Impaired Communicative Functions in Rats." *PLoS One* **11**(10): e0164403.
- Pannek, K., X. Hatzigeorgiou, P. B. Colditz and S. Rose (2013). "Assessment of structural connectivity in the preterm brain at term equivalent age using diffusion MRI and T2 relaxometry: a network-based analysis." *PLoS One* **8**(8): e68593.
- Paolicelli, R. C., G. Bolasco, F. Pagani, L. Maggi, M. Scianni, P. Panzanelli, M. Giustetto, T. A. Ferreira, E. Guiducci, L. Dumas, D. Ragozzino and C. T. Gross (2011). "Synaptic pruning by microglia is necessary for normal brain development." *Science* **333**(6048): 1456-1458.
- Pavlidis, E., R. O. Lloyd and G. B. Boylan (2017). "EEG - A Valuable Biomarker of Brain Injury in Preterm Infants." *Dev Neurosci* **39**(1-4): 23-35.
- Petrelli, F., L. Pucci and P. Bezzi (2016). "Astrocytes and Microglia and Their Potential Link with Autism Spectrum Disorders." *Front Cell Neurosci* **10**: 21.
- Petrenko, V., J. Mihhailova, P. Salmon and J. Z. Kiss (2015). "Apoptotic neurons induce proliferative responses of progenitor cells in the postnatal neocortex." *Exp Neurol* **273**: 126-137.
- Petrenko, V., Y. van de Looij, J. Mihhailova, P. Salmon, P. S. Huppi, S. V. Sizonenko and J. Z. Kiss (2018). "Multimodal MRI Imaging of Apoptosis-Triggered Microstructural Alterations in the Postnatal Cerebral Cortex." *Cereb Cortex* **28**(3): 949-962.
- Pierrat, V., L. Marchand-Martin, C. Arnaud, M. Kaminski, M. Resche-Rigon, C. Lebeaux, F. Bodeau-Livinec, A. S. Morgan, F. Goffinet, S. Marret, P. Y. Ancel and E.-w. 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.
- Pierson, C. R., R. D. Folkerth, S. S. Billiards, F. L. Trachtenberg, M. E. Drinkwater, J. J. Volpe and H. C. Kinney (2007). "Gray matter injury associated with periventricular leukomalacia in the premature infant." *Acta Neuropathologica* **114**(6): 619-631.
- Pizzarelli, R. and E. Cherubini (2011). "Alterations of GABAergic signaling in autism spectrum disorders." *Neural Plast* **2011**: 297153.
- Plomgaard, A. M., A. D. Andersen, T. H. Petersen, Y. van de Looij, T. Thymann, P. T. Sangild, C. Thomsen, S. V. Sizonenko and G. Greisen (2019). "Structural brain maturation differs between preterm and term piglets, whereas brain activity does not." *Acta Paediatr* **108**(4): 637-644.
- Pogledic, I., I. Kostovic, C. Fallet-Bianco, H. Adle-Biasette, P. Gressens and C. Verney (2014). "Involvement of the subplate zone in preterm infants with periventricular white matter injury." *Brain Pathol* **24**(2): 128-141.
- Preston, M., X. Gong, W. Su, S. G. Matsumoto, F. Banine, C. Winkler, S. Foster, R. Xing, J. Struve, J. Dean, B. Baggenstoss, P. H. Weigel, T. J. Montine, S. A. Back and L. S. Sherman (2013). "Digestion products of the PH20 hyaluronidase inhibit remyelination." *Ann Neurol* **73**(2): 266-280.
- Prins, J. R., S. Eskandar, B. J. L. Eggen and S. A. Scherjon (2018). "Microglia, the missing link in maternal immune activation and fetal neurodevelopment; and a possible link in preeclampsia and disturbed neurodevelopment?" *Journal of Reproductive Immunology* **126**: 18-22.
- Quezada, S., M. Castillo-Melendez, D. W. Walker and M. Tolcos (2018). "Development of the cerebral cortex and the effect of the intrauterine environment." *J Physiol* **596**(23): 5665-5674.
- Ramlackhansingh, A. F., D. J. Brooks, R. J. Greenwood, S. K. Bose, F. E. Turkheimer, K. M. Kinnunen, S. Gentleman, R. A. Heckemann, K. Gunanayagam, G. Gelosa and D. J. Sharp (2011). "Inflammation after trauma: microglial activation and traumatic brain injury." *Ann Neurol* **70**(3): 374-383.
- Ranasinghe, S., G. Or, E. Y. Wang, A. Ievins, M. A. McLean, C. M. Niell, V. Chau, P. K. Wong, H. C. Glass, J. Sullivan and P. S. McQuillen (2015). "Reduced Cortical Activity Impairs Development and Plasticity after Neonatal Hypoxia Ischemia." *J Neurosci* **35**(34): 11946-11959.
- Rathbone, R., S. J. Counsell, O. Kapellou, L. Dyet, N. Kennea, J. Hajnal, J. M. Allsop, F. Cowan and A. D. Edwards (2011). "Perinatal cortical growth and childhood neurocognitive abilities." *Neurology* **77**(16): 1510-1517.
- Reynolds, L. C., R. G. Pineda, A. Mathur, C. Vavasseur, D. K. Shah, S. Liao and T. Inder (2014). "Cerebral maturation on amplitude-integrated electroencephalography and perinatal exposures in preterm infants." *Acta Paediatr* **103**(3): e96-e100.
- Robinson, S., C. J. Corbett, J. L. Winer, L. A. S. Chan, J. R. Maxwell, C. V. Anstine, T. R. Yellowhair, N. A. Andrews, Y. Yang, L. O. Sillerud and L. L. Jantzie (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.
- Roessmann, U. and P. Gambetti (1986). "Astrocytes in the developing human brain." *Acta Neuropathologica* **70**(3): 308-313.
- Rogers, C. E., D. M. Barch, C. M. Sylvester, D. Pagliaccio, M. P. Harms, K. N. Botteron and J. L. Luby (2014). "Altered gray matter volume and school age anxiety in children born late preterm." *J Pediatr* **165**(5): 928-935.
- Romero, R., R. Gomez, T. Chaiworapongsa, G. Conoscenti, J. C. Kim and Y. M. Kim (2001). "The role of infection in preterm labour and delivery." *Paediatr Perinat Epidemiol* **15 Suppl 2**: 41-56.
- Rousset, C. I., S. Chalon, S. Cantagrel, S. Bodard, C. Andres, P. Gressens and E. Saliba (2006). "Maternal exposure to LPS induces hypomyelination in the internal capsule and programmed cell death in the deep gray matter in newborn rats." *Pediatr Res* **59**(3): 428-433.



- Rylaarsdam, L. and A. Guemez-Gamboa (2019). "Genetic Causes and Modifiers of Autism Spectrum Disorder." *Front Cell Neurosci* **13**: 385.
- Sajdel-Sulkowska, E. M., M. Makowska-Zubrycka, K. Czarzasta, K. Kasarello, V. Aggarwal, M. Bialy, E. Szczepanska-Sadowska and A. Cudnoch-Jedrzejewska (2019). "Common Genetic Variants Link the Abnormalities in the Gut-Brain Axis in Prematurity and Autism." *The Cerebellum* **18**(2): 255-265.
- Sato, K. (2015). "Effects of Microglia on Neurogenesis." *Glia* **63**(8): 1394-1405.
- Sato, W., T. Kochiyama, S. Uono, S. Yoshimura, Y. Kubota, R. Sawada, M. Sakihama and M. Toichi (2017). "Reduced Gray Matter Volume in the Social Brain Network in Adults with Autism Spectrum Disorder." *Front Hum Neurosci* **11**: 395.
- [Sauer AK, Bockmann J, Steinestel K, Boeckers TM, Grabrucker AM \(2019\) Altered Intestinal Morphology and Microbiota Composition in the Autism Spectrum Disorders Associated SHANK3 Mouse Model. Int J Mol Sci 20 10.3390/ijms20092134.](#)
- Scafidi, J., T. R. Hammond, S. Scafidi, J. Ritter, B. Jablonska, M. Roncal, K. Szigeti-Buck, D. Coman, Y. Huang, R. J. McCarter, Jr., F. Hyder, T. L. Horvath and V. Gallo (2014). "Intranasal epidermal growth factor treatment rescues neonatal brain injury." *Nature* **506**(7487): 230-234.
- Schafer, D. P., E. K. Lehrman, A. G. Kautzman, R. Koyama, A. R. Mardinly, R. Yamasaki, R. M. Ransohoff, M. E. Greenberg, B. A. Barres and B. Stevens (2012). "Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner." *Neuron* **74**(4): 691-705.
- Scheuer, T., V. Brockmoller, M. Blanco Knowlton, J. H. Weitkamp, T. Ruhwedel, S. Mueller, S. Endesfelder, C. Buhner and T. Schmitz (2015). "Oligodendroglial maldevelopment in the cerebellum after postnatal hyperoxia and its prevention by minocycline." *Glia* **63**(10): 1825-1839.
- Schmitz, T., S. Endesfelder, M. C. Reinert, F. Klinker, S. Muller, C. Buhner and D. Liebetanz (2012). "Adolescent hyperactivity and impaired coordination after neonatal hyperoxia." *Exp Neurol* **235**(1): 374-379.
- [Sgritta, M., Dooling, S. W., Buffington, S. A., Momin, E. N., Francis, M. B., Britton, R. A., & Costa-Mattioli, M. \(2019\). Mechanisms Underlying Microbial-Mediated Changes in Social Behavior in Mouse Models of Autism Spectrum Disorder. Neuron, 101\(2\), 246–259.e6.](#)
- [Sharon G, Cruz NJ, Kang DW, et al. Human Gut Microbiota from Autism Spectrum Disorder Promote Behavioral Symptoms in Mice. Cell. 2019;177\(6\):1600-1618.e17. doi:10.1016/j.cell.2019.05.004.](#)
- Shaw, J. C., M. J. Berry, R. M. Dyson, G. K. Crombie, J. J. Hirst and H. K. Palliser (2019). "Reduced Neurosteroid Exposure Following Preterm Birth and Its' Contribution to Neurological Impairment: A Novel Avenue for Preventative Therapies." *Front Physiol* **10**: 599.
- Shiow, L. R., G. Favrais, L. Schirmer, A.-L. Schang, S. Cipriani, C. Andres, J. N. Wright, H. Nobuta, B. Fleiss, P. Gressens and D. H. Rowitch (2017). "Reactive astrocyte COX2-PGE2 production inhibits oligodendrocyte maturation in neonatal white matter injury." *Glia* **65**(12): 2024-2037.
- Sidman, R. L. and P. Rakic (1973). "Neuronal migration, with special reference to developing human brain: a review." *Brain Res* **62**(1): 1-35.
- Song, J., F. Xu, L. Wang, L. Gao, J. Guo, L. Xia, Y. Zhang, W. Zhou, X. Wang and C. Zhu (2015). "Early amplitude-integrated electroencephalography predicts brain injury and neurological outcome in very preterm infants." *Sci Rep* **5**: 13810.
- Southwell, D. G., H. Seifkar, R. Malik, K. Lavi, D. Vogt, J. L. Rubenstein and V. S. Sohal (2020). "Interneuron Transplantation Rescues Social Behavior Deficits without Restoring Wild-Type Physiology in a Mouse Model of Autism with Excessive Synaptic Inhibition." *The Journal of Neuroscience* **40**(11): 2215-2227.
- Souza, R. T., E. J. McKenzie, B. Jones, J. V. de Seymour, M. M. Thomas, E. Zarate, T. L. Han, L. McCowan, K. Sulek, S. Villas-Boas, L. C. Kenny, J. G. Cecatti and P. N. Baker (2019). "Trace biomarkers associated with spontaneous preterm birth from the maternal

- serum metabolome of asymptomatic nulliparous women - parallel case-control studies from the SCOPE cohort." *Sci Rep* **9**(1): 13701.
- Stavropoulos, K. K.-M. (2017). "Using neuroscience as an outcome measure for behavioral interventions in Autism spectrum disorders (ASD): A review." *Research in Autism Spectrum Disorders* **35**: 62-73.
- Stojanovska, V., A. Atik, I. Nitsos, B. Skiöld, S. K. Barton, V. A. Zahra, K. Rodgers, S. B. Hooper, G. R. Polglase and R. Galinsky (2018). "Effects of Intrauterine Inflammation on Cortical Gray Matter of Near-Term Lambs." *Frontiers in pediatrics* **6**: 145-145.
- Stolp, H. B., G. Ball, P. W. So, J. D. Tournier, M. Jones, C. Thornton and A. D. Edwards (2018). "Voxel-wise comparisons of cellular microstructure and diffusion-MRI in mouse hippocampus using 3D Bridging of Optically-clear histology with Neuroimaging Data (3D-BOND)." *Sci Rep* **8**(1): 4011.
- Stolp, H. B., B. Fleiss, Y. Arai, V. Supramaniam, R. Vontell, S. Birtles, A. G. Yates, A. A. Baburamani, C. Thornton, M. Rutherford, A. D. Edwards and P. Gressens (2019). "Interneuron Development Is Disrupted in Preterm Brains With Diffuse White Matter Injury: Observations in Mouse and Human." *Front Physiol* **10**: 955.
- Stolp, H. B., C. Turnquist, K. M. Dziegielewska, N. R. Saunders, D. C. Anthony and Z. Molnar (2011). "Reduced ventricular proliferation in the foetal cortex following maternal inflammation in the mouse." *Brain* **134**(Pt 11): 3236-3248.
- Striedter, G. F., S. Srinivasan and E. S. Monuki (2015). "Cortical folding: when, where, how, and why?" *Annu Rev Neurosci* **38**: 291-307.
- Sucksdorff, M., L. Lehtonen, R. Chudal, A. Suominen, P. Joelsson, M. Gissler and A. Sourander (2015). "Preterm Birth and Poor Fetal Growth as Risk Factors of Attention-Deficit/ Hyperactivity Disorder." *Pediatrics* **136**(3): e599-608.
- Tahraoui, S. L., S. Marret, C. Bodenat, P. Leroux, M. A. Dommergues, P. Evrard and P. Gressens (2001). "Central role of microglia in neonatal excitotoxic lesions of the murine periventricular white matter." *Brain Pathol* **11**(1): 56-71.
- [Sullivan, S. M., S. T. Bjorkman, S. M. Miller, P. B. Colditz and D. V. Pow \(2010\). "Structural remodeling of gray matter astrocytes in the neonatal pig brain after hypoxia/ischemia." \*Glia\* \*\*58\*\*\(2\): 181-194.](#)
- Tataranno, M. L., N. H. P. Claessens, P. Moeskops, M. C. Toet, K. J. Kersbergen, G. Buonocore, I. Isgum, A. Leemans, S. Counsell, F. Groenendaal, L. S. de Vries and M. Benders (2018). "Changes in brain morphology and microstructure in relation to early brain activity in extremely preterm infants." *Pediatr Res* **83**(4): 834-842.
- Tay, T. L., J. C. Savage, C. W. Hui, K. Bisht and M. E. Tremblay (2017). "Microglia across the lifespan: from origin to function in brain development, plasticity and cognition." *J Physiol* **595**(6): 1929-1945.
- Thomason, M. E., D. Scheinost, J. H. Manning, L. E. Grove, J. Hect, N. Marshall, E. Hernandez-Andrade, S. Berman, A. Pappas, L. Yeo, S. S. Hassan, R. T. Constable, L. R. Ment and R. Romero (2017). "Weak functional connectivity in the human fetal brain prior to preterm birth." *Sci Rep* **7**: 39286.
- Tibrewal, M., B. Cheng, P. Dohare, F. Hu, R. Mehdizadeh, P. Wang, D. Zheng, Z. Ungvari and P. Ballabh (2018). "Disruption of Interneuron Neurogenesis in Premature Newborns and Reversal with Estrogen Treatment." *J Neurosci* **38**(5): 1100-1113.
- [Truttmann, A. C., V. Ginot and J. Puyal \(2020\). "Current Evidence on Cell Death in Preterm Brain Injury in Human and Preclinical Models." \*Front Cell Dev Biol\* \*\*8\*\*: 27-](#)
- Ure, A. M., K. Treyvaud, D. K. Thompson, L. Pascoe, G. Roberts, K. J. Lee, M. L. Seal, E. Northam, J. L. Cheong, R. W. Hunt, T. Inder, L. W. Doyle and P. J. Anderson (2016). "Neonatal brain abnormalities associated with autism spectrum disorder in children born very preterm." *Autism Res* **9**(5): 543-552.
- van de Looij, Y., A. Chatagner, C. Quairiaux, R. Gruetter, P. S. Hüppi and S. V. Sizonenko (2014). "Multi-modal assessment of long-term erythropoietin treatment after neonatal hypoxic-ischemic injury in rat brain." *PLoS one* **9**(4): e95643-e95643.
- van den Heuvel, L. G., M. Fraser, S. L. Miller, G. Jenkin, E. M. Wallace, J. O. Davidson, C. A. Lear, R. Lim, G. Wassink, A. J. Gunn and L. Bennet (2019). "Delayed intranasal infusion of

- human amnion epithelial cells improves white matter maturation after asphyxia in preterm fetal sheep." J Cereb Blood Flow Metab **39**(2): 223-239.
- Van Essen, D. C., C. J. Donahue, T. S. Coalson, H. Kennedy, T. Hayashi and M. F. Glasser (2019). "Cerebral cortical folding, parcellation, and connectivity in humans, nonhuman primates, and mice." Proceedings of the National Academy of Sciences **116**(52): 26173-26180.
- Van Steenwinckel, J., A. L. Schang, M. L. Krishnan, V. Degos, A. Delahaye-Duriez, C. Bokobza, Z. Csaba, F. Verdonk, A. Montané, S. Sigaut, O. Hennebert, S. Lebon, L. Schwendimann, T. Le Charpentier, R. Hassan-Abdi, G. Ball, P. Aljabar, A. Saxena, R. K. Holloway, W. Birchmeier, O. Baud, D. Rowitch, V. Miron, F. Chretien, C. Leconte, V. C. Besson, E. G. Petretto, A. D. Edwards, H. Hagberg, N. Soussi-Yanicostas, B. Fleiss and P. Gressens (2019). "Decreased microglial Wnt/ $\beta$ -catenin signalling drives microglial pro-inflammatory activation in the developing brain." Brain **142**(12): 3806-3833.
- Vasistha, N. A., M. Pardo-Navarro, J. Gasthaus, D. Weijers, M. K. Muller, D. Garcia-Gonzalez, S. Malwade, I. Korshunova, U. Pfisterer, J. von Engelhardt, K. S. Hougaard and K. Khodosevich (2019). "Maternal inflammation has a profound effect on cortical interneuron development in a stage and subtype-specific manner." Mol Psychiatry.
- Verderio, C., L. Muzio, E. Turola, A. Bergami, L. Novellino, F. Ruffini, L. Riganti, I. Corradini, M. Francolini, L. Garzetti, C. Maiorino, F. Servida, A. Vercelli, M. Rocca, D. Dalla Libera, V. Martinelli, G. Comi, G. Martino, M. Matteoli and R. Furlan (2012). "Myeloid microvesicles are a marker and therapeutic target for neuroinflammation." Ann Neurol **72**(4): 610-624.
- Verma, S., R. Nakaoke, S. Dohgu and W. A. Banks (2006). "Release of cytokines by brain endothelial cells: A polarized response to lipopolysaccharide." Brain Behav Immun **20**(5): 449-455.
- Verney, C., I. Pogledic, V. Biran, H. Adle-Biassette, C. Fallet-Bianco and P. Gressens (2012). "Microglial reaction in axonal crossroads is a hallmark of noncystic periventricular white matter injury in very preterm infants." J Neuropathol Exp Neurol **71**(3): 251-264.
- Vezzani, A., S. Balosso and T. Ravizza (2019). "Neuroinflammatory pathways as treatment targets and biomarkers in epilepsy." Nat Rev Neurol **15**(8): 459-472.
- Vinall, J., R. E. Grunau, R. Brant, V. Chau, K. J. Poskitt, A. R. Synnes and S. P. Miller (2013). "Slower postnatal growth is associated with delayed cerebral cortical maturation in preterm newborns." Sci Transl Med **5**(168): 168ra168.
- Volpe, J. J. (2009). "The encephalopathy of prematurity--brain injury and impaired brain development inextricably intertwined." Semin Pediatr Neurol **16**(4): 167-178.
- Volpe, J. J. (2019). "Dysmaturation of Premature Brain: Importance, Cellular Mechanisms, and Potential Interventions." Pediatr Neurol **95**: 42-66.
- Vontell, R., V. Supramaniam, J. Wyatt-Ashmead, P. Gressens, M. Rutherford, H. Hagberg and C. Thornton (2015). "Cellular mechanisms of toll-like receptor-3 activation in the thalamus are associated with white matter injury in the developing brain." J Neuropathol Exp Neurol **74**(3): 273-285.
- Vottier, G., H. Pham, J. Pansiot, V. Biran, P. Gressens, C. Charriaut-Marlangue and O. Baud (2011). "Deleterious effect of hyperoxia at birth on white matter damage in the newborn rat." Dev Neurosci **33**(3-4): 261-269.
- Wang, Y., X. Cheng, Q. He, Y. Zheng, D. H. Kim, S. R. Whittemore and Q. L. Cao (2011). "Astrocytes from the Contused Spinal Cord Inhibit Oligodendrocyte Differentiation of Adult Oligodendrocyte Precursor Cells by Increasing the Expression of Bone Morphogenetic Proteins." The Journal of Neuroscience **31**(16): 6053.
- Wassink, G., J. O. Davidson, S. K. Dhillon, M. Fraser, R. Galinsky, L. Bennet and A. J. Gunn (2017). "Partial white and grey matter protection with prolonged infusion of recombinant human erythropoietin after asphyxia in preterm fetal sheep." J Cereb Blood Flow Metab **37**(3): 1080-1094.
- Wellmann, S., C. Bührer and T. Schmitz (2014). "Focal necrosis and disturbed myelination in the white matter of newborn infants: a tale of too much or too little oxygen." Front Pediatr **2**: 143.

- Whitehead, K., L. Jones, M. P. Laudiano-Dray, J. Meek and L. Fabrizi (2020). "Altered cortical processing of somatosensory input in pre-term infants who had high-grade germinal matrix-intraventricular haemorrhage." Neuroimage Clin **25**: 102095.
- Whitehead, K., R. Pressler and L. Fabrizi (2017). "Characteristics and clinical significance of delta brushes in the EEG of premature infants." Clin Neurophysiol Pract **2**: 12-18.
- Wiebe, S., A. Nagpal, V. T. Truong, J. Park, A. Skalecka, A. J. He, K. Gamache, A. Khoutorsky, I. Gantois and N. Sonenberg (2019). "Inhibitory interneurons mediate autism-associated behaviors via 4E-BP2." Proc Natl Acad Sci U S A **116**(36): 18060-18067.
- Wolosin, S. M., M. E. Richardson, J. G. Hennessey, M. B. Denckla and S. H. Mostofsky (2009). "Abnormal cerebral cortex structure in children with ADHD." Hum Brain Mapp **30**(1): 175-184.
- Won, H., W. Mah and E. Kim (2013). "Autism spectrum disorder causes, mechanisms, and treatments: focus on neuronal synapses." Front Mol Neurosci **6**: 19.
- Wood, N. S., K. Costeloe, A. T. Gibson, E. M. Hennessy, N. Marlow, A. R. Wilkinson and E. P. S. Group (2005). "The EPICure study: associations and antecedents of neurological and developmental disability at 30 months of age following extremely preterm birth." Arch Dis Child Fetal Neonatal Ed **90**(2): F134-140.
- Xie, D., F. Shen, S. He, M. Chen, Q. Han, M. Fang, H. Zeng, C. Chen and Y. Deng (2016). "IL-1beta induces hypomyelination in the periventricular white matter through inhibition of oligodendrocyte progenitor cell maturation via FYN/MEK/ERK signaling pathway in septic neonatal rats." Glia **64**(4): 583-602.
- Yekhlef, L., G. L. Breschi, L. Lagostena, G. Russo and S. Taverna (2015). "Selective activation of parvalbumin- or somatostatin-expressing interneurons triggers epileptic seizurelike activity in mouse medial entorhinal cortex." J Neurophysiol **113**(5): 1616-1630.
- Zhan, Y., R. C. Paolicelli, F. Sforzini, L. Weinhard, G. Bolasco, F. Pagani, A. L. Vyssotski, A. Bifone, A. Gozzi, D. Ragozzino and C. T. Gross (2014). "Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior." Nat Neurosci **17**(3): 400-406.
- Zhang, Y., T. E. Inder, J. J. Neil, D. L. Dierker, D. Alexopoulos, P. J. Anderson and D. C. Van Essen (2015). "Cortical structural abnormalities in very preterm children at 7 years of age." Neuroimage **109**: 469-479.
- Zikopoulos, B. and H. Barbas (2013). "Altered neural connectivity in excitatory and inhibitory cortical circuits in autism." Front Hum Neurosci **7**: 609.
- Zubiaurre-Elorza, L., S. Soria-Pastor, C. Junque, D. Segarra, N. Bargallo, N. Mayolas, C. Romano-Berindoague and A. Macaya (2011). "Gray matter volume decrements in preterm children with periventricular leukomalacia." Pediatr Res **69**(6): 554-560.

**Table 1.** Summary of post-mortem studies of preterm born infants including analysis of the grey matter, highlighting the case characteristics, regions of interest and the grey and white matter injuries described. White matter injury provided as context for overall injury severity.

Reference / Study location / Years of sample collection	Number of cases (n) / Gestational age at birth / Survival time	Pathologists description of injury / Post-mortem delay	Nature of cases designated as controls	Regions of interest	Grey matter pathology (description of what was analysed)	White matter pathology (description of what was analysed)
Andiman <i>et al.</i> (2010) / Dept. of Pathology, Children's Hospital Boston, MA, USA / 1993 - 2007	20 WMI 15 controls / WMI = 33.9 ± 4.3 Control = 33.1 ± 6.2 / WMI = 5.9 ± 14.0 weeks Controls = 13.2 ± 23.6. (NS diff)	PVL or diffuse WMI as diagnosed by a histopathologist / Post-mortem delay not reported	Prematurity with respiratory distress syndrome, n=4; congenital heart disease, n=2; primary pulmonary hypertension, n=1; hydrops fetalis due to placental chorioangiomas, n=1; hydrops fetalis, n=1; sacral teratoma, n=1; cystic lymphatic malformation of the neck, n=1; Werdnig-Hoffmann disease, n=1; foreign body aspiration, n=1; Blackfan-Diamond syndrome, n=1; & bronchiolitis, n=1  No difference in mean Apgar scores at 5 minutes (6.8 for both groups) or in other disorders / confounders	In the WMI cases, the cortex overlying WMI and compared it to similar cortical areas in control cases	No sig. difference in the presence of fractin-immunopositive neurons in any cortical layer  No sig. difference in the incidence of the percent of MAP2-stained pyramidal cells in layer V or obvious cortical anomalies.  Significant reduction (67%) in the density (MAP2) of layer V pyramidal neurons  No sig. difference in the cortical or laminar thickness (MAP2, H&E)	Periventricular focal necrosis in the deep white matter with surrounding diffuse reactive gliosis and microglial activation (previous neuropathologic studies)
Haynes and van Leyen (2013) 12/15-Lipoxygenase / Dept. of Pathology, Children's Hospital Boston, MA, USA. / Collection epoch not reported	13 PVL 17 controls / PVL - 29 to 43 PC weeks (median=35.5) & 0-8 PN weeks (median=1.5) controls - 20 to 43 PC weeks (median=33.5) and 0-2.5 PN weeks (median=1) / Survival time not reported	PVL as diagnosed by a histopathologist / PVL = 6 to 25 h Control = 4 to 25 h	Control cases did not have PVL or other significant brain pathology upon standard histologic examination. Autopsy reports were reviewed for major clinical findings, systemic autopsy diagnoses, and neuropathologic findings.	Subcortical white matter and the cortex overlying WMI and compared it to similar cortical areas in control cases	No increase in 12/15-LOX expression in neurons of the cerebral cortex in PVL.  Cell death or total cell number not assessed in the grey matter.	PVL had "focal" necrotic component in the periventricular region, and "diffuse" component characterized by reactive gliosis and activated microglia in the surrounding white matter  Increased 12/15-LOX expression in large round CD68+ cells, lectin double positive and O4 double positive cells and scattered TUNEL- positive cells.
Haynes <i>et al.</i> (2008) Diffuse axonal	13 PVL 17 Control	PVL as diagnosed by a histopathologist / PVL = 6-44 hours (median=17)	Control cases did not have PVL or other significant brain pathology upon standard histologic examination.	The area of	Approx. 1/3 <sup>rd</sup> PVL cases had thalamic gliosis, neuronal loss, and/or microinfarcts as determined by conventional histopathologic examination.	PVL based on histopathologic Criteria – periventricular focal

<p>/ Dept. of Pathology, Children's Hospital Boston, MA / <b>Collection epoch not reported</b></p>	<p>(spread across acute and later stages) Mean gestational age (wks) PVL = 36 +/- 3 Controls = 32 +/- 7 Mean postnatal age (wks) – PVL = 7.5 +/- 17 Control = 10.5 +/- 27</p>	<p>Controls = 1.5–132 hours (median=14)</p>	<p>Causes of death included Noonan's syndrome 1, Fetal hydrops 1, Neonatal hepatic disease 1, Immune thrombocytopenia 1, Possible mitochondrial disorder 1, Sudden unexplained death in childhood, 1, Trisomy 21 1, Unexplained stillbirth.</p>	<p>study for axonal damage in PVL was distant from the infarct, i.e., in a separate section with no overlying cortical damage.</p>	<p>Visually appreciable neuronal loss was present in the overlying cerebral cortex in 15% of the PVL cases. None of the non-PVL, non-axonal controls examined showed evidence of thalamic and/or cerebral cortical damage</p>	<p>necrosis in association with diffuse reactive gliosis and microglial activation  Diffuse axonal injury, as determined by the apoptotic marker fraction, in the gliotic (non-necrotic) cerebral white matter in the acute and organizing stages of focal PVL</p>
<p>Ligam et al. (2009) / Dept. of Pathology, Children's Hospital Boston, MA, USA. / <b>Collection epoch not reported</b></p>	<p>22 PVL 16 non-PVL Gestational age in PVL=32.5+/-4.8 gw Controls =36.7+/-5.2 gw Sig dif in gw. <b>PVL = approx. 4 weeks</b> <b>Controls = approx. 20 weeks (P=0.07)</b></p>	<p>PVL as diagnosed by a histopathologist / <i>Post-mortem delay not described</i></p>	<p>Control cases did not demonstrate white matter features. Lower rates of NEC and sepsis in controls than in PVL.</p>	<p>Thalamic sections were analysed at one of the following levels: I (anterior), level of the mammillary bodies; II (mid), level of the red nucleus; and III (posterior), level of the lateral geniculate nucleus</p>	<p>Increased thalamic pathology via neuropathologist assessment (H&amp;E) Trend to decreased neuronal density with H&amp;E (p=0.07) – criteria for neuronal discrimination not described Increased density of reactive astrocytes (GFAP) in the mediodorsal nucleus and the lateral posterior nucleus No significant increase in the density of CD68 + cells and numbers overall low. No difference in the density of MDA-immunopositive neurons or percent of MDA-immunopositive neurons</p>	<p>Histopathology to confirm (or not) PVL, with "focal" necrotic component in the periventricular region, and "diffuse" component characterized by reactive gliosis and activated microglia in the surrounding white matter</p>
<p>Kinney et al. (2012) / Dept. of Pathology, Children's Hospital Boston, MA, USA. / <b>1998-2012</b></p>	<p>15 PVL 10 controls <i>The mean gestational age PVL = 32.8+/-4.1 weeks in the Control = 30.1+/-5.9 weeks</i> <b>PVL = 34.+/- 4.6 postconceptional weeks</b> <b>Controls = 31.6+/-6.6 postconceptional weeks</b></p>	<p>PVL as diagnosed by a histopathologist / <i>Causes of death in PVL: respiratory distress syndrome (n=7); congenital heart disease (n=3); primary skeletal disorders (n=2); congenital diaphragmatic hernia (n=1); inborn error of metabolism (n=1); &amp; VOGM (n=1)</i> PVL = median 14 hours Control = median 16.5 hours</p>	<p>Controls did not demonstrate white matter abnormalities. Causes of death in controls respiratory distress syndrome (n=5); congenital heart disease (n=1); hydrops fetalis due to placental chorioangiomas (n=1); hydrops fetalis due to parvovirus (n=1); primary pulmonary hypertension (n=1); and bronchiolitis (n=1).</p>	<p>Neurons in the ventricular/subventricular region, periventricular white matter, central white matter, and subplate region in PVL cases and controls – including five subtypes of subcortical neurons: granular, unipolar, bipolar, inverted pyramidal, and multipolar</p>	<p>The neuronal density of the granular neurons in each of the four regions was 54–80% lower (p≤0.01) in the PVL cases compared to controls adjusted for age and post-mortem interval. The overall densities of unipolar, bipolar, multipolar, and inverted pyramidal neurons did not differ significantly between the PVL cases and controls.</p>	<p>Analysis grouped neurons in the subplate and white matter collectively. PVL was characterised by necrotic foci in the periventricular and/or central white matter; and diffuse astrogliosis and microglial activation in the surrounding white matter</p>

<p>Pierson et al. (2007) / Dept. of Pathology, Children's Hospital Boston, MA, USA / 1997-1999</p>	<p>17 PVL 17 DWMI 7 Negative (controls) / PVL=3.7+/-4.1 (median=2.3) DWMI=3.4+/-14.0 (median=1.2) Negative=0.8+/-1.2 (median)</p>	<p>PVL or diffuse white matter gliosis (DWMI) without necrosis; / Post-mortem delay not described</p>	<p>"Negative" white matter group with no diffuse gliosis or focal periventricular necrosis in the cerebral white matter</p>	<p>Seventeen grey matter regions, across the limbic system, cerebral cortex, deep grey nuclei, cerebellum and relay nuclei  Seven white matter regions – frontal lobe, temporal lobe, parietal lobe, occipital lobe, corpus callosum, internal capsule and cerebellum.</p>	<p>Sig increased neuronal injury in the cerebellar cortex and frontal cortex of PVL compared with DWMI or Negatives (H&amp;E).  No increase in astrogliosis (GFAP)</p>	<p>Focal periventricular necrosis; diffuse white matter gliosis</p>
<p>Haldipur et al. (2011) / National Brain Research Centre, Manesar, India / 2007 - 2010</p>	<p>40 cases / Across the window of 28 weeks of gestation to 8 postnatal months / 4 controls with 0 days survival &amp; 32 cases of varying age at birth and survival.</p>	<p>All cases are those in which the autopsy indicated minimum or no damage to the brain and cerebellum in particular / Delay = &lt;24 h</p>	<p>Still birth cases – with no obvious signs of injury as per cases with post-natal survival</p>	<p>Cerebellum</p>	<p>EGL cell density significantly increased by preterm birth  EGL thickness reduced by preterm birth</p>	<p>None described</p>
<p>Marin-Padilla (1997) / Paediatric Autopsy Service, Dartmouth-Hitchcock Medical Center, Hannover, New Hampshire, USA / (via ref 23). / Collection epoch not reported</p>	<p>33 cases total / 5 cases born preterm who all had short survival time / 3 months through to 5 years survival</p>	<p>PVL as diagnosed by a histopathologist / Post-mortem delay not described</p>	<p>No controls – description of changes over time after WM injury only.</p>	<p>Grey matter overlying frank WM injury</p>	<p>No changes visible in the acute cases – which were the pre-term born infants.  In the cases surviving longer – late term and term born infants, no change in the upper cortical layer vascular and cellular distribution and morphology (H&amp;E, Golgi).  Axomatized pyramidal neurons change from being long projecting to being local-circuit (Golgi). These cells have increased circuitry and altered neuronal morphology – populations of larger and smaller cells with altered distributions (Golgi, H&amp;E, GFAP).</p>	<p>Cystic white matter lesions</p>

<p>Stolp et al. (2019) / Perinatal Pathology Department, Imperial Health Care Trust, London, UK. / <b>Collection epoch not reported</b></p>	<p><b>Non-WMI group , n=7</b> <b>WMI group, n=6</b> / Non-WMI group = 23<sup>+2</sup> to 28<sup>+1</sup> gw WMI group 26<sup>+5</sup> to 29<sup>+3</sup> gw / <b>Non-WMI group = 5 min to 43 hours.</b> <b>WMI group 1 min to 5 weeks (comparison p = 0.002)</b></p>	<p>Evidence of diffuse (non-cystic) white matter injury (WMI cases) including white matter gliosis and focal lesions. / 1–3 days – bodies stored at 4°C</p>	<p>Seven cases showed no significant brain pathology, = non-neuropathologic controls (no WMI cases).</p>	<p>Interneurons of the frontal cortex and underlying white matter</p>	<p>No change in the total number of cortical neurons, identified by HuC/HuD immunoreactivity, with 53,104 ± 11,009 immunopositive cells/mm<sup>2</sup> found in the control brains (n = 5), and 52,120 ± 6,327 cells/mm<sup>2</sup> in the cortex of the white matter injury cases (n = 4).  Significant decrease in the cortical calretinin+ cells  Calbindin- and parvalbumin-positive cells were observed in low numbers in both cases, insufficient for determining statistically significant changes. Somatostatin and Neuropeptide Y only found in the white matter.</p>	<p>No statistical differences in the number of SST or NPY subpopulations in the white matter between preterm infants with or without white matter injury. Significant decrease in the arborization of Somatostatin and Neuropeptide Y interneurons in both of these interneuron classes  As previously reported Vontell et al., 2015</p>
<p>Panda et al. (2018) / New York Medical College and Albert Einstein College of Medicine, USA / <b>2002–2016</b></p>	<p>Fetuses: 20–22 gw, n=5 Infants: 23-28 gw, n=5 Infant: 29-34 gw, n=5  20–40 gestational weeks (gw): 26–27 gw infants surviving for 4–6 weeks were compared with 32–33 gw infants who lived for approx. 3 days. Therefore, both had PMA33 gw at their death.</p>	<p>Excluded = moderate to severe intraventricular haemorrhage, major congenital anomalies, history of neurogenetic disorder, chromosomal defects, culture-proven sepsis, meningitis, or hypoxic-ischemic encephalopathy and infants receiving extracorporeal membrane oxygenation treatment / post-mortem interval of approx. 18 h.</p>	<p>None. Comparisons of effects of varying degrees of prematurity.</p>	<p>Cortex (cortical plate), white matter (embryonic intermediate layer), and ganglionic eminences, which were cut at the level of the head of caudate nucleus</p>	<p>More prematurely born infants have fewer GAD67+ neurons in upper and not lower cortical layers  More prematurely born infants have fewer Parvalbumin+ neurons in upper and not lower cortical layers  More prematurely born infants have greater numbers of Somatostatin+ neurons in upper and not lower cortical layers  Calretinin+ and neuropeptide Y+ interneurons not effected by preterm birth</p>	<p>No analysis undertaken.</p>
<p>Vontell et al. (2015) / Perinatal Pathology Department, Imperial Health Care Trust, London, UK. / <b>Collection epoch not reported</b></p>	<p>7 WMI and 7 controls. / All &lt;32 weeks' gestational age, vaginally delivered / <b>Survival time not reported</b></p>	<p>Cerebral white matter gliosis, lipid-laden macrophages, and focal lesions with evidence of WMI on pathologic examination (WMI cases) / 1–3 days – bodies stored at 4°C</p>	<p>Also, extremely preterm, but with no significant brain pathology on gross and microscopic examination from post-mortem examination and had no visible brain abnormalities on post-mortem magnetic resonance imaging</p>	<p>Thalamus (medial dorsal (MD) nucleus, ventral lateral posterior (VLp) nucleus, ventral posterior lateral (VPL) nucleus)  White matter (posterior limb of the internal capsule (PLIC) adjacent to the VLp (PLIC-VLp) and PLIC adjacent to the VPL (PLIC-VPL))</p>	<p>No difference in the total average cell density in thalamic regions (H&amp;E)  Significant decrease in neurons in WMI cases in the MD, VLp, and VPL (HuC/HuD)  Significant increase in the ratio of astrocytes (GFAP+) to total cell count in thalamic regions in WMI cases, compared with MD (p &lt; 0.01) and VLp (p &lt; 0.01)—but not VPL  Significant increase in IBA1+ cells in WMI cases in the MD, VLp, and VPL.</p>	<p>No difference in the average total cell density in white matter regions  Significant increases in neurons in PLIC-VPL but not in PLIC-VLp  Significant increase in IBA1+ cells in the PLIC-VPL (p &lt; 0.05), but not in PLIC-VLp</p>



<p>Pogledic <i>et al.</i> (2014) / Hôpital Robert Debré, Paris, France. / Collection epoch not reported</p>	<p>Cystic (c)-WMI, n=7 Controls, n=18 / c-WMI = 24+4 to 27 gw controls = 24+2 to 34 gw / c-WMI = 0-4 weeks and 1 day Control = 0-11 days</p>	<p>Cystic cases including focal lesions with macroscopic cysts associated or not with necrosis and/or calcifications surrounded by diffuse pallor / post-mortem interval &lt;48 h</p>	<p>Non-cystic cases without tissue loss displayed pallor and gliosis (18/18 cases) associated with microscopic necrotic foci in a few cases (4/18 cases) and were considered to consist of diffuse lesions</p>	<p>Cortical regions located in the posterior part of the superior, middle and inferior frontal gyri and sulci, and the precentral gyrus and central sulcus corresponding to corresponding to the presumptive premotor and motor areas (areas 8-6-4) and contiguous prefrontal areas.</p>	<p>Significantly increased cortical plate and subplate astrogliosis (GFAP) in c-WMI versus control preterm WMI (no change in very preterm cystic and control cases)  No increase in cortical plate and subplate microgliosis (IBA1) in c-WMI versus control preterm WMI (for very preterm or just preterm cases)</p>	<p>White matter cysts were confined to the white matter without extending into superficial layers of the cerebral wall such as the subplate and cortical plate</p>
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Note: Of the 12 studies identified, those highlighted in yellow (n=6) report studies performed on tissues drawn from the same pool of post-mortem samples – between the years of 1993-2007. It is unclear, and undeterminable from the case reports, how many times a single case appears across the 6 studies and as such how co-dependent the findings are. *Abbreviations:* 12/15-LOX, 12/15-lipoxygenase; DWMI, diffuse white matter injury; EGL, external granule cell layer; h, hours; GAD67, glutamate decarboxylase 67; GFAP, glial fibrillary protein; gw, gestational weeks; H&E, hematoxylin and eosin; MAP2, microtubule associated protein 2; MD, Medial dorsal nucleus (thalamus); MDA, malondialdehyde; NEC, necrotising enterocolitis; PC, post-conceptual; PLIC, posterior limb of the internal capsule; PVL, periventricular leukomalacia; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labelling; WMI, white matter injury; VLp, ventral lateral, posterior (thalamus); vPL, Ventral posterolateral nucleus (thalamus).