



Published in final edited form as:

*Nat Neurosci.* 2014 March ; 17(3): 341–346. doi:10.1038/nn.3604.

## Neurobiology of premature brain injury

**Natalina Salmaso<sup>1,2</sup>, Beata Jablonska<sup>3</sup>, Joseph Scafidi<sup>3</sup>, Flora M Vaccarino<sup>1,2,4,5</sup>, and Vittorio Gallo<sup>3</sup>**

<sup>1</sup>Child Study Center, Yale University, New Haven, Connecticut, USA

<sup>2</sup>Program in Neurodevelopment and Regeneration, Yale University, New Haven, Connecticut, USA

<sup>3</sup>Center for Neuroscience Research, Children's National Medical Center, Washington, DC, USA

<sup>4</sup>Kavli Institute for Neuroscience, Yale University, New Haven, Connecticut, USA

<sup>5</sup>Department of Neurobiology, Yale University, New Haven, Connecticut, USA

### Abstract

Every year in the United States, an estimated 500,000 babies are born preterm (before 37 completed weeks of gestation), and this number is rising, along with the recognition of brain injuries due to preterm delivery. A common underlying pathogenesis appears to be perinatal hypoxia induced by immature lung development, which causes injury to vulnerable neurons and glia. Abnormal growth and maturation of susceptible cell types, particularly neurons and oligodendrocytes, in preterm babies with very low birth weight is associated with decreased cerebral and cerebellar volumes and increases in cerebral ventricular size. Here we reconcile these observations with recent studies using models of perinatal hypoxia that show perturbations in the maturation and function of interneurons, oligodendrocytes and astroglia. Together, these findings suggest that the global mechanism by which perinatal hypoxia alters development is through a delay in maturation of affected cell types, including astroglia, oligodendroglia and neurons.

### Prematurity and brain injury in the newborn

The incidence of live preterm births in the USA has increased by 14% between 1990 and 2002 and continues to rise steadily. This is believed to be due at least in part to the increased number of births to mothers over 35, increased use of fertility treatments and a myriad of environmental factors, including smoking. Nevertheless, medical, pharmacological and technological advances have enhanced the survival rate in increasingly younger infants, particularly those born at a very low birth weight (VLBW); that is, under 1,500 g and less than 32 gestational weeks of age. Because lower birth weight and earlier gestational age are predictors of pathology, this improved survival rate of VLBW children is accompanied by

© 2014 Nature America, Inc. All rights reserved.

Correspondence should be addressed to V.G. (vgallo@cnmcresearch.org) or F.M.V. (flora.vaccarino@yale.edu).

Reprints and permissions information is available online at <http://www.nature.com/reprints/index.html>.

#### COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

increased morbidity in this population: their developmental trajectory remains complicated and plagued with disabilities, even in the absence of focal brain injury. For example, VLBW infants typically show neurological sequelae including cerebral gray and white matter abnormalities, ventriculomegaly and decreased cortical and basal ganglia volumes. Long-term longitudinal follow-up studies of this population have demonstrated long term cognitive difficulties, particularly those involved with language and executive functions, and psychiatric illnesses including autistic spectrum and anxiety disorders<sup>1,2</sup>. Many aspects of cognitive and neurological sequelae improve over time when extra resources are provided, including special education services and occupational and physical therapy. Under these conditions, many VLBW children show no appreciable differences from term-born children by the time they reach adolescence<sup>3,4</sup>. Unfortunately, there are few prognostic indicators of the likelihood that a particular child will suffer long-term neurological and neuropsychological consequences. Neuroimaging studies in adolescents who were born prematurely have delineated abnormal brain asymmetries and aberrant connectivity, suggesting that cognitive improvements might be achieved by establishing unusual patterns of connectivity<sup>5</sup>, perhaps due to a prolonged period of plasticity.

Premature birth has a negative impact on structural and functional integrity of the brain, and serial magnetic resonance imaging studies clearly have demonstrated a reduction in gray and white matter volumes in premature infants as compared to term controls<sup>6,7</sup>. Diffusion tensor imaging analysis has revealed differences in fractional anisotropy between prematurely born and term infants, indicating compromised microstructural organization and integrity of white matter, which are correlated with gestational age. These alterations in structural organization last until adolescence and correlate with a broad spectrum of cognitive and neurological deficits<sup>8</sup>. Diffuse white matter injury, together with gray matter and hippocampal abnormalities<sup>9,10</sup>, are the most common types of cerebral abnormalities associated with prematurity, as focal necrotic lesions characteristic of cystic periventricular leukomalacia are now rarely observed in premature infants<sup>11</sup>.

These observations raise important questions about the cellular substrates and cellular dynamics, in both neurons and glia, associated with premature brain injury. Yet it has been difficult to reproduce in animal models alterations in the trajectory of brain development and maturation due to early brain injury and prematurity, and we still are unable to discriminate alterations that are beneficial from those that are deleterious. The ever-rising rate of premature births invokes the necessity of animal models that recapitulate the phenotype of VLBW-associated injury to further delineate causative factors and potential therapeutics for recovery from injury associated with premature birth. We propose that there is a delay in maturation following injury associated with premature birth.

## Models of VLBW premature birth

While there are focal ischemic injuries associated with VLBW, the most common injuries observed clinically are a global decrease in cortical volume and ventriculomegaly, which are attributable to chronic hypoxic injury, sustained as a consequence of immature lung development. Many aspects of this global injury are recapitulated through the use of a rodent model of chronic perinatal hypoxia in which mice are reared under low oxygen between

postnatal day (P) 3 and P11. In this model (see ref. 12 for a review), hypoxically reared pups initially show decreases in cortical gray and white matter volumes and increased ventricle size. Subsequently, many aspects of this early brain phenotype show substantial recovery; for example, cortical and hippocampal volumes are reestablished to levels observed in normoxic controls by early adulthood, as are the total number of excitatory neurons and oligodendrocytes (OLCs) in the cortex and hippocampus. However, there are also important aspects of this injury that do not show recovery, most notably the maturation of GABA interneurons in the cortex and myelin structure (see below). These cellular deficits correlate with long-lasting deficits in learning and memory, increased anxiety, and decreases in fine motor skills well into adulthood, which is quite similar to the observed clinical phenotype<sup>13</sup>.

Cellular, functional and behavioral abnormalities have been found in models of perinatal hypoxic-ischemic injury such as the Vannucci model (Table 1), in which carotid artery occlusion is followed by exposure to hypoxia, typically from P3 to P9 (for a review, see ref. 14). In contrast to the chronic hypoxic, the hypoxic-ischemic model induces focal and diffuse injury that leads to a more typical glial reactive response and the formation of a glial scar. Recent studies have shown that mice exposed to the hypoxic-ischemic Vannucci model also show cognitive and motor deficits that last well into adulthood. Imaging studies have suggested that the extent of behavioral deficits is correlated to that of hippocampal and white matter injury, as well as to the expression of myelin basic protein and neurofilament protein in the white matter<sup>15,16</sup>.

Although substantially more time-consuming and expensive than rodent models, global ischemia models in preterm fetal sheep provide opportunities for more thorough and integrated analysis of the complex pathophysiological events that contribute to injury in the premature human brain during critical periods in development. This is because large animal brains more faithfully recapitulate developmental events that occur in humans<sup>17</sup>. The fetal sheep model, in which global ischemia is induced at a gestational time point roughly corresponding to the third trimester in humans, results in global brain volume loss and necrotic and non-necrotic white matter injury characterized by reactive astrogliosis and/or reactive microglia and macrophages<sup>17</sup>.

### **Perturbed maturation of interneurons in models of prematurity**

Unlike the focal and diffuse necrosis induced by hypoxia-ischemia, chronic postnatal hypoxia induces a general loss of cortical and hippocampal gray and white matter volume in the absence of necrosis and detectable neuronal cell death. The mechanism by which this massive volume loss occurs has been rather mysterious, and even more puzzling is how the recovery of this volume can ensue. Neurons are mostly generated in the first part of gestation in humans and during embryogenesis in rodents; hence, perturbations induced by prematurity in humans and by perinatal hypoxic injury in rodent models are unlikely to affect neurogenesis. Loss of neurons due to apoptosis may explain in part the decrease in gray matter volumes of cortex and basal ganglia in both human and mice; however, it would be necessary to invoke massive cell genesis to explain the subsequent recovery. Indeed, there is an increase in both cortical and hippocampal neurogenesis from astroglial progenitors in the period of recovery from hypoxia. Unlike astroglia residing in the dentate

gyrus or subventricular zone (SVZ), which maintain stem cell-like potential throughout life, astroglial cells in the cortex and hippocampus rapidly lose pluripotency in the early postnatal period; by 2 weeks after birth, cortical astroglial cells are no longer able to give rise to self-renewing neural stem cells, indicating their progressive maturation to protoplasmic astrocytes<sup>18</sup>. In contrast, cortical astroglial cells in 2-week-old mice reared under hypoxic conditions from P3 to P11 are able to generate neurons and OLCs both *in vitro* and *in vivo*<sup>18</sup>.

Although striking, the extent of cortical neurogenesis in hypoxically reared mice is relatively small and unlikely to entirely explain the recovery of cortical gray matter volume that is observed. Other contributing mechanisms are likely to be increased growth of axons, dendrites and synaptic connections. Although dendritic growth and pruning has not been yet examined in rodent models of hypoxia, it has been recently demonstrated that the cortical volume loss in the sheep model of ischemia is associated with impaired expansion of dendritic arbors and reduced synaptic density of cortical pyramidal cells and that the abnormal growth and orientation of the apical dendrites of pyramidal cells can explain the decrease in cortical functional anisotropy observed in these animals<sup>19</sup>. This is suggestive of a neuronal maturation defect in both hypoxia and ischemia animal models of prematurity.

In addition to a loss of volume, a 25–30% decrease in cortical parvalbumin (PrV)- and somatostatin (SST)-positive GABAergic inhibitory neurons in the cerebral cortex of adult mice reared under hypoxia has been noted, which could be due to either a delay in maturation of these cells or to cell death (Fig. 1)<sup>20</sup>. However, no evidence was found of increased GABA interneuron cell death following hypoxia. Furthermore, experiments using a transgenic mouse line expressing GFP under the *Gad1* promoter, which allows visualization of all GABAergic cells regardless of stage of maturation or subtype, found no differences between normoxically and hypoxically reared mice in the total numbers of all GFP<sup>+</sup> interneurons, but rather a decrease in the subsets of GABAergic cells that express PrV and SST<sup>20</sup>.

Because there are no changes in interneuron genesis or apoptosis in hypoxic mice, at least at the time points examined, we propose that decreases in PrV and SST protein expression are due to perturbed maturation of these interneuron subtypes. Under normal conditions in mice, expression of PrV protein begins to be detectable toward the end of the second postnatal week and is contingent on excitatory input and neurotrophin signaling<sup>21</sup>. In adulthood, PrV<sup>+</sup> and SST<sup>+</sup> interneurons form a dense inhibitory network of synapses on adjacent pyramidal cells<sup>22,23</sup>. PrV<sup>+</sup> neuron-mediated regulation of cortical excitability improves the efficiency of cortical information processing; firing of these neurons generates gamma oscillations, which are disrupted in several neuropsychiatric disorders<sup>24,25</sup>. In mice, a decreased number of hippocampal PrV<sup>+</sup> interneurons correlates with the degree of deficit in spatial memory in the Morris water maze task<sup>26</sup>. Hence, the lasting decrease in PrV and SST expression and therefore dampened inhibitory neurotransmission may be responsible at least in part for the deficits in learning and spatial memory observed after hypoxic insults<sup>13</sup>.

In humans, premature birth is associated with increased incidence of seizure disorders, which has been related to decreases in cortical GABA content (for a review, see ref. 27). Interestingly, animals that are deficient in PrV neurons show increased seizures activity<sup>28</sup>.

Therefore, similar differences may also occur in this model of chronic hypoxia, although this remains to be demonstrated. Nevertheless, these findings provide exciting opportunities for therapeutic interventions because we can use these mice to select pharmacotherapeutic compounds that increase GABAergic cell differentiation and maturation. Discovering compounds that enhance the maturation of GABAergic neurons in the postnatal period presents therapeutic opportunities for a variety of disorders that have shown GABAergic neuron dysregulation, including schizophrenia and perhaps autism.

## **Perturbed astrocyte function and aberrant myelination**

In contrast to neurogenesis, gliogenesis occurs mostly during the first and second postnatal weeks in rodents and during the last part of gestation in humans. Thus, the ongoing genesis and initial maturation of these cells coincides with the timing of injury in mouse models of premature brain damage and in children born prematurely. Moreover, because this is a critical time for gliogenesis, recovery from premature brain injury also critically involves glial function. Indeed, we have found that glia, both astrocytes and OLCs, are integral to the recovery from hypoxic injury (see below). Furthermore, results from different laboratories provide converging evidence that supports the hypothesis that, as for neurons, a global delay in glial cell maturation is also observed after premature brain injury.

Astrocyte maturation is delayed by perinatal hypoxia, as demonstrated by decreased GFAP and increased nestin expression, which suggests a more immature phenotype<sup>29</sup>. Furthermore, astrocyte function is also compromised, as glial-specific glutamate transporter expression and function are reduced after hypoxia<sup>29</sup>, pointing to defective glutamate clearance and increased extracellular levels of the amino acid as likely contributors to excitotoxic death of OLCs. Conversely, another function of astrocytes that is thought to be a protective reaction to injury, reactive gliosis, was revealed in a mouse model of lipopolysaccharide-induced neonatal white matter injury and in human neonatal tissue. Activated STAT-3 signaling in astrocytes prevents TGF $\beta$ -1 production by microglial cells, which in turn inhibits OLC maturation<sup>30</sup>. Deletion of STAT-3 in astrocytes enhances TGF $\beta$ -1 in microglia and exacerbates white matter injury by further delaying OLC maturation<sup>30</sup>. In summary, astrocytes appear to play multiple roles depending on the type of injury and specific cellular interactions with other glial cell types.

In the postnatal brain, OLCs originate from progenitor cells (OPCs) located in the parenchyma and in the SVZ. In rodents, these cells proliferate, migrate and differentiate during the first 2 postnatal weeks, whereas in humans they mostly develop between 23 and 37 weeks of gestation (Fig. 2). Previous studies in human tissue have associated OLC susceptibility with pre-OLCs or immature OLCs that are present in large numbers between 23 and 32 weeks of postconceptional age<sup>31</sup>. However, more recent analysis in animal models and in human tissue has revealed multiple cellular and molecular mechanisms underlying the effects of premature brain injury on OLCs. These include (i) induction of pre-OLC or OLC death that occurs during early phases of the injury<sup>32</sup>, (ii) a parallel robust regenerative or proliferative response of OPCs in white matter and SVZ during early phases of the injury, (iii) delayed or arrested OLC maturation<sup>31,33</sup>, and (iv) delayed myelination<sup>33</sup> (Fig. 2).

What causes pre-OLC and OLC death in premature-birth white matter injury? Several factors appear to be involved, including excitotoxicity and oxidative stress<sup>32</sup>. In animal models of hypoxia-ischemia, both apoptotic and excitotoxic cell death of immature OLCs have been observed<sup>34</sup>. Immature OLCs are particularly susceptible to oxidative stress and free-radical mediated injury<sup>32</sup>, and, in postmortem brain tissue from human premature infants, apoptosis was detected in the OLC lineage<sup>35</sup>. A still-open issue is whether death of mature OLCs occurs after perinatal hypoxia and prematurity or whether cell loss is limited to immature OLCs.

In mice exposed to chronic hypoxia, OLCs regenerate and myelin proteins return to normal levels within a few weeks after the injury, but myelin structure, as detected by electron microscopy, is abnormal<sup>33</sup>. Altogether, these results point to a delay in maturation of the OLC lineage during a critical developmental time window as a major cause of white matter dysfunction in premature brain injury. Future studies should test the hypothesis that delayed OLC maturation caused by hypoxia occurs because of a cellular environment that is no longer conducive to proper functional myelination. As specific cellular interactions (OLCs-axons and OLCs-astrocytes) are crucial for functional myelination, it is possible that developmentally regulated changes in electrical activity of subcortical white matter axons might contribute to the delayed and abnormal myelination after hypoxia.

These findings raise important questions about the molecular mechanisms underlying the effects of hypoxia on OLC development. On the basis of the hypothesis that at least some of the effects of hypoxia on delayed OLC differentiation and increased OPC proliferation could be related, cell cycle regulatory mechanisms were examined, in particular regulators of G1-S phase transition. Major changes in the Cdk2-p27<sup>Kip1</sup> pathway were found in white matter OLCs after hypoxia: in particular, an activation of Cdk2 in OPCs, which promotes their proliferation, as well as a transient reduction in p27<sup>Kip1</sup> during phases of delayed differentiation<sup>33</sup>. Consistent with these findings, reduced p27<sup>Kip1</sup> was also observed in subcortical white matter of preterm neonates with hypoxic-ischemic encephalopathy<sup>33</sup>. Finally, loss- and gain-of-function analysis also demonstrated direct involvement of Cdk2 and p27<sup>Kip1</sup> in the delay of OLC maturation observed after hypoxia<sup>33</sup>. Both Cdk2 and p27<sup>Kip1</sup> are crucial in timing OLC development under normal physiological conditions (for a review, see ref. 36). These results, together with studies from other laboratories<sup>37,38</sup>, indicate that inappropriate reactivation of developmental mechanisms is likely involved in the delayed OLC maturation observed in neonatal brain injury.

Another mechanism likely to be crucial in preventing OLC differentiation after premature-birth brain injury involves OPC-astrocyte interactions, particularly reactive astrocyte-derived high-molecular-weight hyaluronic acid<sup>39</sup>. Both this proteoglycan and its putative receptor CD44 are highly expressed in diffuse white matter injury<sup>31</sup>, and a specific hyaluronidase, PH20, has been identified that digests hyaluronic acid to generate bioactive fragments that actively inhibit OLC differentiation and myelination<sup>40</sup>.

Further analysis in animal models having white matter structures that more closely resemble their human counterparts and more extensive analysis of human tissue will provide crucial information about selective sensitivities of different stages of the OLC lineage to premature

white matter injury. Finally, improved functional imaging techniques will allow more detailed analysis of developmental myelination under pathological conditions in the human brain and will define the relevance of animal models to premature injury in infants.

## Potential interventions

The cellular findings described above demonstrate the considerable complexity of gray and white matter injury observed in the premature brain and identify a need for global interventions that involve not only preventing cell death but also resynchronizing neuronal and glial developmental programs to promote timely establishment of neuronal maturation and connectivity (particularly for inhibitory neurons), as well as functional myelination. Although it is most likely that no single approach will prevent all neurocognitive difficulties and promote repair at multiple levels, findings in premature infants and in animal models point to some avenues that deserve further exploration.

Results obtained in premature infants indicate that the environment affects plasticity and recovery after neonatal brain injury. Two of the strongest predictors of long-term neurological outcome of VLBW infants are level of maternal education and the presence of a two-parent household, even when controlling for variables such as income. The fact that these factors mediate long-term outcome suggests that there are environmental moderators of recovery; hence, knowledge of the underlying mechanisms may lead to new therapeutics. Studies have tried to mimic these environmental factors by providing a stimulating environment to mice following hypoxic injury. Results showed that even only 2 weeks of enrichment reverses deficits in learning and memory observed in hypoxically reared mice<sup>41</sup>. Moreover, hypoxic mice reared under enriched conditions show increased levels of PrV and SST protein expression that are similar to those of normoxic controls by the time they reach early adulthood<sup>20</sup>. Hence, the behavioral improvement observed after environmental enrichment<sup>41</sup> might in part be mediated by normalization of interneuron neurochemical properties.

We hypothesize that enrichment may support or even accelerate maturation processes and therefore act to reverse delayed maturation observed in central cell types. Certainly, in addition to increased cell proliferation and survival, environmental enrichment has been demonstrated to increase angiogenesis, dendritic complexity and synaptic connectivity and to increase maturation in other neural systems, including the visual system<sup>42</sup>. Furthermore, enrichment has been demonstrated to upregulate several trophic factors, among them BDNF and its receptor TrkB, which are crucial in the postnatal maturation of PV<sup>+</sup> interneurons<sup>21</sup>. Research focused on elucidating the molecular mechanisms that induce these factors under enriched conditions and experimental manipulations to understand their specific and causal role in the beneficial effects of enrichment during functional recovery are necessary and will undoubtedly reveal potential therapeutic avenues for a myriad of injuries. Unveiling these mechanisms is particularly crucial because, in spite of the important beneficial effects of environmental enrichment, the cost, feasibility and labor intensiveness of enrichment interventions in premature infants is challenging. Therefore, the discovery of key molecules that could potentially be targeted for pharmacotherapy of premature brain injury is imperative.

Exploratory studies have already led to the identification of other promising therapeutic avenues, whose interaction and potential synergism with environmental enrichment is not yet known. Wnt- $\beta$ -catenin signaling is important in OLC development and regeneration<sup>37</sup>. Axin 2 is an essential element of this pathway, is highly expressed in OPCs and negatively regulates the Wnt signaling pathway by promoting  $\beta$ -catenin degradation. Fancy *et al.* (2011) demonstrated that Axin2 functional stability is essential for timely myelination after hypoxic injury. Treatment with a small-molecule inhibitor (XAV939) that targets the enzyme tankyrase to stabilize Axin2 levels promotes oligodendrogenesis and myelination, indicating that small compounds that easily cross the blood-brain barrier could be incorporated into future therapeutic approaches aimed at promoting premature brain injury. Small compounds could also be designed to target PH20 hyaluronidase and prevent hyaluronic acid breakdown, and/or activate the p27<sup>Kip1</sup> pathway to promote OLC maturation.

There is also evidence for the importance of fibroblast growth factors (FGF) for stimulating the recovery of neuron number and gray matter volume after hypoxic injury. FGF2 is a growth factor involved in neurogenesis and gliogenesis both during embryonic and postnatal development<sup>43</sup>. FGF2 is normally upregulated in parenchymal astrocytes in the first 3 weeks after birth in rodents, and these GFAP<sup>+</sup> cells express the FGF receptors Fgfr1, Fgfr2 and Fgfr3. The conditional knockout of Fgfr1 or Fgfr2 in GFAP<sup>+</sup> cells *per se* produces a 30% loss of cortical PrV GABAergic interneurons<sup>44</sup>, possibly owing to abnormal astrocyte-to-interneuron signaling. Furthermore, the expression of FGF2 and Fgfr1 is increased in mice recovering from chronic hypoxia<sup>45</sup>, and the deletion of Fgfr1 in GFAP<sup>+</sup> cells precludes the recovery of cortical volume and cortical excitatory neurons and worsens the decrease in PV<sup>+</sup> interneurons in hypoxic mice<sup>46</sup>. Given this evidence, we hypothesize that FGF signaling in astroglial cells is sufficient to stimulate the recovery of excitatory neurons but insufficient to boost the deficient maturation of inhibitory neurons.

## Future directions

Analysis in animal models of premature brain injury has been critical in beginning to identify specific cellular and functional abnormalities in gray and white matter regions of the brain. Using a model of chronic neonatal hypoxia has allowed the identification of different phases of the injury and the characterization of the progenitor and stem cells involved in neuronal and glial regeneration after injury. Overall, these findings are consistent with those obtained from other animal models and support the notion that premature brain injury dysregulates neuronal and glial developmental programs, resulting in delays and abnormalities in functional maturation of different brain regions.

It is important to emphasize that this analysis has not only identified the cellular basis for delayed maturation, but has also defined critical periods of intervention—based on temporal windows of prolonged neural plasticity—to promote cellular, structural and functional recovery. Characterization of specific signaling pathways activated during these critical periods will also help in identifying cellular and molecular targets that can be manipulated for therapeutic purposes. Furthermore, manipulation of the environment during brain development to provide an enriched experience appears to be a viable approach, either by



itself or in combination with cellular and pharmacological manipulations to maximize functional recovery after neonatal brain injury.

In light of the complexity of neonatal brain injuries, future approaches to promote regeneration and functional recovery will have to be tested in different animal models that more closely resemble the human brain. For example, producing white matter injury in rodents has been challenging because they have less white matter than species with larger brains, and the distribution of the white matter differs from that of larger mammals because of the lissencephalic nature of the rodent brain. Both sheep and baboons have been used to model premature brain injury, and furthermore, we have recently extended analyses of developing white matter injury to early postnatal piglets, in which white matter development closely resembles that of its human counterpart<sup>47</sup>. The sheep and baboon models show many similarities to the clinical situation observed in premature infants, including enlarged ventricles, neuronal and glial death, and microglia activation in white matter. Although these larger animal models have several drawbacks, including high cost and very limited potential for the genetic manipulations that are routinely performed in rodents, they allow cell dynamics studies that are not feasible in the developing human brain and better physiologic control and monitoring of several variables, including cerebral blood flow. Therefore, they might represent better testing grounds for human drugs. In the future, information from different experimental designs and tissue analysis from distinct animal models will need to become more integrated to define feasible and successful therapeutic approaches that will combine molecular and cellular results with the more complex physiology and pathology of higher mammals. Finally, it will be essential to integrate findings from animal models with analysis in postmortem human tissue. For this purpose, banking brain tissue that is representative of the broad spectrum of injuries in premature neonates is a high priority.

The issues associated with the use of human tissue could be at least in part overcome by exploiting cellular models that utilize human neural progenitor/stem cells or inducible pluripotent stem cell (iPSC) technology. Although neuronal and glial progenitors are attractive cellular elements for developing cell replacement strategies after neonatal brain injury, it has been very difficult to obtain homogeneous cell populations capable of generating neurons or myelinating glia suitable for clinical use. Nevertheless, preclinical studies have shown that human progenitors can be (i) widely dispersed after grafting in the host brain, (ii) able survive at a high density after transplantation and (iii) able to properly myelinate host axons to promote functional recovery<sup>48</sup>. The use of human cord blood cells and their potential to repair neonatal hypoxic-ischemic brain injury has recently been investigated in both preclinical studies and clinical trials<sup>49</sup>. The main advantages of iPSCs with respect to other sources of human precursor cells is that they are an unlimited source of isogenic cells that would not be subjected to rejection. Very recent studies are encouraging in that they have suggested that neuronal precursors derived from human iPSCs can populate the cerebral cortex and the basal ganglia and can ameliorate symptoms in a rodent model of Huntington disease<sup>50</sup>. Hence, there is hope that iPSCs can be used therapeutically in neonatal hypoxic injury.

## Acknowledgments

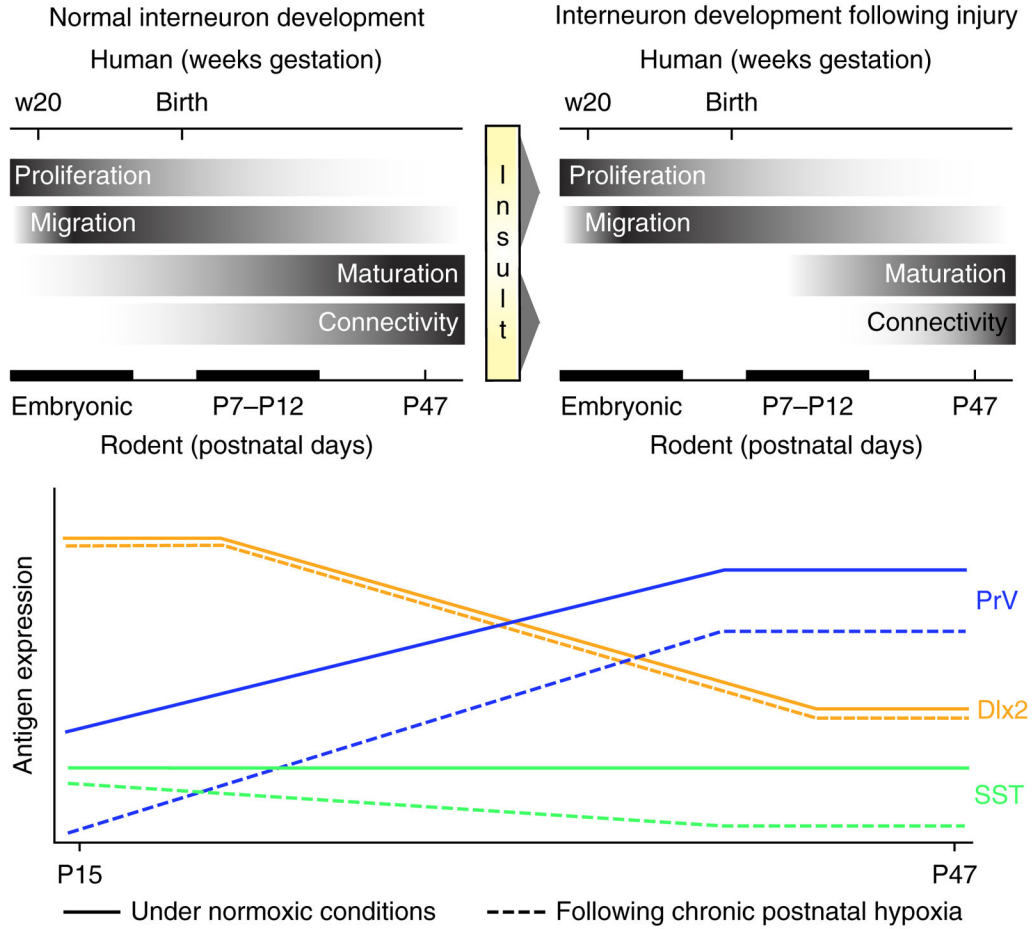
This research has been supported by the US National Institutes of Health NINDS (P01 NS062686 and R01 NS060750 to F.M.V. and R01NS045702 to V.G.), NICHD (P30HD040677 to V.G.) and NIA (R21 AG034495 to FMV), and by grants from the Brain and Behavior Research Foundation (F.M.V.), the state of Connecticut (F.M.V.), Women's Health Research at Yale (F.M.V.) and the Cerebral Palsy International Research Foundation (V.G.). N.S. is recipient of a Canadian Institute of Health Research fellowship. J.S. is the recipient of K08NS073793 (NINDS). We are particularly grateful to L. Ment for support and discussion. We thank J. Ritter for help with figures.

## References

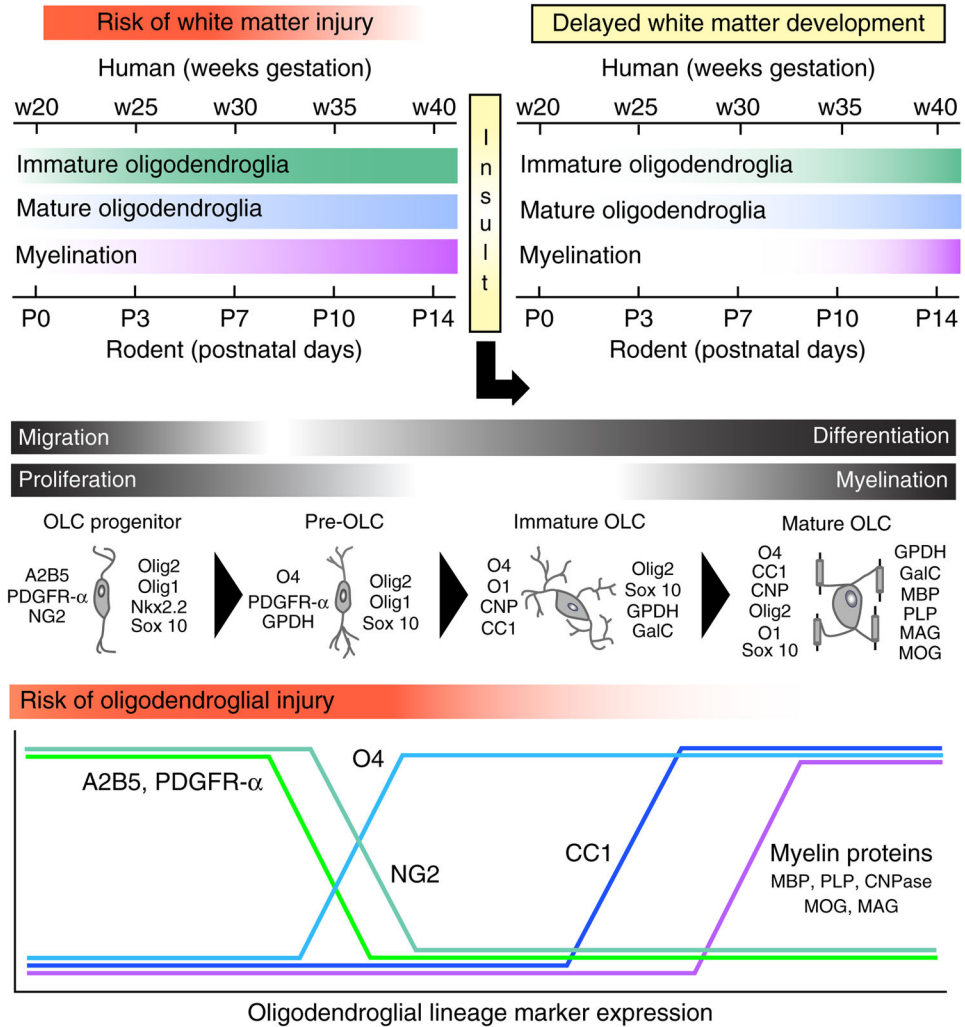
1. Anderson P, Doyle LW. Victorian Infant Collaborative Study Group. Neurobehavioral outcomes of school-age children born extremely low birth weight or very preterm in the 1990s. *J Am Med Assoc.* 2003; 289:3264–3272.
2. Johnson S, et al. Psychiatric disorders in extremely preterm children: longitudinal finding at age 11 years in the EPICure study. *J Am Acad Child Adolesc Psychiatry.* 2010; 49:453–463. [PubMed: 20431465]
3. Luu TM, et al. Evidence for catch-up in cognition and receptive vocabulary among adolescents born very preterm. *Pediatrics.* 2011; 128:313–322. [PubMed: 21768322]
4. Ment LR, et al. Change in cognitive function over time in very low-birth-weight infants. *J Am Med Assoc.* 2003; 289:705–711.
5. Myers EH, et al. Functional connectivity to a right hemisphere language center in prematurely born adolescents. *Neuroimage.* 2010; 51:1445–1452. [PubMed: 20347043]
6. Ball G, et al. The effect of preterm birth on thalamic and cortical development. *Cereb Cortex.* 2012; 22:1016–1024. [PubMed: 21772018]
7. Inder TE, et al. Periventricular white matter injury in the premature infant is followed by reduced cerebral cortical gray matter volume at term. *Ann Neurol.* 1999; 46:755–760. [PubMed: 10553993]
8. Eikenes L, et al. Young adults born preterm with very low birth weight demonstrate widespread white matter alterations on brain DTI. *Neuroimage.* 2011; 54:1774–1785. [PubMed: 20965255]
9. Beauchamp MH, et al. Preterm infant hippocampal volumes correlate with later working memory deficits. *Brain.* 2008; 131:2986–2994. [PubMed: 18799516]
10. Brown NC, et al. Neurobehavior at term and white and gray matter abnormalities in very preterm infants. *J Pediatr.* 2009; 155:32–38. [PubMed: 19394041]
11. Volpe JJ. Cerebral white matter injury of the premature infant—more common than you think. *Pediatrics.* 2003; 112:176–180. [PubMed: 12837883]
12. Scafidi J, et al. Modeling premature brain injury and recovery. *Int J Dev Neurosci.* 2009; 27:863–871. [PubMed: 19482072]
13. Salmaso N, et al. Environmental enrichment increases the GFAP<sup>+</sup> stem cell pool and reverses hypoxia-induced cognitive deficits in juvenile mice. *J Neurosci.* 2012; 32:8930–8939. [PubMed: 22745493]
14. Vannucci RC, Vannucci SJ. Perinatal hypoxic-ischemic brain damage: evolution of an animal model. *Dev Neurosci.* 2005; 27:81–86. [PubMed: 16046840]
15. Cengiz P, et al. Chronic neurological deficits in mice after perinatal hypoxia and ischemia correlate with hemispheric tissue loss and white matter injury detected by MRI. *Dev Neurosci.* 2011; 33:270–279. [PubMed: 21701150]
16. Fan LW, et al. Hypoxia-ischemia induced neurological dysfunction and brain injury in the neonatal rat. *Behav Brain Res.* 2005; 165:80–90. [PubMed: 16140403]
17. Back SA, et al. The instrumented fetal sheep as a model of cerebral white matter injury in the premature infant. *Neurotherapeutics.* 2012; 9:359–370. [PubMed: 22399133]
18. Bi B, et al. Cortical glial fibrillary acidic protein-positive cells generate neurons after perinatal hypoxic injury. *J Neurosci.* 2011; 31:9205–9221. [PubMed: 21697371]
19. Dean JM, et al. Prenatal cerebral ischemia disrupts MRI-defined cortical microstructure through disturbances in neuronal arborization. *Sci Transl Med.* 2013; 5:168ra7.

20. Komitova M, et al. Hypoxia-induced developmental delays of inhibitory interneurons are reversed by environmental enrichment in the postnatal mouse forebrain. *J Neurosci*. 2013; 33:13375–13387. [PubMed: 23946395]
21. Patz S, et al. Parvalbumin expression in visual cortical interneurons depends on neuronal activity and TrkB ligands during an early period of postnatal development. *Cereb Cortex*. 2004; 14:342–351. [PubMed: 14754872]
22. Packer AM, Yuste R. Dense, unspecific connectivity of neocortical parvalbumin-positive interneurons: a canonical microcircuit for inhibition? *J Neurosci*. 2011; 31:13260–13271. [PubMed: 21917809]
23. Fino E, Yuste R. Dense inhibitory connectivity in neocortex. *Neuron*. 2011; 69:1188–1203. [PubMed: 21435562]
24. Bartos M, Vida I, Jonas P. Synaptic mechanisms of synchronized gamma oscillations in inhibitory interneuron networks. *Nat Rev Neurosci*. 2007; 8:45–56. [PubMed: 17180162]
25. Sohal VS, et al. Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature*. 2009; 459:698–702. [PubMed: 19396159]
26. Stevens HE, et al. Learning and memory depend on fibroblast growth factor receptor 2 functioning in hippocampus. *Biol Psychiatry*. 2012; 71:1090–1098. [PubMed: 22541947]
27. Jensen FE. Neonatal seizures: an update on mechanisms and management. *Clin Perinatol* vii. 2009; 36:881–900.
28. Schwaller B, et al. Parvalbumin deficiency affects network properties resulting in increased susceptibility to epileptic seizures. *Mol Cell Neurosci*. 2004; 25:650–663. [PubMed: 15080894]
29. Raymond M, et al. Chronic perinatal hypoxia reduces glutamate-aspartate transporter function in astrocytes through the Janus kinase/signal transducer and activator of transcription pathway. *J Neurosci*. 2011; 31:17864–17871. [PubMed: 22159101]
30. Nobuta H, et al. STAT3-mediated astrogliosis protects myelin development in neonatal brain injury. *Ann Neurol*. 2012; 72:750–765. [PubMed: 22941903]
31. Buser JR, et al. Arrested preoligodendrocyte maturation contributes to myelination failure in premature infants. *Ann Neurol*. 2012; 71:93–109. [PubMed: 22275256]
32. Back SA, et al. Maturation-dependent vulnerability of oligodendrocytes to oxidative stress-induced death caused by glutathione depletion. *J Neurosci*. 1998; 18:6241–6253. [PubMed: 9698317]
33. Jablonska B, et al. Oligodendrocyte regeneration after neonatal hypoxia requires FoxO1-mediated p27Kip1 expression. *J Neurosci*. 2012; 32:14775–14793. [PubMed: 23077062]
34. Ness JK, et al. Perinatal hypoxia-ischemia induces apoptotic and excitotoxic death of periventricular white matter oligodendrocyte progenitors. *Dev Neurosci*. 2001; 23:203–208. [PubMed: 11598321]
35. Back SA, et al. Selective vulnerability of late oligodendrocyte progenitors to hypoxia-ischemia. *J Neurosci*. 2002; 22:455–463. [PubMed: 11784790]
36. Nguyen L, et al. The Yin and Yang of cell cycle progression and differentiation in the oligodendroglial lineage. *Ment Retard Dev Disabil Res Rev*. 2006; 12:85–96. [PubMed: 16807909]
37. Fancy SP, et al. Axin2 as a regulatory and therapeutic target in newborn brain injury and remyelination. *Nat Neurosci*. 2011; 14:1009–1016. [PubMed: 21706018]
38. Reid MV, et al. Delayed myelination in an intrauterine growth retardation model is mediated by oxidative stress upregulating bone morphogenetic protein 4. *J Neuropathol Exp Neurol*. 2012; 71:640–653. [PubMed: 22710965]
39. Back SA, et al. Hyaluronan accumulates in demyelinated lesions and inhibits oligodendrocyte progenitor maturation. *Nat Med*. 2005; 11:966–972. [PubMed: 16086023]
40. Preston M, et al. Digestion products of the PH20 hyaluronidase inhibit remyelination. *Ann Neurol*. 2013; 73:266–280. [PubMed: 23463525]
41. Salmaso N, et al. Environmental enrichment increases the GFAP+ stem cell pool and reverses hypoxia-induced cognitive deficits in juvenile mice. *J Neurosci*. 2012; 32:8930–8939. [PubMed: 22745493]

42. Baroncelli L, et al. Nurturing brain plasticity: impact of environmental enrichment. *Cell Death Differ.* 2010; 17:1092–1103. [PubMed: 20019745]
43. Vaccarino, FM., et al. Fibroblast growth factor signaling regulates growth and morphogenesis at multiple steps during brain development. In: Pedersen, RA.; Shatten, G., editors. *Current Topics in Developmental Biology*. Academic; San Diego: 1999. p. 179-200.
44. Stevens HE, et al. Fgfr2 is required for the development of the medial prefrontal cortex and its connections with limbic circuits. *J Neurosci.* 2010; 30:5590–5602. [PubMed: 20410112]
45. Ganat Y, et al. Chronic hypoxia up-regulates fibroblast growth factor ligands in the perinatal brain and induces fibroblast growth factor-responsive radial glial cells in the sub-ependymal zone. *Neuroscience.* 2002; 112:977–991. [PubMed: 12088755]
46. Fagel DM, et al. Fgfr1 is required for cortical regeneration and repair after perinatal hypoxia. *J Neurosci.* 2009; 29:1202–1211. [PubMed: 19176828]
47. Ishibashi N, et al. White matter protection in congenital heart surgery. *Circulation.* 2012; 125:859–871. [PubMed: 22247493]
48. Goldman SA, Nedergaard M, Windrem MS. Glial progenitor cell-based treatment and modeling of neurological disease. *Science.* 2012; 338:491–495. [PubMed: 23112326]
49. Carroll J. Human cord blood for the hypoxic-ischemic neonate. *Pediatr Res.* 2012; 71:459–463. [PubMed: 22278181]
50. Jeon I, et al. Neuronal properties, in vivo effects, and pathology of a Huntington's disease patient-derived induced pluripotent stem cells. *Stem Cells.* 2012; 30:2054–2062. [PubMed: 22628015]



**Figure 1.** Chronic perinatal hypoxia causes a delay in maturation of interneurons. Top, relative time course of normal interneuron development (left) in the brains of both humans and rodents. Exposure to hypoxic injury (right) during the early postnatal period does not affect interneuron proliferation or migration but induces a delay of interneuron maturation and connectivity. Bottom, developmental pattern of interneuron antigen expression for parvalbumin (PrV), somatostatin (SST) and distal-less homeobox 2 (Dlx2) both under normal conditions (solid lines) and following hypoxic injury (dashed lines). Immature interneuron markers such as Dlx2 are unaffected by injury at this stage of development, but proteins upregulated with maturation such as PrV are vulnerable and show long-lasting perturbations in expression levels. For more details, see ref. 20.



**Figure 2.** Perinatal white matter injury causes delays in OLC maturation and in developmental myelination. Top, time course of oligodendroglia development and myelination in the human and rodent brain identifies a specific time window for risk of white matter injury. A perinatal insult causes a delay in OLC maturation and myelination. Middle, OLC lineage progression under normal physiological conditions. Antigenic markers identify distinct stages of OLC maturation from the OLC progenitor to the mature, myelinating OLC stage. OLC progenitors and pre-OLCs are most abundant during the developmental time window corresponding to high risk of white matter injury. Bottom, developmental regulation of major developmental markers at different stages of OLC lineage progression. Markers: PDGFR- $\alpha$ , platelet-derived growth factor receptor- $\alpha$ ; A2B5, a cell surface ganglioside epitope expressed on oligodendrocyte progenitors; NG2, neuron-glia antigen 2; Olig1, oligodendrocyte transcription factor 1; Olig2, oligodendrocyte transcription factor 2; GPDH, glycerol-3-phosphate dehydrogenase; O1, oligodendrocyte antibody 1, a mature oligodendrocyte marker; O4, oligodendrocyte antibody 4, an immature oligodendrocyte marker; CNP, cyclic nucleotide phosphodiesterase; CC1, antibody that detects the APC

(adenomatous polyposis coli) protein; Sox10, SRY-related HMG-box 10 transcription factor; GalC, galactocerebroside; MBP, myelin basic protein; PLP, proteolipid protein; MAG, myelin-associated glycoprotein; MOG, myelin oligodendrocyte glycoprotein.

**Table 1**

## Animal models of VLBW premature birth

Model	Species	Features	Advantages	Disadvantages
Hypoxia-ischemia (Vannucci model)	Mouse, rat, pig	Necrotic injury OLC death Axon damage Microglial activation Astrogliosis	Focal injury Widely used	Difficult to perform in the very young pup Variability in phenotype Unilateral injury
Chronic intermittent hypoxia	Mouse, rat	Neuronal and OLC death	Diffuse moderate to severe injury	Severe injury
Chronic hypoxia	Mouse, rat	Diffuse gray and white matter injury Ventriculomegaly Moderate neuronal cell death and altered white matter development	Mild injury Global diffuse injury similar to that in most very preterm children Cognitive deficits and hyperactivity	Mild injury Lack of gliosis
LPS-induced inflammation	Mouse, rat, rabbit, sheep	Diffuse gliosis Microglial activation Neuronal and OLC death	Inflammatory insult to the developing brain HI can be added to increase degree of injury	Type of induction (LPS) is not similar to human (infection)
Interleukin-1 $\beta$	Mouse	Systemic inflammation that results in hypomyelination	Impaired OLC development Axonal damage	No increased cell death No astrogliosis
Premature delivery	Baboon	Diffuse gliosis, subarachnoid hemorrhage, gray and white matter injury; microcystic PVL, moderate ventriculomegaly	Baboons are delivered prematurely and treated similarly to infants	Difficulty in performing behavioral testing Cost

HI, hypoxia-ischemia; LPS, lipopolysaccharide; PVL, periventricular leukomalacia.