

# Towards improved animal models of neonatal white matter injury associated with cerebral palsy

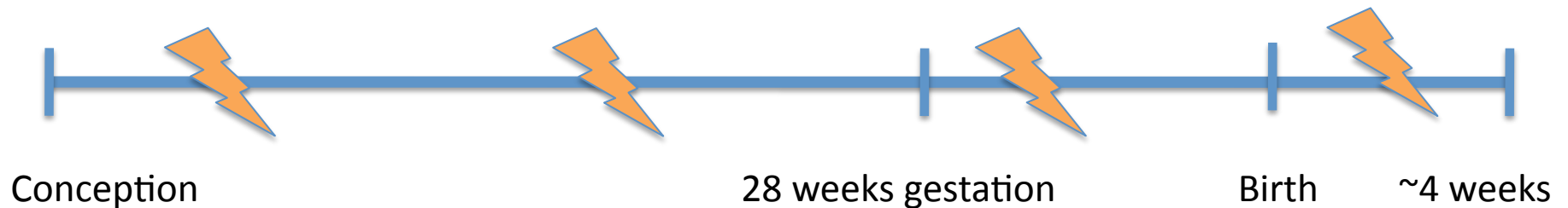
John C. Silbereis et al., 2010

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# What is Cerebral Palsy?

“CP denotes a condition of the brain reflected by movement limitation that is often associated with some degree of cognitive impairment. It is generally considered a fixed, non-progressive condition resulting from neurological injury in the antenatal or perinatal period”



# CP rates are increasing in the United States

Late 1970's, **1.3/1000**

Currently, **>3/1000**

→ Increasing survival of ELBW premature infants born at gestational ages <28 weeks



## Events associated with acquired brain injuries that lead to CP:

In utero hypoxic-ischemia (HI) events:

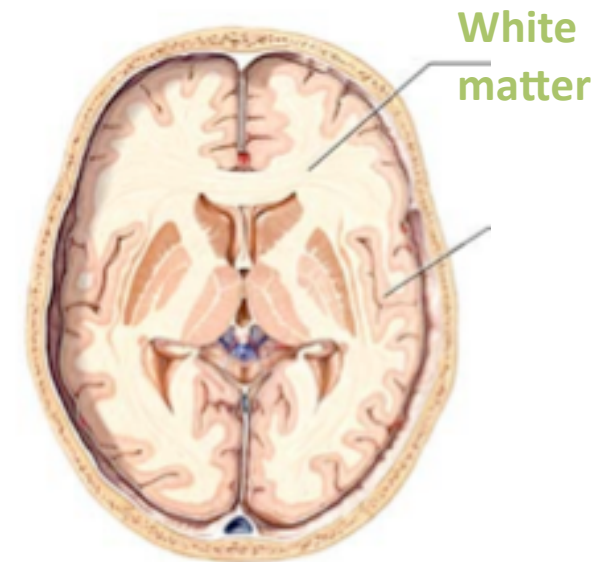
- *placental insufficiency, stroke, infection*

Perinatal events:

- *respiratory failure*

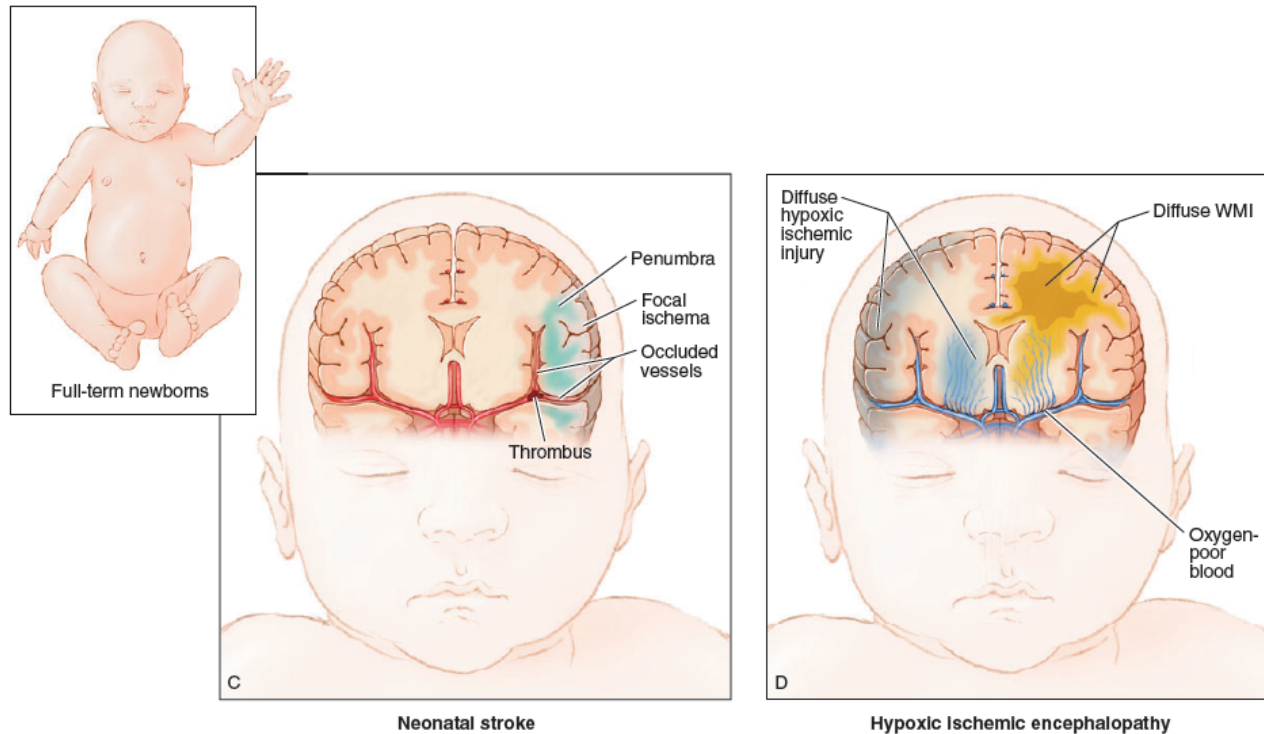
Neonatal disorders

- *chronic lung disease*



**\*Damage to white matter (WMI) has emerged as an increasingly common cause of CP**

# Common Brain Injuries Associated with Later Development of Cerebral Palsy



**Neonatal Stroke:** focal region of cortex is affected

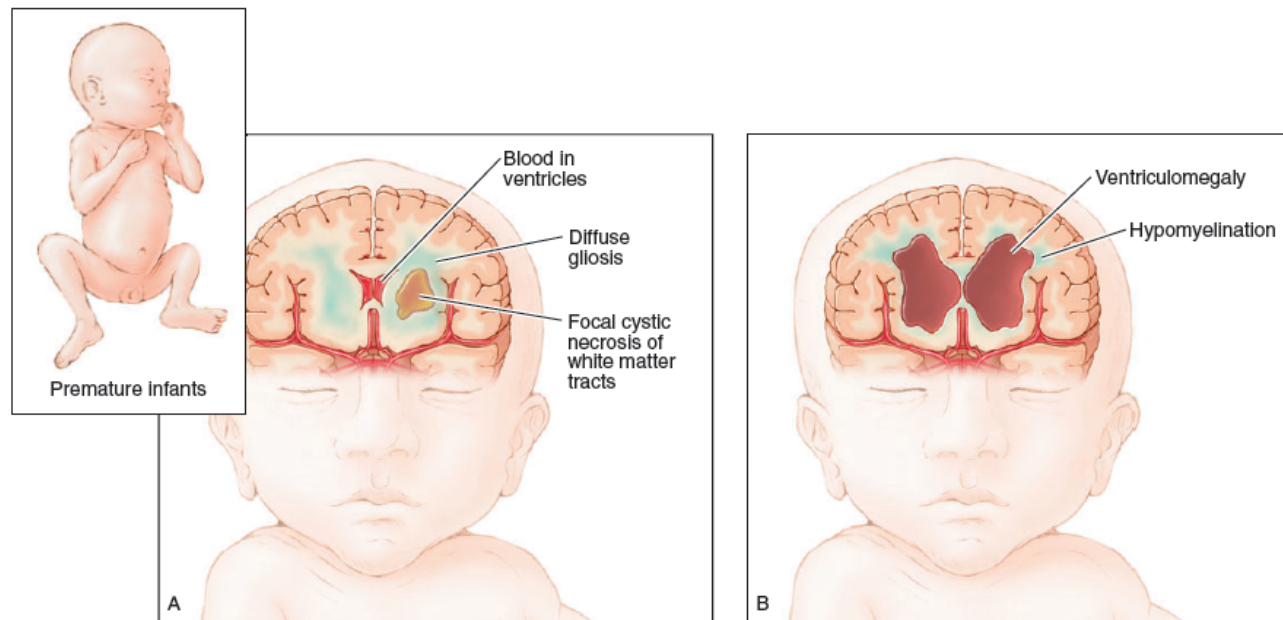
- Ischemic: blood supply decreased

**Hypoxic Ischemic Encephalopathy:**

results in global HI injury to the brain

- neurons of the cortical plate, basal ganglia and white matter tracts

# Common Brain Injuries Associated with Later Development of Cerebral Palsy



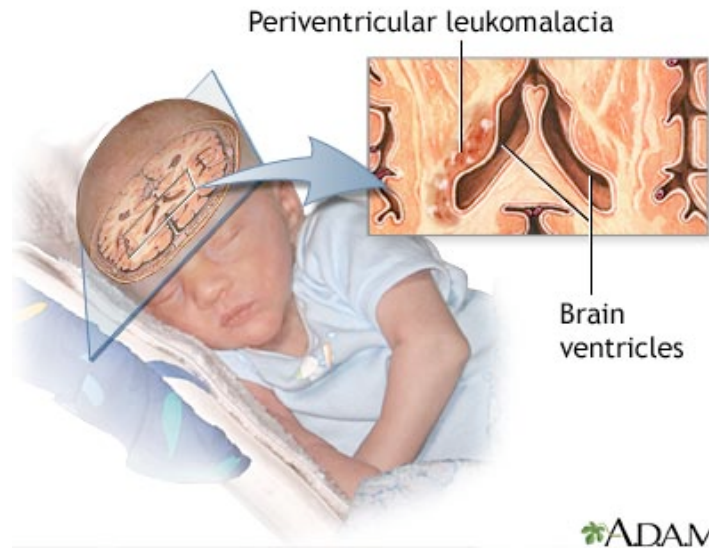
Cerebral palsy and periventricular leukomalacia

**Intraventricular hemorrhage:**  
results from germinal matrix  
hemorrhage into the ventricles

**Long term effects of brain injury:**  
hypomyelination results from failure of  
lesion repair

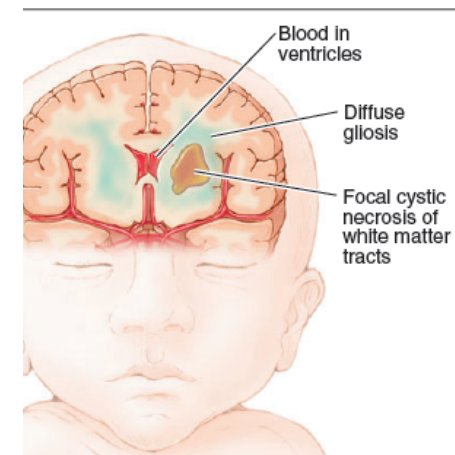
- Also lateral ventricles dilated  
(ventriculomegaly)

# White Matter Injury



- Periventricular Leukomalacia (PVL):** macroscopic cystic or microscopic non-cystic lesions with cell degeneration
- Results from immaturity of autoregulation and blood supply to white matter (reperfusion failure)

Focal and diffuse non-cystic lesions that trigger oligodendrocyte degeneration and disturbances in myelination



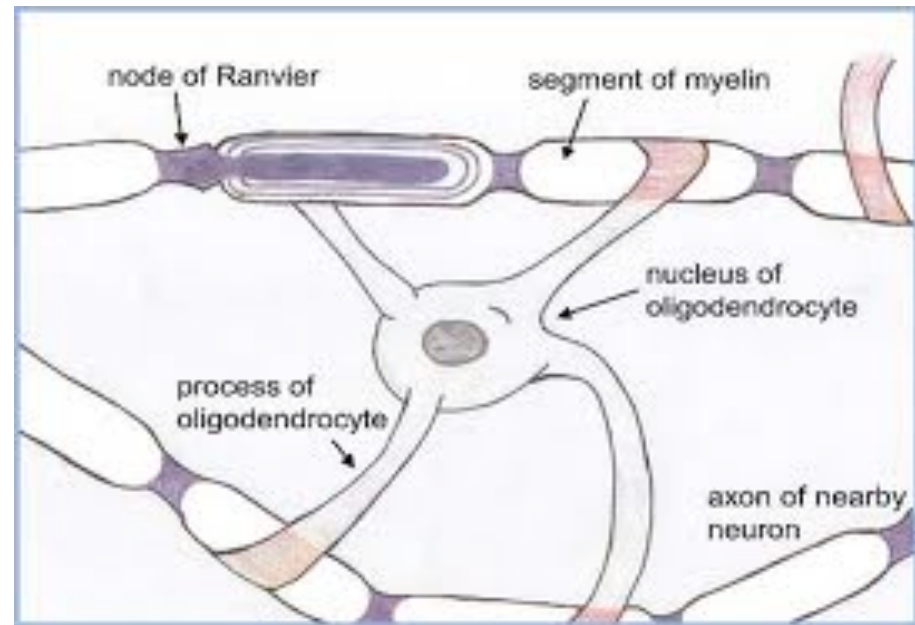
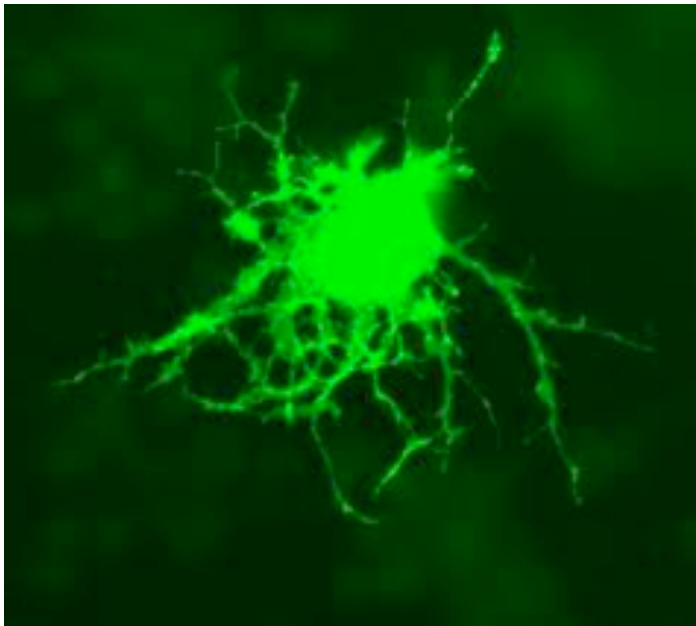
# Preventing WMI

“Development of therapies to prevent neonatal white matter injury leading to Cerebral Palsy is hindered by a poor understanding of the underlying cellular, molecular and genetic mechanisms”

## How can we learn more?

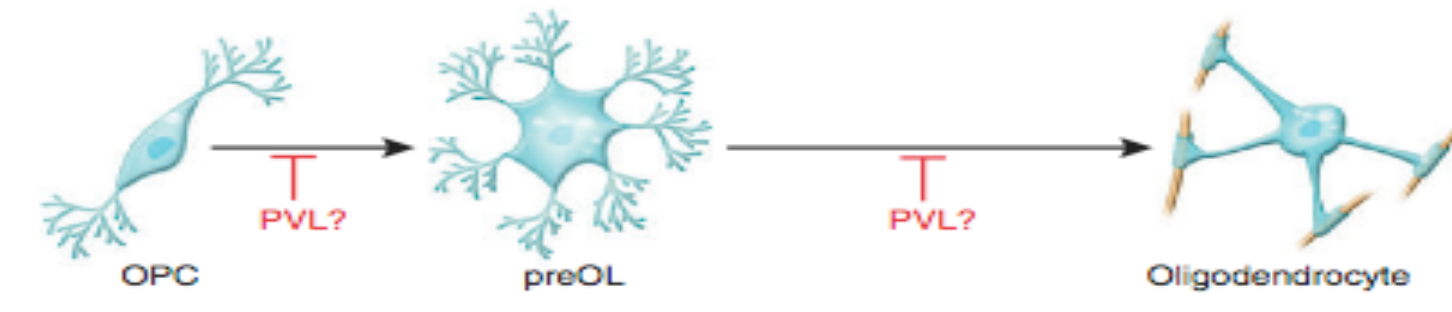
- Need for improved animal models to provide clues about cellular intrinsic and extrinsic pathways that regulate responses of oligodendrocytes during initial and progressive phases of injury
- Also need to understand impact of neonatal insults on neuronal populations

Oligodendrocytes enable the formation of myelin and Nodes of Ranvier, allowing optimal conduction of the nervous impulse



Recently discovered genes that are necessary for oligodendrocyte development: Olig1 and Olig2

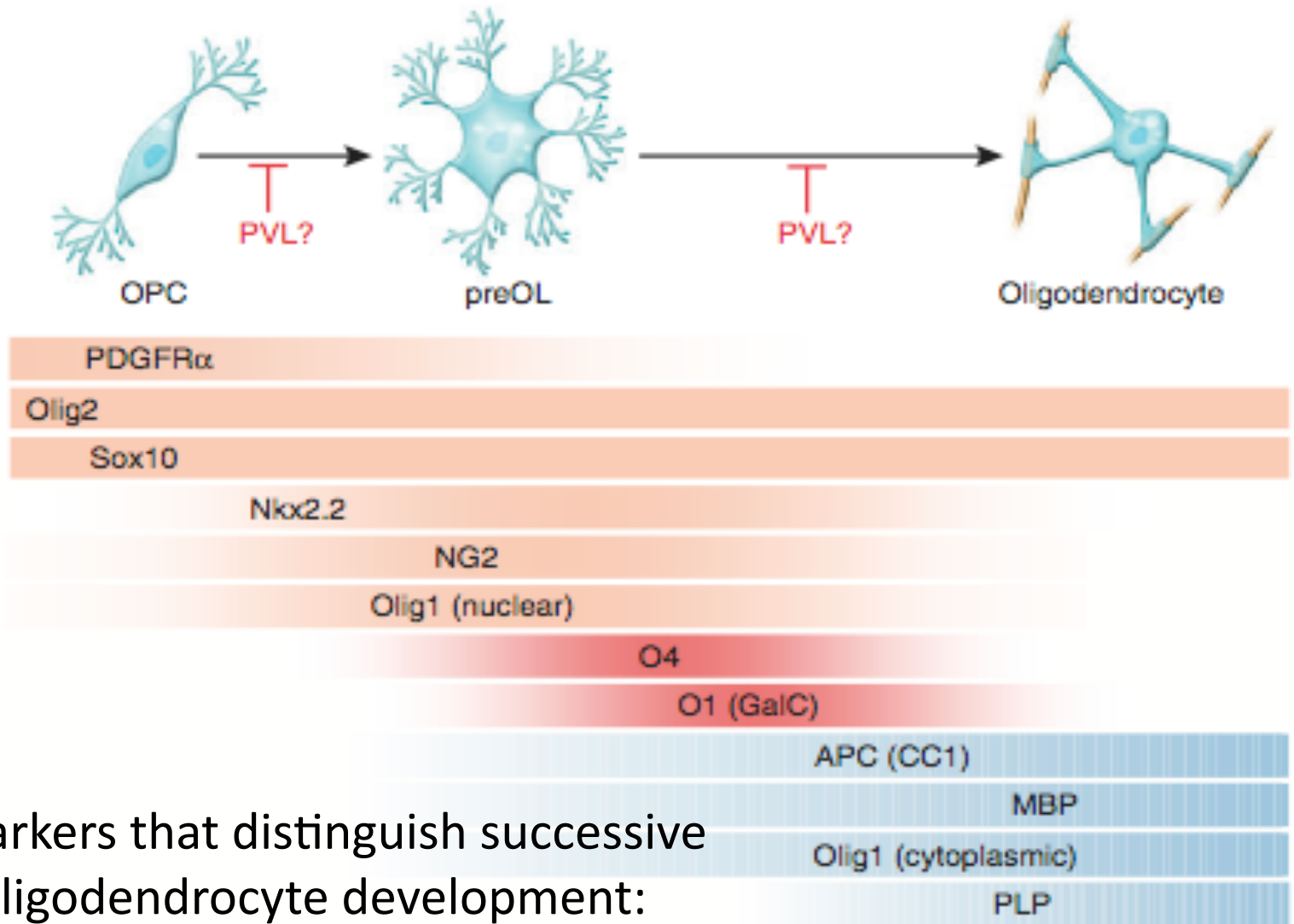
# Oligodendrocyte development:



**Neural stem cells** (NSCs) give rise to **oligodendrocyte precursor cells** (OPCs) during embryonic development

OPCs proliferate and migrate throughout the brain and then differentiate into late oligodendrocyte progenitors, **premyelinating oligodendrocytes** (preOLs)

PreOLs undergo extensive cellular growth and process elaboration and mature into **myelinating oligodendrocytes**



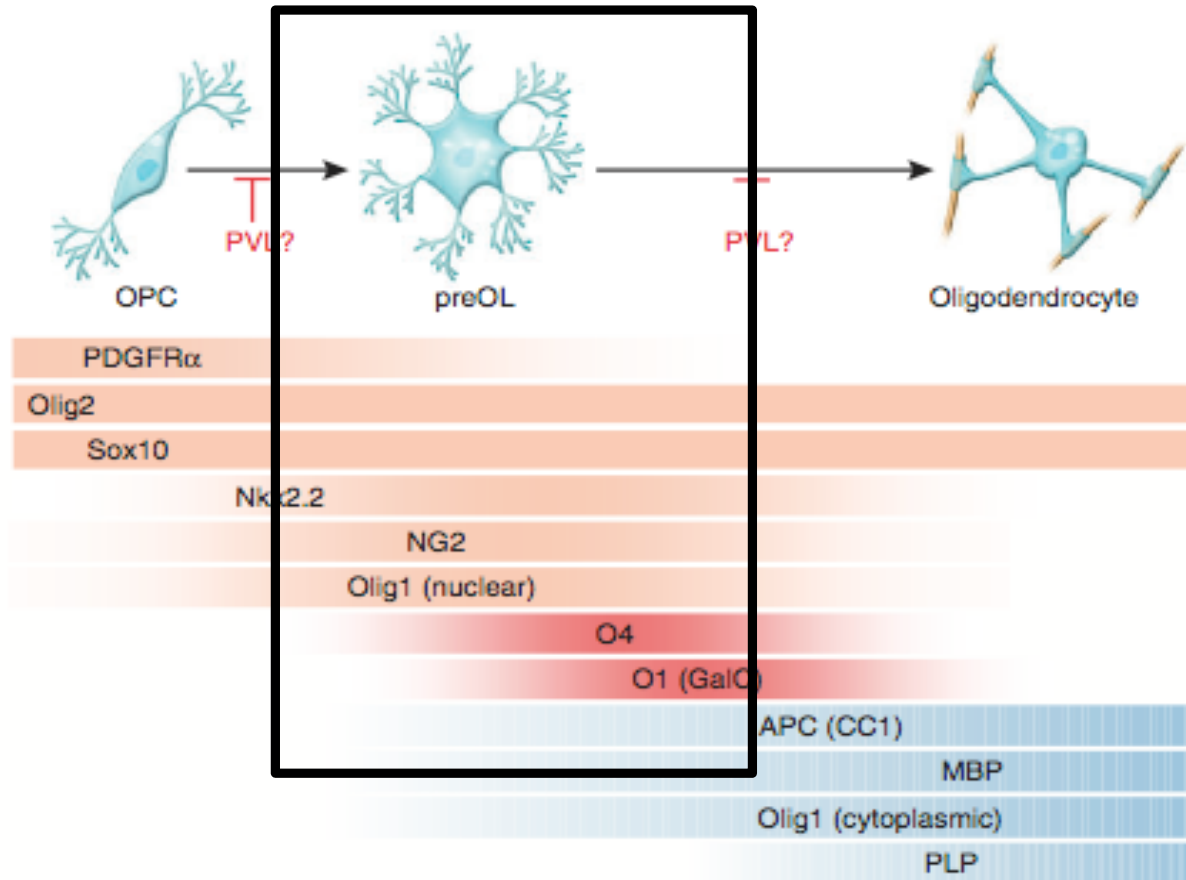
Various markers that distinguish successive stages of oligodendrocyte development:

\**Sonic hedgehog (Shh)*

\**Bone morphogenetic protein (BMP)*

\**Platelet-derived growth factor receptor- $\alpha$  (PDGFR $\alpha$ )*

In both human patients and rodent injury models, white matter is most susceptible to injury at the ages at which preOLs predominate in the forebrain



Not well understood, but suggested that 4 main factors play a role:

1. Their greater sensitivity to oxidative stress

*White matter lesions in the brains of premature infants demonstrate significant levels of oxidative damage that are accompanied by depletion of preOLs*

2. Cytokine-induced cell deaths related to inflammation

*Controversial, elevated levels of TNF $\alpha$  and IL1 $\beta$  observed in cystic PVL lesions, but has not been studied in more common diffuse WMI form of PVL*

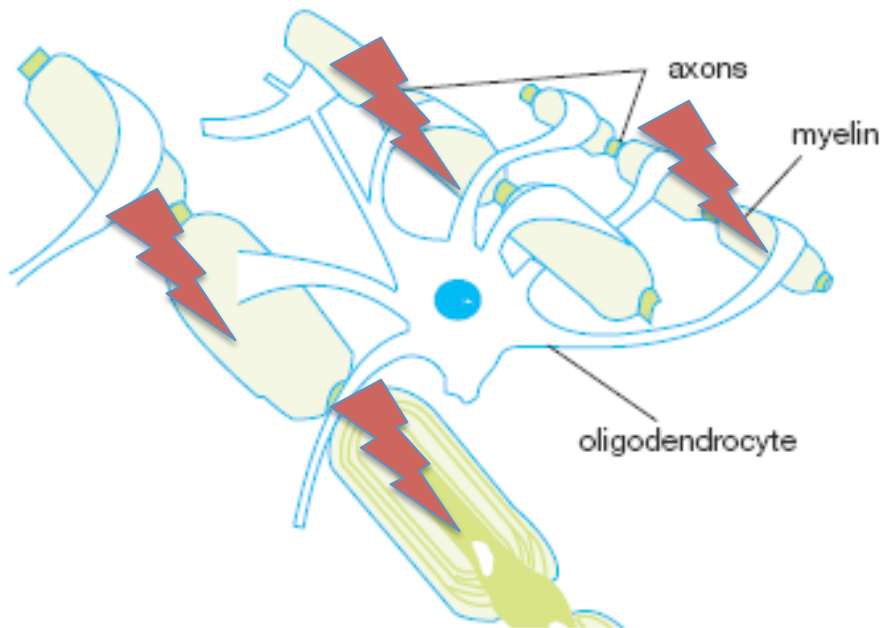
### 3. Perturbations of oligodendrocyte development coupled with ineffective repair mechanisms

*Billards et al used Olig2 and other early oligodendrocyte markers, and found that OPCs were present in acute and chronic human PVL lesions, suggesting a block in differentiation*

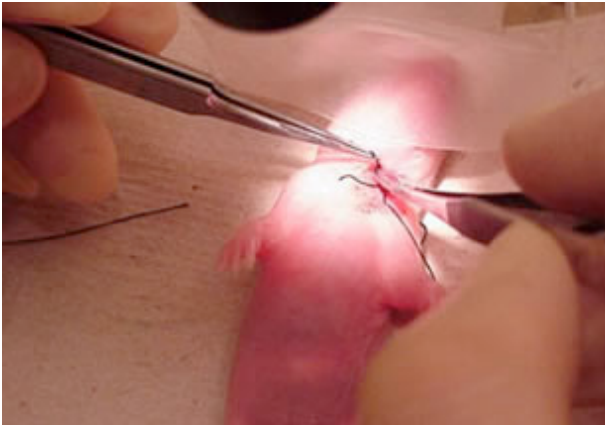
*Findings in a neonatal rat model confirmed that remyelination after chronic HI injury was delayed owing to a failure of preOL differentiation*

## 4. Axonal damage

*Little research- axons release important growth factors, such as **PDGF** and **BDNF**, that promote oligodendrocyte survival and myelination. Possible that a lack of normal signaling between axons and oligodendrocytes contributes to myelination failure.*



# Existing Animal Models of WMI



- Pups anesthetized
- Vertical incision made on the neck
- Right common carotid artery is cauterized to restrict blood flow

- HIs were subjected to 8% oxygen for 120 minutes in airtight container

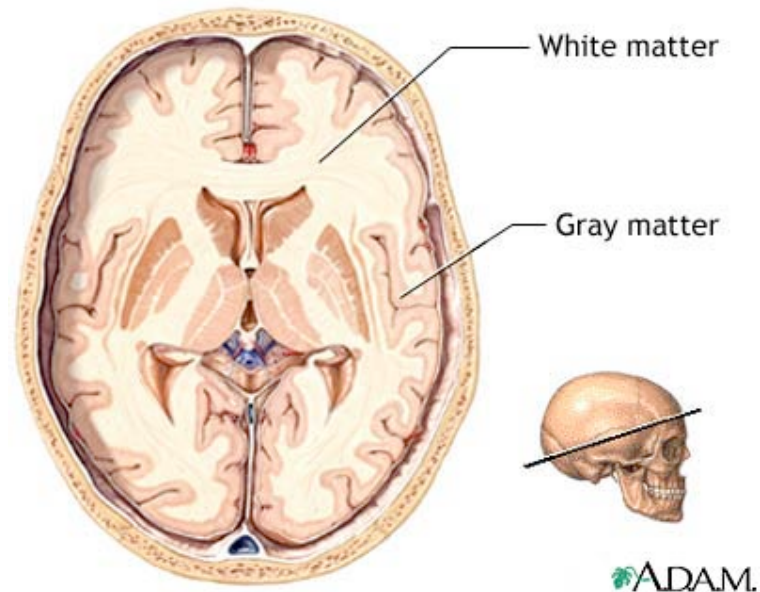


## P7 Induction of HI

- Injury resembling what we see in **full term** infants with HIE
- More gray matter damage

## P2 Induction of HI

- Injury resembling what we see in **preterm** infants
- High number of preOLs so highly vulnerable to injury
- BUT OPCs and myelinating oligodendrocytes more resistant to single and recurrent episodes of HI



# Overview of HI Model

Table 1. Overview of common animal models for studying developmental white matter injury

Model	Species	Pathological features of WMI	Advantages	Disadvantages	References
Rice-Vannucci (HIE)	Mouse, rat	Oligodendrocyte cell death Alterations of oligodendrocyte development Necrotic cysts of white matter and cortex Axonal damage Forebrain neural cell death Microglial activation Ventriculomegaly	Widely used and well characterized Use of transgenic mice	Not adapted to large animals Variability between animals Stroke and global ischemia rare in premature infants	Vannucci and Vannucci, 2005; Segovia et al., 2008

# Chronic Intermittent Perinatal and Intrauterine HI Models

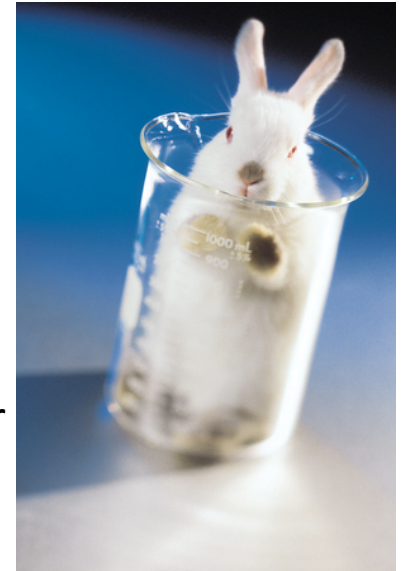
- ELBW infants with chronic lung disease due to premature birth suffer hypoxic episodes and are at risk of developing CP
  - Rodent models involving continuous or intermittent hypoxic rearing are ideal models of this type of injury

Chronic hypoxia	Mouse, rat	Oligodendrocyte cell death is minimal Alterations of oligodendrocyte development Moderate neural cell death Ventriculomegaly	Generates chronic global hypoxia that is common in premature human infants Consistency between specimens Use of transgenic mice	Lacks gliosis Inflammatory response poorly characterized Relatively mild WMI	Back et al., 2006a; Fagel et al., 2006; Chahboune et al., 2009; Scafidi et al., 2009
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# Rabbit Models

- Abruption of placental blood flow by inflation of a balloon catheter inserted into the uterine artery
  - Procedure induces lesions throughout cerebral gray and white matter as well as intraventricular hemorrhage and periventricular white matter loss
- Surviving rabbits display range of motor deficits resembling CP
  - Only model that yields correlations with neurobehavioral outcome and assessment



In utero ischemia	Rabbit	Lesions to multiple forebrain structures (cortex, thalamus and basal ganglia) and white matter tracts Intraventricular hemorrhage	Rabbits exhibit hypertonic motor deficits similar to those observed in CP	Not amenable to genetic experiments Requires specialized infrastructure and expertise	Cai et al., 1998; Drobyshevsky et al., 2005
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# Large animal models of preterm WMI provide significant translational potential

**Primate model of prematurity:** baboons were delivered by induced labor and provided ventilator-support and neonatal intensive care

Pathological features include:

- Cystic PVL
- Diffuse white matter gliosis
- Ventriculomegaly
- Intraventricular hemorrhage



Premature delivery

Baboon

Cystic necroses, diffuse white matter gliosis  
Ventriculomegaly  
Intraventricular hemorrhage

Highest similarity to human development  
Includes premature birth and neonatal intensive care with no need for experimental insult  
Allows for chronic instrumentation

High cost  
Requires highly specialized infrastructure and expertise  
Animals do not survive long term

Dieni et al., 2004; Inder et al., 2005a; Inder et al., 2005b

# Fetal sheep is most widely studied large animal model of developmental brain injury



WMI is induced by:

Reversible carotid artery occlusion

Umbilical cord compression

Maternal hypoxemia between ~90 days gestation (65%) and near term

1. Neurodevelopment of the preterm sheep fetus (65% gestation) is comparable to that of the preterm human between approximately 24 and 28 weeks gestation
2. The size of the fetal sheep permits monitoring of fetal physiological status (EEG, analysis of cerebral blood flow)

Sheep	Relatively selective graded cerebral white matter lesions that resemble the spectrum seen in human Cortical and subcortical gray matter injury is a major feature if ischemia is severe	Brain development and neurovasculature more similar to human Chronic instrumentation allows physiological monitoring, EEG and analysis of fetal cerebral blood flow
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McClure et al., 2008

# Maternal infection is correlated with an increased incidence of CP

Ovine and rat models: bacteria or the lipopolysaccharide (LPS) endotoxin are administered either systemically or uteroplacentally to the mother

In the child:

- *Robust microglial activation*
- *WMI injury*
- *Death of deep layer cortical neurons in the child*



LPS-induced inflammation

Mouse, rat, sheep, rabbit

Microglial activation  
Diffuse white matter gliosis  
Oligodendrocyte cell death  
Deep cortical layer neuronal cell death  
More severe when coupled with HI

Specifically creates an inflammatory insult to the developing brain  
Can be coupled with HI-based models  
Feasible in transgenic mice

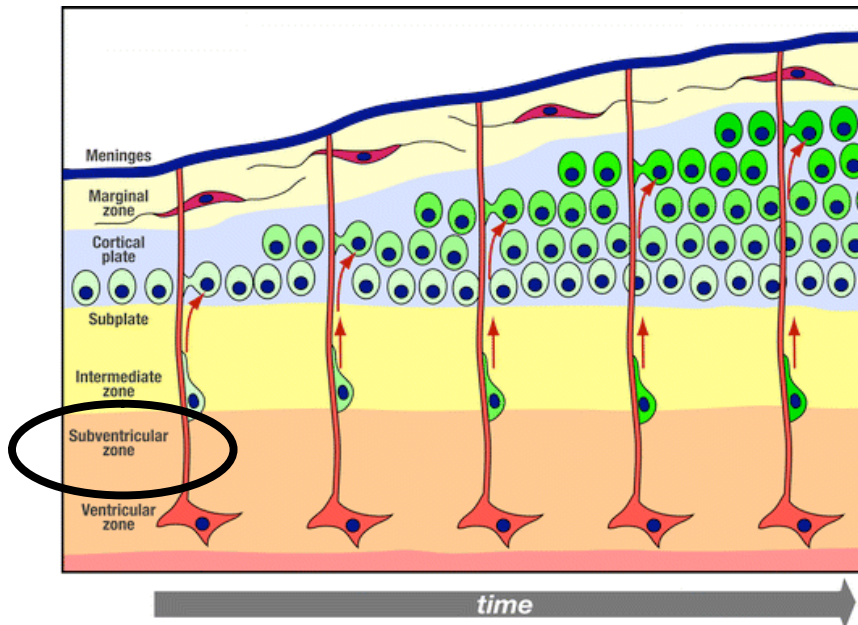
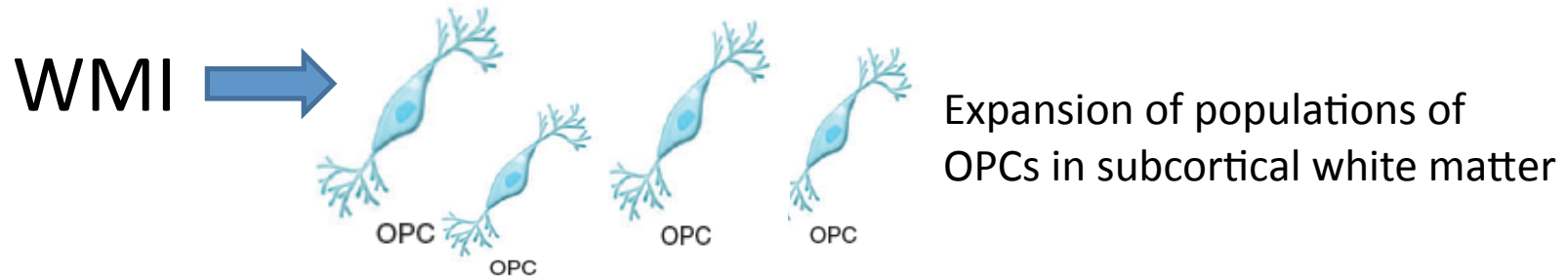
Infection of LPS-containing bacteria rarely causes inflammation in clinical CP  
Unclear whether LPS crosses the blood-brain barrier

Wang et al., 2006

# Future Directions: Insights from Developmental Neurobiology

- Need to define impact of WMI on neural progenitor populations present in white matter or in germinal zones (subventricular zone)
- Define mechanisms of myelination failure in preterm infants

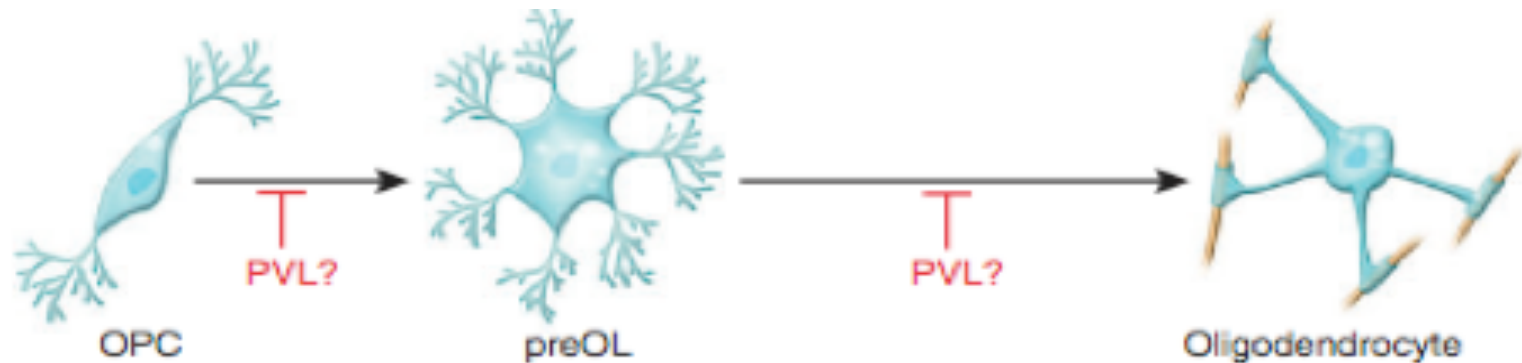
# What we do know about OPCs



- OPCs make up small amount of SV Z progenitors in healthy brain but expands significantly due to WMI
- SVZ-derived OPCs migrate to regions of WMI

Migration of OPCs is an important component of the response to WMI

## Use of new genetic tools:

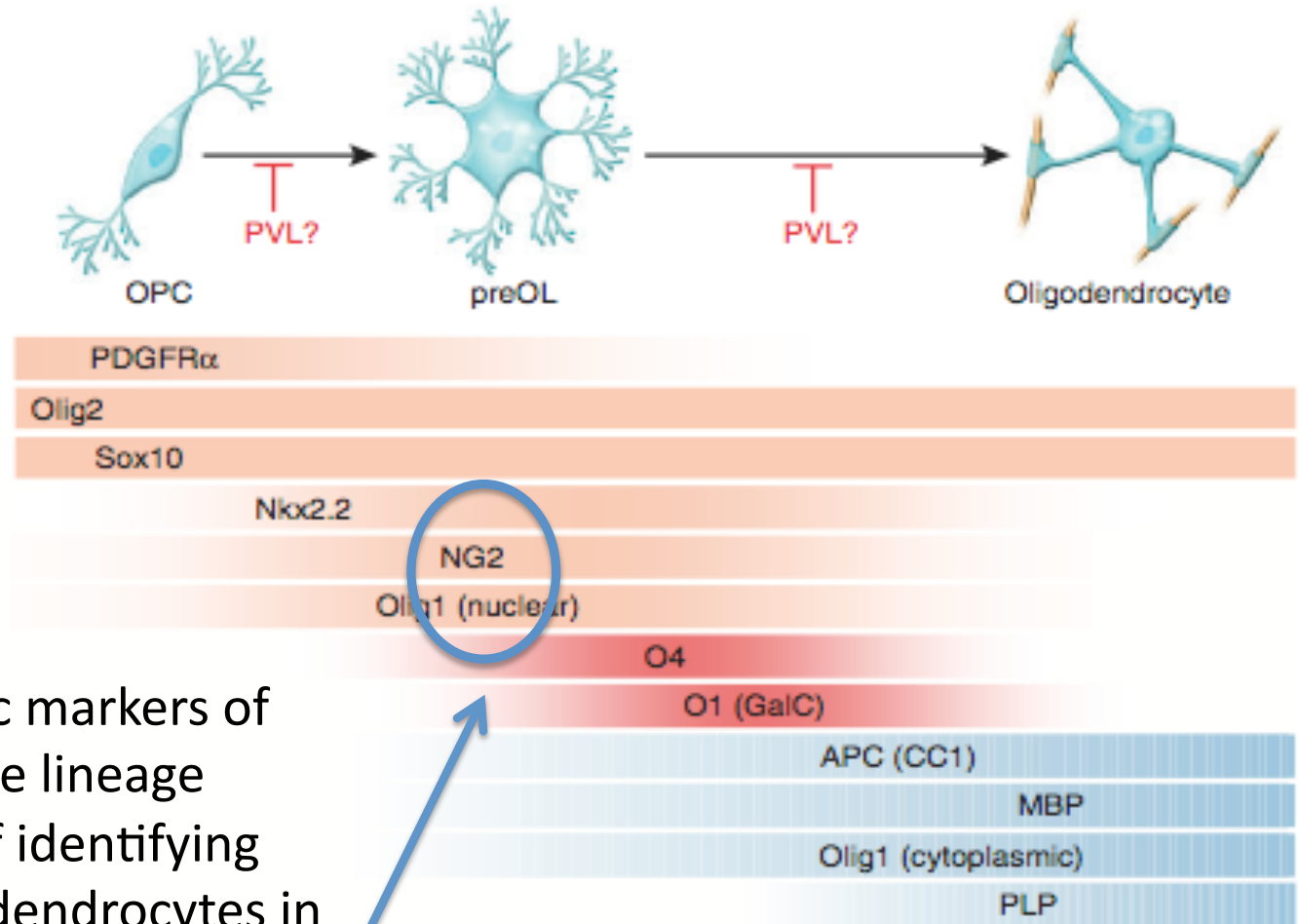


In the past decade research has enabled the knockout of oligodendrocyte lineages in a developmental-stage specific manner

Transgenic lines take control of oligodendrocyte-lineage-specific enhancers, which enable targeting of oligodendrocytes at precise stages of development

**Provides powerful tools for studying WMI at distinct stages of development**

New markers for stage-specific study of the oligodendrocyte lineage in vivo and in vitro:

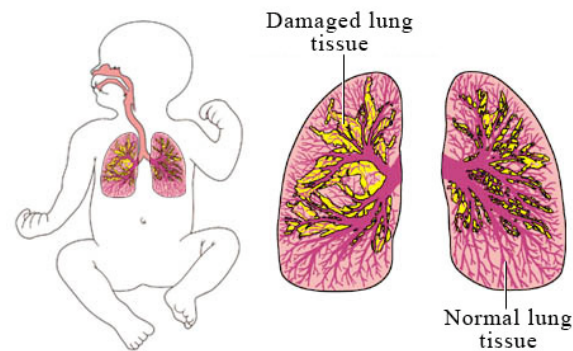


Maturation-specific markers of the oligodendrocyte lineage provide a means of identifying and studying oligodendrocytes in neonatal WMI

Ex. NG2 used in research to identify OPCs and preOLs

# Postnatal Damage Due to Prematurity

- Premature infants with chronic lung disease who might be chronically hypoxemic are at highest risk for poor long term developmental outcomes



- Standard treatment practices in neonatal care might cause injury on developing brain



Postnatal dexamethasone associated with development of CP



Potential of inhaled anesthetics can cause neuronal apoptosis in neonate

# Conclusions

- WMI continues to increase worldwide
  - Main form of brain injury in preterm survivors
- Crucial need for human neuropathological studies to better understand cellular and molecular events that occur with WMI
- Small animal models provide initial answers to cellular and molecular questions
  - Models that more closely resemble humans will provide opportunities for more progress toward understanding WMI