The white matter damage in neonates (is always) mediated by inflammation: an important situation and an interesting «story»
Some important « apples »

- Pathology of leucomalacia: « end product »
- Early and late neuronal migrations: their importance
- Cerebral blood flow autoregulation impairment
- Biochemical properties of brain cells
- Metabolic differences between neurons and glial cells
- pH, pCO2, BP, O2 intervention
- Cerebral blood flow, CMRG, CMRO2 and extractions measurements
- US diagnosis of PVL and classification
- Epidemiological studies
- The inflammatory components
data from our group concerning « pvl or periventricular leucomalacia »

- Soc Bel Ped 1991:
  « Hyperechogenic brain densities in 56 neonates < 34 w : relevance of perinatal factors » and the endotoxins hypothesis

- 2d WordCongr perinat med 1993
  « haemorrhage and ischaemia lesions of the brain in a cohort of 474 babies under 1.5 kg: neonatal features »
From epidemiology:
DISCOVERIES FROM THREE LARGE
COHORT STUDIES OF PREMATURING

Nigel Paneth MD MPH
Michigan State University
http://www.epi.msu.edu/faculty/paneth.htm
Neonatology 2006
Miami, Nov 10th, 2006
(Paneth) White Matter Damage and Cerebral Palsy

Percent with CP

No WMD

WMD

(+ later impairments !): Cognitive, learning

Antenatal **steroids** may prevent brain damage.
- Magnesium sulfate probably does not prevent brain injury.
- Vaginal delivery predisposes to brain damage, but this may be because of its role as a marker of placental inflammation.
(Paneth) SUMMARY OF 5 KEY FINDINGS ABOUT PREMATURE INFANTS IN DEN AND NBH

1. Brain damage is widespread in infants who die, and can be diffuse or focal, but **white matter is the tissue most affected.**
2. US evidence of white matter injury ... is the most important determinant of long-term outcome.
3. **Thyroid hormone** is the single most predictive measure of outcome obtainable on serum in the first week of life.
4. Hypocapnia (PCO2 < 25) and perhaps hyperoxia (PO2 > 60) should be avoided.
5. The finding in the **placenta** most predictive of brain injury is fetal **vasculitis**; membrane inflammation alone is not associated with brain injury.

**Comment:**
Amnionitis gives:
- an increased release of cytokines and
- a decreased expression of angiogenic factors
Anatomic aspects
The quantitative brain in newborns

- Brain is 14-16% of body weight
- Brain is 60% of body metabolism (see glucose) and O2 consumption
- 10% of brain is CSF of which 6/7 is coming from choroid plexus and 1/7 from capillaries
- 3-5% of brain is blood
- 40% of brain is glial cells
- 4% of brain is neurons
- 40% of brain is EC fluid
Important developmental aspects in structures and metabolism

Figure 1.23 Human cerebral capillary obtained at biopsy. Blood–brain barrier (BBB) capillaries are characterized by the presence of transcytotic vesicles in endothelial cells (E), a high mitochondrial content (large arrow), and the formation of tight junctions (small arrows) between endothelial cells that restrict the transport of solutes through the interendothelial barrier. The capillary endothelium is ensheathed within a basement membrane (arrowheads), which also houses pericytes (P). The basement membrane reacts with cytoplasmic foot processes (asterisk), which may be responsible for induction of BBB cells on the endothelial cells. L, lumen of the capillary. Bar = 1 μm. From Claudio et al. (1995).

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Neuronal migration, blood brain barrier, cells’ biochemistry, Cerebral blood flow autoregulation

Prof Oreste Battisti
The late neuronal migration

24 weeks

28 weeks
Ultrastructure of blood-brain-barrier: this very important structure is not efficient before 27 weeks, and definitely not before 32 weeks.

**FIGURE 1.22** Electron micrograph of a blood–brain barrier (BBB) capillary. Endothelial cells joined by tight junctions form continuous capillaries with no fenestrations and restrict the passage of solutes between blood and brain. Pericytes (P) are present within the basement membrane (arrowheads) of these capillaries, serve to control vascular tone, and can also be phagocytic in the brain. Astrocyte foot processes (A) surround the basement membrane and are responsible for the induction of BBB properties on endothelial cells. Bar = 2 μm.

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The blood-brain barrier

- Brain cells: neurons, astrocytes, microcytes, radial cells, oligodendrocytes;
- Microcirculation;
- Ependyma and villi;
- Arachnoids;
The cortex microcirculation: observe the differences between arteries and veins.

**FIGURE 2.21** Microvasculature of the human neocortex. (A) Primary visual cortex (area 17). Note the presence of segments of deep penetrating arteries that have a larger diameter than the microvessels and run from the pial surface to the deep cortical layers, as well as the high density of microvessels in the middle layer (layers IVV and IVCa). (B) Prefrontal cortex (area 9). Cortical layers are indicated by Roman numerals. The microvessels are stained using an antibody against laminin (a component of the extracellular matrix).

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**Fig. 2.22.** Another area of the brain shown in Fig. 2.21. There is a mass of deep vein branches (red).
Fetal and neonatal Brain development: histology and biochemistry

- Radial cells;
- oligodendrocytes
- astrocytes
- microglia

Microcirculation: observe differences between A and V

40 % glial cells, 4 % neurons, 4 % blood,
10 % CSF, 35 % ECF, 10 % variance
Disturbances of late neuronal migration:
axonal retraction, pericapillaries congruence, rosettes, ependymal disruption,

And also of volume and Myelination
Excitotoxicity: apoptosis and necrosis; neuroplasticity

**FIGURE 14.5** Aspects of a model that relates glutamate exposure to hippocampal synapses to long-term synaptic strengthening. Glutamate can act through postsynaptic glutamate receptors (mGluR) to activate G proteins (G). Glutamate also acts through NMDA and AMPA receptors to increase intracellular levels of free Ca"^2+" and Ca"^2+" bound to calmodulin. These events lead to activation of phospholipase C (PLC), CAMKII, calmodulin (CaN), adenyl cyclase (AC), and PKA. Two forms of cross-talk between these signaling pathways are illustrated. As discussed in the text, PKA activation leads to the inhibition of PP1. This inhibition releases dephosphorylation of CAMKII by PP1, thus helping to sustain CAMKII activity. Also, MAPK activates phospholipase A_2 (PLA_2) and the resulting increase in arachidonic acid activates PKC. PKC in turn activates MAPK, which further activates MAPK. As illustrated, MAPK, PKA, and CAMKII regulate gene expression and cytosolic components, such as the cytoskeleton, that are essential for long-term synaptic strengthening.
When brain blood flow is in low range and even « dependent »

CBF, CBV, CMRO2 and CMRG

<table>
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<tr>
<th>CBF</th>
<th>[G]a</th>
<th>% w</th>
<th>[O2]a</th>
<th>% w</th>
<th>CBV</th>
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<td>30</td>
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<td>40</td>
<td>25</td>
<td>32</td>
<td>18</td>
<td>75</td>
<td>4.6</td>
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</tbody>
</table>

Sources: Volpe, Wyatt, Michenfelder, Greisen and Battisti
Summary of Main features concerning brain cytology and biochemistry

- Function and integrity of cells, temperature;
- 10 glial cells for one neuron;
- Neurone-glial cells unit;
- Surrounding capillaries and the BBB;
- Differences in density of capillaries;
- Differences in veins and arteries networks;
- Differences in CMRO2 and CMRG between neurons and glial cells
- Late neuronal migration and transitory period of hypersensitivity

- Neurons:
  similarity of reactions
- Oligodendrocytes:
  oxydative stress protection ; trophins; myelin synthesis; non phagocytic
- Radial cells:
  guiders and helpers
- Astrocytes:
  nutrition of neurons, reservoir of beta-amyloid protein and chondroitine sulfate proteoglycan glutamate and TNF;« fibrous » in WM, « protoplasmic » in GM;
- Microglia (< mesoderm):
  macrophage, reservoir of cytokins
From protecting to damaging « biochemical attitudes »

Depending on the environmental conditions, actual and preceding, factors can protect or damage

iCe and iNu Ca++
FR CREB, CAMD, caspase 3 and 6

**Appoptotic cascade:**
- if > 1h of Low pCO2 ( < 27 mmHg; )
- if > 6 h of high pCO2 ( > 65 mmHg )
- if hypoxia ( > 65 mmHg? pO2 )
- for > 1 h ? Time ?

---

**TABLE 1**

The Relationship of Some Presumably Protective Substances to Oligodendrocytes and Neurons

<table>
<thead>
<tr>
<th>Category</th>
<th>Molecule</th>
<th>Promotion of Oligodendrocyte Differentiation</th>
<th>Promotion of Oligodendrocyte or-Precur ac- or Survival</th>
<th>Protection of Oligodendrocyte or Promotion of Remyelination</th>
<th>Promotion of Neurotrophic Survival or Protection</th>
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<tr>
<td>Hormones</td>
<td>Corticosteroids</td>
<td>42, 121</td>
<td>121</td>
<td>122</td>
<td>123-125</td>
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<td></td>
<td>Thyroid Hormones</td>
<td>42, 43, 126</td>
<td>127</td>
<td>128, 129</td>
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<td>Neurotrophins</td>
<td>BDNF</td>
<td>37</td>
<td>35 killer: 135</td>
<td>115, 130-134</td>
<td>136-140</td>
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<td></td>
<td>Nerve Growth</td>
<td></td>
<td>35, 37, 59</td>
<td>132, 133, 141</td>
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<tr>
<td></td>
<td>Neurotrophic</td>
<td></td>
<td>37, 105</td>
<td>132, 133, 141</td>
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<tr>
<td>IL-6 family</td>
<td>IL-6</td>
<td>58 astrocytic (105)</td>
<td>37, 58</td>
<td>143-145</td>
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<td></td>
<td>LIF</td>
<td></td>
<td>37, 58</td>
<td>142</td>
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<tr>
<td></td>
<td>CNTF</td>
<td>58 astrocytic (105)</td>
<td>37, 58</td>
<td>143-145</td>
<td></td>
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<tr>
<td>Angiogenic</td>
<td>VEGF</td>
<td></td>
<td></td>
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<tr>
<td>cytokines</td>
<td>bFGF</td>
<td>Inhibitor: 64, 67</td>
<td>149-151</td>
<td>152, 153</td>
<td>136-154-157</td>
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<tr>
<td>Other cytokines</td>
<td>Inhibitor: 158</td>
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<td></td>
<td>155-157, 160-162</td>
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<td>and growth factors</td>
<td>IGF1</td>
<td>25, 55, 163, 164</td>
<td>37, 66, 165</td>
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<td></td>
<td>Inhibitor: 158</td>
<td>66, 150</td>
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<td>132, 174, 175</td>
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<td></td>
<td>PDGF</td>
<td>25, 166, 167</td>
<td>25, 166, 167</td>
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<td></td>
<td>TGFβ</td>
<td>176</td>
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<td>GGF/Neuregulin</td>
<td>183</td>
<td></td>
<td>178-182</td>
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<td></td>
<td>IL-2</td>
<td>184-187</td>
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<td></td>
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</tbody>
</table>

The numbers identify relevant references (controversial issues in italics). Abbreviations: NGF, nerve growth factor; LIF, leukocyte inhibitory factor; EGF, epidermal growth factor; IGF, insulin-like growth factor; PDGF, platelet-derived growth factor; TGF-β, T-cell growth factor-β.
From an « explanatory cascade of events »

From outside brain

Inflammation or

From inside brain

Endotoxins coming from outside brain affects heart Function and possibly CBF = « dual effects »
This is in about 15-25% of cases

A. Insult(s) component
- Exposure related...
- Infection
- Hypoxia - Ischemia - Reperfusion
- Inflammation

B. Developmental component
- Developmentally-regulated...
  - a. vascular / ependymal factors
  - b. oligodendroglia development
  - c. endogenous protectors (trophic factors)
- Increased vulnerability

White Matter Damage

Extrinsic inflammation

In 25 – 33% cases
Metabolic aspects
Intracerebral Consequences of iCDO2 and/or iCDG

• **At BBB level**: entry of small molecules (15 ′) and big molecules (120 ′); of neutrophils and monocytes (120 ′)

• **At neurons level**: axones then dendrites edema (30-55 ′), followed by retraction and hypersensitivity to EAA; body edema (50-75 ′), action on peptides and nucleus;

• **At vessels’ level**: capillaries surrounded, thrombosis;

• **At OL level**:

• **At astrocytes level**: glutamate, NO, FR, proteolytic enzymes

• **At microglia level**:

• **Energy failure and oxidative stress**:
  → Free radicals (OFR, NO, Fe+++)
  → EAA
  → Release of NA from locus ceorulus
  → Activation of microglia and LycT4
  → Genes activation (CREB, JUN)
  → Relesa of toxins: AOAA, MPP, 3NPA
  → Inflammatory products:
    - Proteolytic enzymes on matrix: from neurons, astrocytes, microglia;
    - Il 1,6,8,9, TNFa, complement, antithrombin III, factor V, protein C, antipohospholipid antibody
Cytokines liberation and storage

- Density of small vessels in germinal > cortex > white matter;
- Capillaries in BBB are very rich in mitochondria;
- All Brain cells can produce complement, cytokines and coagulation proteins;
- MHC system in brain = HLA system outside brain;
- Microglia works with CD4+ TH1 cells; can command astrocytes and endothelial cells.
- Astrocytes can store toxins
- Ly B works with CD4+TH2 cells;
- Any cell works with CD8+T cells
- Fibroblasts can produce cytokines and stem cells.

Distant intervention

By locus coeruleus
And hippocampus
The cellular effects of hypoxia or iCDO2

EAA, Free radicals and cytokins

- In about 75% of cases, first trigger is iCDO2 giving rise to excitotoxycity and inflammatory cascade;
- In about 25% of cases, first trigger is inflammation cascade;
- The importance of iCDG per se is qualitatively well known, but its confounded and proportional place can’t be quantified.
Cell temperature, brain perfusion, function, integrity and metabolism

**HYPOTHERMIA AND CMRO₂**

- **37°C**
  - Function = 3.3 ml • 100 g⁻¹ • min⁻¹
  - Integrity = 2.2 ml • 100 g⁻¹ • min⁻¹
  - Q10 = 2.4

- **27°C**
  - Function = 1.4 ml • 100 g⁻¹ • min⁻¹
  - Integrity = 0.9 ml • 100 g⁻¹ • min⁻¹
  - Q10 = 5.8

- **17°C**
  - Function = 0 ml • 100 g⁻¹ • min⁻¹
  - Integrity = 0.4 ml • 100 g⁻¹ • min⁻¹

Fig. 2-5. Theoretical interaction of temperature, brain function, CMRO₂, and calculated Q10 values. In reducing the temperature from 37 to 27°C, function is maintained, and both of the energy-consuming processes (i.e., function and integrity) are presumed to be affected equally with a slightly more than 50 percent reduction in CMRO₂, thus generating a Q10 value of about 2.4. With a further 10°C reduction in temperature to 17°C, function is abolished, resulting in a step decrease in CMRO₂ such that the calculated Q10 value is 5.0 or greater. At this point the total oxygen consumed by the brain is reduced to less than 8 percent of the normothermic value.

**Anesthesia and the Brain**

Fig. 1-14. Thresholds for changes in cerebral electrical activity. The shaded area at the base of the triangle represents a deficiency in neuroelectric monitoring capabilities, that is, that exists between the last measurable electrical change and the development of irreversible tissue damage. During arterial hypoxia, brain oxygen extraction, CBF, and CPP are progressively reduced as the impedance increase as a normal physiologic response to oxygen deprivation. Oxygen extraction decreases when CBF and CPP are reduced. (From Shapiro, with permission.)
neuroprotection: why hypothermy will not be possible in premature infants

Values at 27 °C!
Protective factors: we are still at an experimental stage

Attention to deleterious effects of exogenous steroids and morphine

• « Good » deliveries of O2 and G < good CBF and good [concentrations]
• Good distribution of cells in network (radial cells till 60 PCA, alignment of OL at 30 PCA, of astrocytes at 34 PCA
• DHEA < hippocampus protects against glutamate;
• Oligotrophins < glial cells;
• Growth and angiogenic factors < glial and endothelial cells;
• T4
• Dopamine, endorphins, cortisol, cytC:
• Neurotrophins, neuropoietic haemopietic factors, neuropeptides, melatonin, a-MSH
• Pharmacology: IGF-1, GPE (caspase inhibitor), melatonin, interleukin inhibitor
CNS damage

- Similarity of reactions by neurons;
- more dependency of environment
- damage from inside CNS:
  --> astrocytes,
  loss of nutrition, of cytoskeletal compound, activation of GAP3 and release of toxins
  --> microglia,
  release of toxins and activation of MHCII and CR3
  --> neurons:
  loss of targeted-derived factors;
  dendritic and axons atrophy and loss of molecules transport
  --> low synthesis of growth factors and low leuellar guidance
- impairment of BBB and arrival of cytokines (lungs, digestive tract, blood cells, bone marrow)

CNS repair

- Neurotrophic factors have specific targets;
- their main roles are to prevent neuronal loss and maintenance of axons regrowth;
  1. Neurotrophins family (NGF, BDNF)
  2. Cytokins growth factors (LIF, CNTF, CT-1)
  3. Fibroblasts growth factors (FGF1 and 2); mainly for layers II and III, hippocampus.
  4. The insulin growth factors
  5. The transforming factor beta family of growth factors;
  6. Epidermal growth factor
  7. Hepatocyte GF and Immunophilins
<< Brain protection >>