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 impairement
- Biochemical properties of brain cells
- Metabolic differences between neurons and glial cells
- pH, pCO_2, BP, O_2 intervention
- Cerebral blood flow, CMRG, $CMRO_2$ 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**

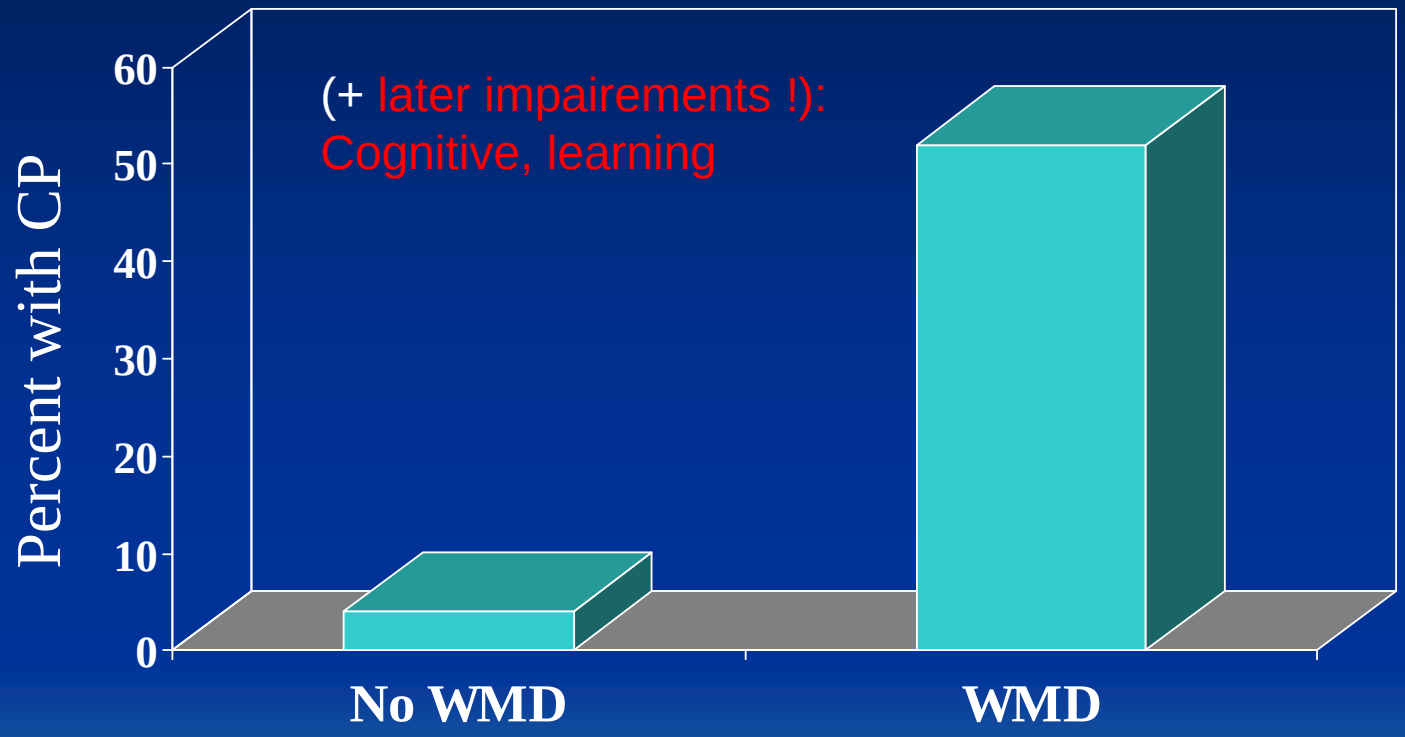
« haemorrhage and ischaemia lesions of the brain in a cohort of 474 babies under 1.5 kg: neonatal features »

From epidemiology: DISCOVERIES FROM THREE LARGE COHORT STUDIES OF PREMATURES

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



Pinto-Martin et al. Pediatrics 1995; 95:249

(Paneth) SUMMARY OF LESS CERTAIN FINDINGS

- 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

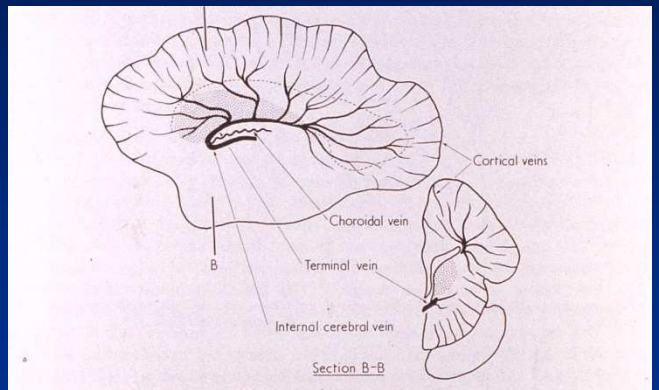
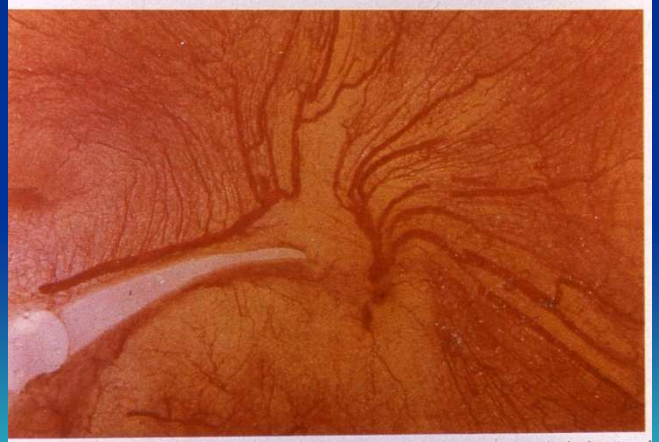


Fig. 2:23. Veins related to caudate nucleus at 30 weeks gestation. (From Hambleton and Wigglesworth 1976.)



ig. 2:24. Barium-gelatin injection of cerebrum at 36 weeks gestation, showing transcerebral veins around teral ventricle.

- Brain is 14-16 % of body weight
- Brain is 60 % of body metabolism (see glucose) and 02 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

See position

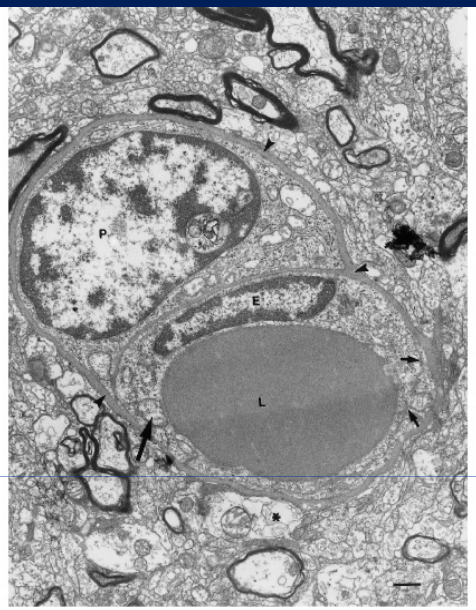


FIGURE 1.23 Human cerebral capillary obtained at biopsy. Blood–brain barrier (BBB) capillaries are characterize
paucity of transcytotic vesicles in endothelial cells (E), a high mitochondrial content (large arrow), and the formati
junctions (small arrows) between endothelial cells that restrict the transport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

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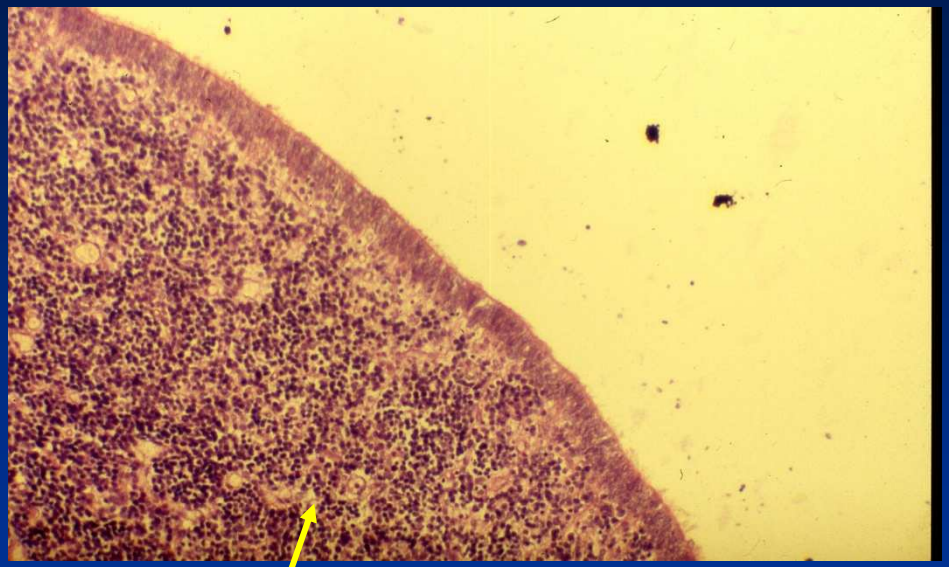
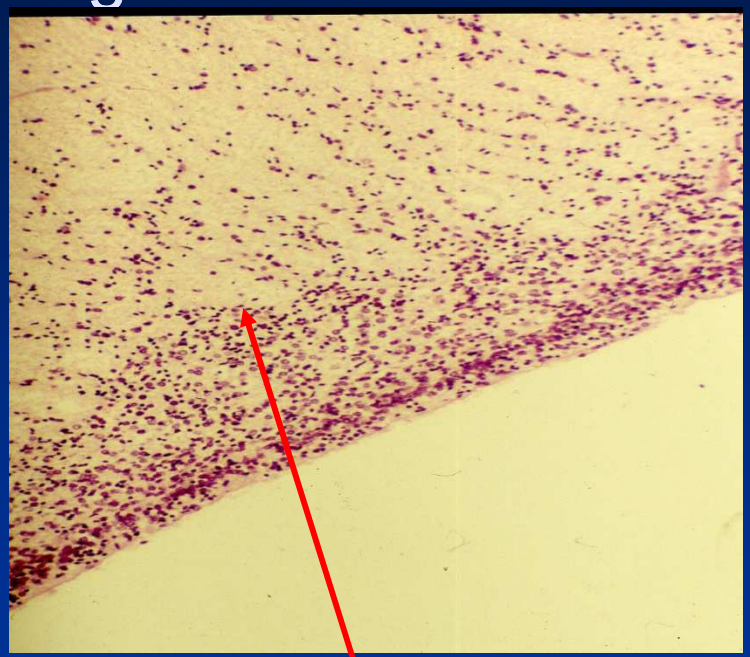
Neuronal migration, blood brain barrier, cells ' biochemistry, Cerebral blood flow autoregulation

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

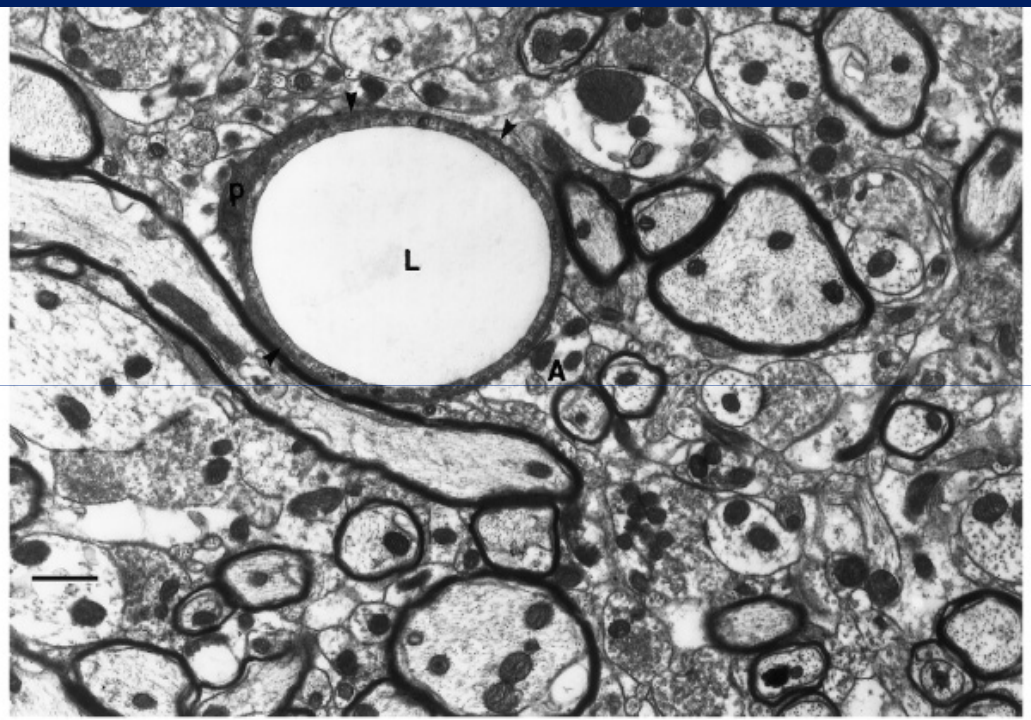


FIGURE 1.22 Electron micrograph of a blood–brain barrier (BBB) capillary. Endothelial cells joined by tight junctions form continuous capillaries with no fenestrations and restrict the passage of solutes between blood and brain. Pericytes (P) are present within the basement membrane (arrowheads) of these 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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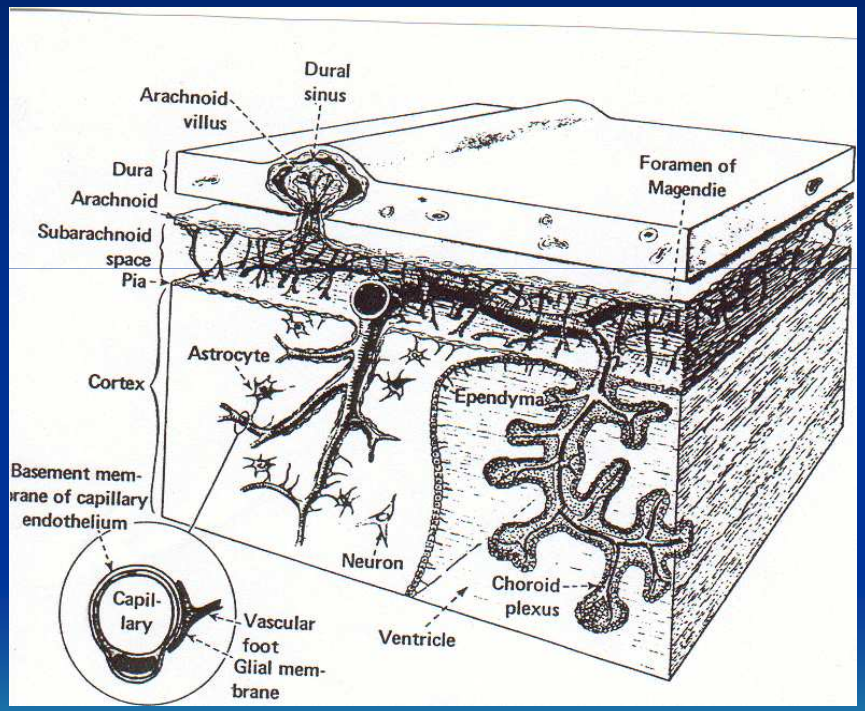
The blood-brain barrier



- Brain cells: neurons, astrocytes, microcytes,radial cells, oligodendrocytes;
- Microcirculation;
- Ependyma and villi;
- Arachnoids;

The cortex microcirculation: observe the differences bewteen arteries and veins

FIGURE 1.21 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 IVB and IVCb). (B) Prefrontal cortex (area 9). Cortical layers are indicated by Roman numerals. The microvessels are stained using an antibody against heparan sulfate proteoglycan core protein, a component of the extracellular matrix.

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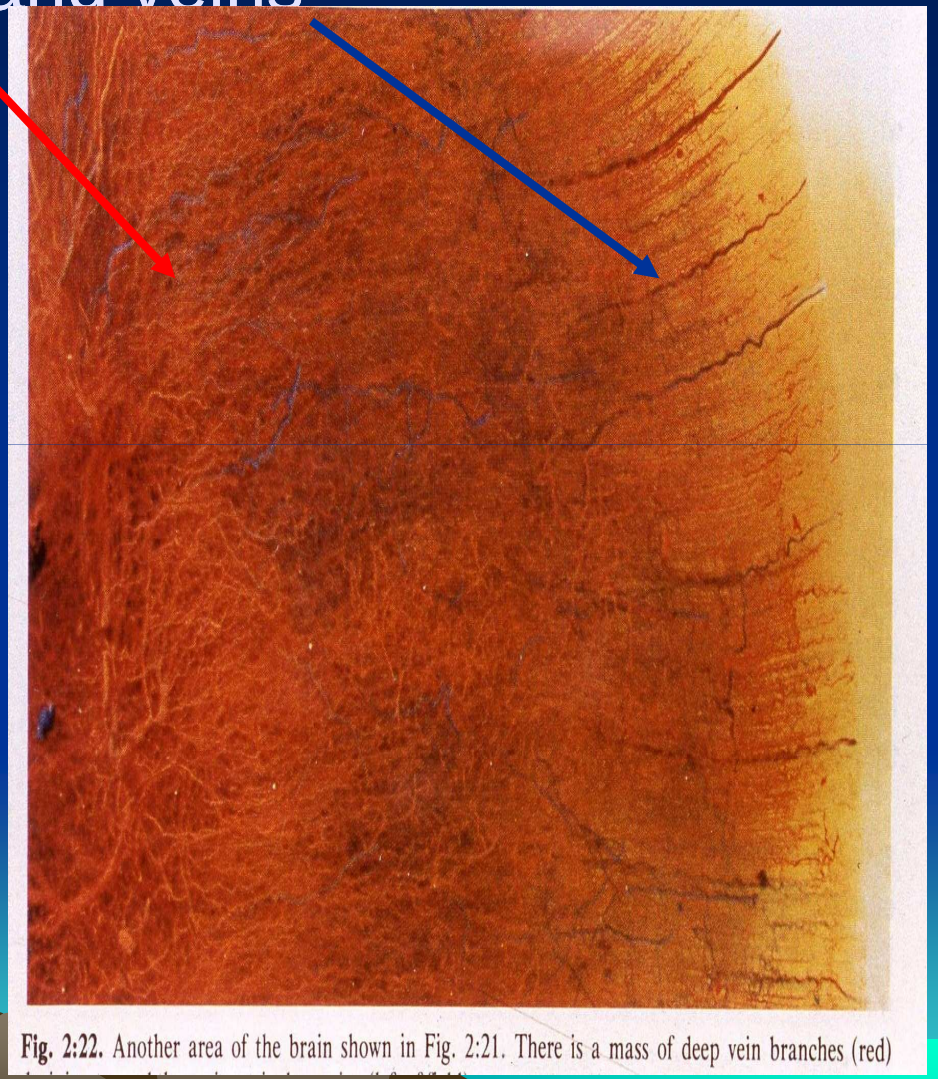


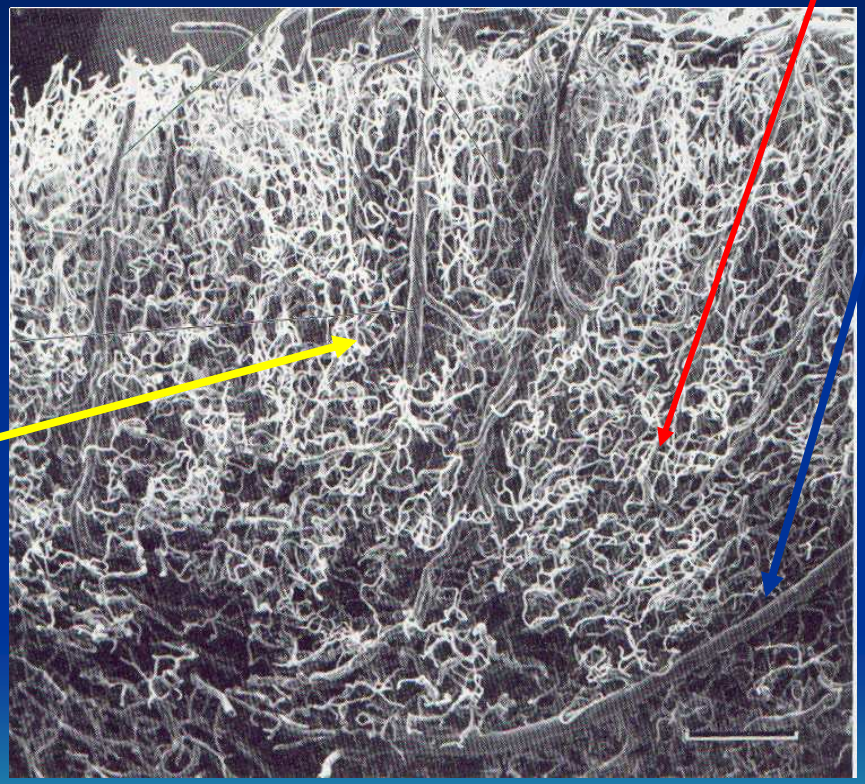
Fig. 2:22. Another area of the brain shown in Fig. 2:21. There is a mass of deep vein branches (red)

Fetal and neonatal Brain development: histology and biochemistry

- Radial cells;
- oligodendrocytes
- astrocytes
- microglia

40 % glial cells , 4 % neurons, 4 % blood, 10 % CSF, 35 % ECF, 10 % variance

Microcirculation: observe differences between A and V



Disturbances of late neuronal migration:

axonal retraction, pericapillaries congruence, rosettes, ependymal dysruption,

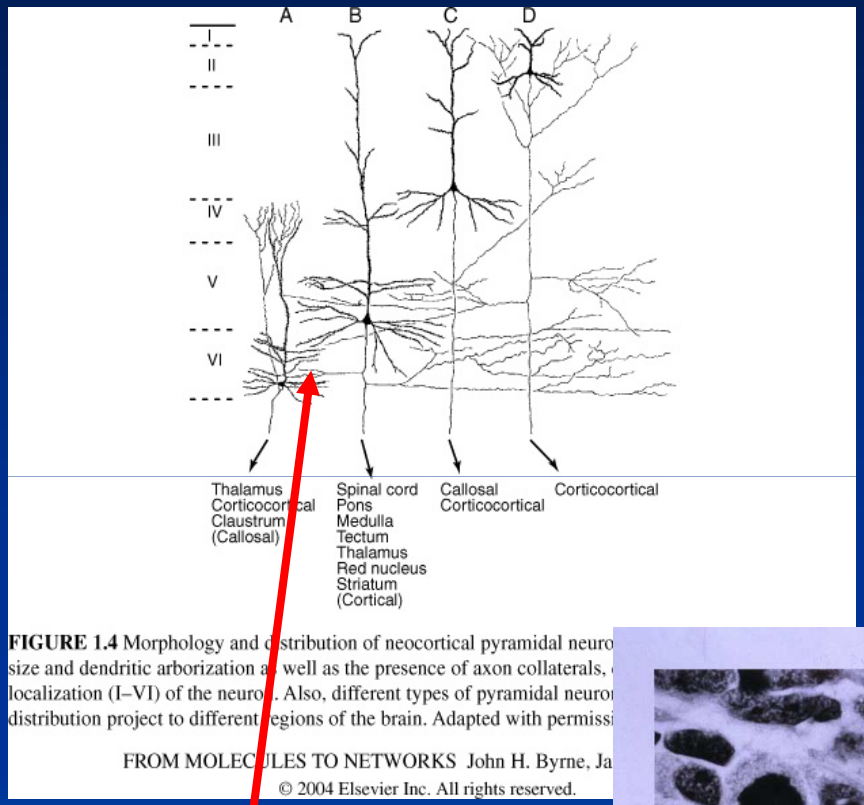


FIGURE 1.4 Morphology and distribution of neocortical pyramidal neuro
size and dendritic arborization as well as the presence of axon collaterals,
localization (I–VI) of the neuro. Also, different types of pyramidal neuro
distribution project to different regions of the brain. Adapted with permiss

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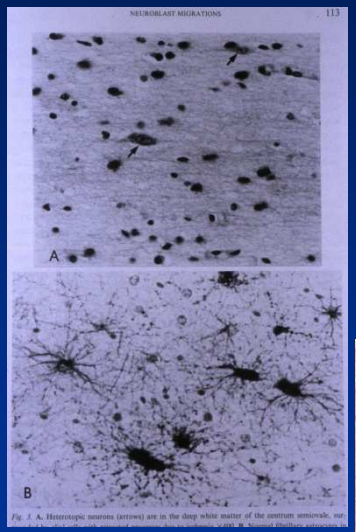

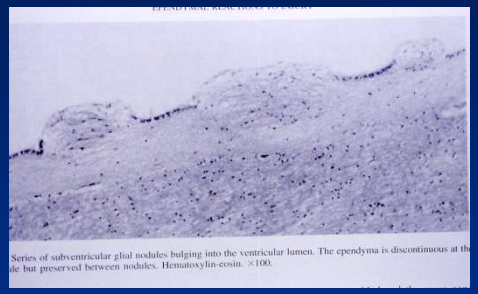

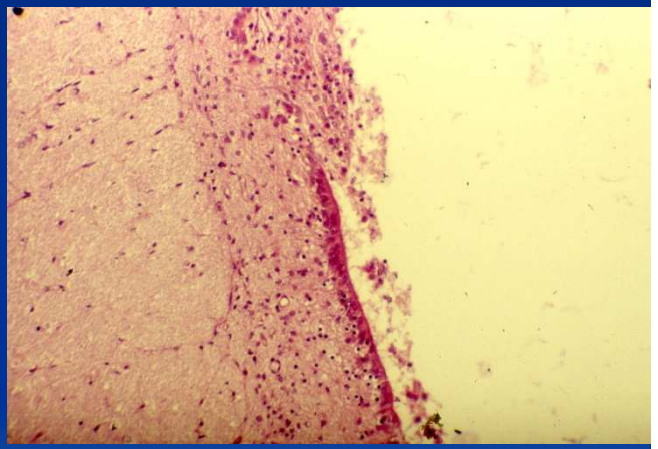

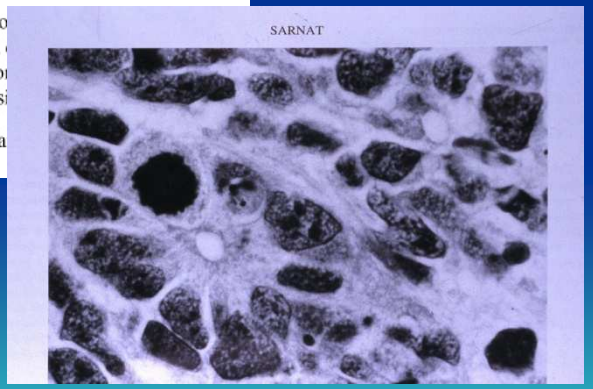

And also of volume and Myelination

Excitotoxicity: apoptosis and necrosis; neuroplasticity

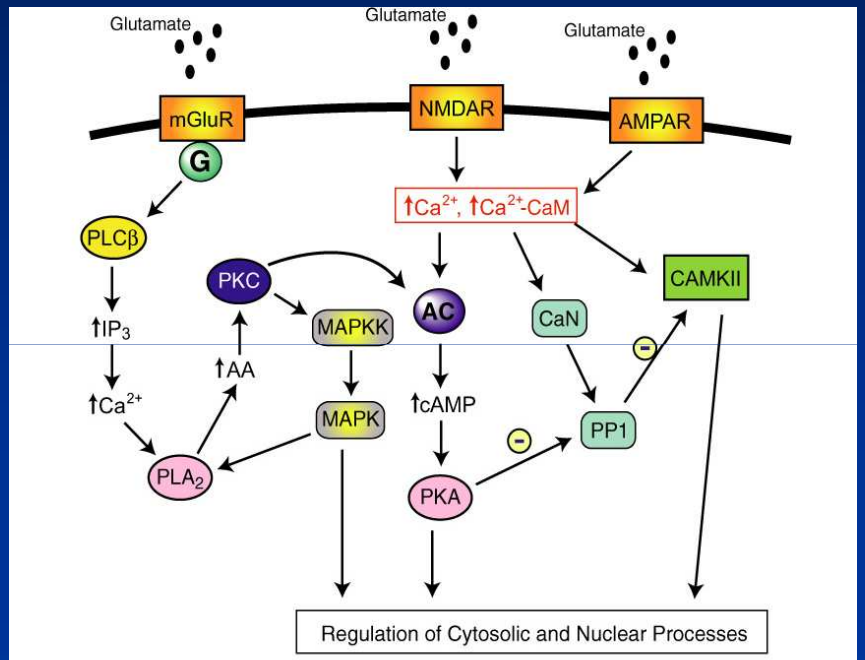

FIGURE 14.5 Aspects of a model that relates glutamate exposure at hippocampal synapses to long-term synaptic strengthening. Glutamate can act through metabotropic glutamate receptors (mGluR) to activate G proteins (G). Glutamate also acts through NMDA and AMPA receptors to increase intracellular levels of free Ca^{2+} and Ca^{2+} bound to calmodulin. These events lead to activation of phospholipase C (PLC), CAMKII, calcineurin (CaN), adenylyl 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 relieves 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 MAPKK, 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.

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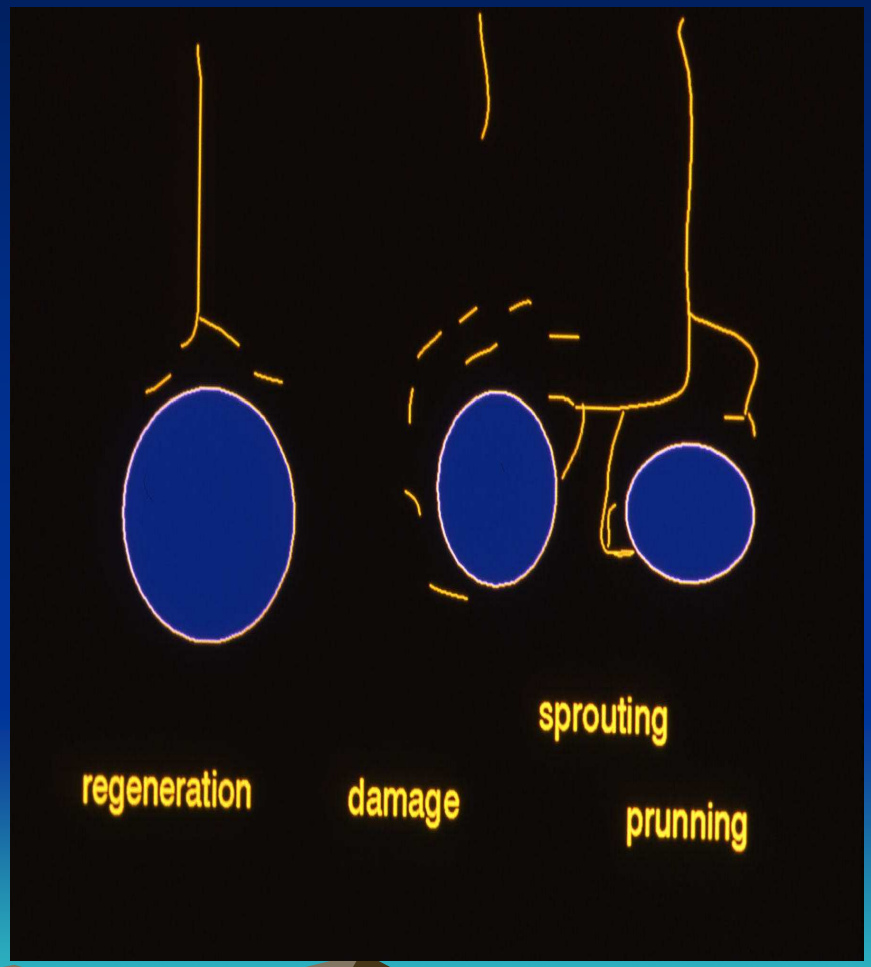


When brain blood flow is in low range and even « dependent »

CBF,CBV,CMRO2 and CMRG

In sick NN

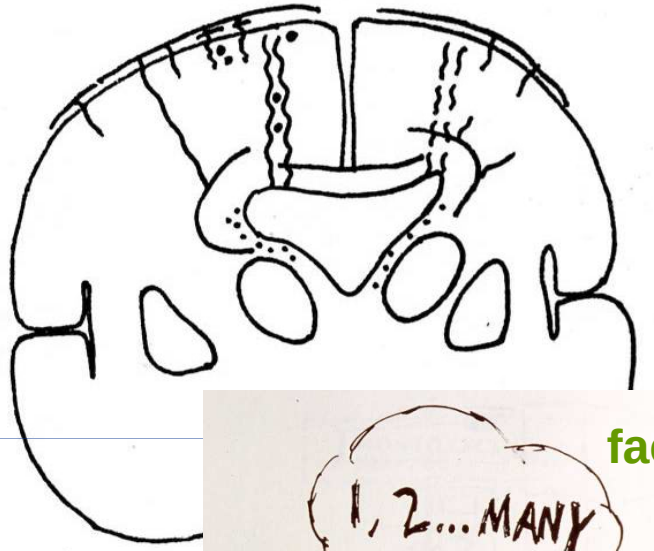
CBF	[G]a	% w	[O2]a	% w	CBV
5	137	30	30	2	2.4
10	99	40	28	5	2.7
15	53	7	26	16	3
20	34	12	24	25	3.3
30	31	22	22	50	4
40	25	32	18	75	4.6

Sources: Volpe, Wyatt, Michenfelder, Greisen and Battisti

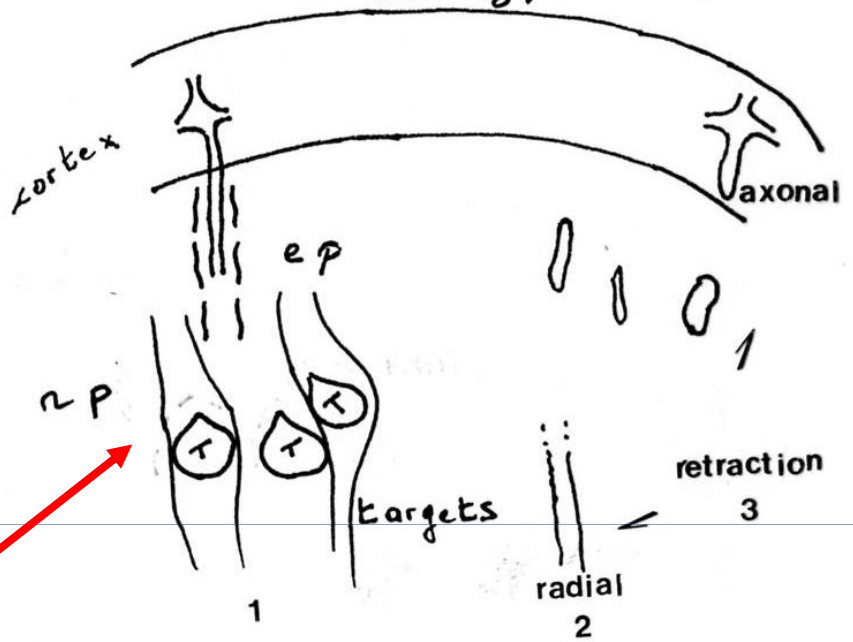
f
r

BRAIN'S DEVELOPMENT

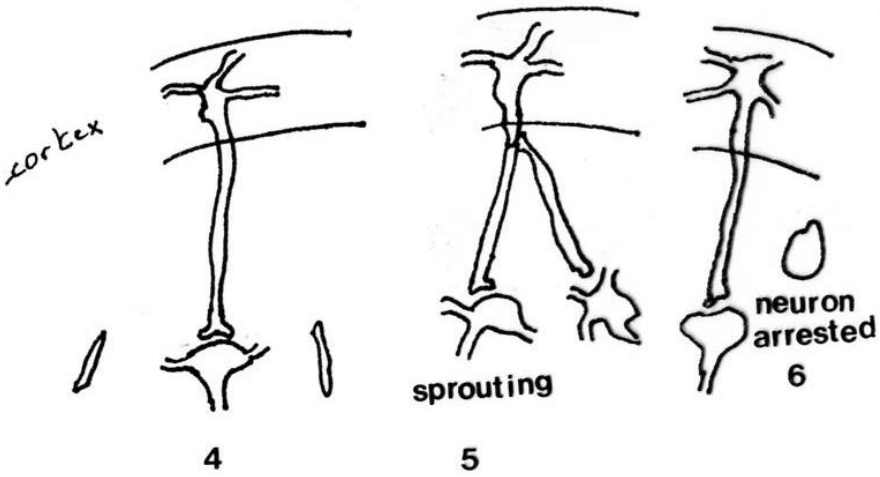
- anatomy -



- histology -



factors



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 ofLow 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

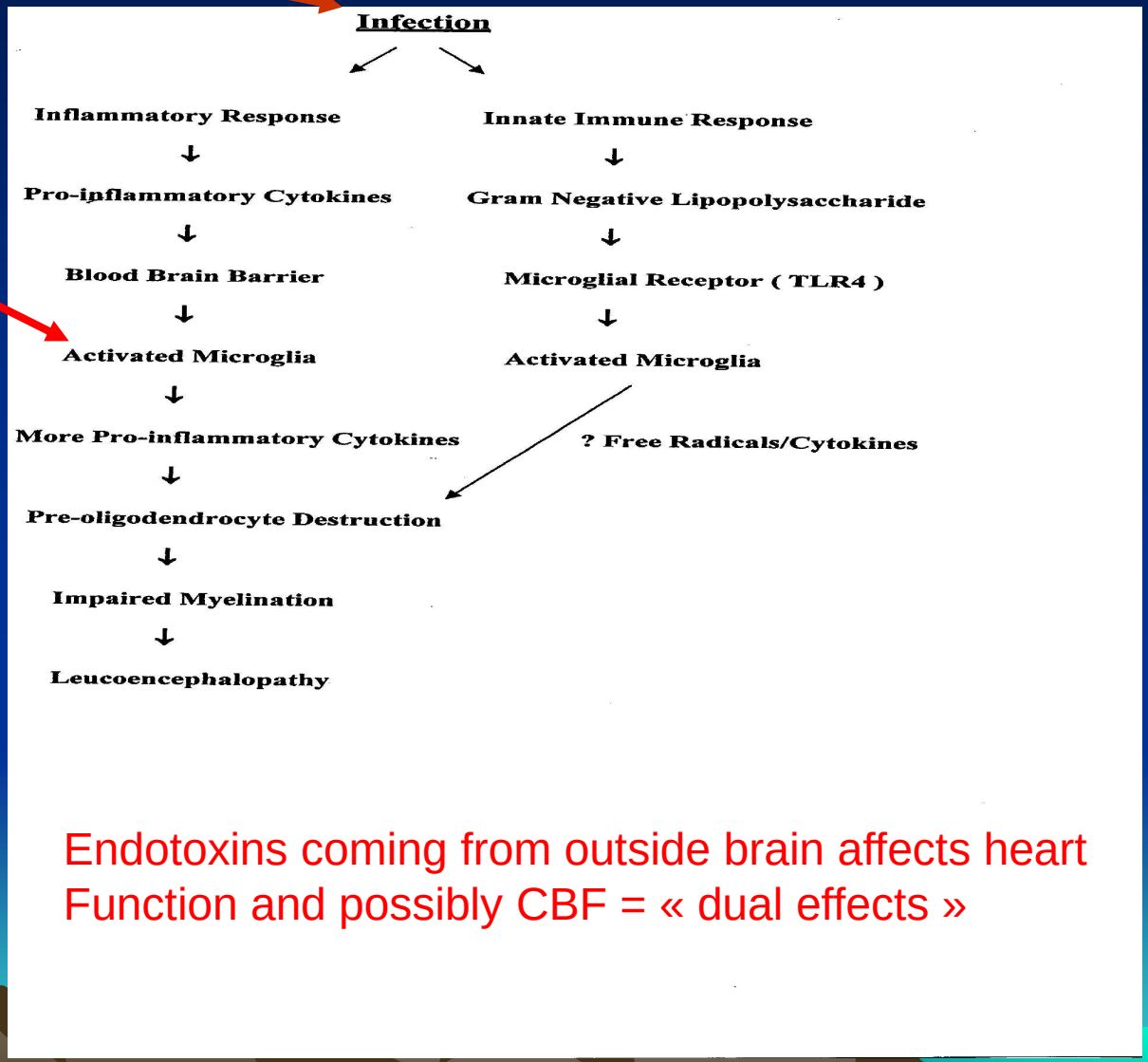
Category	Molecule	Oligotrophic Functions			Neurotrophic Functions
		Promotion of Oligodendrocyte Differentiation	Promotion of Oligodendrocyte or -Precursor Survival	Protection of Oligodendrocyte or Promotion of Remyelination	Promotion of Neuronal Survival or Protection
Hormones	Corticosteroids	42, 121	121	122	123-125
	Thyroid Hormones	42, 43, 126	127	128, 129	44-46
Neurotrophins	BDNF		37		115, 130-134
	NGF		35 *killer: 135*		136-140
	NT-3		35, 37, 59		132, 133, 141
IL-6 family	IL-6		37, 105		
	LIF	58 *astrogenic: 105*	37, 58		142
	CNTF	58 *astrogenic: 105*	37, 58	58, 102, 103	143-145
Angiogenic cytokines	VEGF			*Expressed by glial cells during hypoxia: 146-148*	*Expressed by neurons during hypoxia: 146*
	bFGF	*Inhibitor: 64, 67*	149-151	152, 153	136, 154-157
Other cytokines and growth factors	EGF	*Inhibitor: 158*		159	155-157, 160-162
	IGFs	25, 55, 163, 164	37, 66, 165	25, 166, 167	168-172
	PDGF		66, 150	173	132, 174, 175
	TGFβ	176		177	178-182
	GGF/Neuregulin		183		
	IL-2	184-187			

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

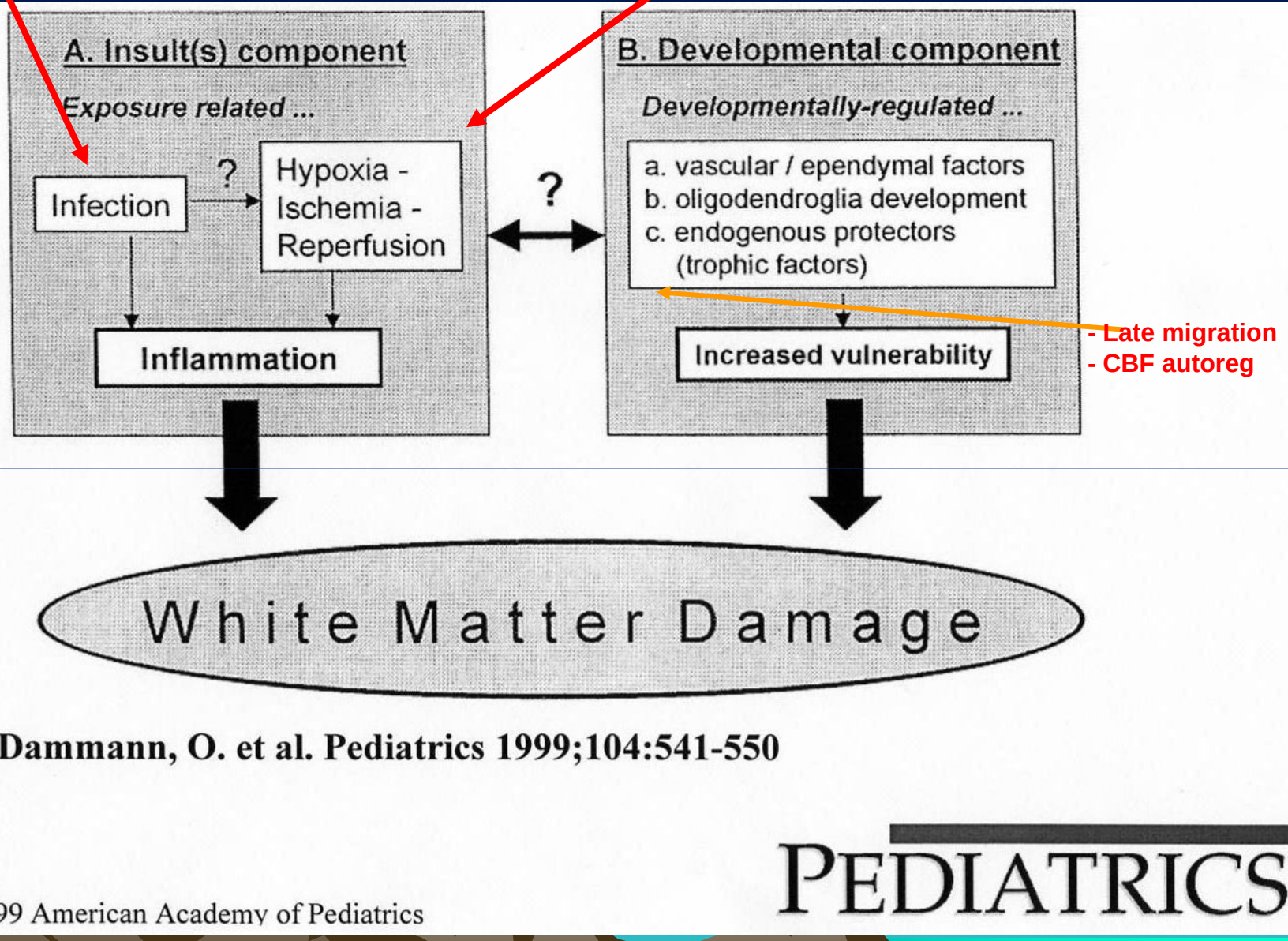
From inside brain



Endotoxins coming from outside brain affects heart Function and possibly CBF = « dual effects »

This is in about 15- 25 % of cases

This is about in 70- 80 % of cases



Dammann, O. et al. Pediatrics 1999;104:541-550

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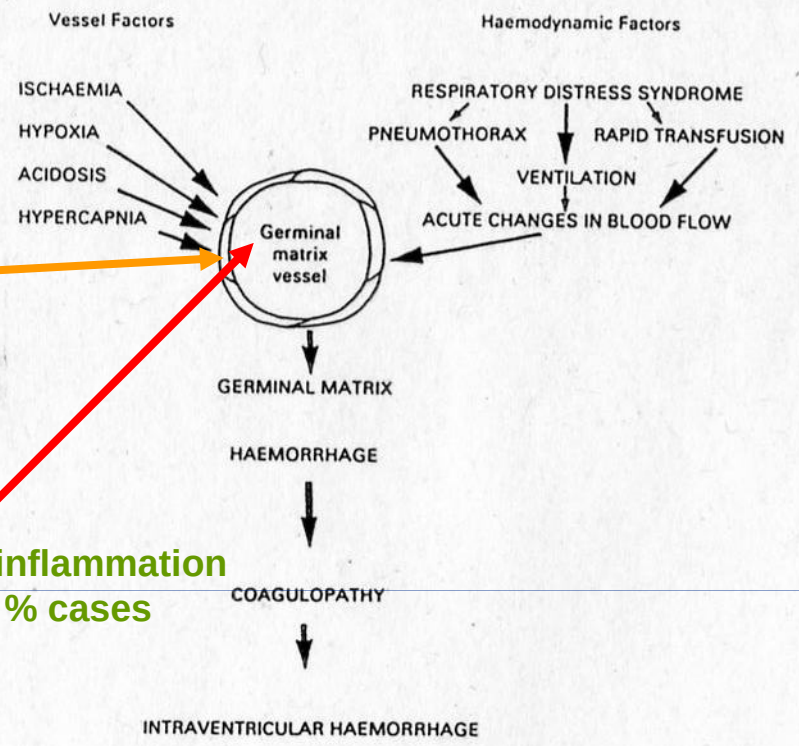

Fig. 28.4 Model for the development of GMH and IVH which is dependent on both vessel and haemodynamic factors.

iCDG

Extrinsic inflammation
In 25 – 33 % cases

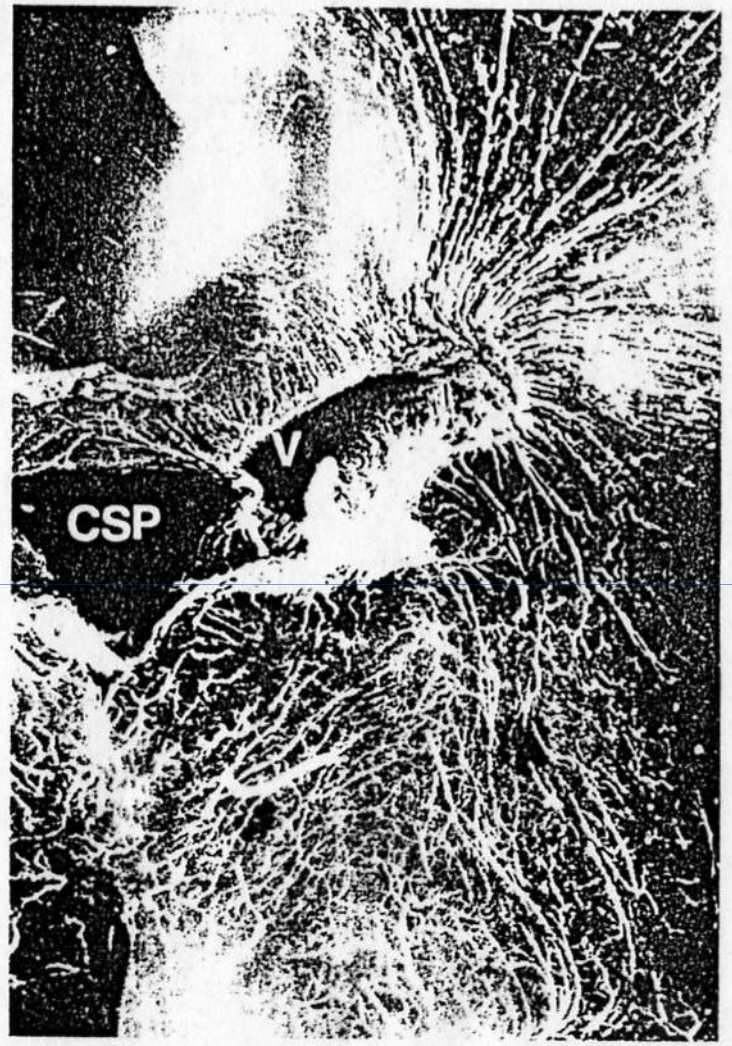

Fig. 28.5 Microvenography showing the fan-shaped leash of veins in the deep periventricular white matter of a 28-weeks-gestation neonate. V = ventricle, CSP = cavum septi pellucidi. (Reproduced with permission from Takashima et al 1986.)

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 oxydative 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, antipohospolipid antibody

Cytokines liberation and storage

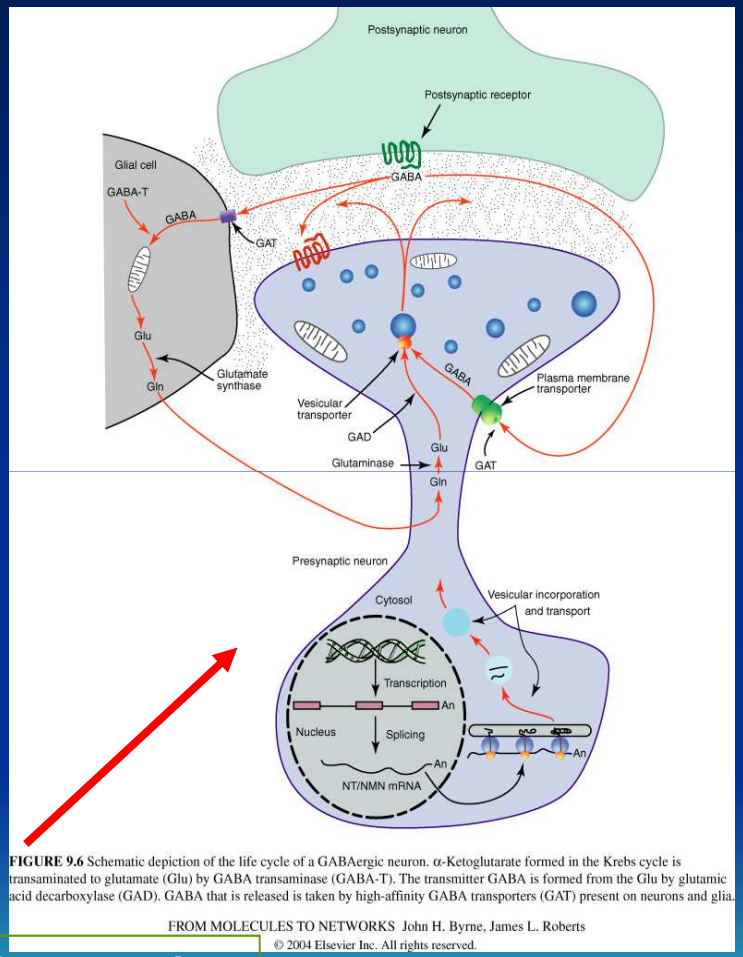

FIGURE 9.6 Schematic depiction of the life cycle of a GABAergic neuron. α-Ketoglutarate formed in the Krebs cycle is transaminated to glutamate (Glu) by GABA transaminase (GABA-T). The transmitter GABA is formed from the Glu by glutamic acid decarboxylase (GAD). GABA that is released is taken by high-affinity GABA transporters (GAT) present on neurons and glia.

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- **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

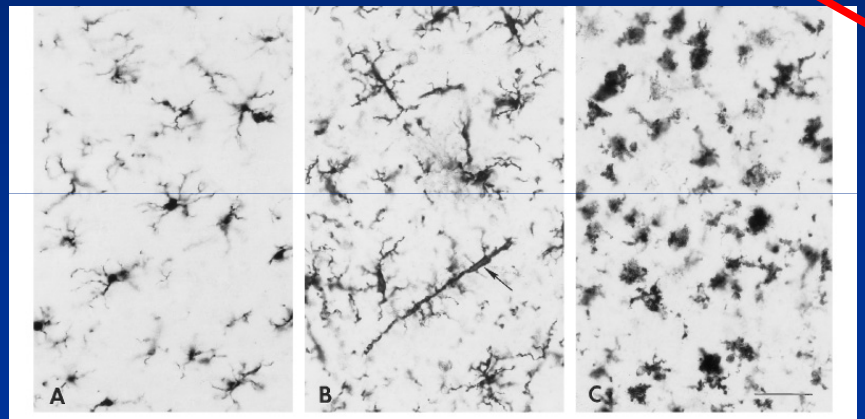

FIGURE 1.20 Activation of microglial cells in a tissue section from human brain. Resting microglia in normal brain (A). Activated microglia in diseased cerebral cortex (B) have thicker processes and larger cell bodies. In regions of frank pathology (C) microglia transform into phagocytic macrophages, which can also develop from circulating monocytes that enter the brain. Arrow in B indicates rod cell. Sections stained with antibody to ferritin. Bar = 40 μm.

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- In about 75 % of cases, first trigger is iCDO2 giving rise to **excitotoxycity and ifnflammatory 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

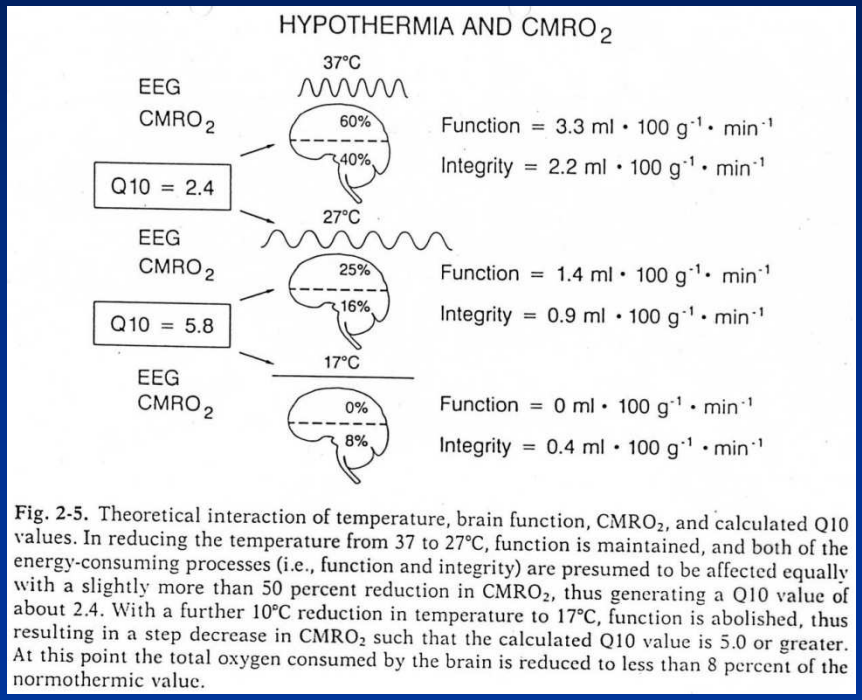

Fig. 2-5. Theoretical interaction of temperature, brain function, $CMRO_2$, and calculated Q10 values. In reducing the temperature from 37 to 27°C, function is maintained, and both of the energy-consuming processes (i.e., function and integrity) are presumed to be affected equally with a slightly more than 50 percent reduction in $CMRO_2$, thus generating a Q10 value of about 2.4. With a further 10°C reduction in temperature to 17°C, function is abolished, thus resulting in a step decrease in $CMRO_2$ 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.

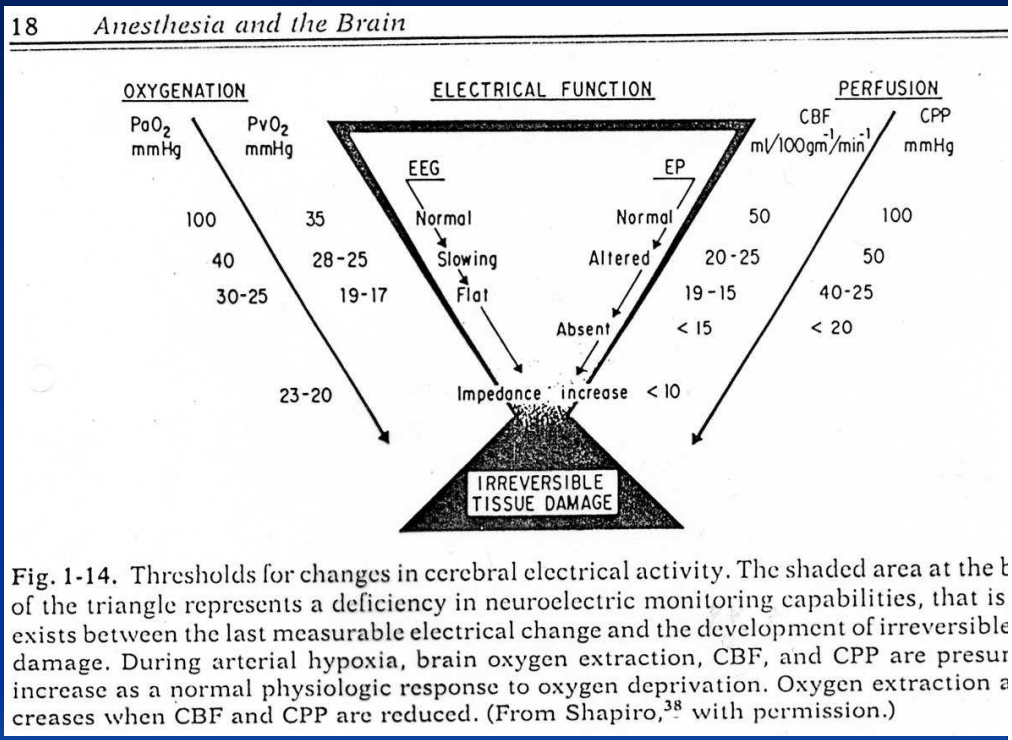

Fig. 1-14. Thresholds for changes in cerebral electrical activity. The shaded area at the b of the triangle represents a deficiency in neuroelectric monitoring capabilities, that is exists between the last measurable electrical change and the development of irreversible damage. During arterial hypoxia, brain oxygen extraction, CBF, and CPP are presur increase as a normal physiologic response to oxygen deprivation. Oxygen extraction a creases when CBF and CPP are reduced. (From Shapiro,[38] with permission.)

neuroprotection: why hypothermy will not be possible in premature infants

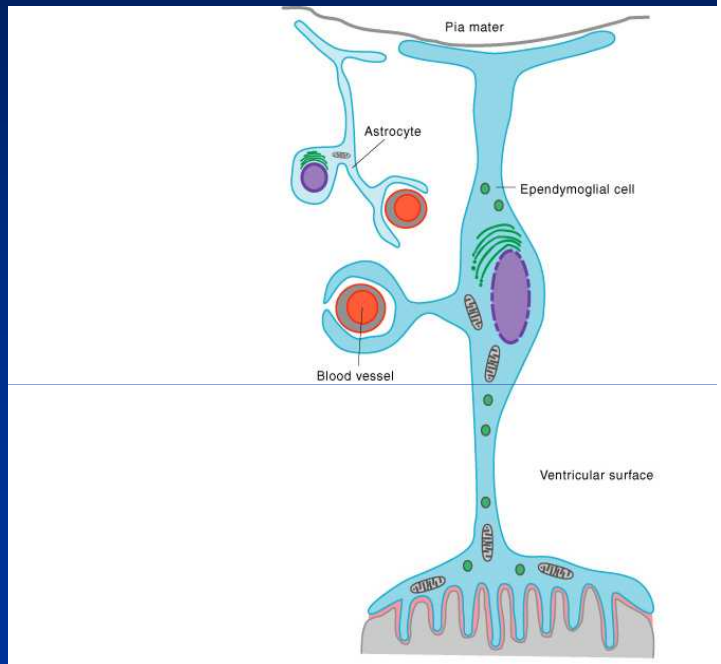

FIGURE 15.8 Ependymoglial cell. The ependyma is a single-layered, ciliated epithelium that covers the surfac brain and central canal of the spinal cord. Ependymoglial cells are polarized, having luminal, lateral, and basal s ependymal cells cover the choroid plexus and the circumventricular organs. In certain regions of the CNS, a sub ependymal cells maintain basal processes of varying lengths. When these basal processes establish direct contac ependymoglial cells resemble in form the radial glia of the developing CNS. The pial end feet of these cells tog astroglial cells participate in the formation of the blood–brain barrier. The ependymoglial basal processes, in passing through neural tissue, usually extend appendages or side-arms that may end in contact with neuronal cell bodies, dendritic, axonal, and synaptic processes, and the walls of the blood vessels.

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	CBF	CMRO	CMRG
	80		
	60	4.8	6.5
Metabolic Zone :		GRAY MATTER	
	40	3.2	4.5
	20	1.6	2.2
EEG		WHITE MATTER	
E		0.8	1.1
K+			
Ca++	0		

Brain's scale.

Values at 27 °C !

Protective factors: we are still at an experimental stage

Attention to deleterious effects of exogenous steroids and morphine

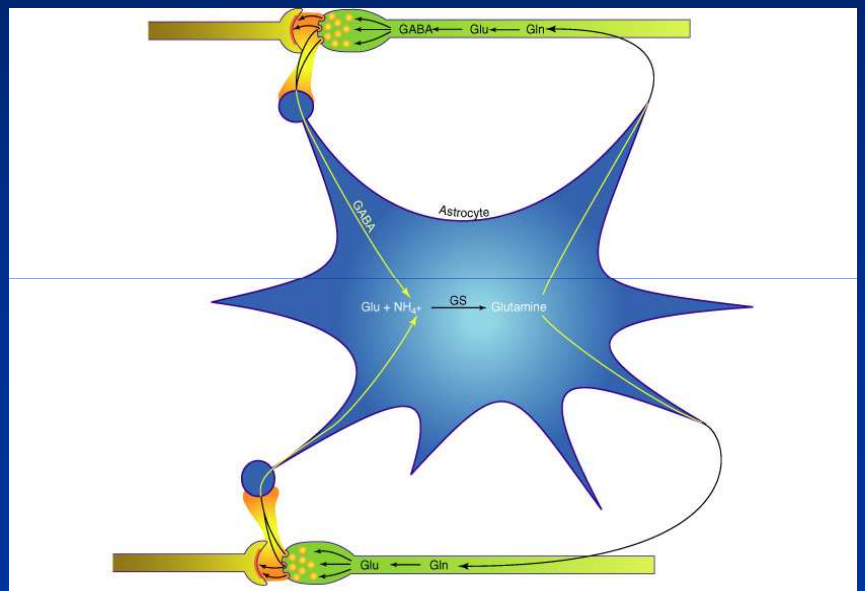

FIGURE 1.19 The glutamate–glutamine cycle is an example of a complex mechanism that involves an active coupling of neurotransmitter metabolism between neurons and astrocytes. The systems of exchange of glutamine, glutamate, GABA, and ammonia between neurons and astrocytes are highly integrated. The postulated detoxification of ammonia and inactivation of glutamate and GABA by astrocytes are consistent with the exclusive localization of glutamine synthetase in the astroglial compartment. Gln, glutamine.

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- « Good » deliveries of O2 and G < good CBF and good [concentrations]
- Good distribution of cells in network (radial cells till 60 PCA, alignement 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, melatonine, a-MSH
- Pharmacology: IGF-1, GPE (caspase inhibitor), melatonine, 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 targed-derived factors;

dendritic and axons atrophy and loss of molecules transport

--> low synthesis of growth factors and low leullar guidance

- **impairement of BBB** and arrival of cytokines (lungs, digestive tract, blood cells, bone marrow)
-

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. **Fibroblats 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 »