The blood-brain barrier

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September 12, 2018

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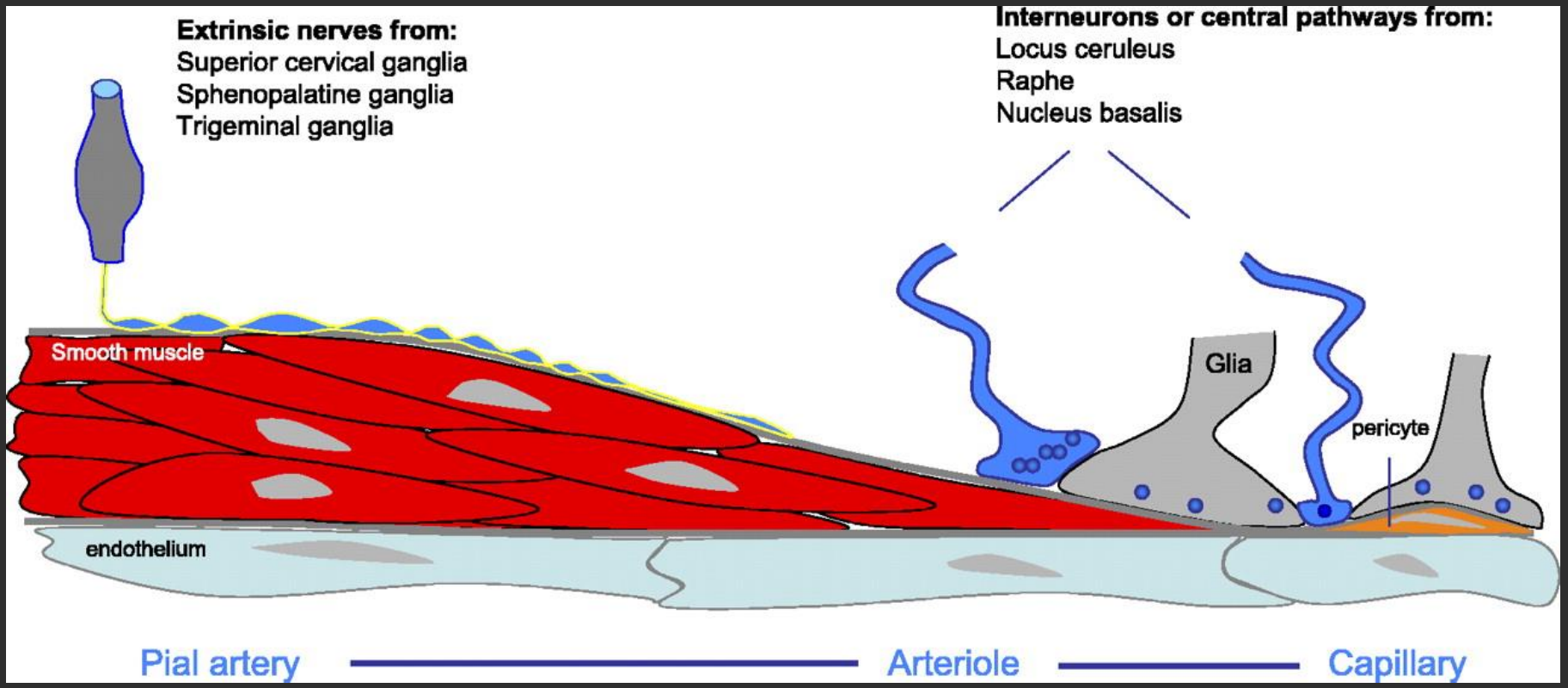
The discovery of the blood-brain barrier

- Paul Ehrlich (1854-1915): injection of anilin-dye into the circulation → every organ stained except the central nervous system

- Edwin Goldmann (1913): injection of the dye into the spinal cord → staining of the brain, but not other organs

  ⇒ **The existence of the blood-brain barrier: the isolation of the brain from the periphery**

Transition from arterioles to capillaries



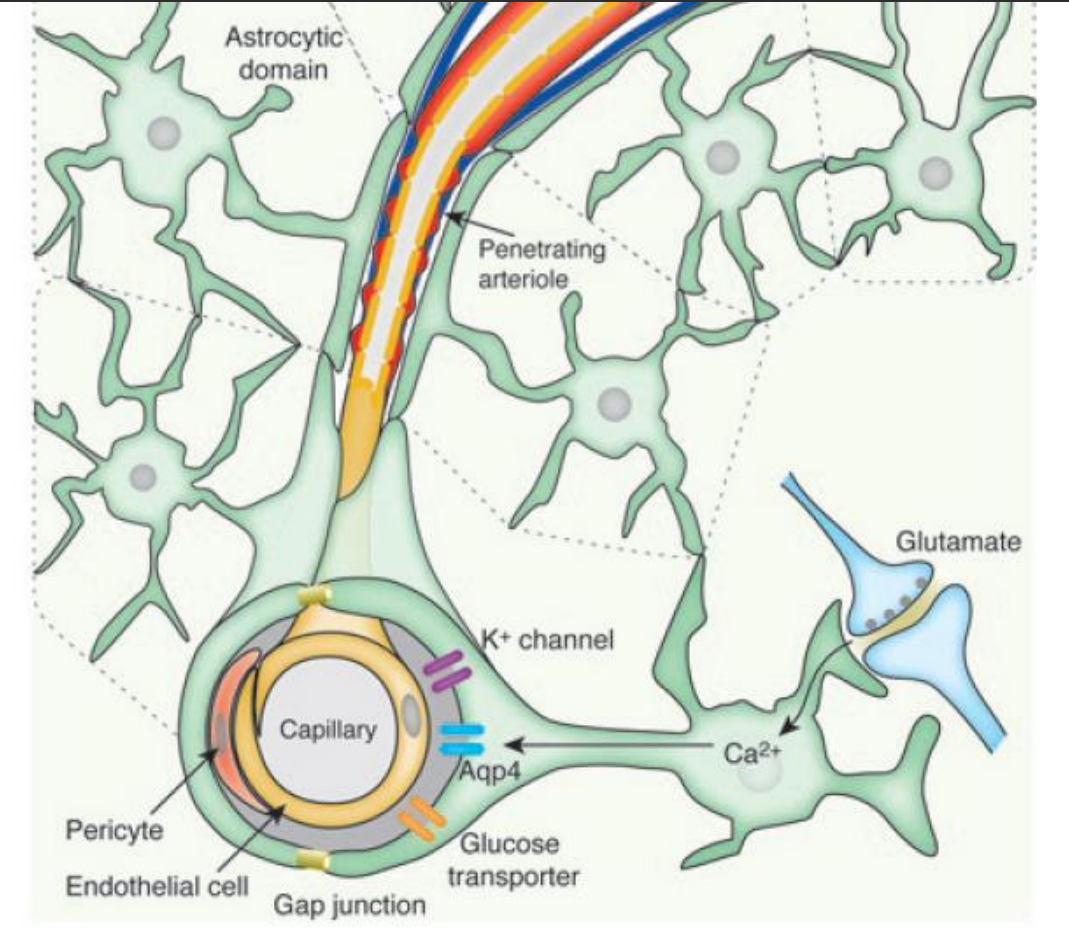
Girouard et al., J Appl Physiol, 2006

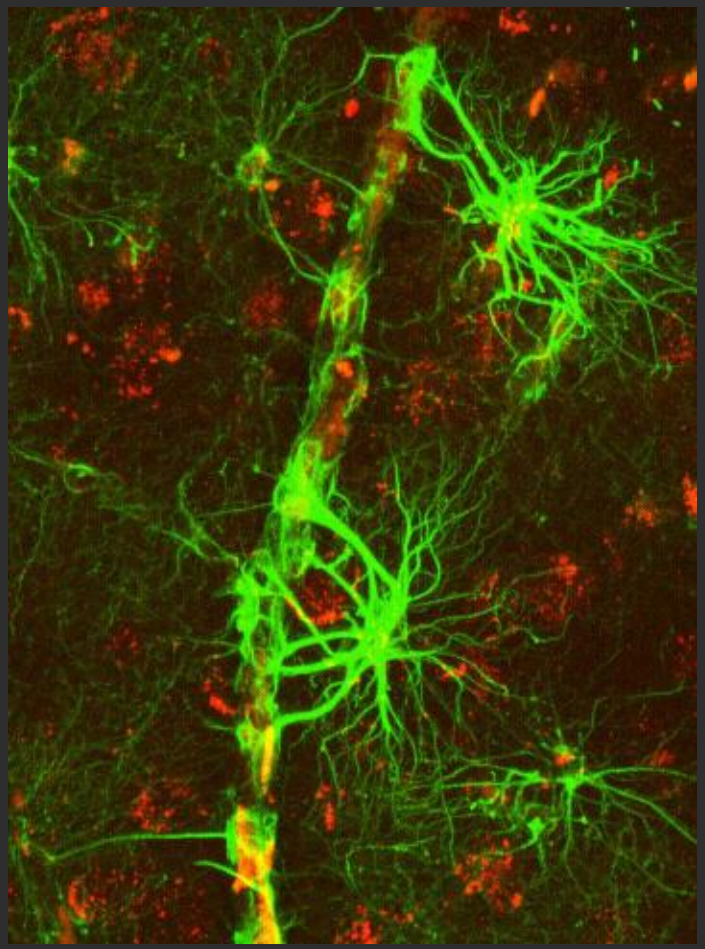
The cerebral capillary network

- Dense capillary network:
  average intercapillary distance: 40µm
- Large surface:
  endothelial layer: 100 cm^2/g
- Volume:
  the mass of endothelial cells
  constitutes 0.1% of the brain tissue

Petzold and Murphy, Neuron, 2011

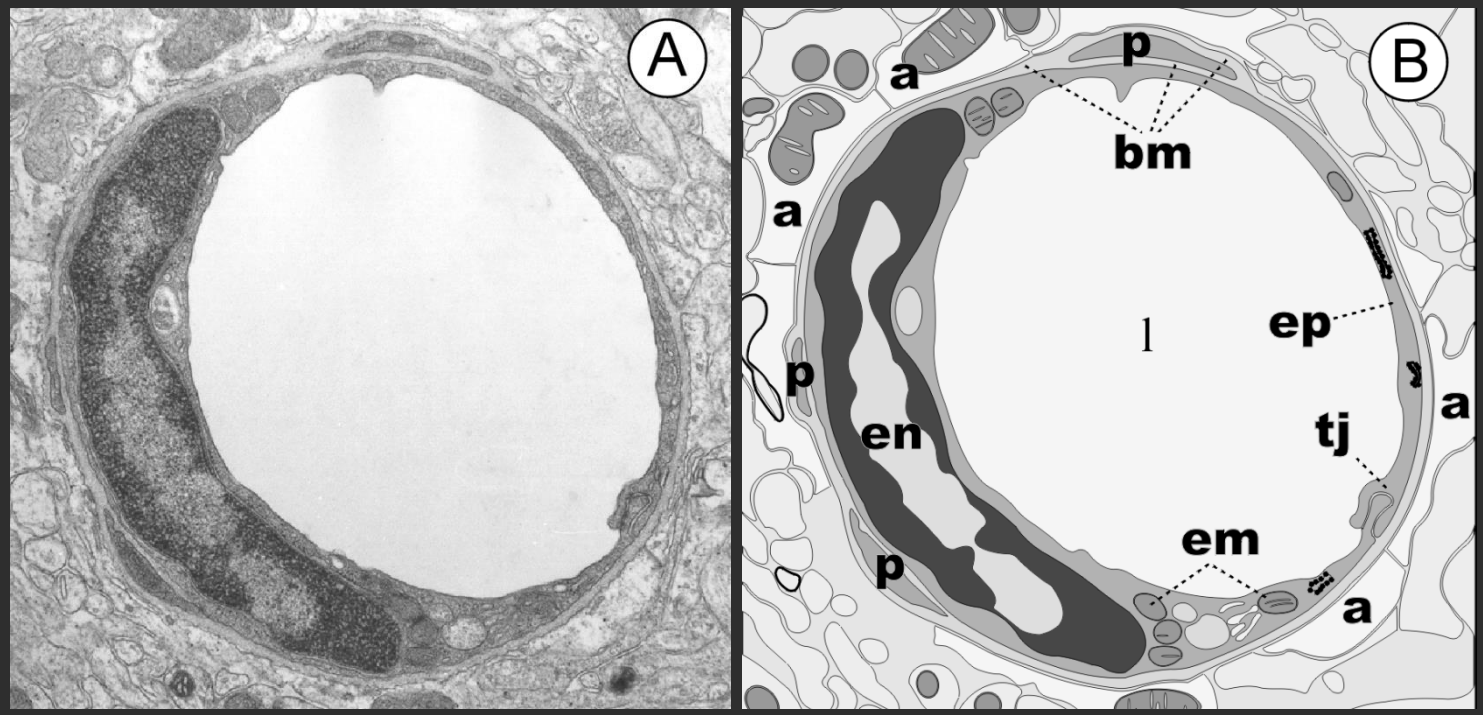
The fine organization of the cerebral capillary network





Ultrastructure of the blood-brain barrier

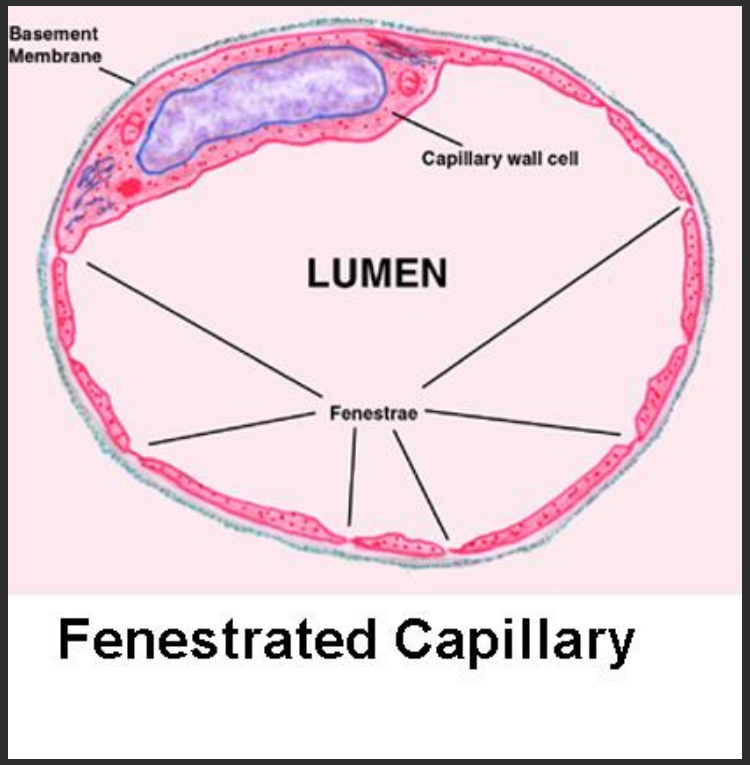
Wall thickness: 40% of that in other endothelial cell types (0.3-0.5 μm) → Shortened transport time for nutrient trafficing



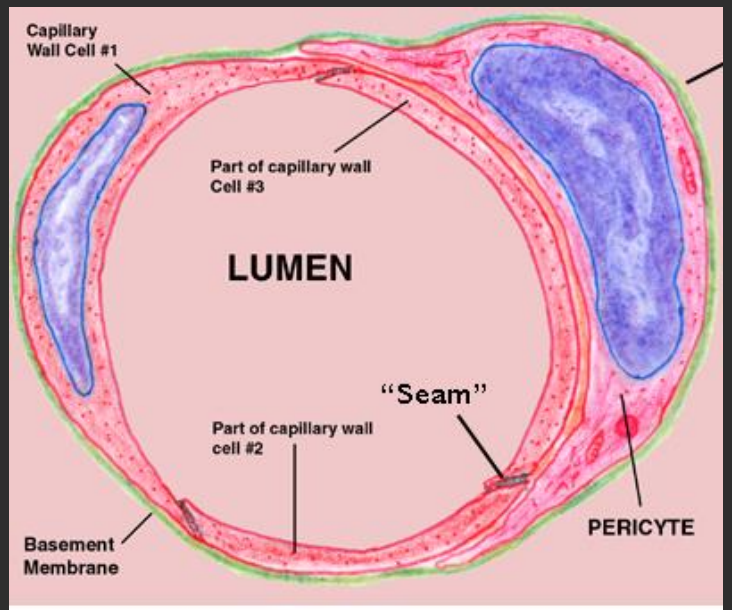
Farkas& Luiten, Progr Neurobiol, 2001

The endothelium of the blood-brain barrier

Endothelium: No fenestrations no free tanscellular diffusion



Fenestrated Capillary

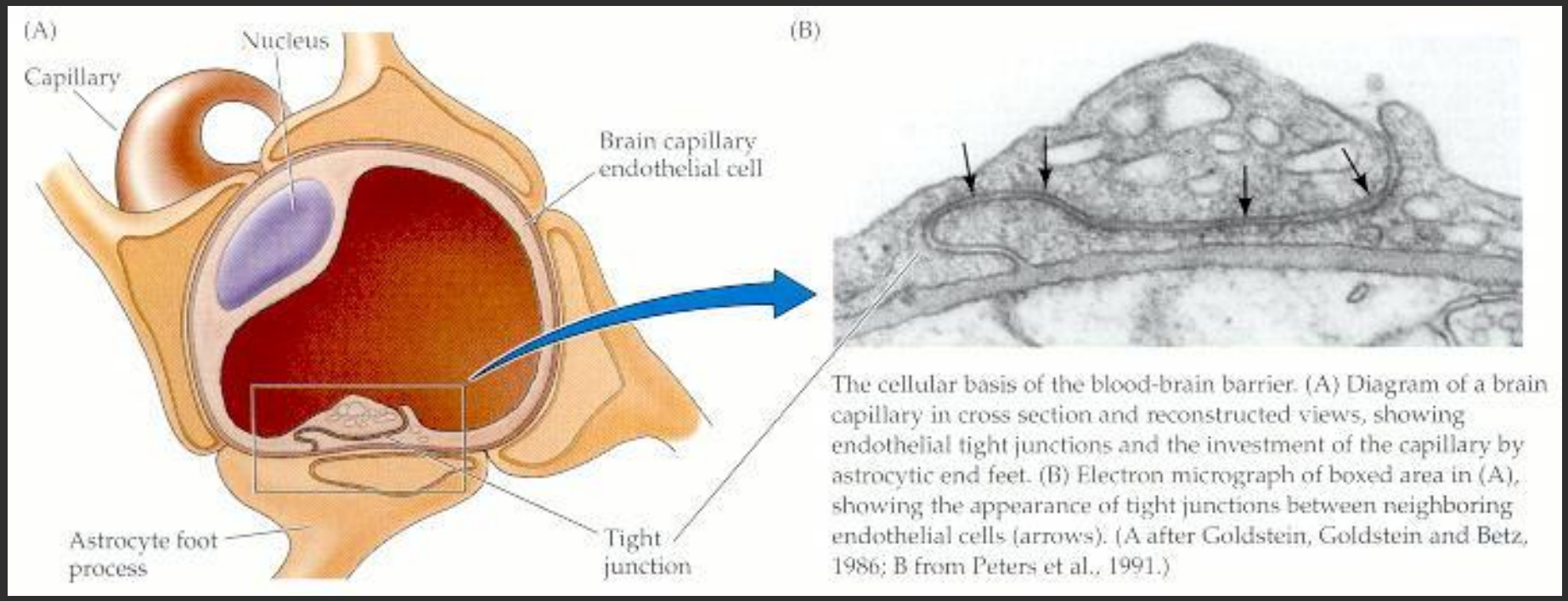


Closed or Continuous Capillary

http://www.vetmed.vt.edu/education/curriculum/vm8054/labs/Lab12b/Lab12b.htm

The endothelium of the blood-brain barrier

Endothelium: Tight junctions → no paracellular diffusion

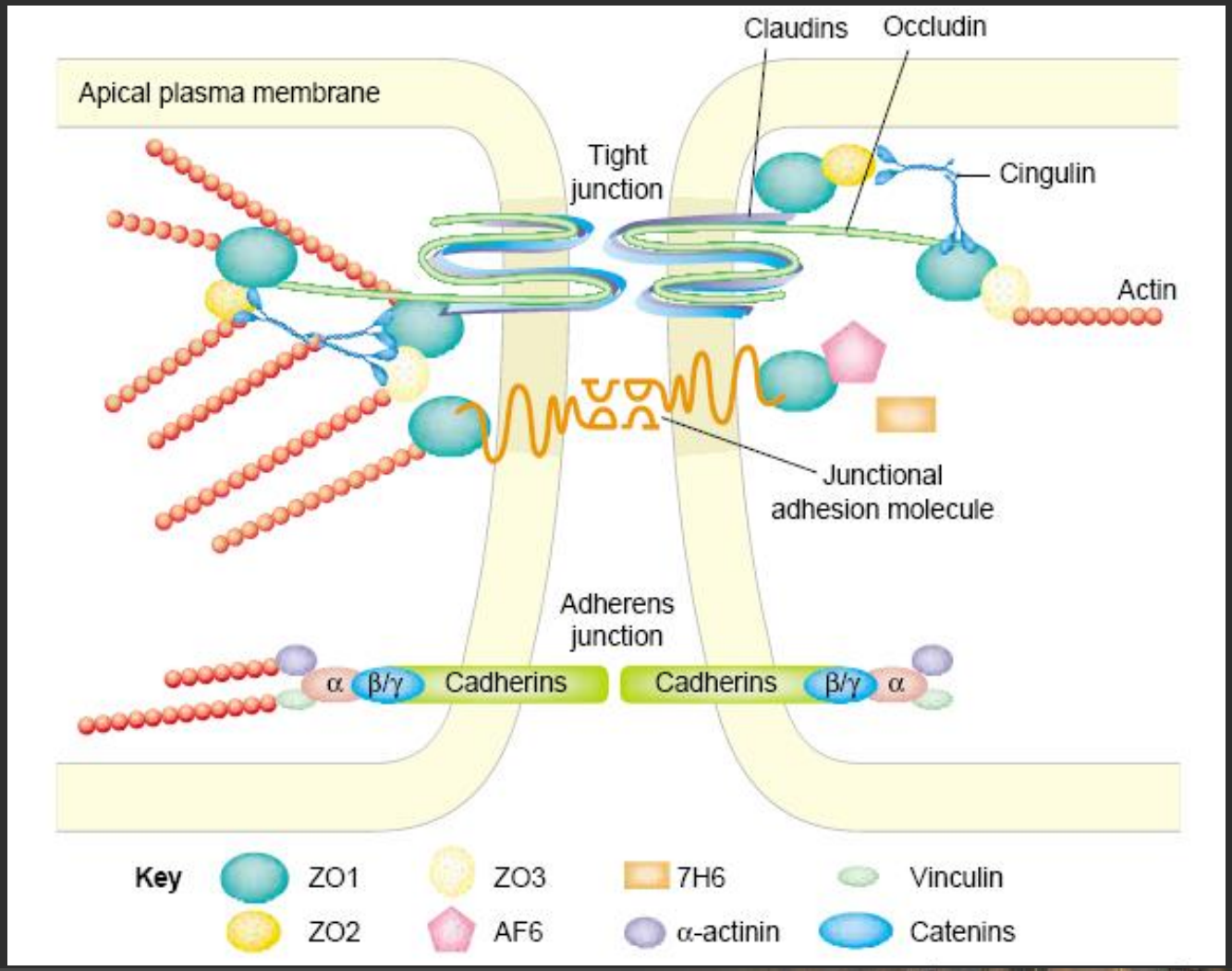


http://www.daviddarling.info/encyclopedia/B/blood-brain_barrier.html

The molecular structure of tight junctions

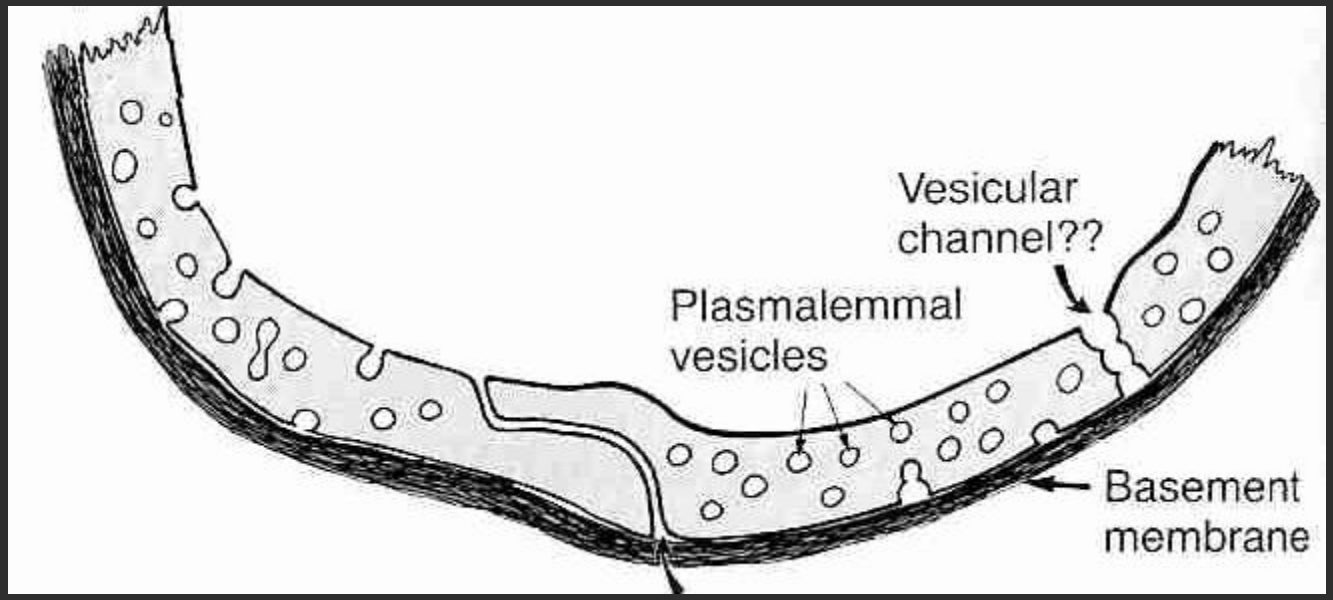
- Cholesterol-rich membrane
- Claudins: skeleton
- Occludin: regulatory protein → electrostatic resistance
- ZO: zonula occludens proteins: anchor that defines the position of the juction

Huber et al., TINS, 2001



The endothelium of the blood-brain barrier

Endothelium: Minor pinocytic transport → reduced penetration

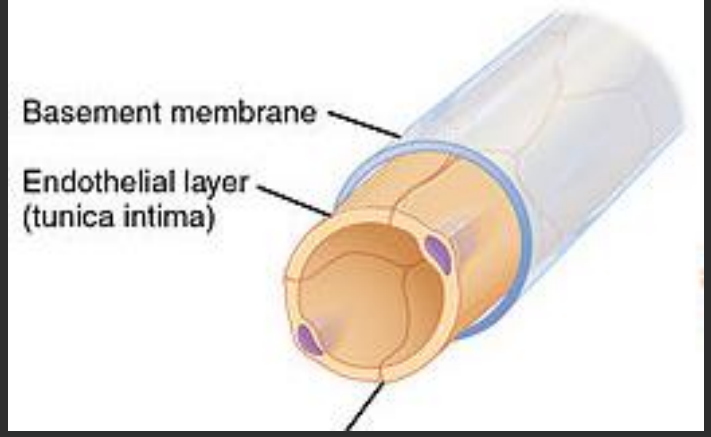


http://www.benbest.com/cryonics/protocol.html

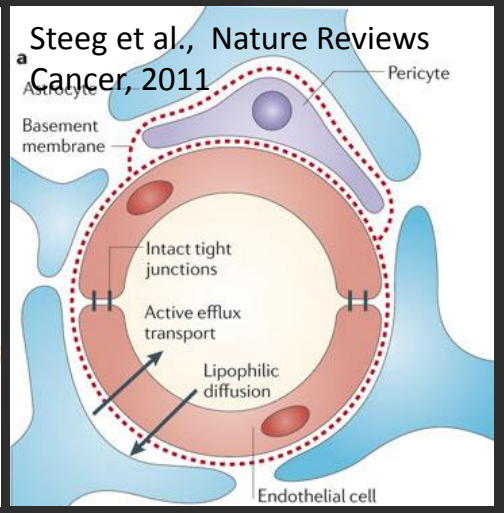
The endothelium of the blood-brain barrier

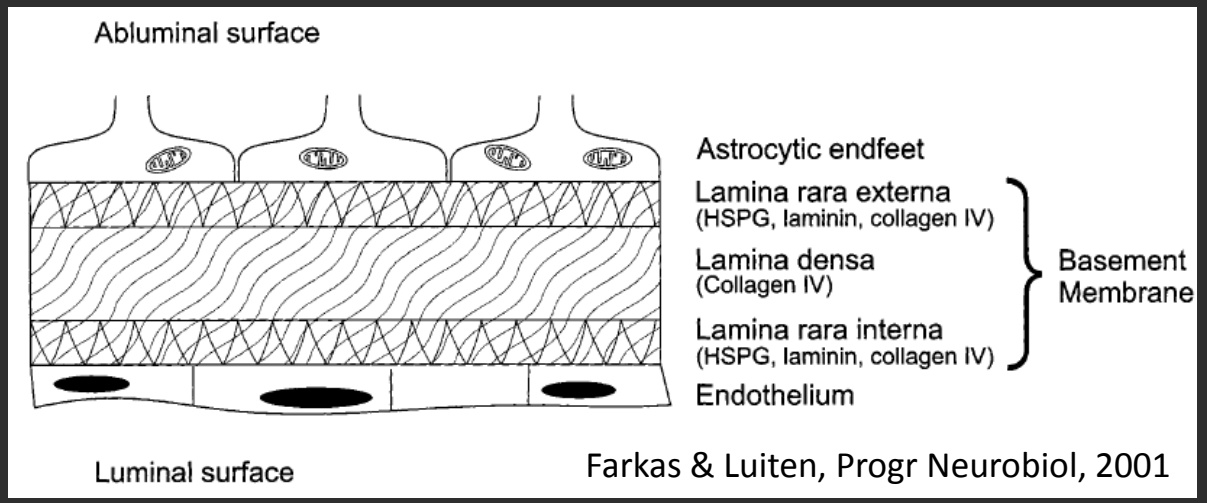
Endothel: thick basement membrane

http://en.wikipedia.org/wiki/Capillary



Steeg et al., Nature Reviews Cancer, 2011

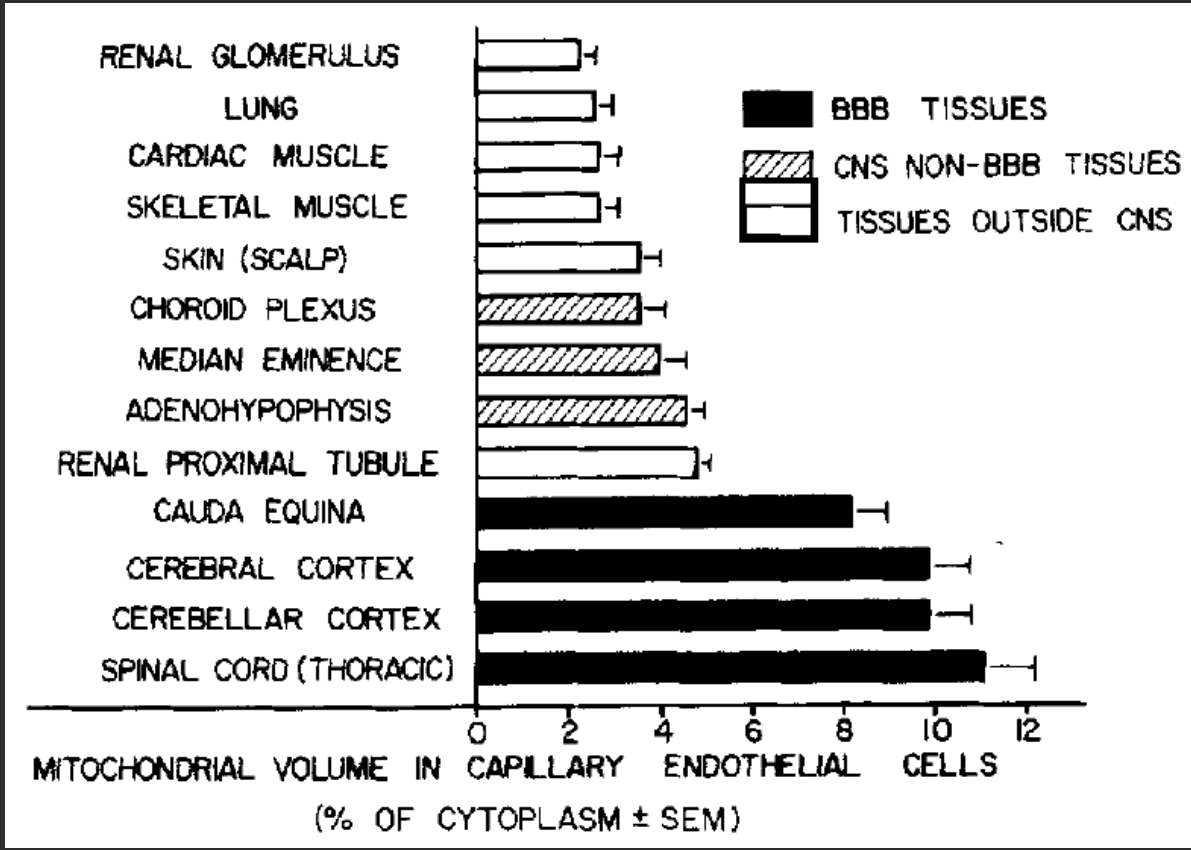




Farkas & Luiten, Progr Neurobiol, 2001

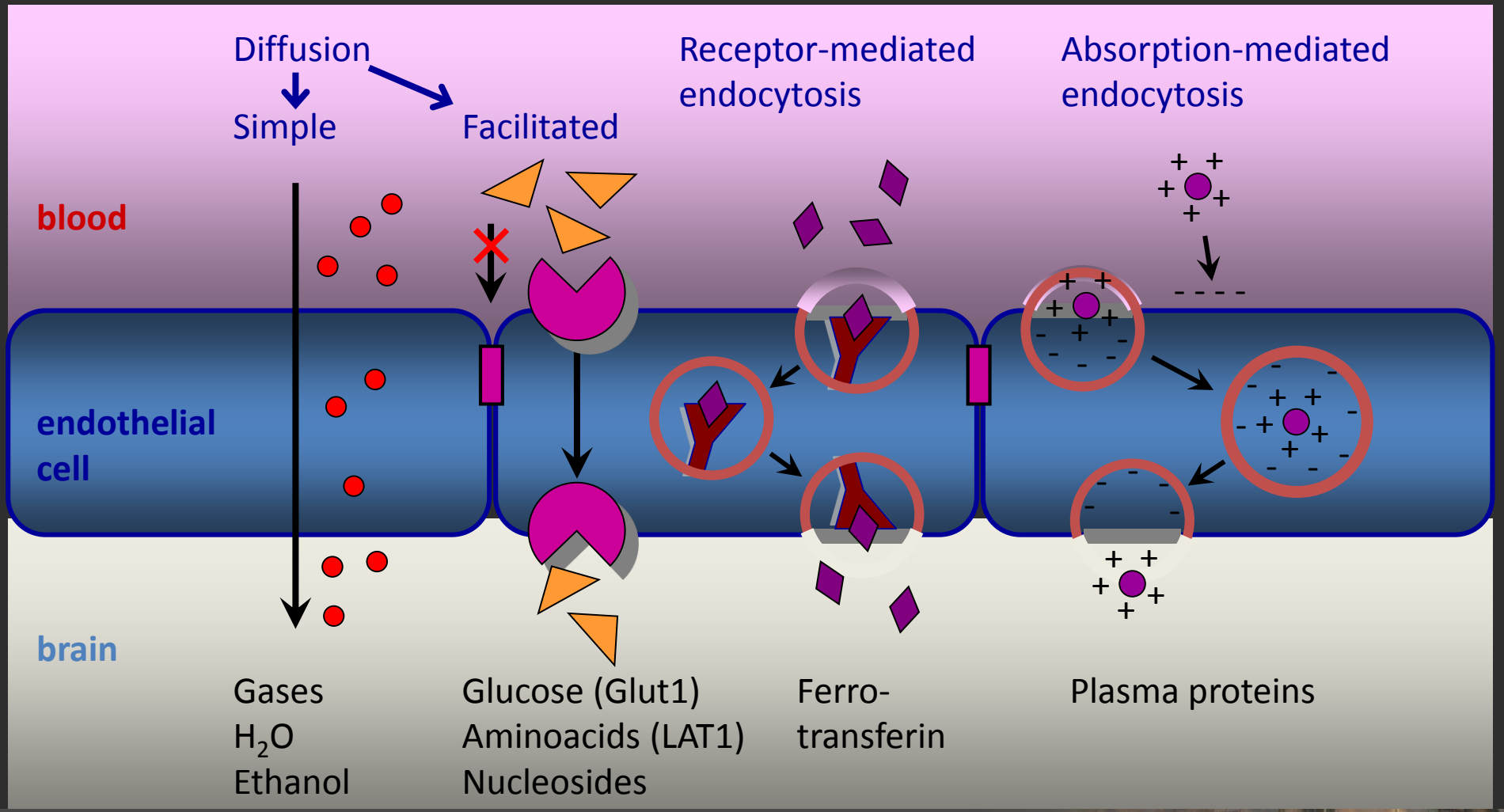
The endothelium of the blood-brain barrier

Endothel: increased amount of mitochondria (up to 10% of the endothelial volume) → metabolic capacity to maintain the barrier



Oldendorf et al., Ann Neurol, 1977

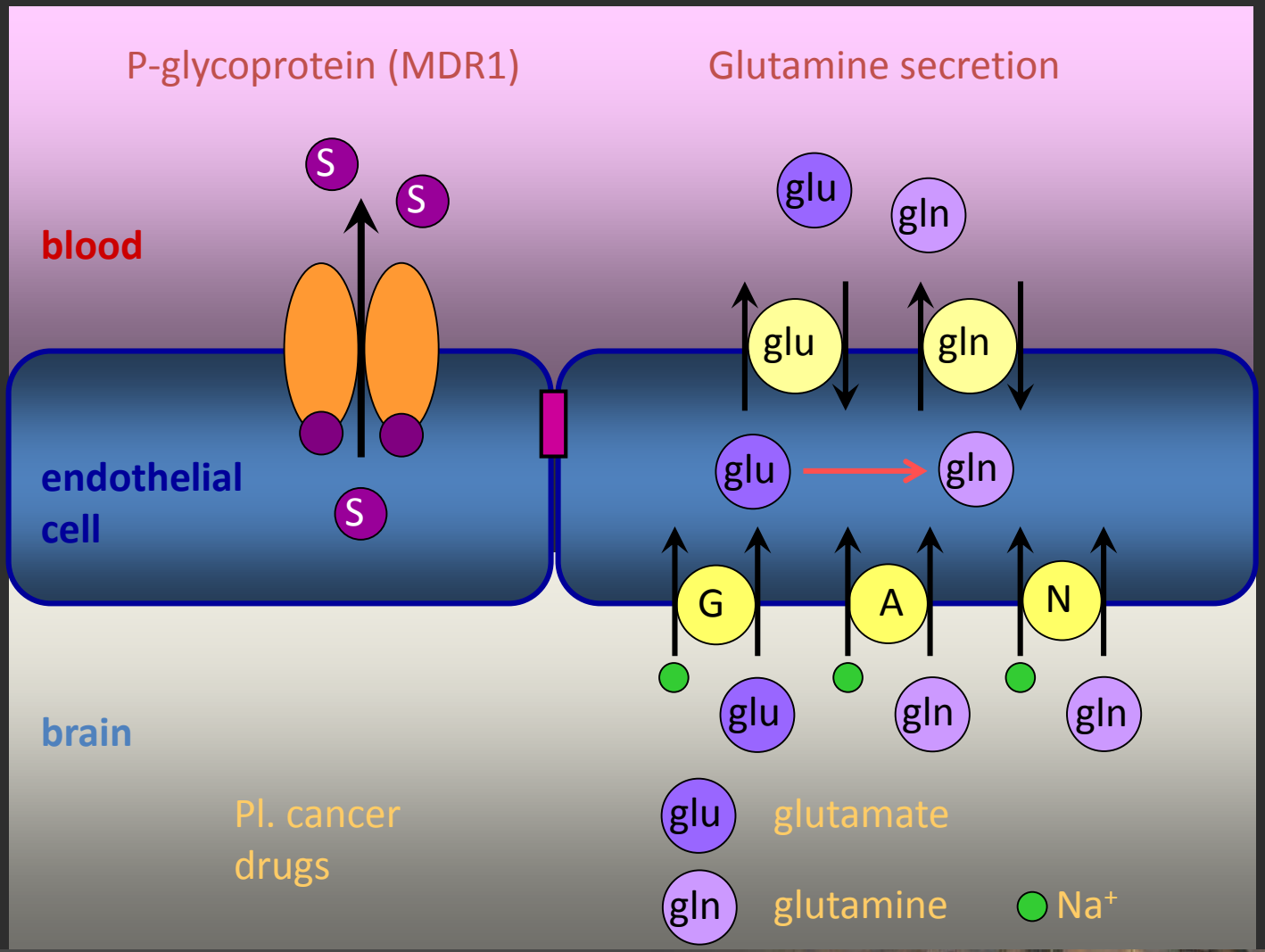
Transport throught the BBB: blood → brain (influx)



Transport throught the BBB: brain → blood (efflux)

P-glycoprotein:
- ATP-dependent
- Active back-transport of hydrophobic drugs

The Na^+-dependent transport systems:
- Elimination of nonessential AAs, toxic AAs,
- Maintainance of the optimal concentrations of all other AAs.



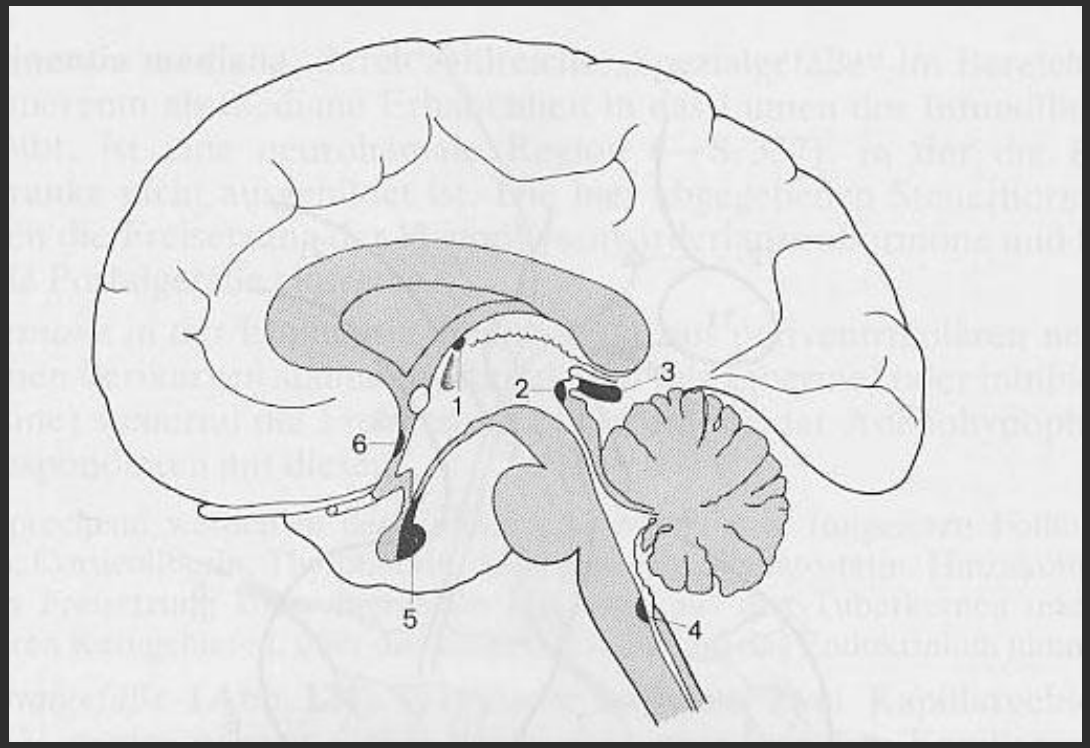
The enzymatic barrier

Enzymatic barrier: degradation of neuroactive substances

(*: general BBB marker)

Enzyme	Function
Alkaline-phosphatase*	Purin & pirimidin metabolism
Monoamine oxidase	Catecolamine inactivation
Aminopeptidase A	Angiotensine metabolism
Endopeptidase	Break-down of neuropeptides (pl. bradykinin, dynorphin, neurotensin)
γ-glutamil-transpeptidase*	Leukotrien conversion C4 $\rightarrow$ D4

Brain areas devoid of blood brain barrier



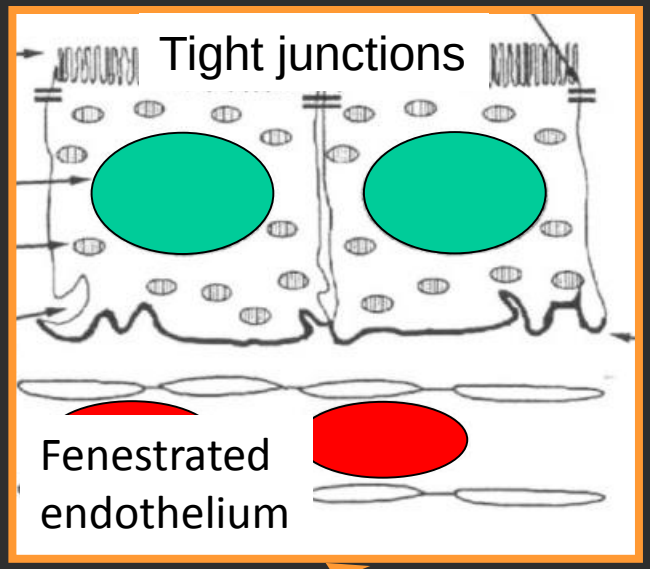
Function:

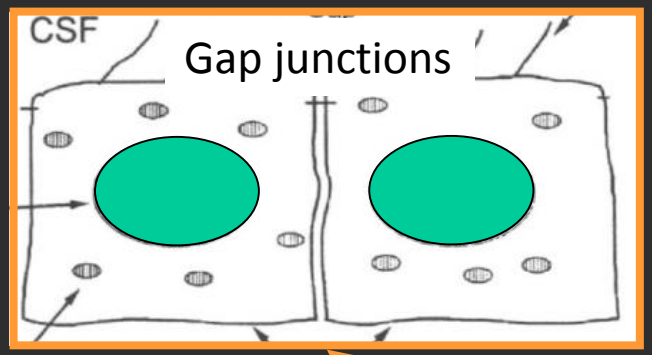
- Hormon production
- Sensory function
- Production of CSF

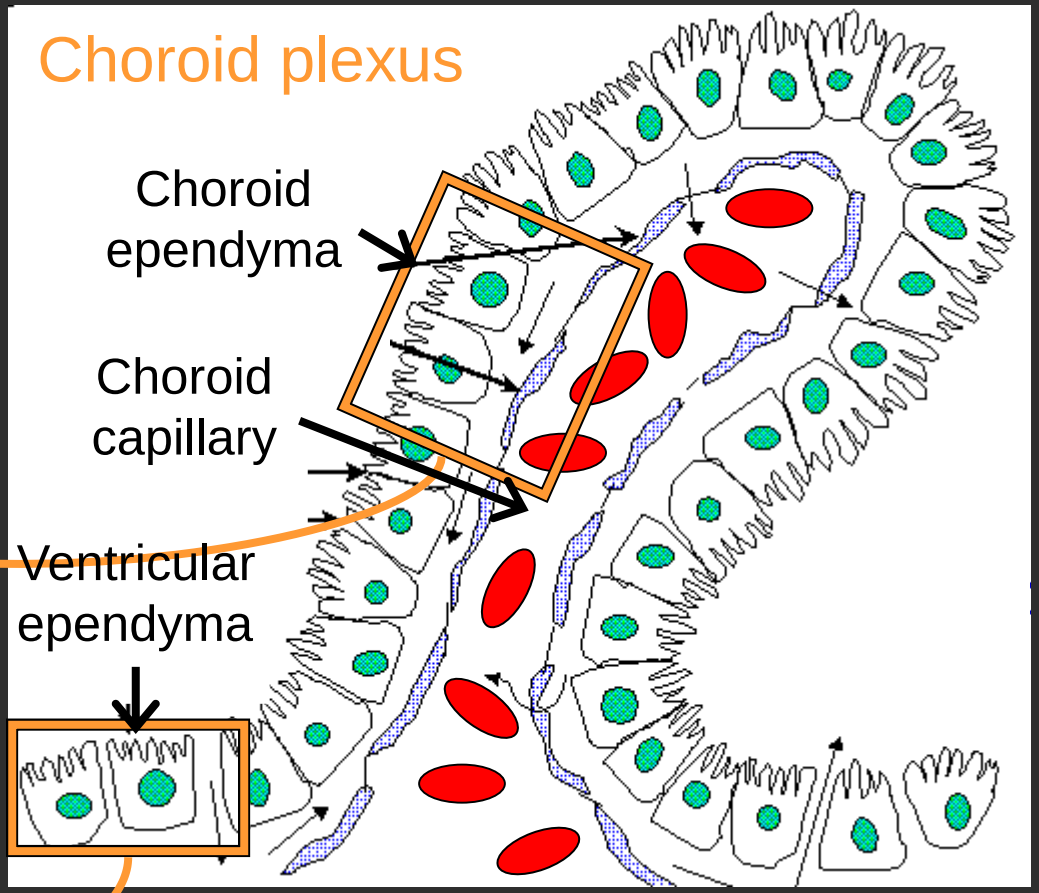
Circumventricular organs:

- Pineal gland (3)
- Median eminence
- Neurohypophysis (5)
- Subfornical organ (1)
- Subcomissural organ (2)
- Area postrema (4)
- Organum vasculosum of lamina terminalis (6)
- Choroid plexus

The blood-CSF barrier

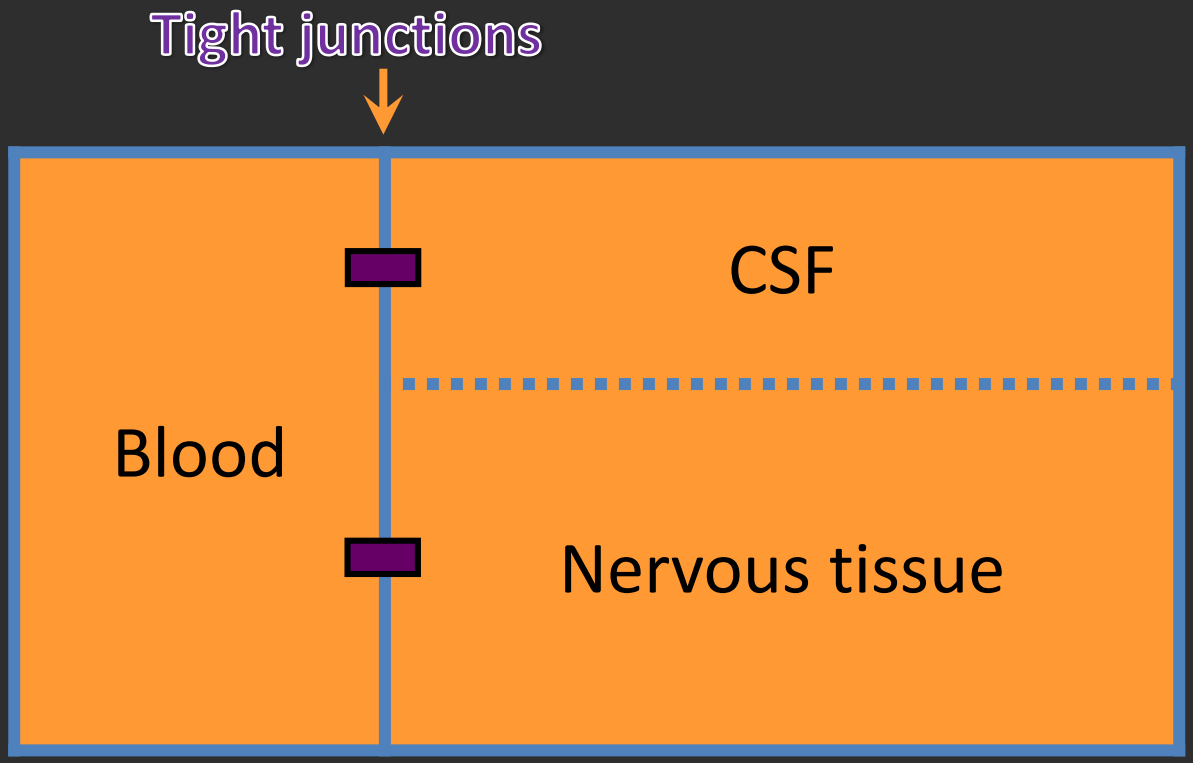






http://www.daviddarling.info/images/choroid_plexus.gif

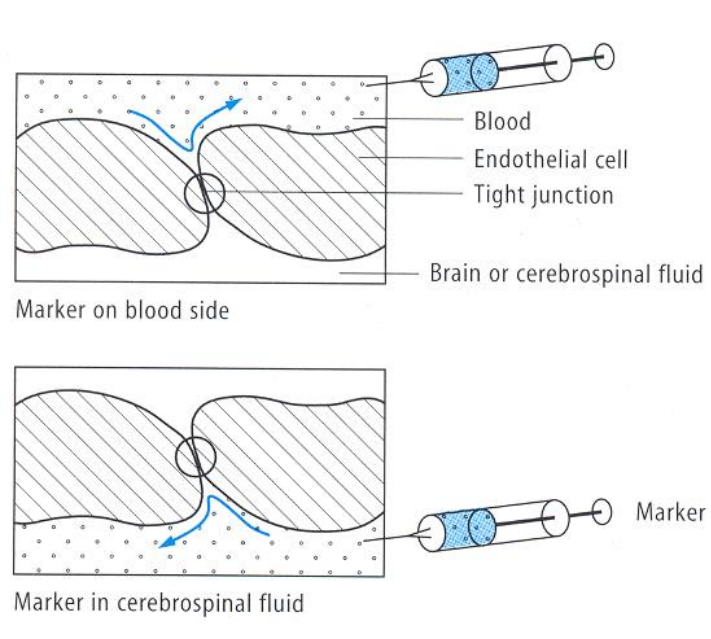
Summary of the barriers of the central nervous system



Tracers to test the integrity of the blood-brain barrier

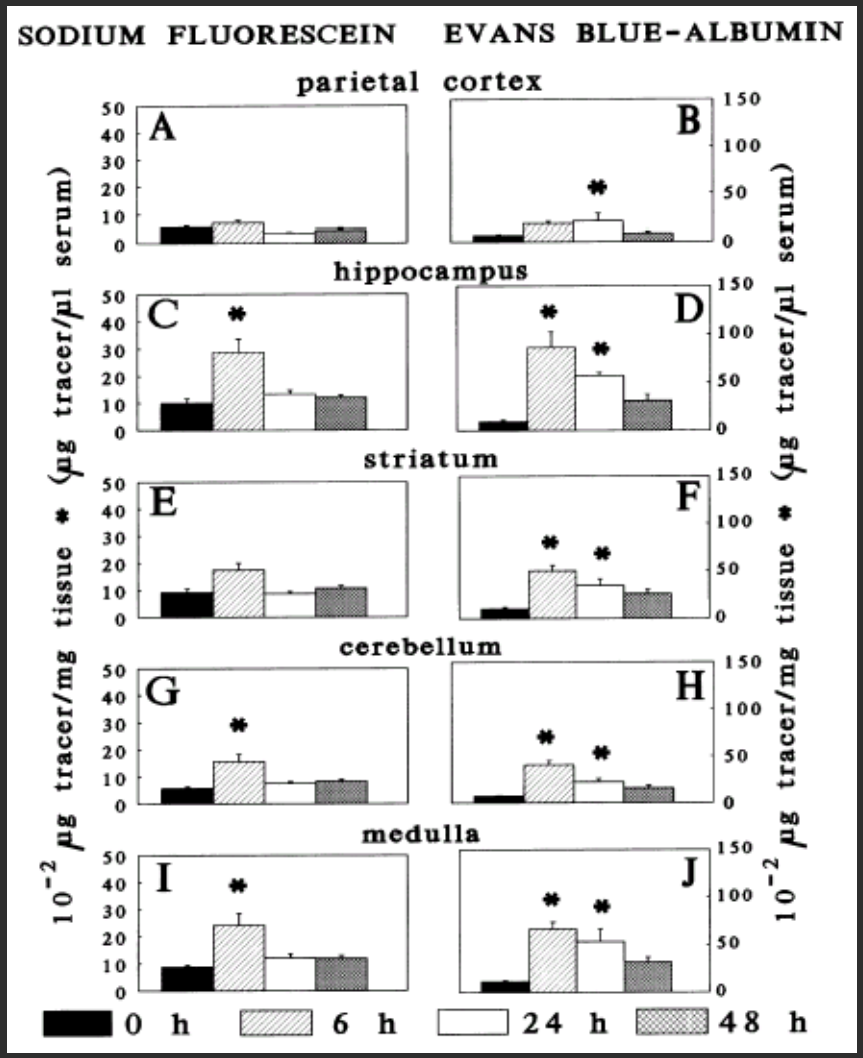
- In experimental animals
- Infusion of Evans-blue: labeling the extravasation of molecules with large molecular weight (60-70 kDa)
- Na^+-fluorescin: labeling the extravasation of molecules with low molecular weight (0,3-0,4 kDa)

- Macroscopic observation: the brain tissue is stained – qualitative analysis
- Spectroscopy: quantitative analysis



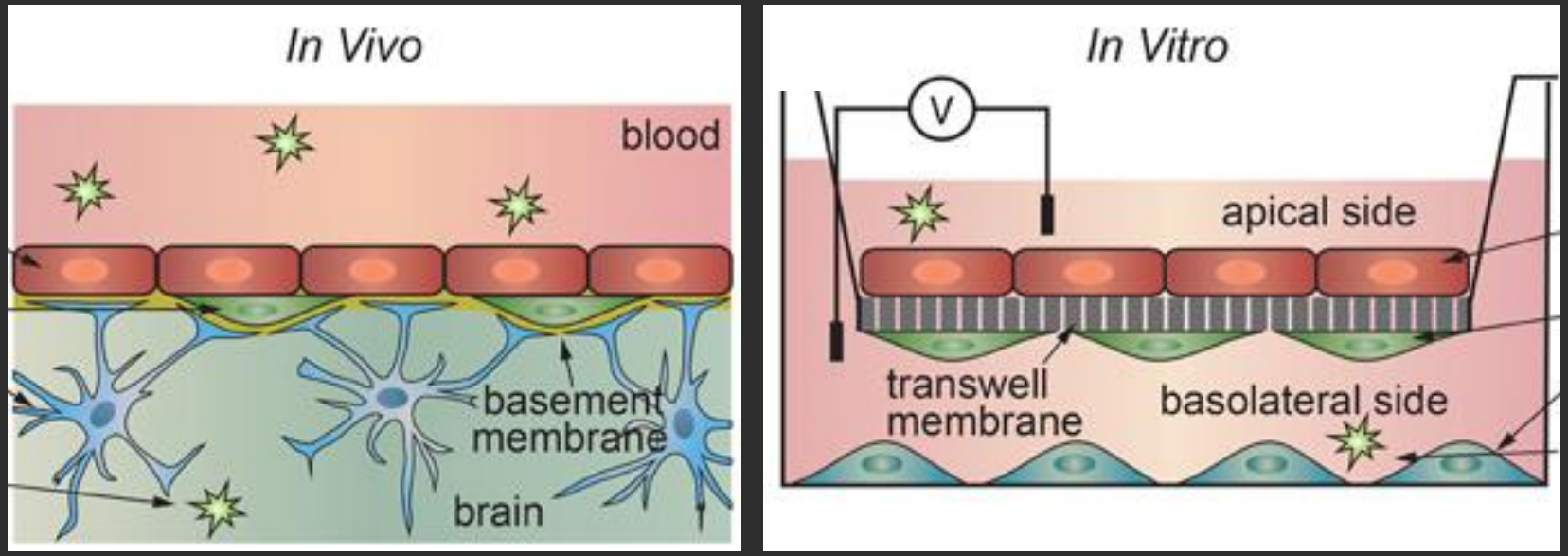
Tracers to test the integrity of the blood-brain barrier

Farkas IG et al.,
Acta Histochem, 2003



Farkas G. et al., Neurosci Lett, 1998

Artificial blood-brain barrier models



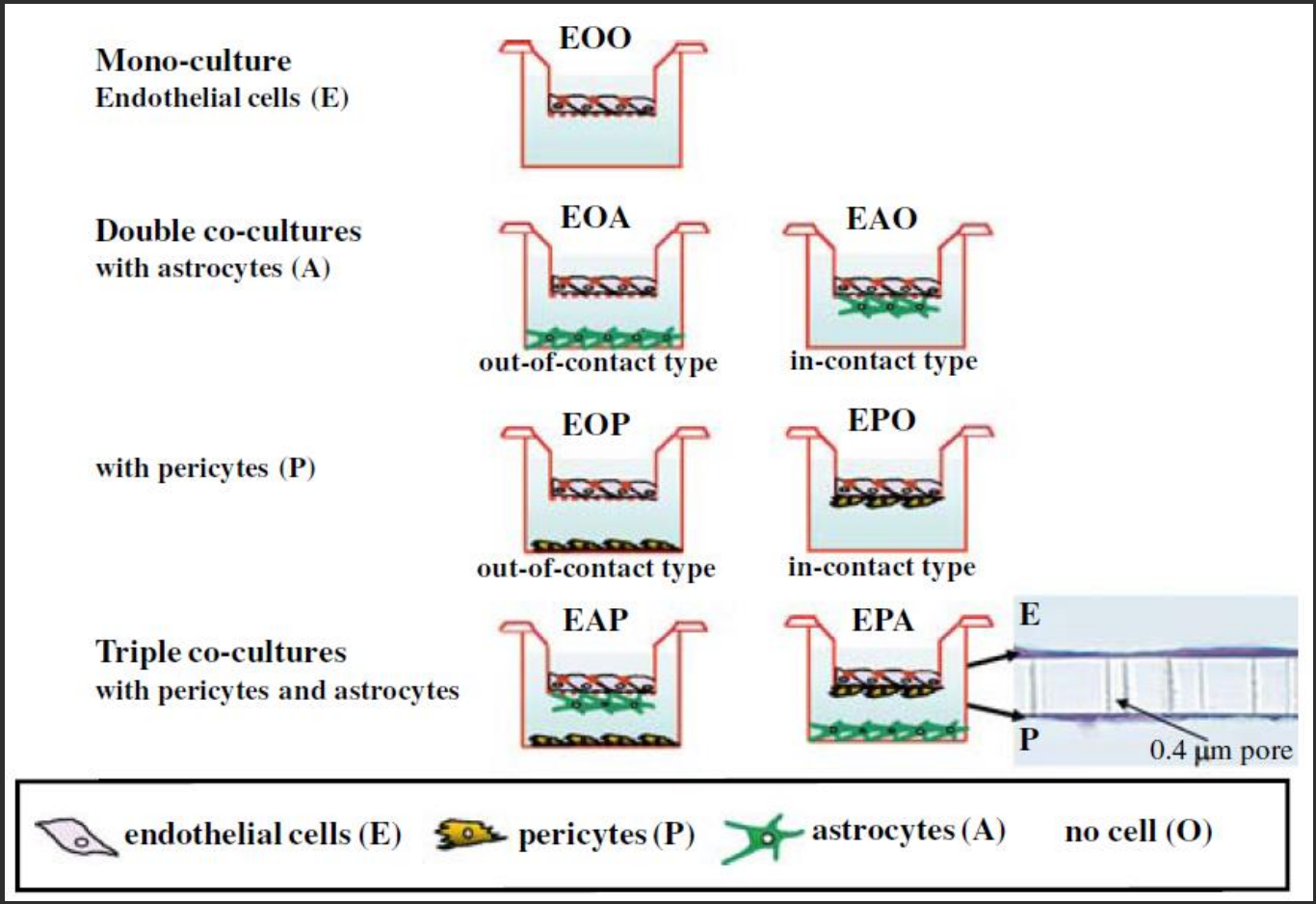
Wong et al., Front Neuroeng, 2013

Endothelial cell

Pericyte

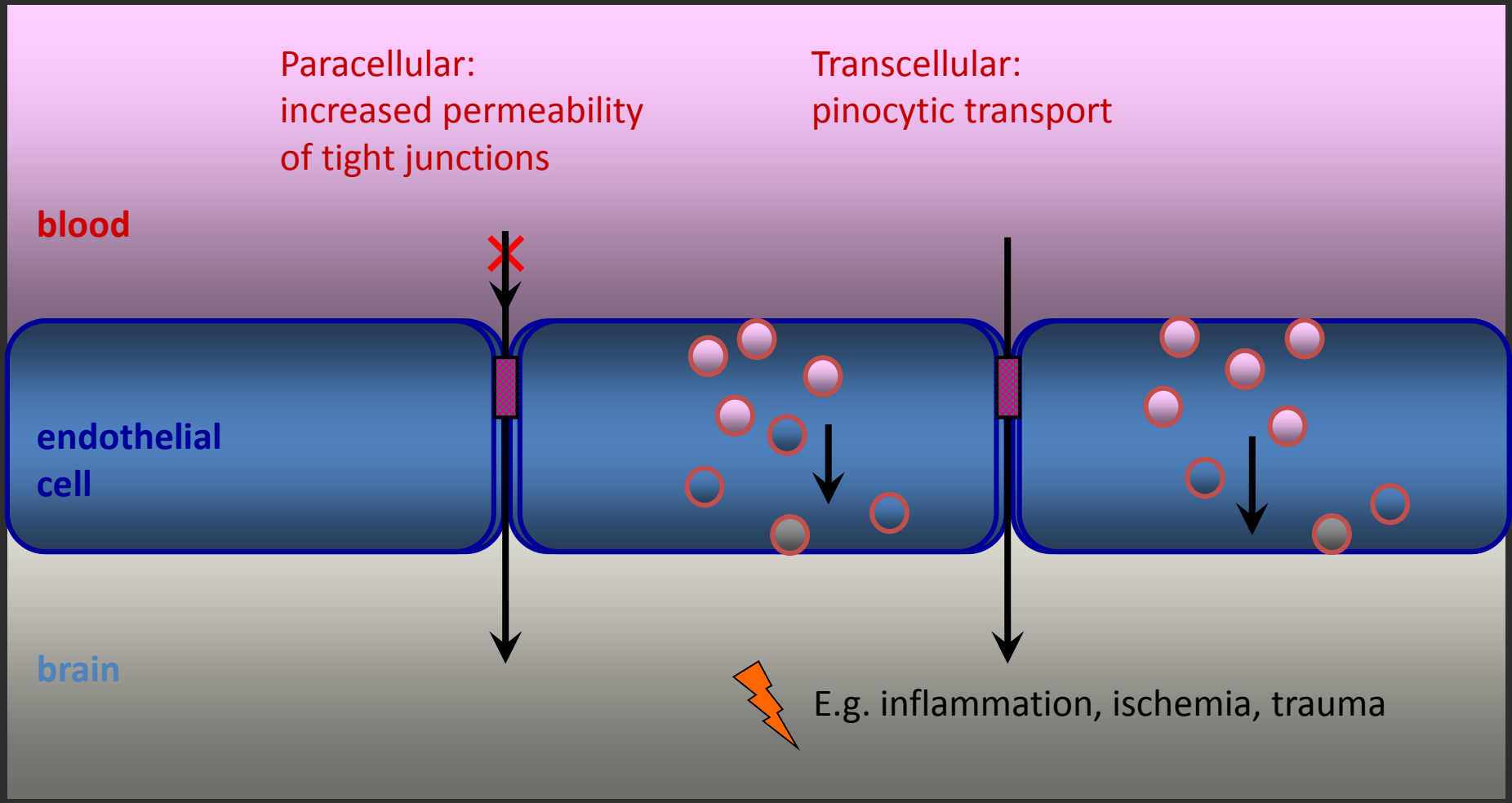
Astrocyte

Artificial blood-brain barrier models



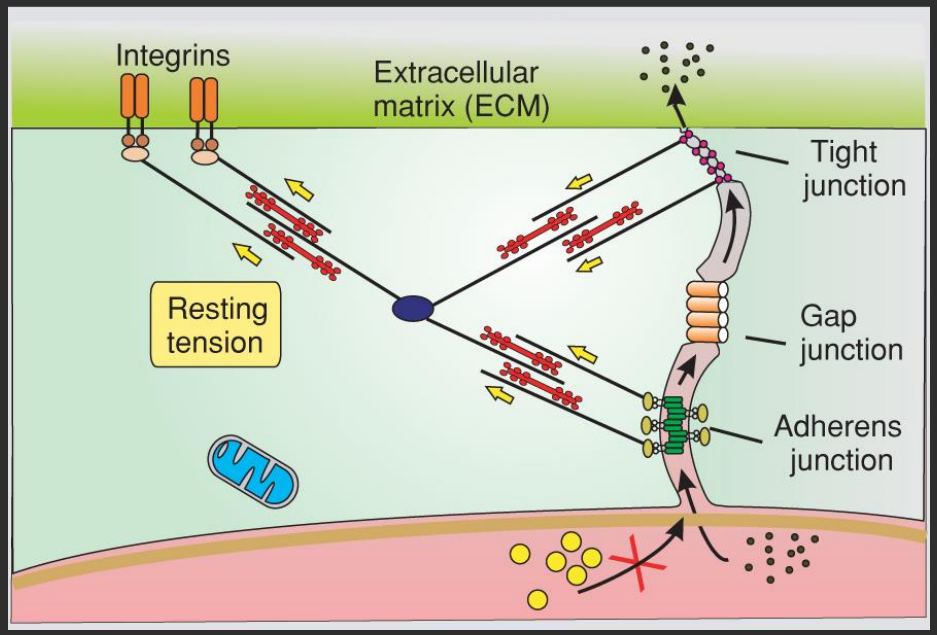
Nakagawa et al., Cell Mol Neurobiol, 2007

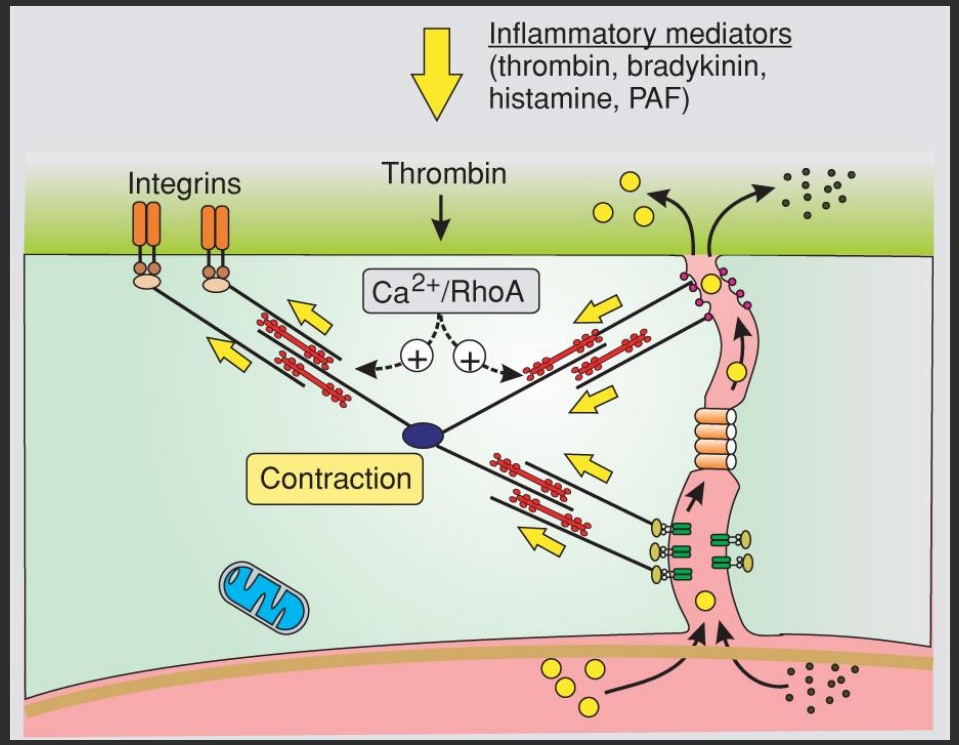
Blood-brain barrier disruption



Inflammation at the BBB

- Causes: infection, trauma, necrosis (stroke)
- Inflammatory mediators activate the contractile machinery of the endothelial cells severing the paracellular junctions.
- Permeability increases + cellular transmigration is made possible.





Consequence of inflammatory BBB opening

Elimination of pathogens, necrotic tissue

But also immune-mediated damage to „innocent bystanders”

Edema formation that can impair blood flow and transcapillary transport

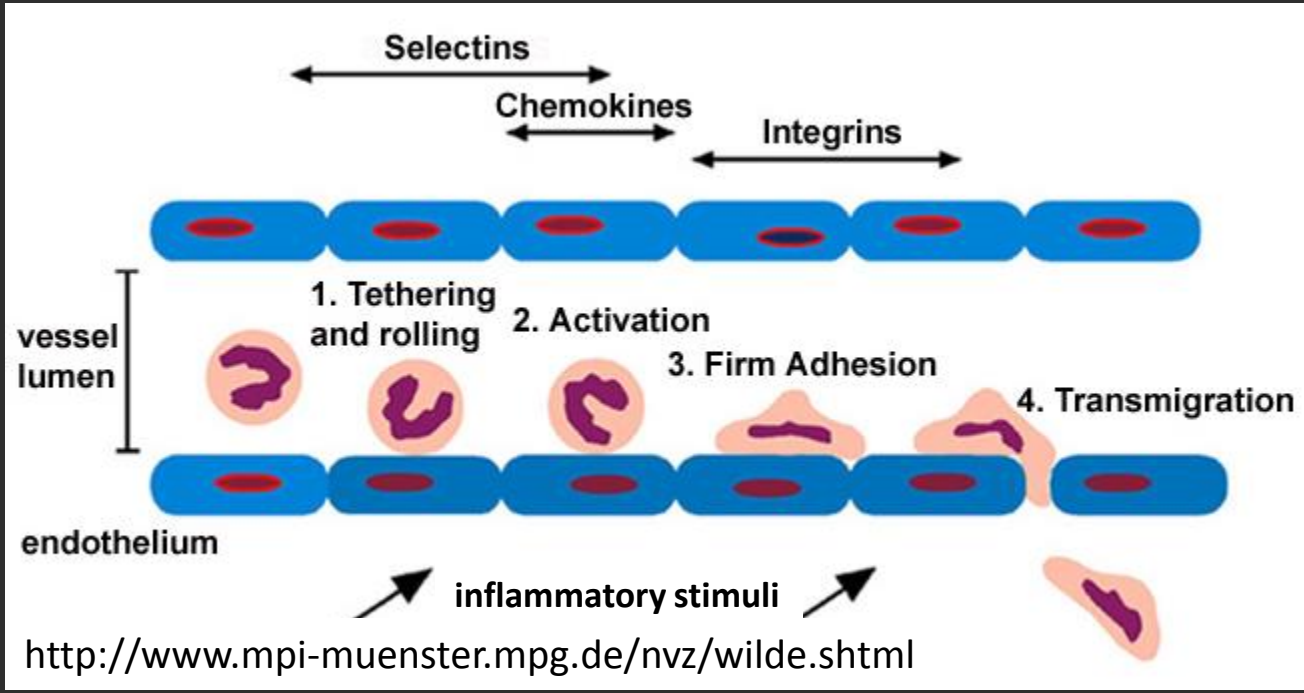
Toxic metabolites or xenobiotics can access the brain tissue

Leukocyte transmigration through the blood-brain barrier

Ransohoff et al., Nat. Rev. Immunol., 2003.

The central nervous system (CNS) has been characterized as an immunologically privileged site in the past, but it should more accurately be viewed as immunologically specialized.

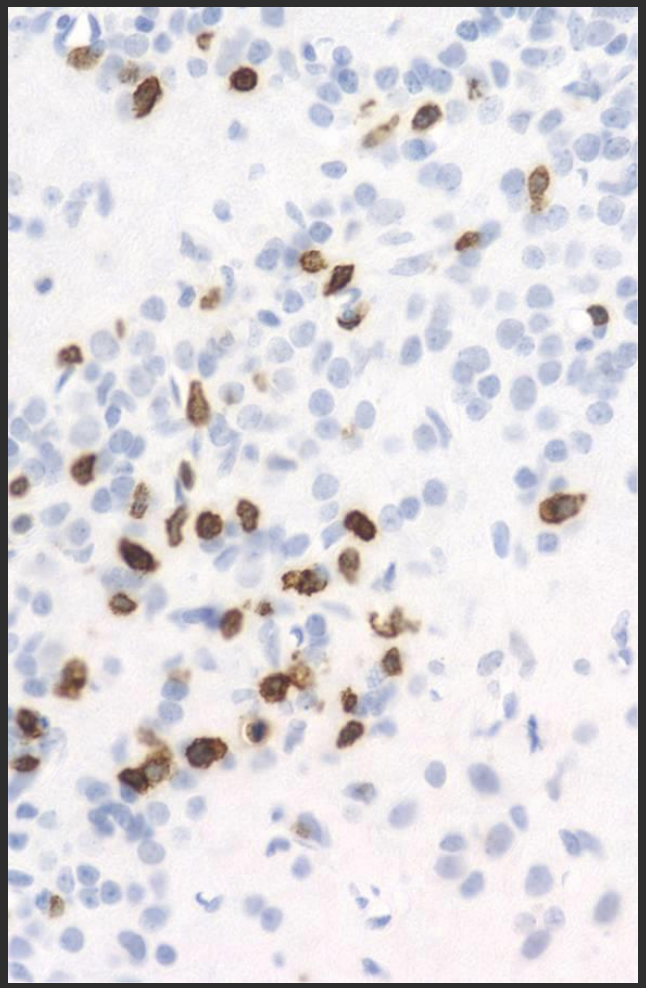
Leukocyte trafficking through the blood vessel wall

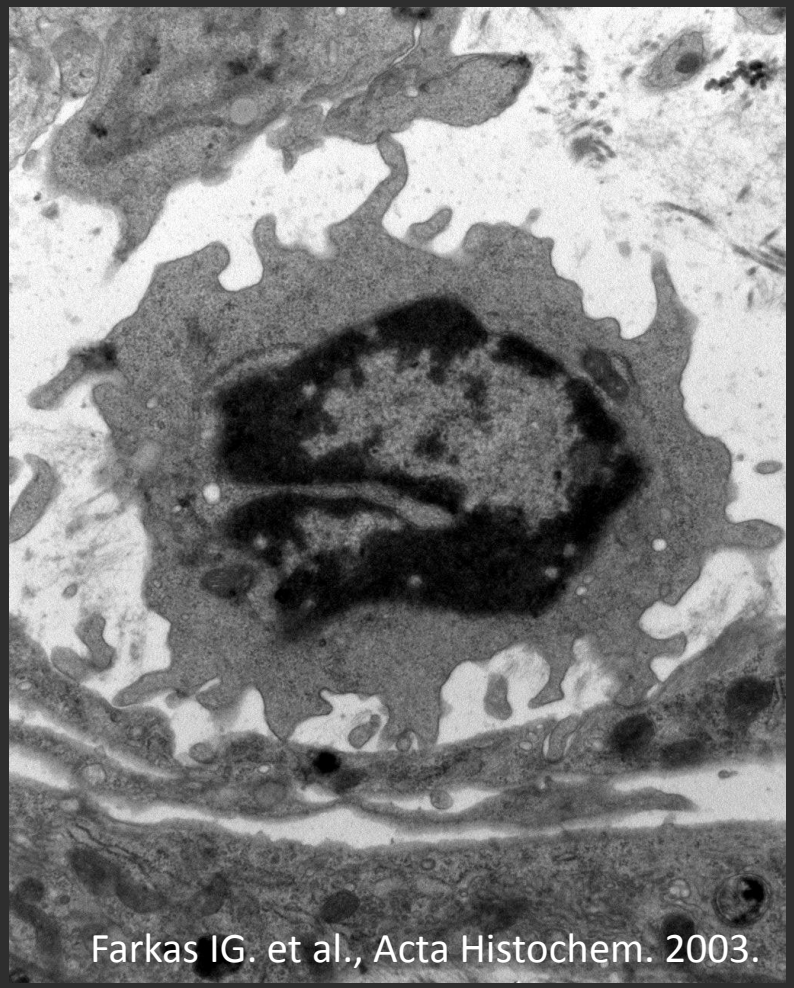


http://www.mpi-muenster.mpg.de/nvz/wilde.shtml

- Selectin-dependent tethering and rolling,
- Chemokine-mediated activation,
- Integrin-dependent firm adhesion and spreading,
- Extravasation into the underlying tissue.

Leukocyte transmigration through the blood-brain barrier





Farkas IG. et al., Acta Histochem. 2003.

Extravasation of tumor cells

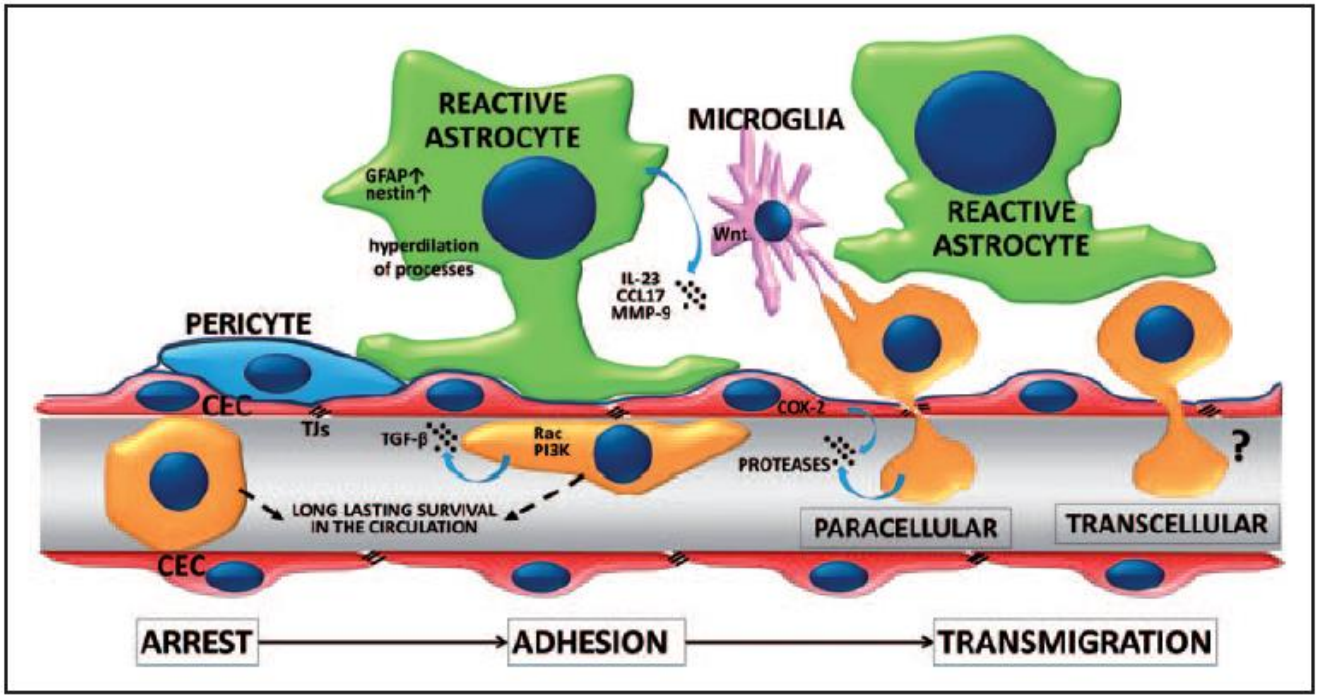


Figure 1. Extravasation of tumor cells through the BBB. Successful metastasis formation is dependent on arrest of tumor cells in the microvessels, followed by the adhesion and transmigration step. Extravasating tumor cells survive for days in the capillary lumen before transmigration is completed. During this process, tumor cells activate the Rac and PI3K signaling pathways and release TGF-β and proteases. CECs may also enhance transendothelial migration of metastatic cells through activation of COX-2 and secretion of MMP-2. Reactive astrocytes and microglia are recruited at initial steps of extravasation. Astrocytes may secrete cytokines, chemokines and proteases to enhance transendothelial migration of tumor cells. Microglia may also enhance invasion of the brain serving as transporters for malignant cells.

Wilhelm et al., JCBFM, 2017

MMPs

↓

BM

TJ

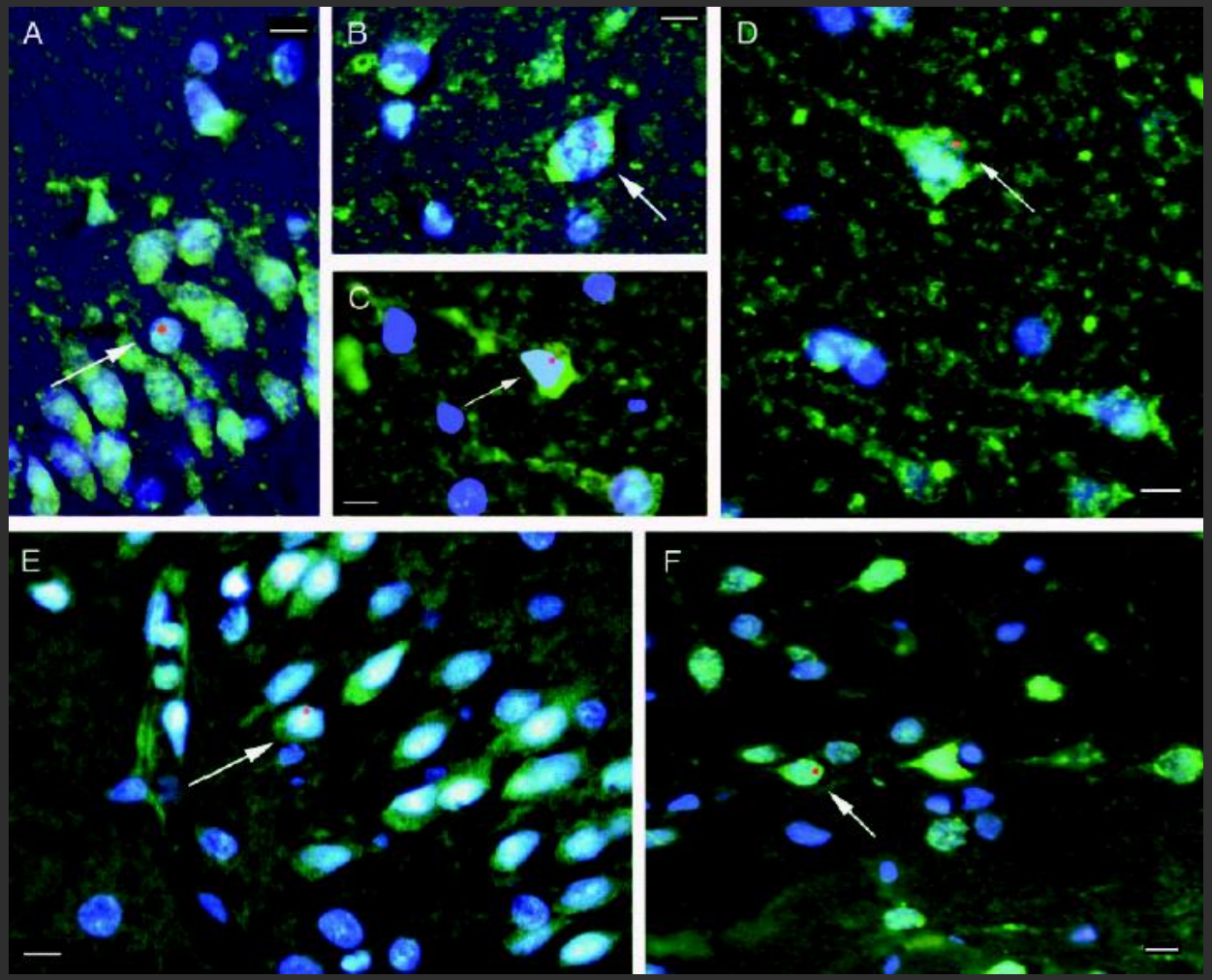
Glycocalyx

Bone marrow stem cell transmigration through the blood-brain barrier

- Female patients with leukemia
- Bone marrow transplantation from a male relative
- Examination of brain tissue

- Cell clusters containing Y-chromosome in the brain
- Most of them are glia
- A small percentage is neuron (7/10 000)

Bone marrow stem cell transmigration through the blood-brain barrier



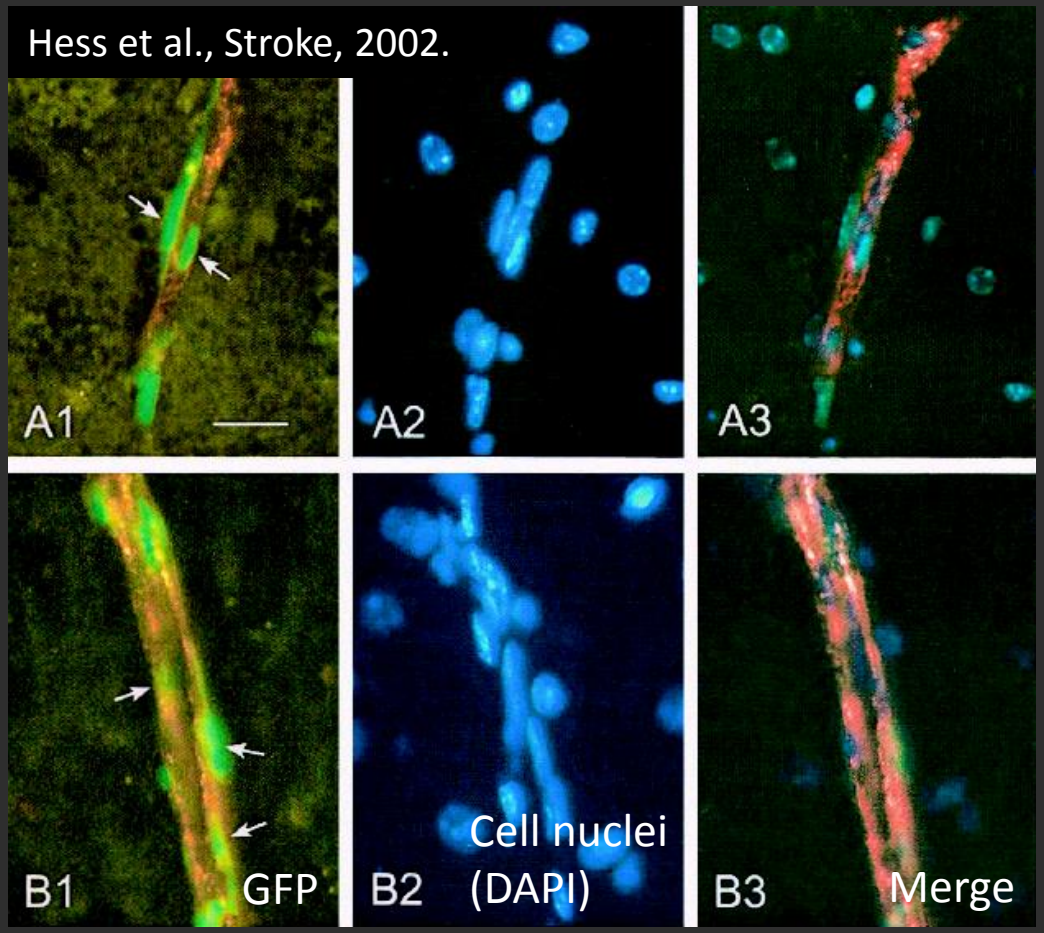
Green: NeuN
Blue: nucleus
Red: Y-chrom.

Bone marrow stem cells: therapeutical potential for stroke?

- Bone marrow transplantation from male mice to females, male bone marrow cells express green fluorescent protein (GFP)
- Middle cerebral artery occlusion $\rightarrow$ stroke
- Histological examination of the brain: labeling/visualization of GFP and Y-chromosomes

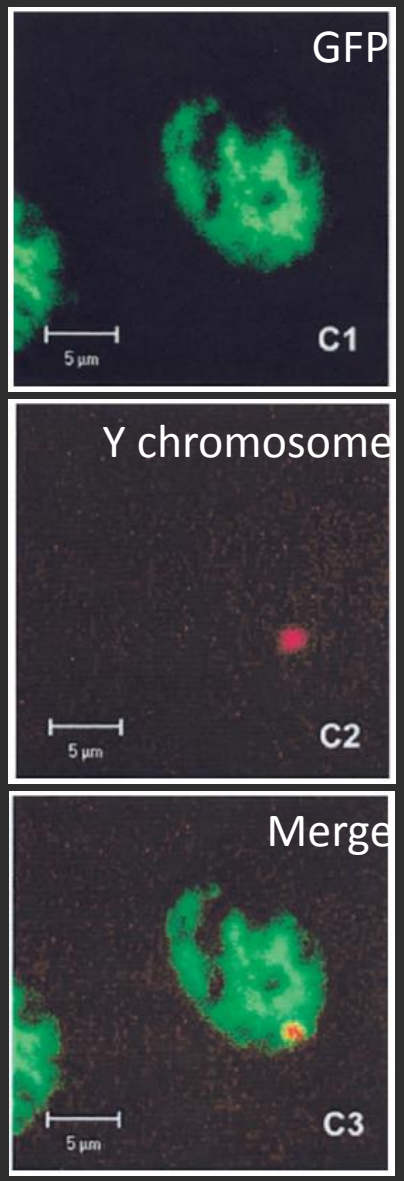
- Labeled cells detected in the cerebral endothelial cells of small vessels
- Labeled, neuron-like cells in the brain

Bone marrow stem cells: therapeutical potential for stroke?



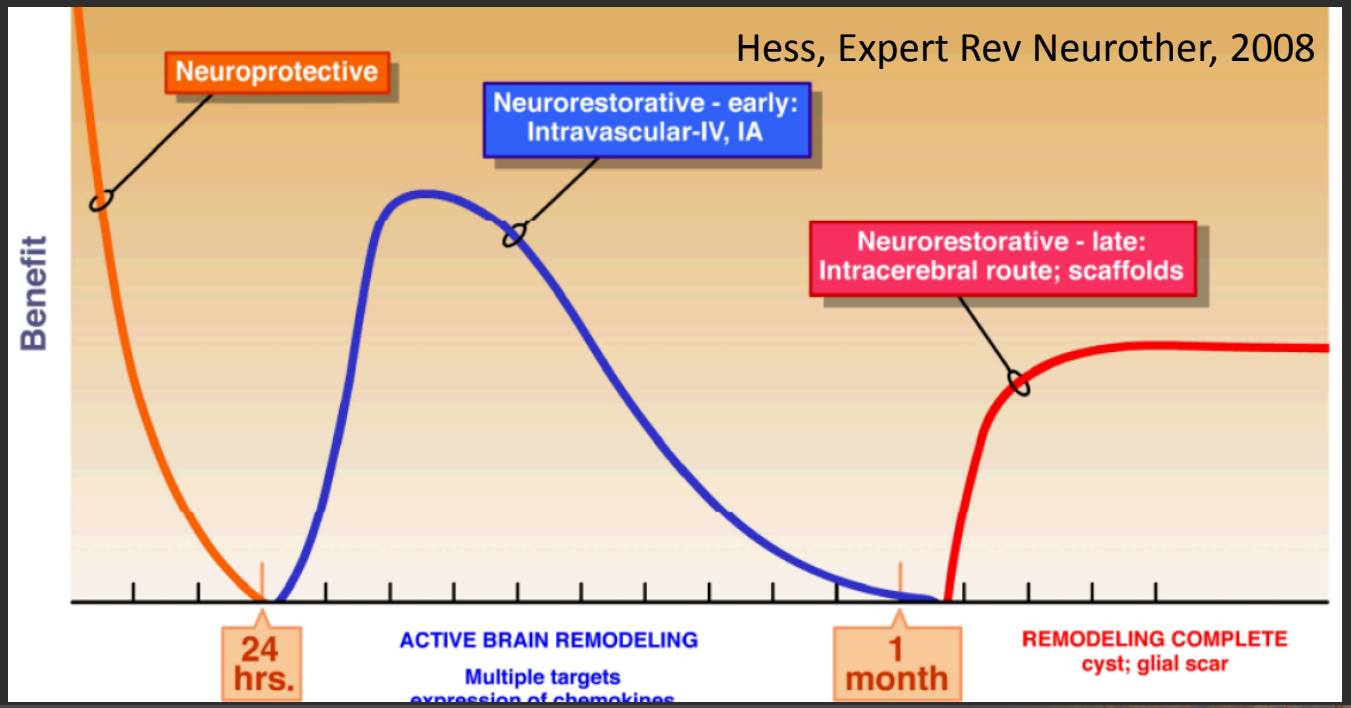
Neurons →

Endothelial cells ←



Bone marrow stem cells: therapeutical potential for stroke?

- Focused on restorative processes
- Longer time window of opportunity than neuroprotective therapies (within the first week after stroke)

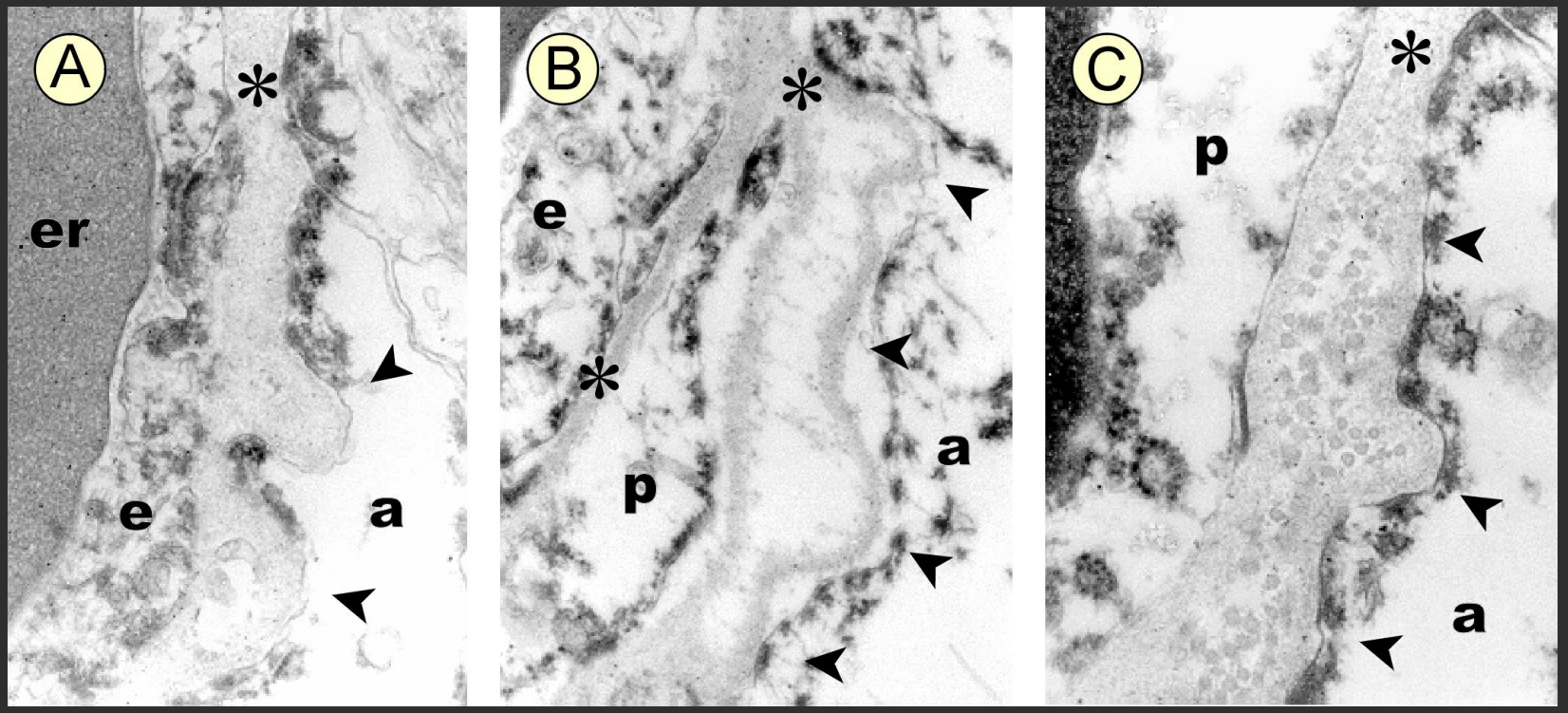


Hindered blood-brain barrier function

- Amyloid angiopathy – Alzheimer's disease
- Basal membrane thickening – aging, neurodegenerative disease, hypertension
- (Atherosclerosis) - hypertension

Basal membrane thickening

- Aging, dementia, hypertension
- Hindered transport

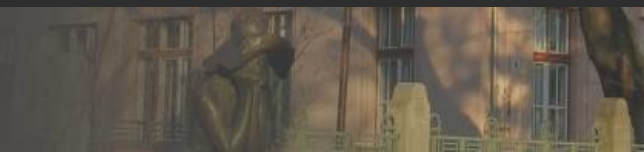


Drugs and the BBB

- The BBB hinders drug delivery to the brain.
- It poses a problem at the treatment of central nervous system diseases.

Potential solutions:

- Increased lipid-solubility of the drug
- Transient opening of the BBB (e.g. osmotic)
- „Wrapping" drugs (liposomes)
- Intranasal pathway



Drugs and the BBB

Methods of Circumventing the Blood-Brain Barrier

Routes and methods of administration

- High dose
- Intrathecal
- Intraventricular
- Intra-arterial (carotid artery)

Non-pharmaceutical methods

- Surgery
- Radiation therapy

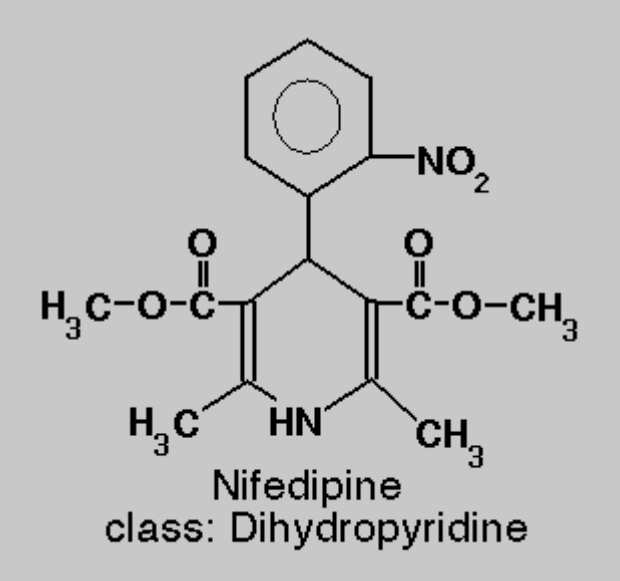
Tailored and carrier drugs

- Increased lipid solubility
- Lipophilic carrier agents (liposomes)
- Cationization
- Glycosylation
- Receptor-mediated transport

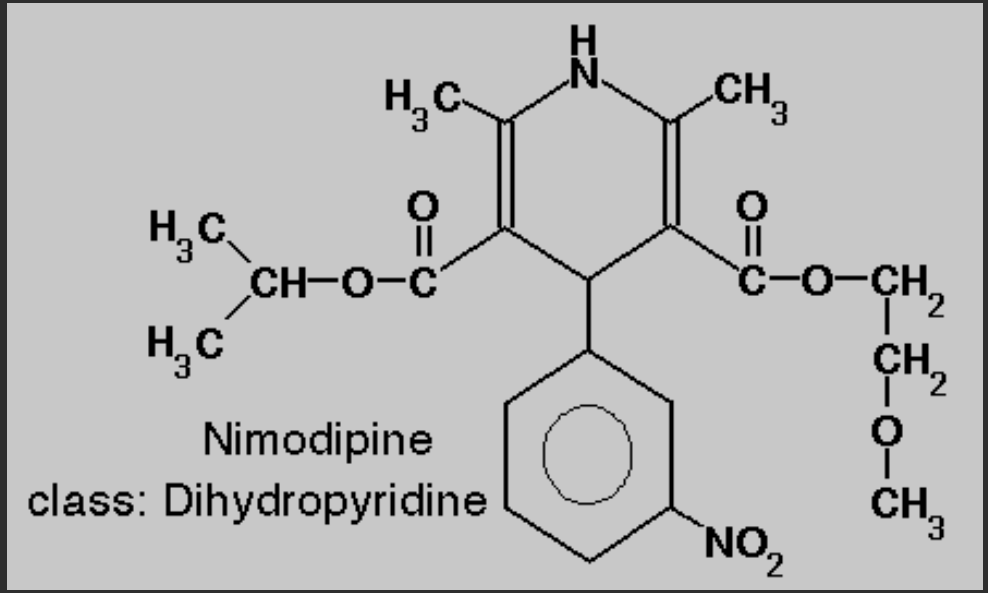
Mechanical Disruption

- BBB disruption
  - Osmotic
  - Pharmacological
  - Biochemical
- Hypertension

Increased lipid solubility



Nifedipine
class: Dihydropyridine



Nimodipine
class: Dihydropyridine

- Dihydropyridines: Ca^{2+} channel antagonists
- For the treatment of hypertension
- Nimodipine: increased lipid solubility: for the treatment of stroke

Drugs and the BBB

Methods of Circumventing the Blood-Brain Barrier

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Mechanical Disruption

- BBB disruption
  - Osmotic
  - Pharmacological
  - Biochemical
- Hypertension

Osmotic opening of the blood-brain barrier

- Intra-carotid infusion of hyperosmotic solutions (for example: mannitol)
- Transient shrinkage of the endothelial cells → losening and opening of the tight junctions
- Defining variables:
  - Length of infusion
  - Osmolarity of the solution
- Influencing physiological parameters:
  - Concentration of blood gases
  - Cardiac output
- Therapeutical target: pl. chemotherapy of brain tumors

Drugs and the BBB

Methods of Circumventing the Blood-Brain Barrier

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Mechanical Disruption

- BBB disruption
  - Osmotic
  - Pharmacological
  - Biochemical
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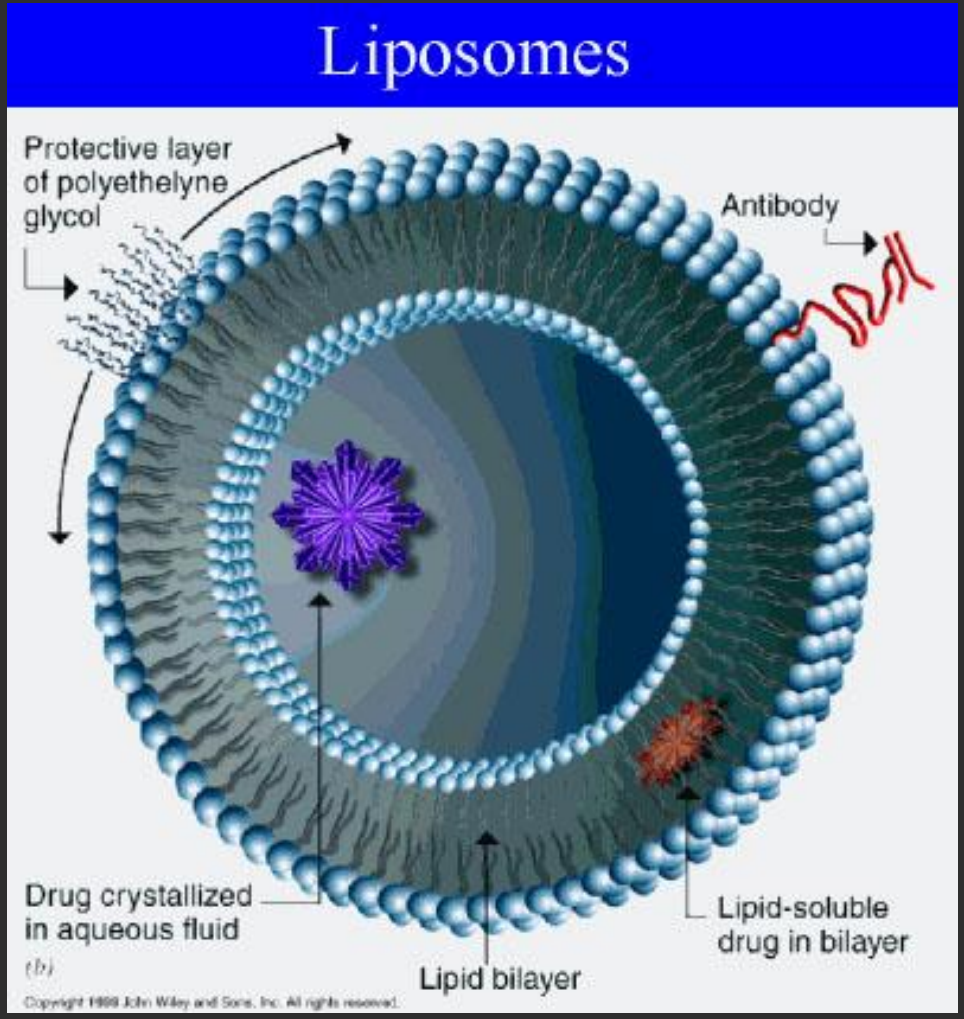
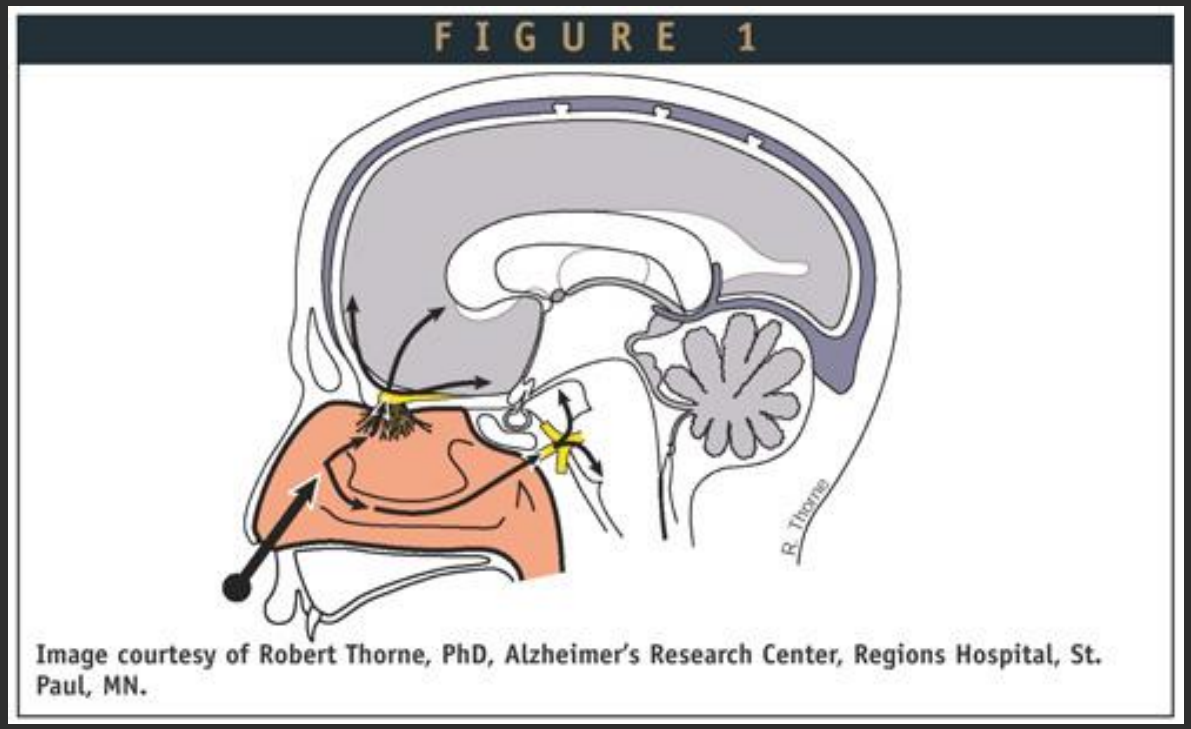
Application of liposomes

- Known for 40 years (Bangham)
- Small, artificial phospholipid vesicles
- For medicines targeting the CNS
- In stroke treatment: e.g. SOD



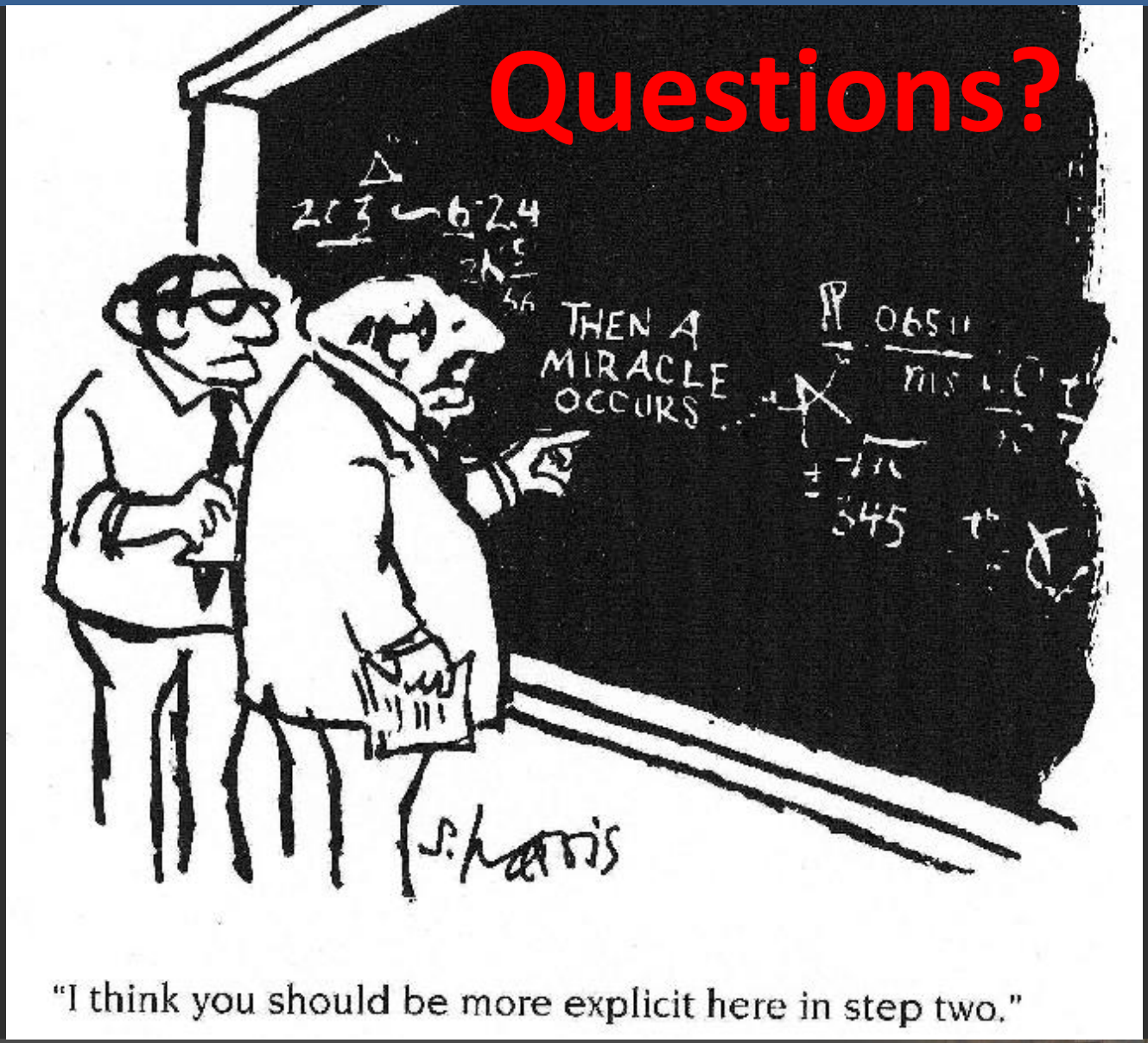
Intranasal treatment strategies

- Sensory nerve endings:
  - n. olfactorius
  - n. trigeminalis
- Nonisnvasive
- NGF, IGF, FGF



- Way of passage:
  - Intraneuronal: axonal transport, hours to days → for specified brain regions
  - Extraneuronal: perineuronal, minutes → brain parenchyma, CSF

Questions?



"I think you should be more explicit here in step two."