Lesson (2)

The cellular organization of the nervous system II (neuroglia)

The neuroglia: structure and functions

In 1856, Rudolf Virchow defined glial cells as a cell population in the brain that is distinct from neurons. In 1919, Pio del Rio Hortega introduced the modern terminology of glial cells:

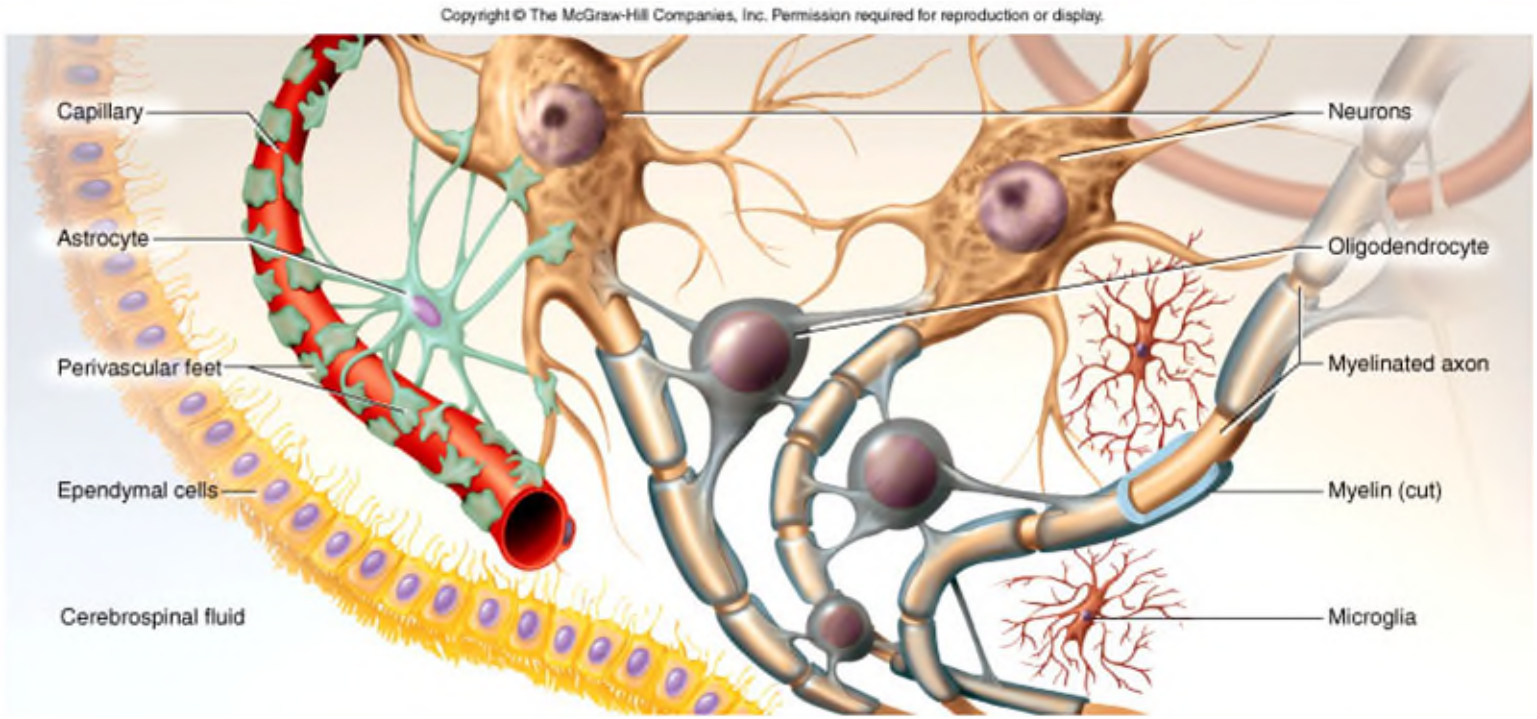
6 main types:

-Astrocytes
-Microglia
-Ependimal cells
-Radial glia
-Oligodendrocytes
-Schwann cells

Functions:

-Filter (Blood-Brain Barrier)
-Physical support
-Protection (sequestration of ion or neurotransmitters in excess; resident immune system)
-Trophic and metabolic support
-Signal transduction (transcytosis, myelin formation)
-Regeneration and degeneration/scar formation (neural stem cells)

Neuroglial Cells of CNS



THE NEUROGLIA: some FUNCTIONs

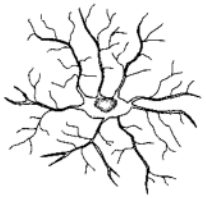
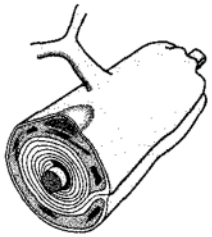
- Astrocytes: Blood brain barrier, support, trophic, signalling, support, homeostasis
- Microglia: resident immune system
- Oligodendrocytes
- Schwann Cells

} Support & myelin, signalling

What are glia?

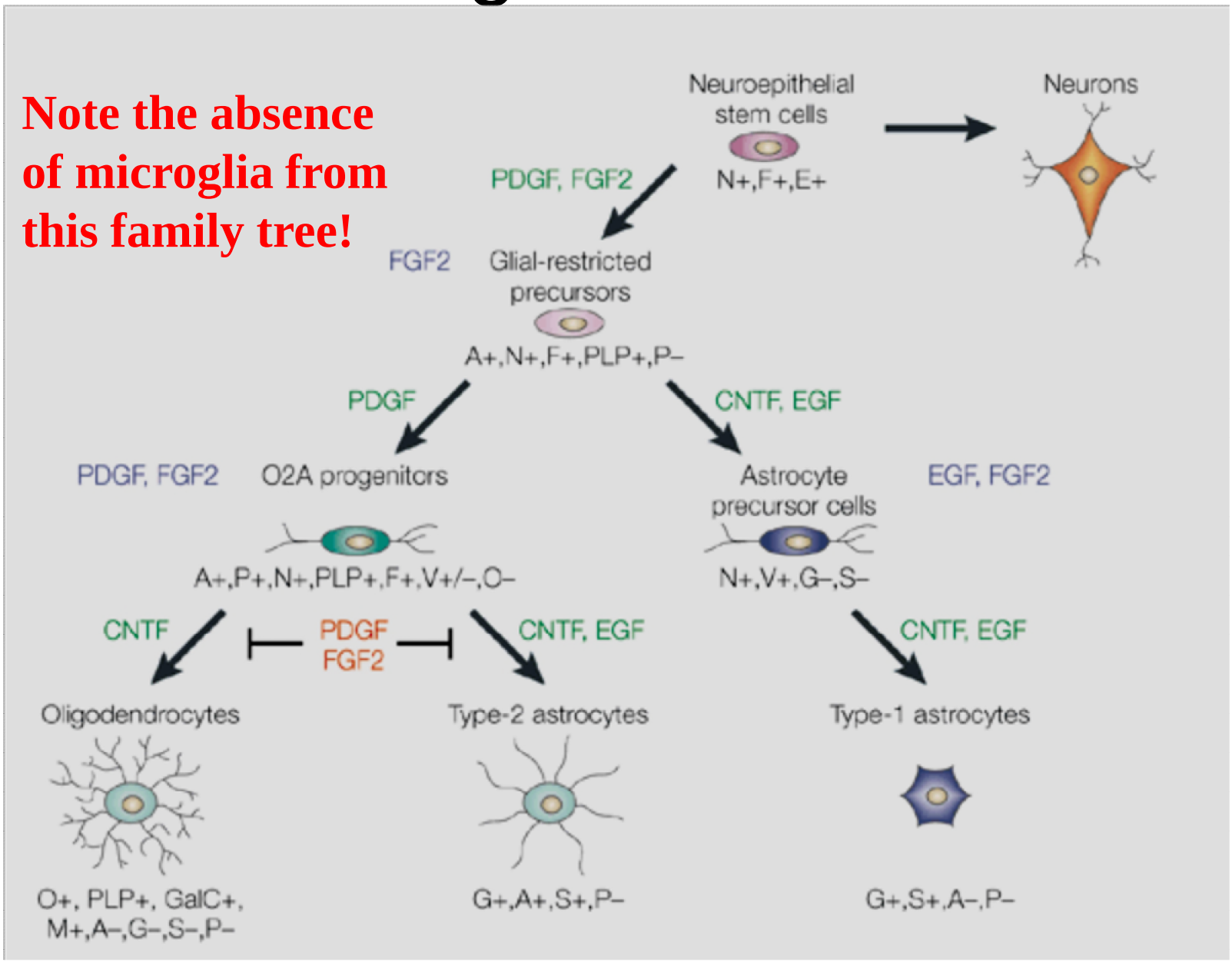
Neuroglia="nerve glue" (Virchow, 1859)
Glia as cells: S. Ramon y Cajal, P. del Rio-Hortega, 1900-1920

TABLE 2-2. Types of Vertebrate Glial Cells

Type	Appearance	Features and Functions
Astroglia		Star-shaped, symmetrical; nutritive and support function
Microglia		Small, mesodermally derived; defensive function
Oligodendroglia		Asymmetrical; form myelin around axons in brain and spinal cord
Schwann cell		Asymmetrical; wraps around peripheral nerves to form myelin

Gliogenesis

Note the absence of microglia from this family tree!



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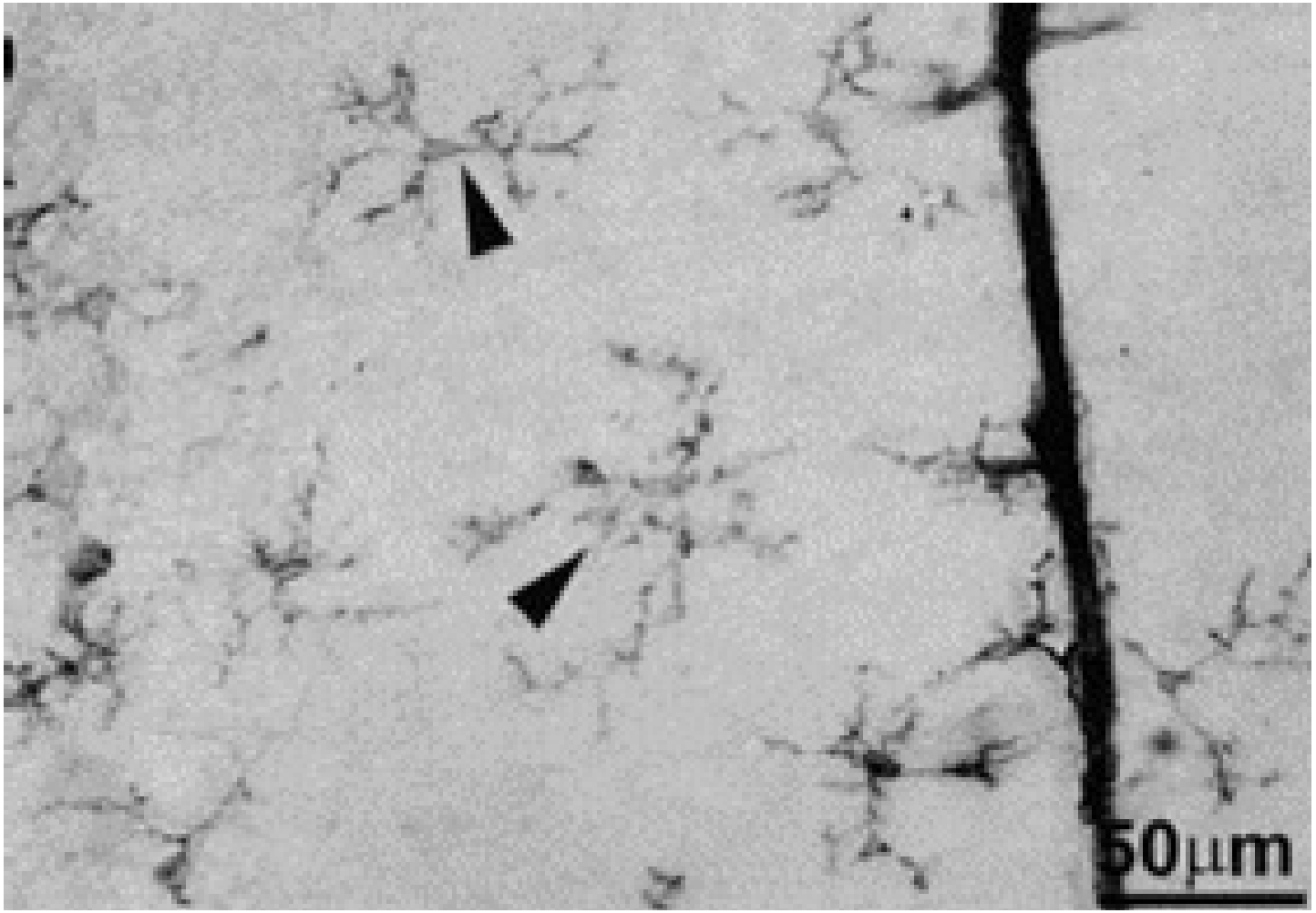
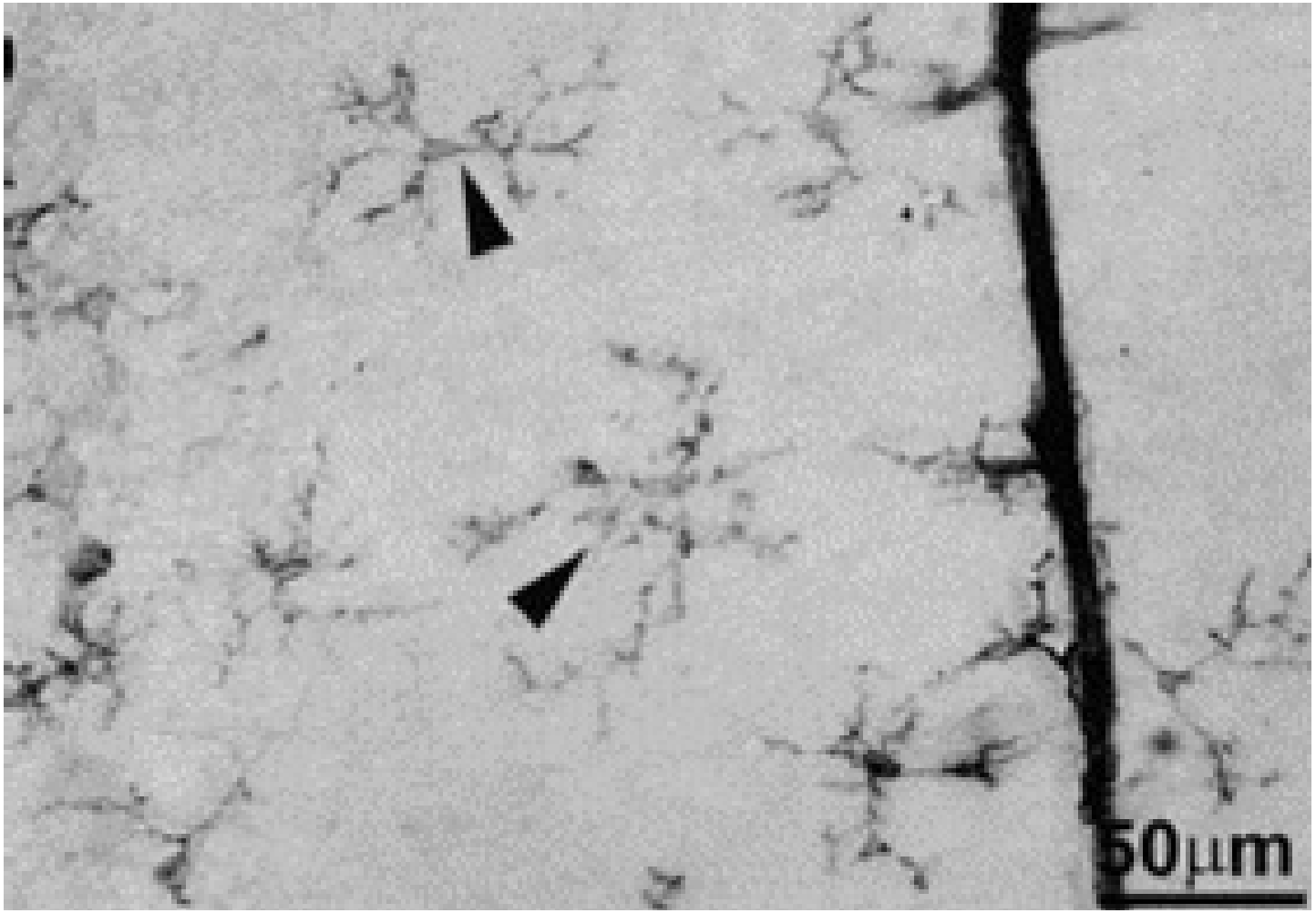

50µm

Microglia: OX-42



Microglia (as opposed to Macroglia=astrocytes, oligod.)

- Professional macrophages
- Microglia derive from bone marrow monocyte lineage, they are not of neuroectodermal origin, like all macroglia
- Chief mediators of immune responses in brain
- CNS is not completely isolated from immune reactions
- Have phenotypic markers similar to tissue macrophages:
  - CD68, HAM-56, IL-1alpha,beta, class II MHC, OX-42

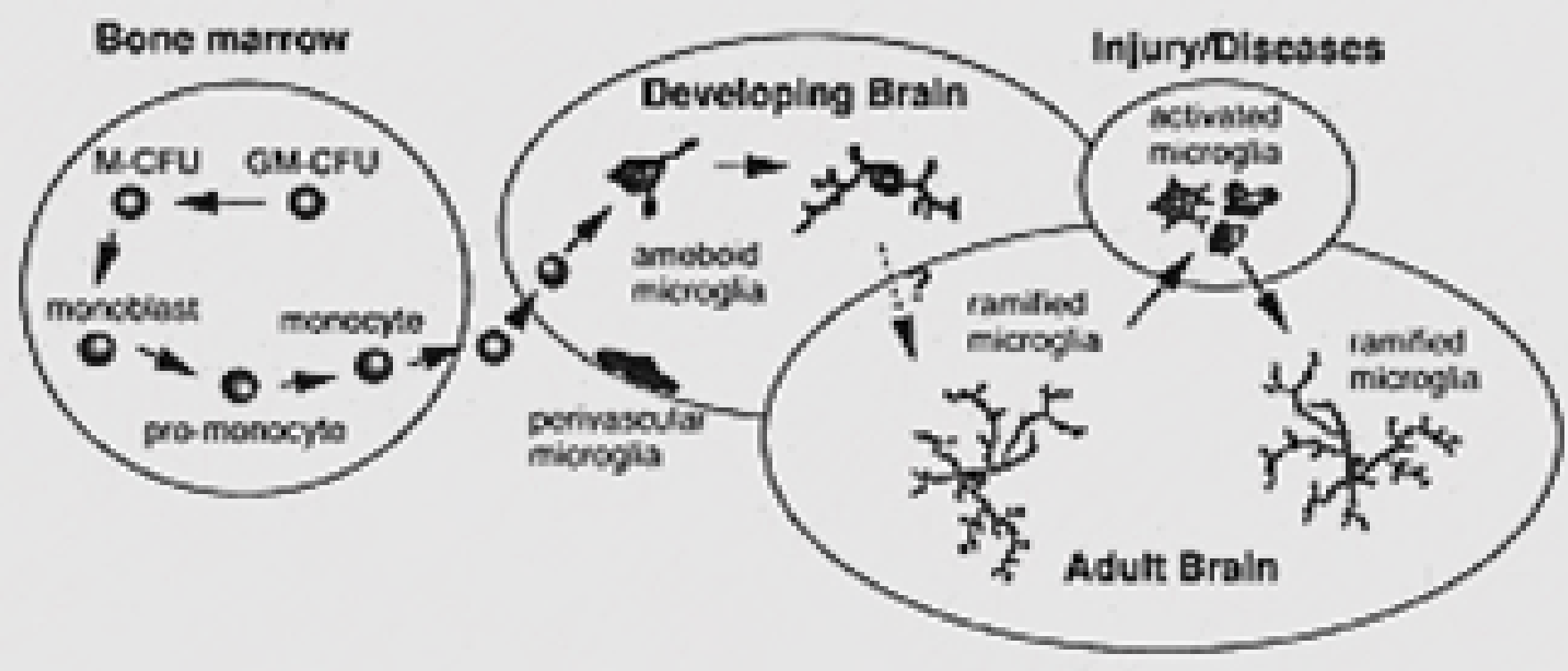
Microglia

- Most roles for microglia in context of CNS pathology; less known yet about normal functions
- Normal functions during development: phagocytosis of apoptotic neurons; secretion of factors.
- Other functions: spines and synapse pruning (plasticity)
- Activated microglia can produce and secrete cytokines capable of activating astrocytes: e.g. IL-1; some think microglia are the primary sensors of CNS damage.

LIKE BODYGUARDS:

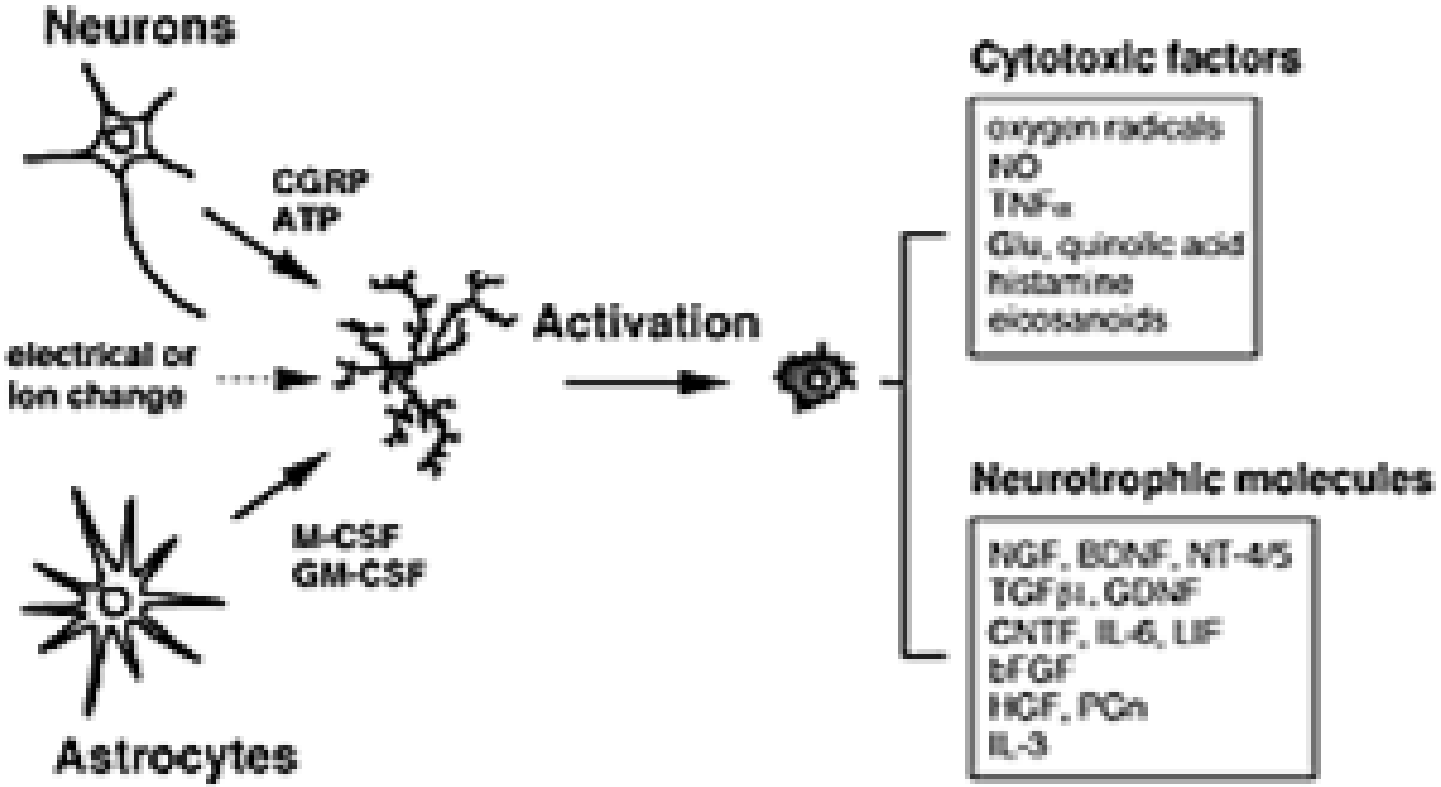
THEY JUST SIT THERE WAITING FOR AN INSULT

Microgliogenesis



How do we know this is true?

Microglial activation

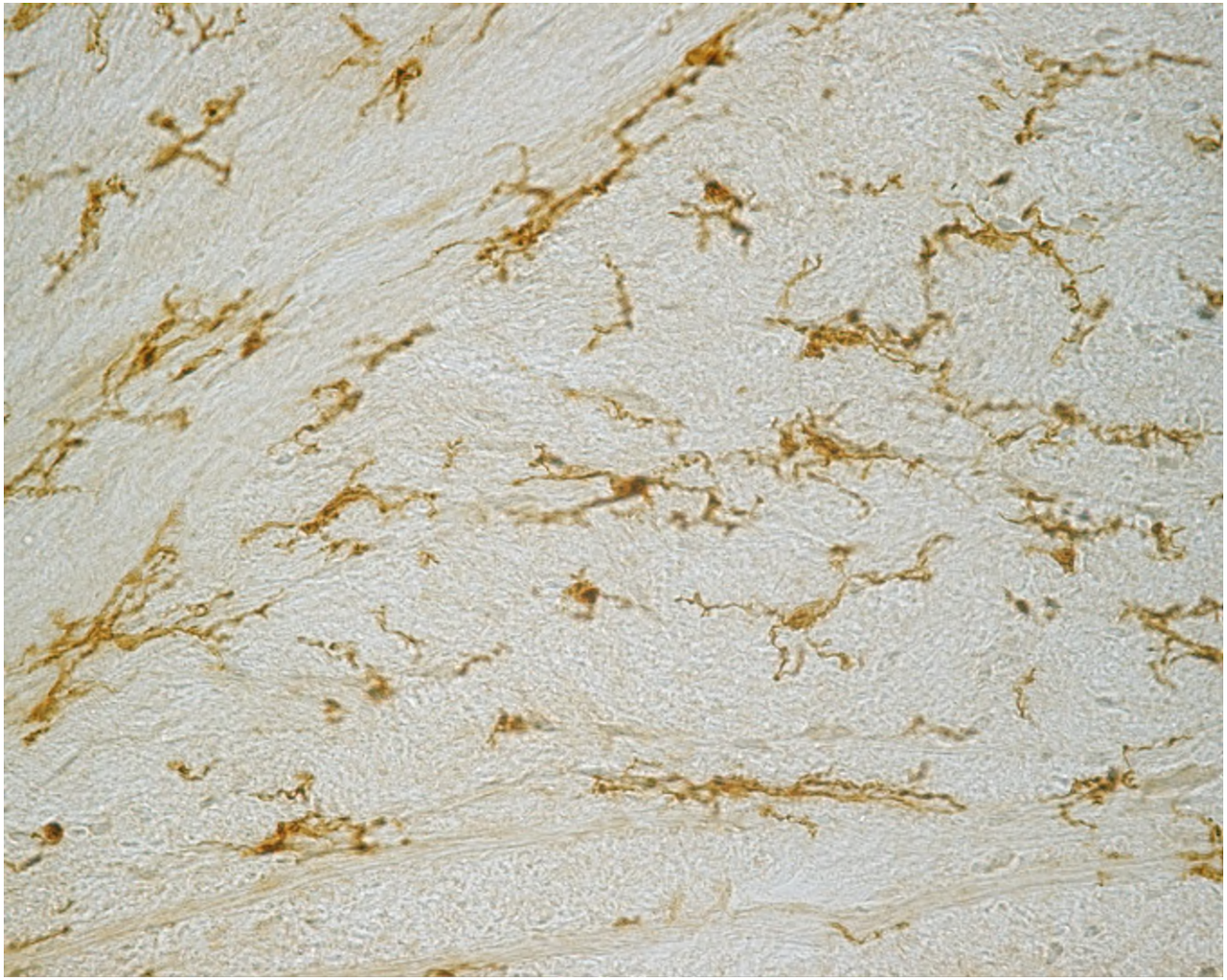


An impressive anagram…

Neurological diseases =
Loses glia. A cure? No! Dies.

Anonymous WWW author

Microglia



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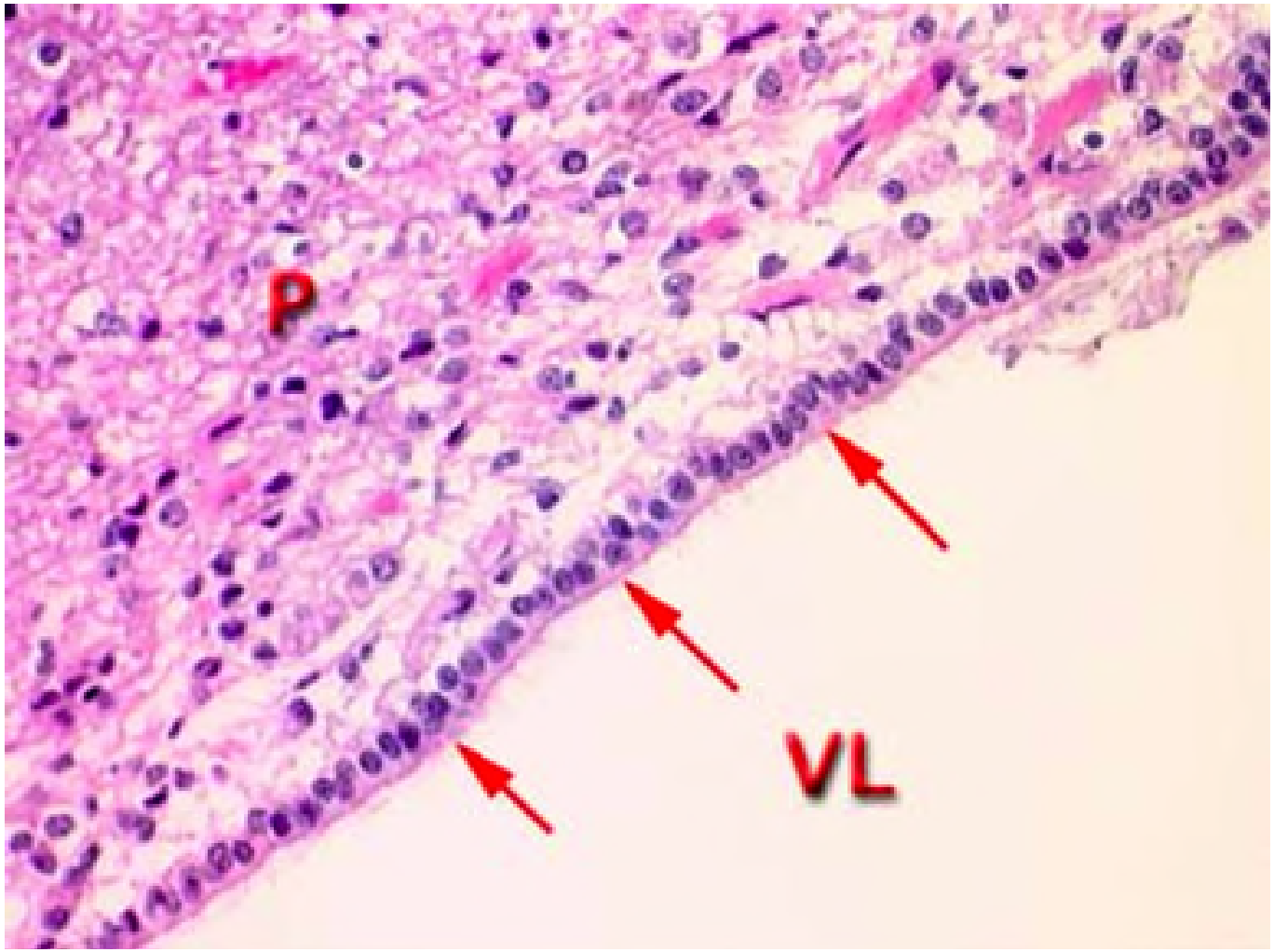
P
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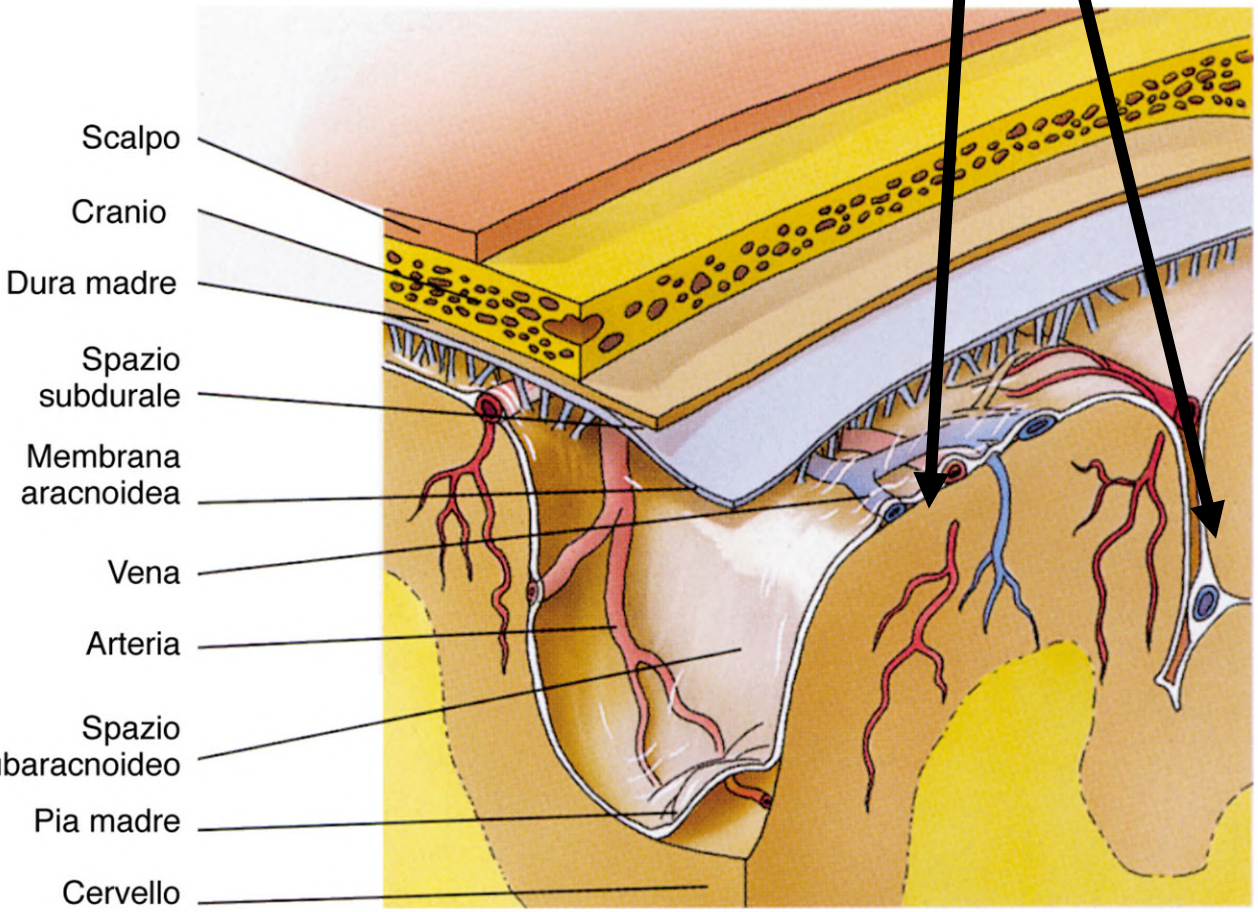
Ependymal cells



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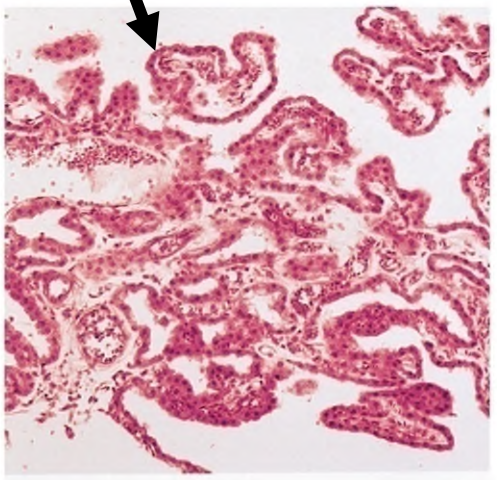


Figura 9–28

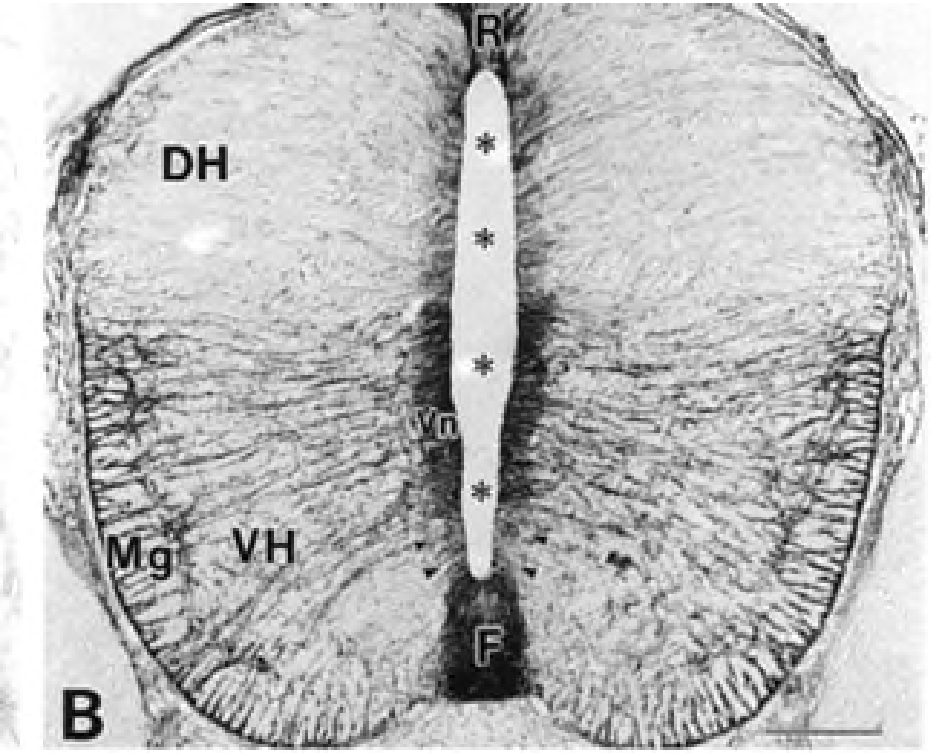
Chroiod plexus

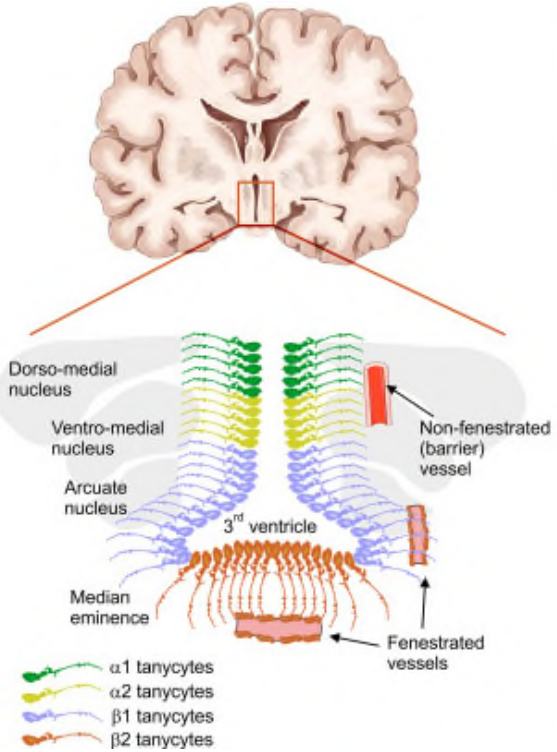
Ependymal cells

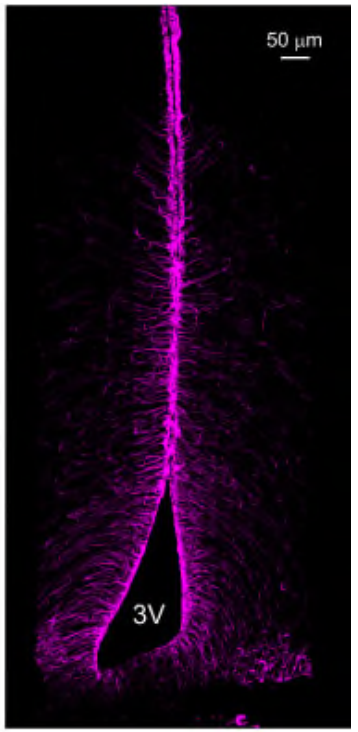
- Line ventricles of brain and spinal cord canal
- Ciliated, columnar epithelium, with cilia and adherens junctions; but express glial markers
- May extend cytoplasmic processes into brain parenchyma
- Recent controversy as to whether Ependymal cells (versus subependymal astrocytes) are adult neural stem cells (resolved: NCS derive from astrocytes)

Radial glia

- Embryonic scaffold throughout CNS
- Guides for radial migration of neurons
- Produce matrix and adhesion proteins
- In adult hypothalamus: Tanycytes – sensitive to photoperiod, glucose conc. Leptin/ghrelin

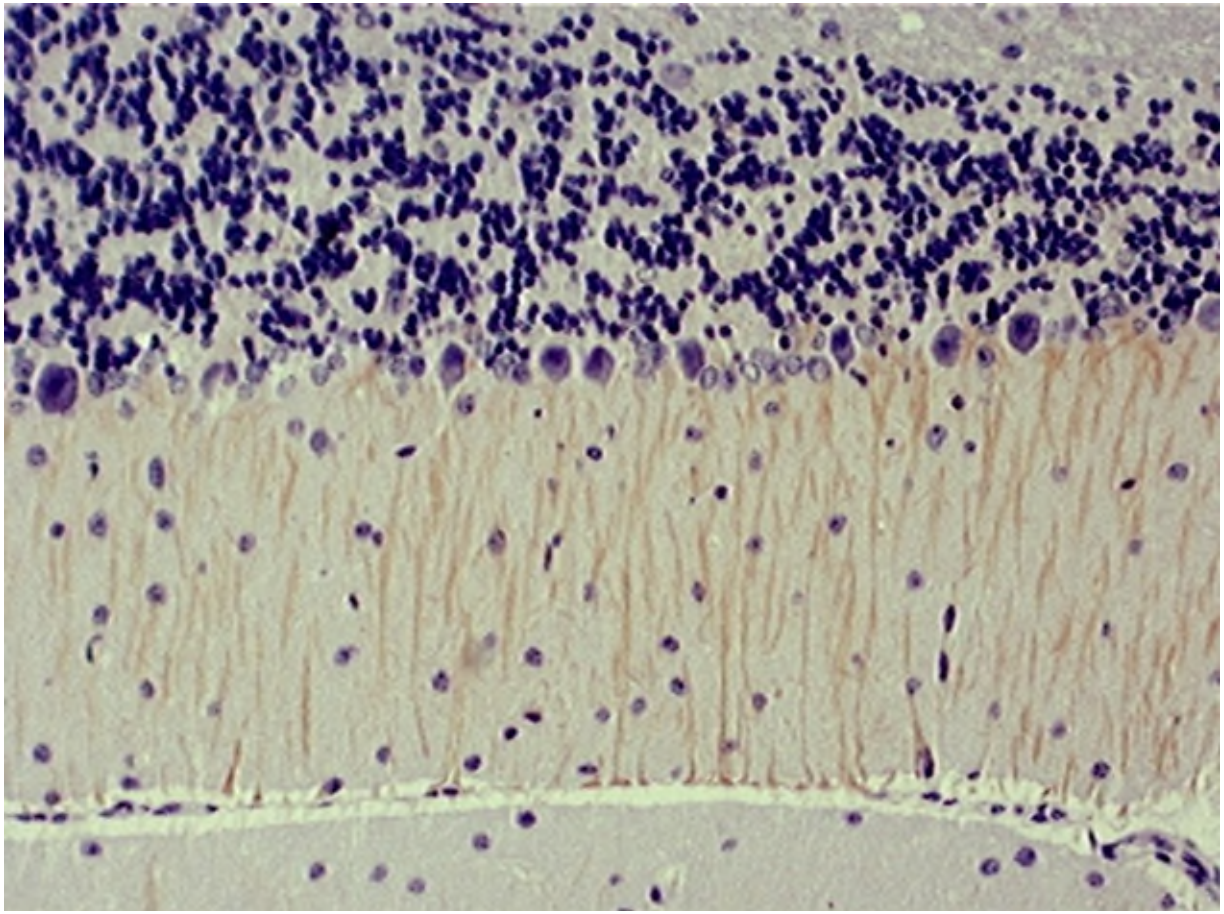






Radial glia

- Adult: radial glia persist in cerebellum (Bergmann glia) and in retina (Muller cells)



GFAP

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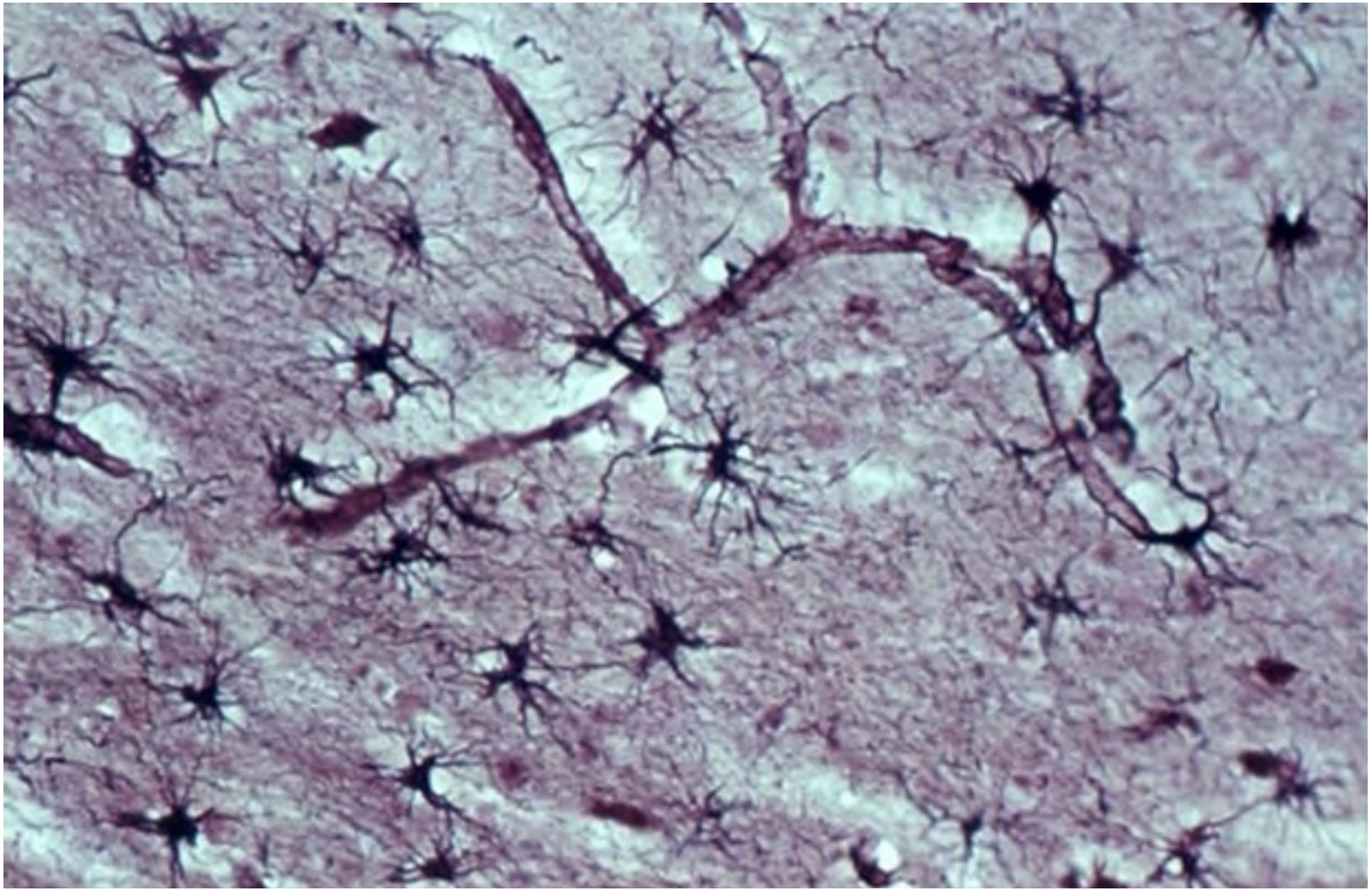
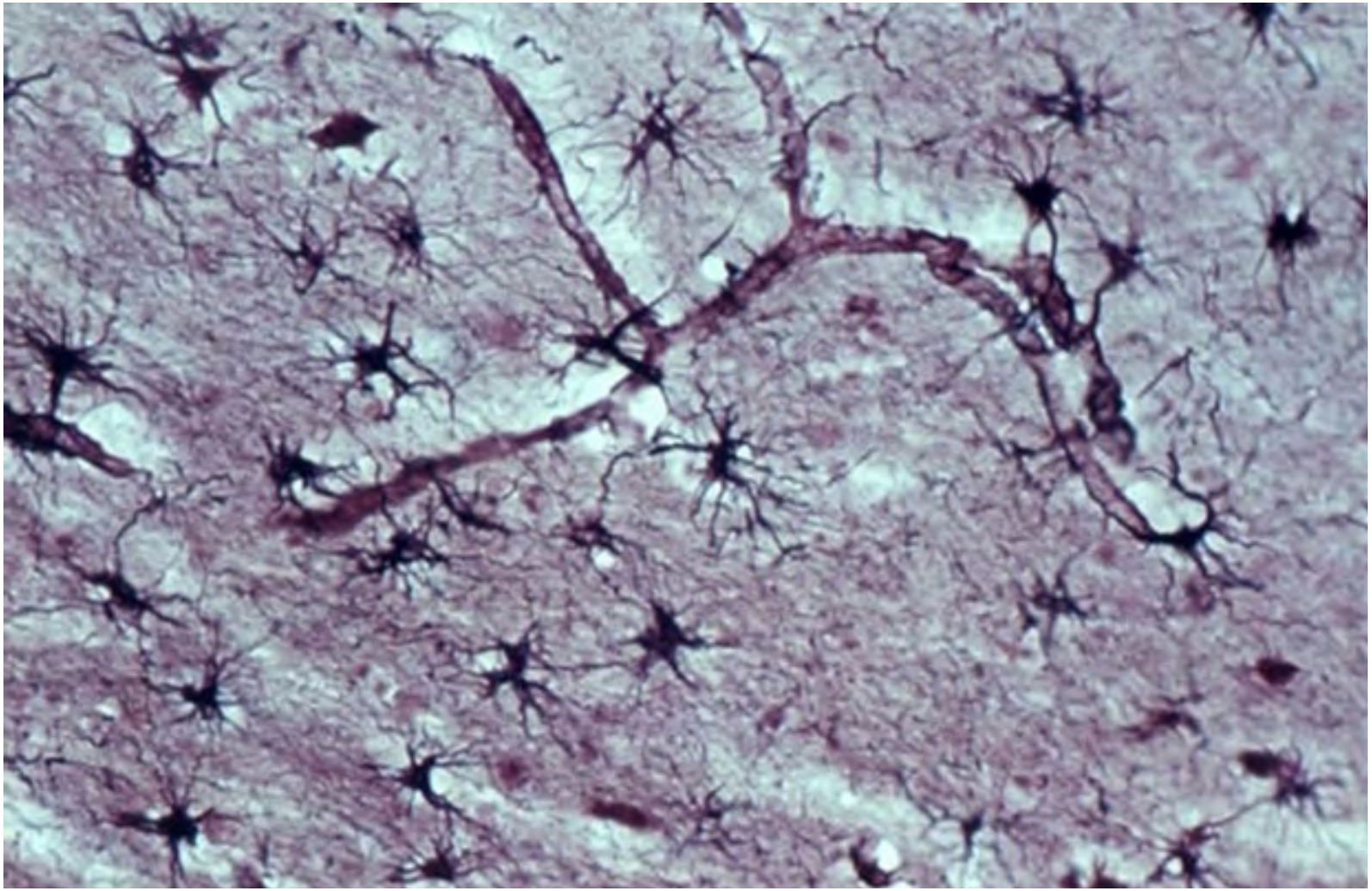
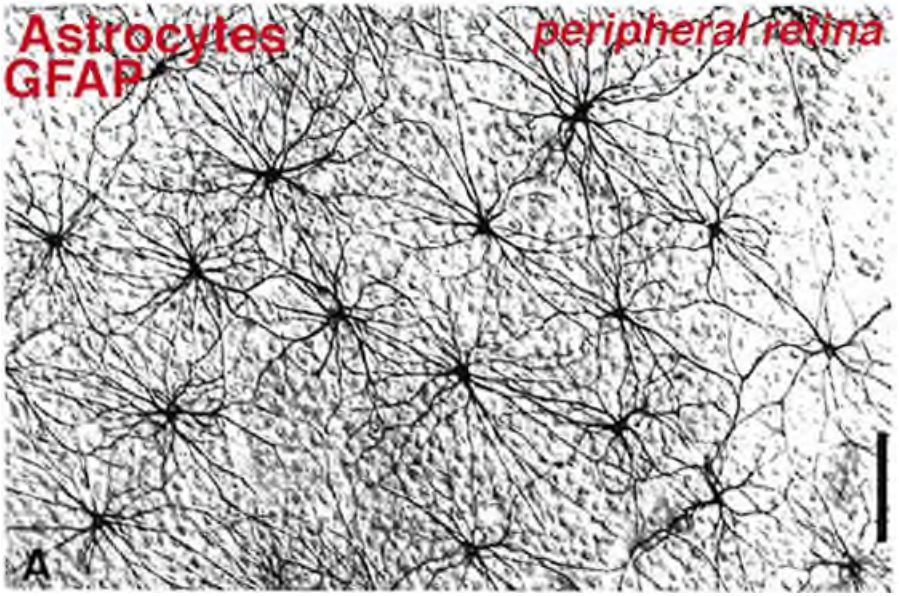


Astrocyte/capillary interactions: foot process



Astrocytes (astroglia) “star-cells”

Most numerous cell type in brain
Constitute ~30-50% of brain volume



NORMAL FUNCTIONS

Developmental: Migrational and Axon guidance of neurons

Homeostasis of neuronal microenvironment

- **Ionic**
- **Metabolic**
- **Neurotransmitter uptake**

Blood-Brain barrier: induction and maintenance

Trophic support of neurons (growth factors)

Synaptogenesis and synaptic remodeling

Two types of astrocytes

-**Protoplasmic (pedunculated) in the grey matter**

- fibrous in the white matter

Fibrous astrocyte in the cerebellum white matter

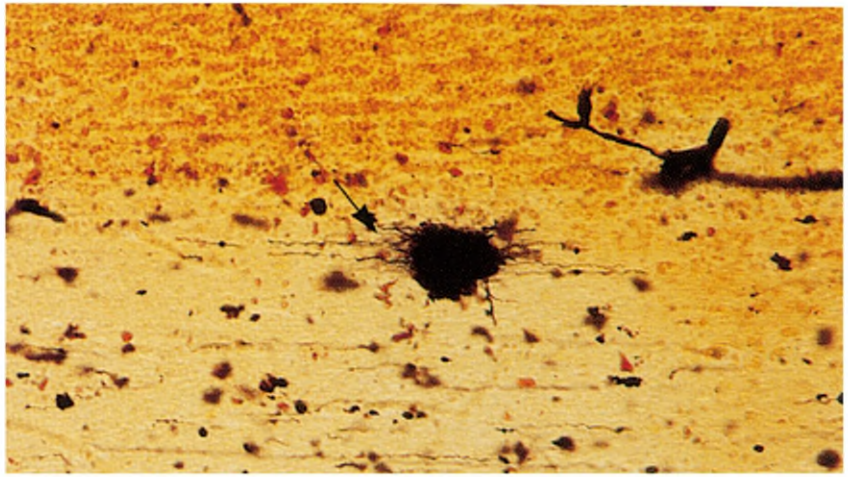
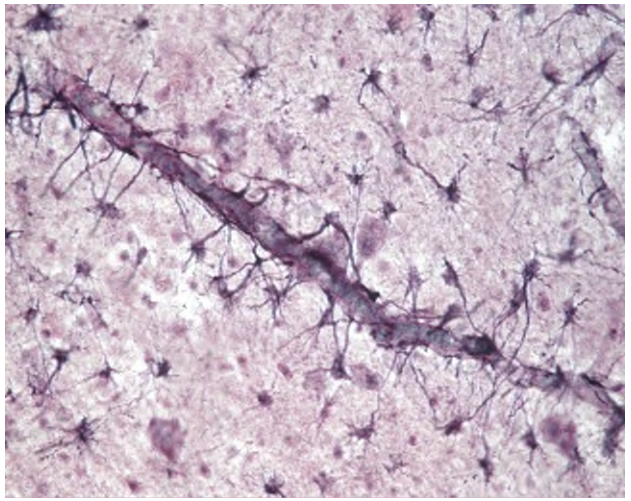


Figure 9–11

Pedunculated astrocytes



Astrocytes

- Astrocytes produce growth factors/neurotrophic factors (NGF, BDNF, GDNF, CNTF, FGFs), especially in development and in regenerative responses to injury
- Buffer extracellular space to maintain homeostasis for neuronal function
- K+ spatial buffering
- Protect neurons from excitoxicity: active glutamate uptake/conversion to glutamine (cycled back to neurons)
- Release gliotransmitters: glutamate, ATP and D-serine, which regulate neuronal excitability and synaptic transmission

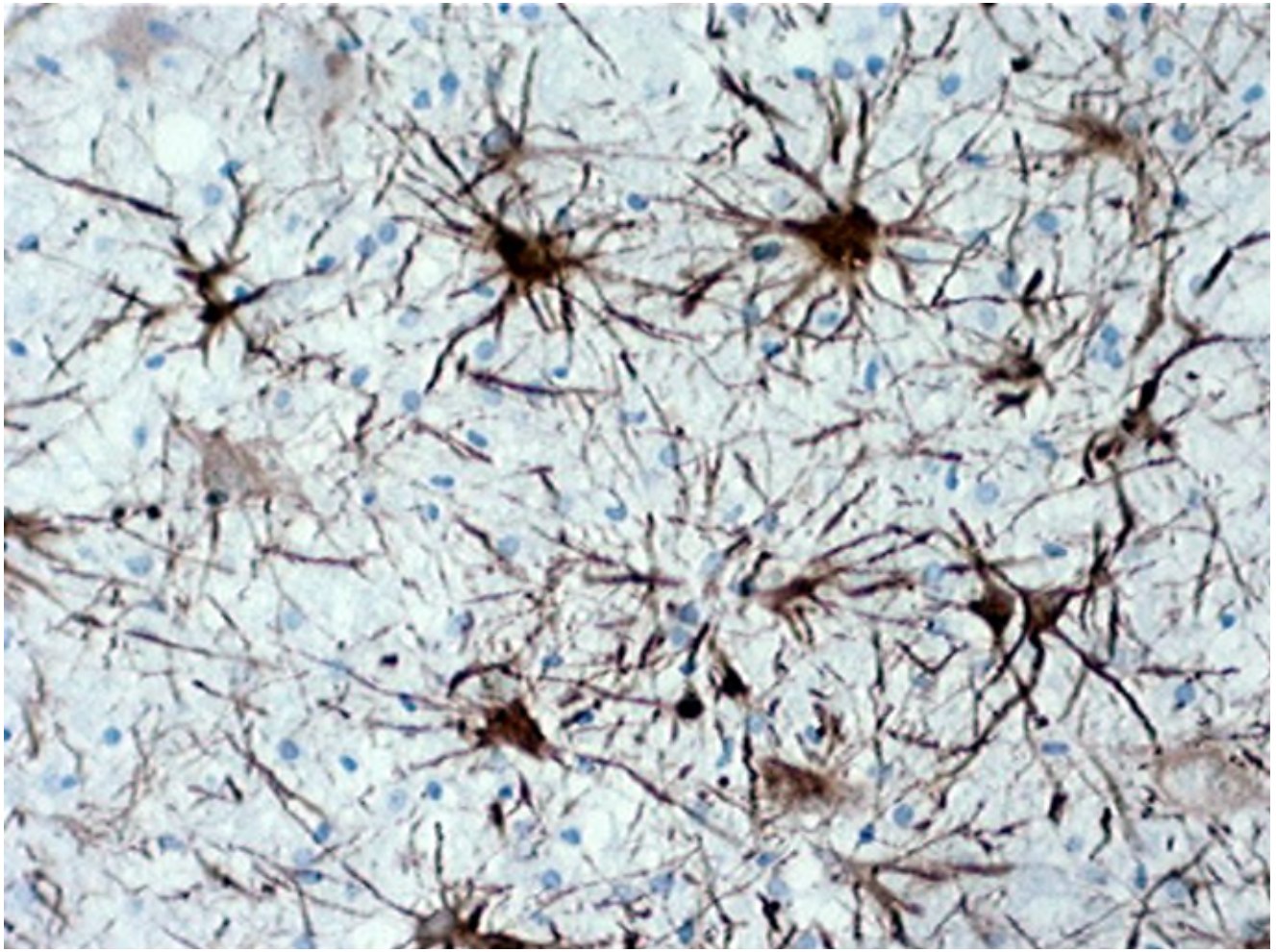
Astrocytes

- Astrocytes contact virtually every cell component in brain
  - Other astrocytes (gap junctions)
  - Ependymal cells
  - Neurons (somas, processes, synapses)
  - Oligodendroglia
  - Capillary endothelial cells

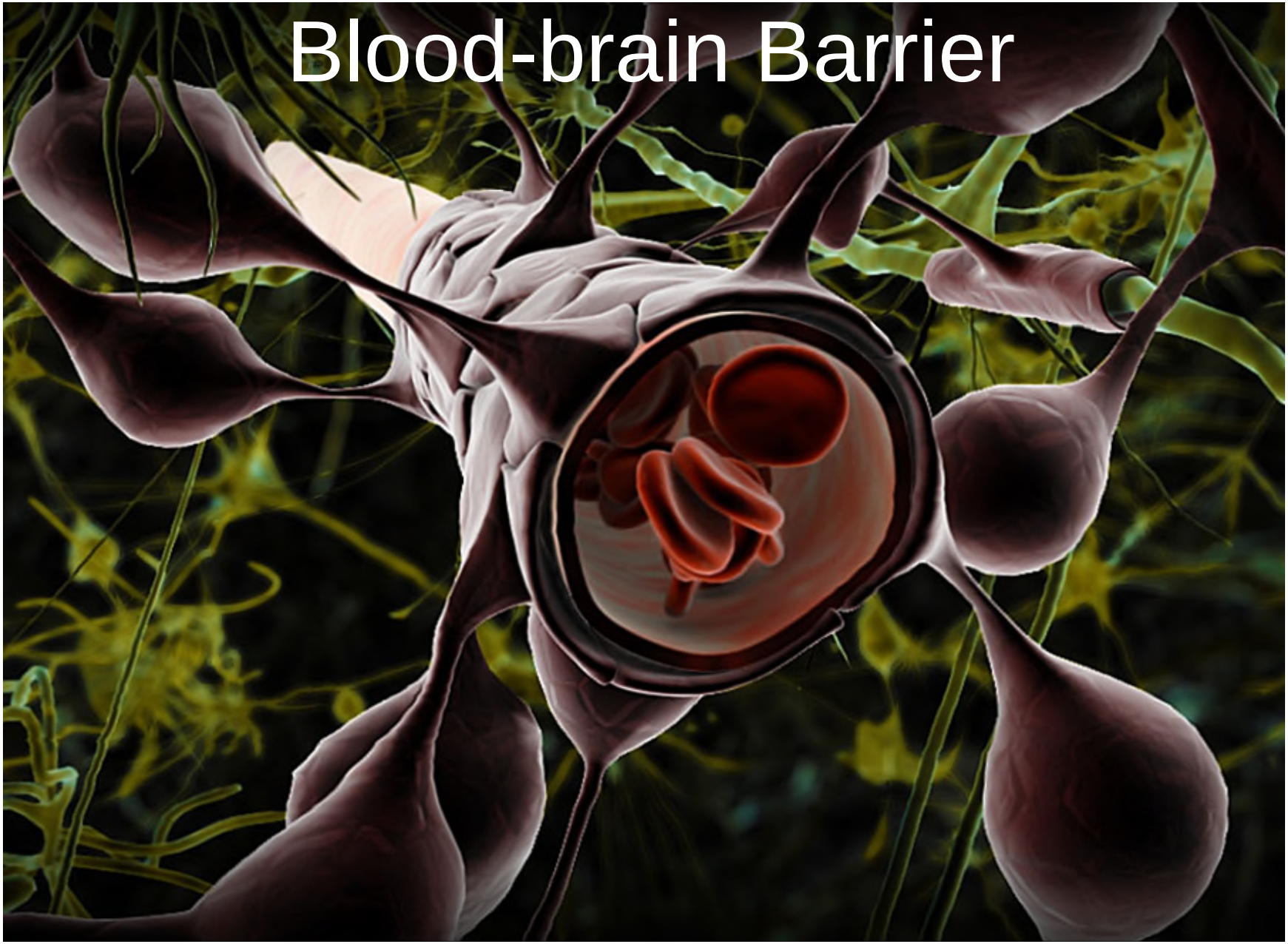
Astrocytes' reactivity: gliosis

- Astrocytosis/gliosis: response of astrocytes to many forms of injury: trauma, inflammation, MS, infection, neurodegeneration
- Classical description of gliosis is hypertrophy, glial filament production +/- proliferation.
- Reality: there must be many distinct forms of astrocyte activation; hundreds or thousands of distinct changes in gene expression

Reactive astrocytes (gliosis)



Blood-brain Barrier



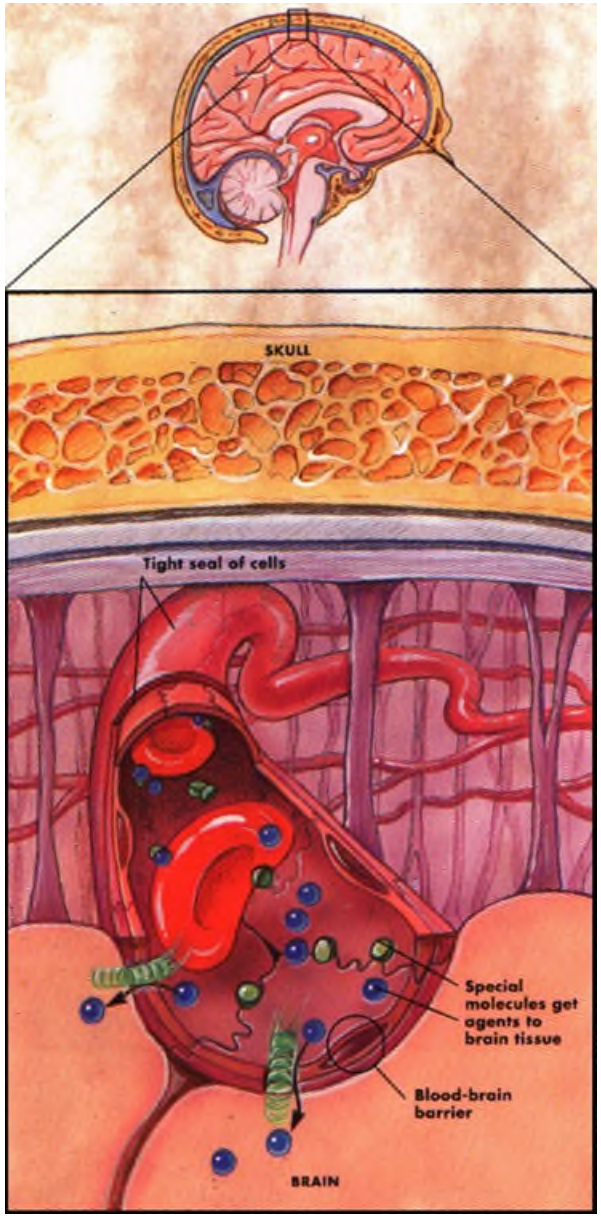
Cerebral vasculature

- High O2 Demand by brain:
- Disproportionately high: 2% of body mass but 20% of O2
- Aerobic metabolism (no energy stores) requires constant blood supply for oxygen and glucose
- cerebral function continues only 10 seconds after ischemia
- Irreversible damage after 6-8 minutes of ischemia
- Demand changes with neuronal activity

- **Ischemia**: decreased blood flow causing temporary or permanent loss of neural function
- **Hypoxia**: oxygen deficiency
- **Infarction**: tissue necrosis due to prolonged ischemia/hypoxia

Cerebral vasculature

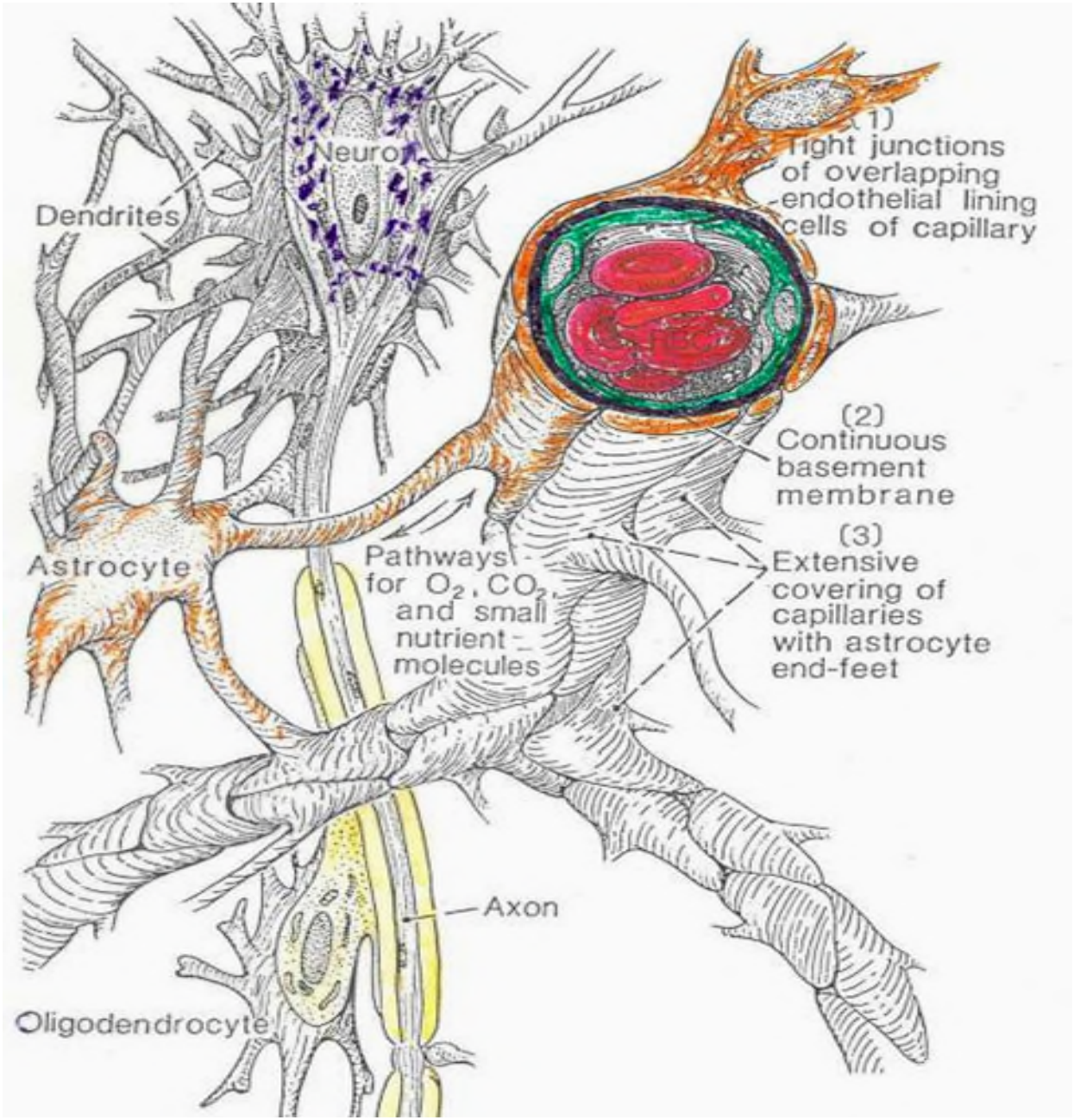
- **Autoregulation (works down to 50 mmHg)**
- Vasculature responds both to pressure and neuronal activity
- **Blood-Brain Barrier (primarily formed by tight junctions between capillary endothelial cells; help from pericytes and astrocyte foot processes)**
- Breaching of BBB due to ischemic damage to endothelium -->edema-->mass effect->herniation



Discovery of the BBB

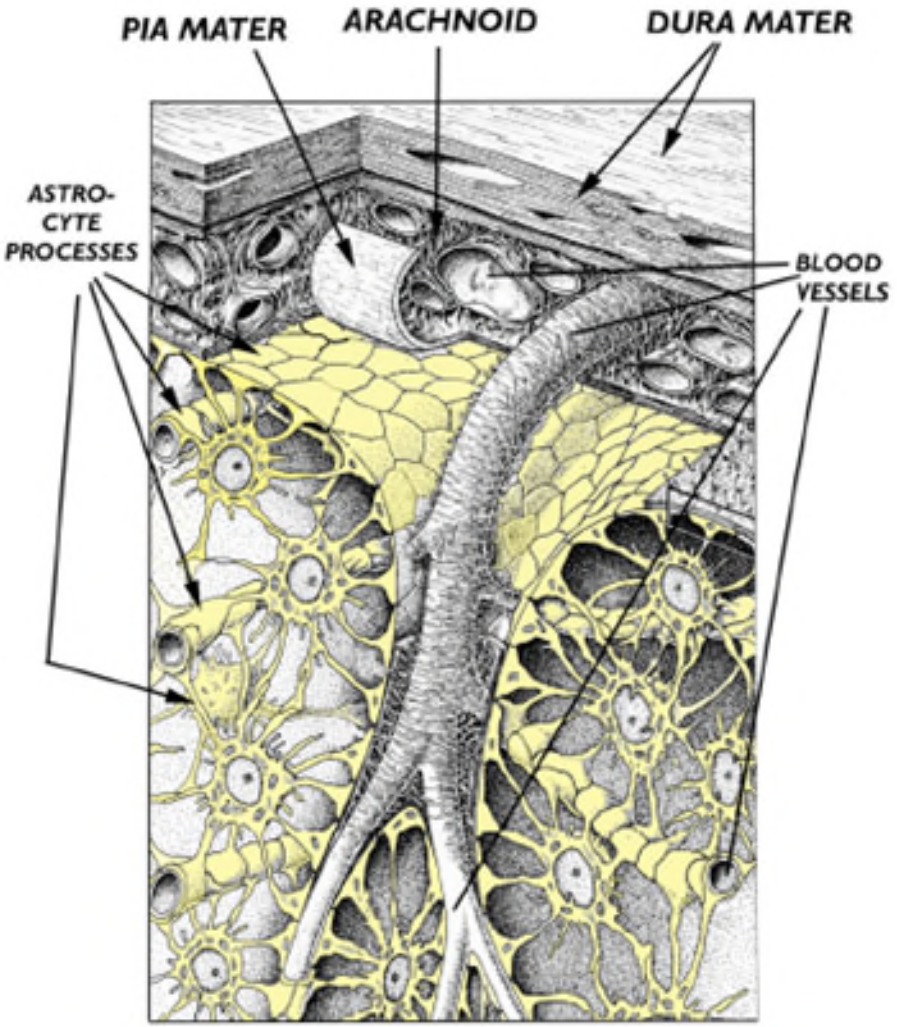
- 1885: Paul Ehrlich discovered that when a particular blue dye was injected into the blood-stream of an animal, tissues of the whole body EXCEPT the brain and spinal cord turned blue.
- To explain this phenomenon, the investigators suggested that a ***"Blood-Brain-Barrier"*** prevented some materials from leaving the brain capillaries and actually entering the brain tissue.

Blood-brain Barrier



THE "GLIA LIMITANS"

- Blood-brain barrier has a structural & physiological basis
  - Astrocytes
  - Spatial buffering of ions
  - Guarding of entry to "brain stuff"



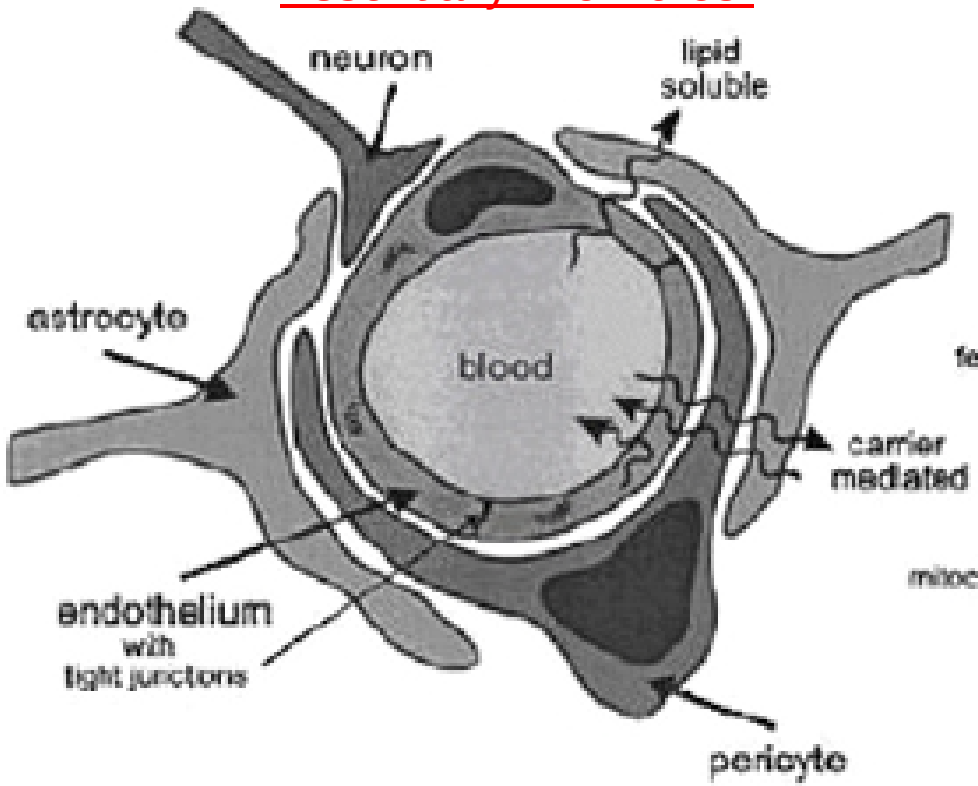
RELATIONSHIP OF ASTROCYTES, BLOOD VESSELS, & MENINGES

What is the Blood Brain Barrier?

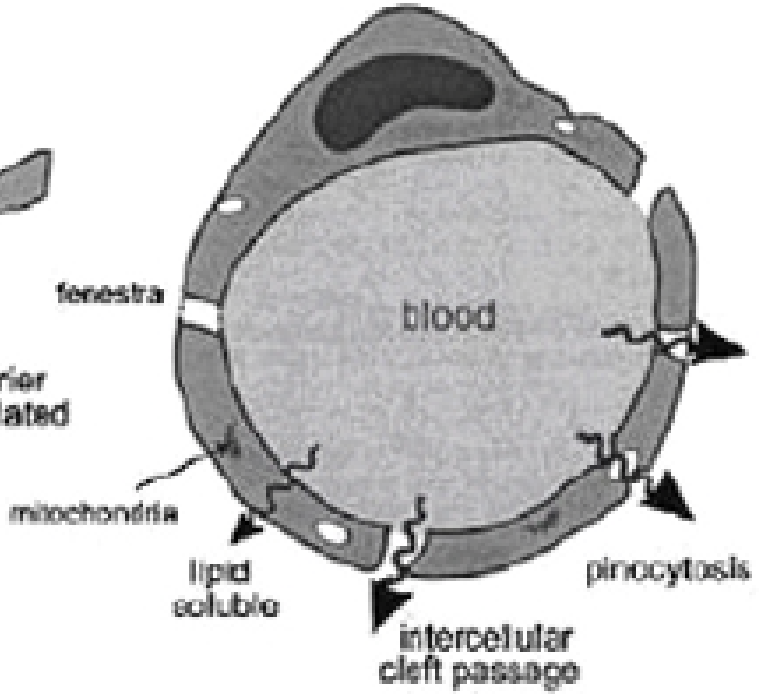
- Structural and functional barrier which impedes and regulates the influx of most compounds from blood to brain
- Formed by brain microvascular endothelial cells (BMEC), astrocyte end feet and pericytes
- Essential for normal function of CNS
- Regulates passage of molecules in and out of brain to maintain neural environment.
- Responsible for metabolic activities such as the metabolism of L-dopa to regulate its concentration in the brain.

Side-by-Side Comparison
Brain & Somatic Capillaries

Brain Capillary with BBB
Essentially "No Pores"



Somatic Capillary, Many Pores



Structure of Blood Brain Barrier

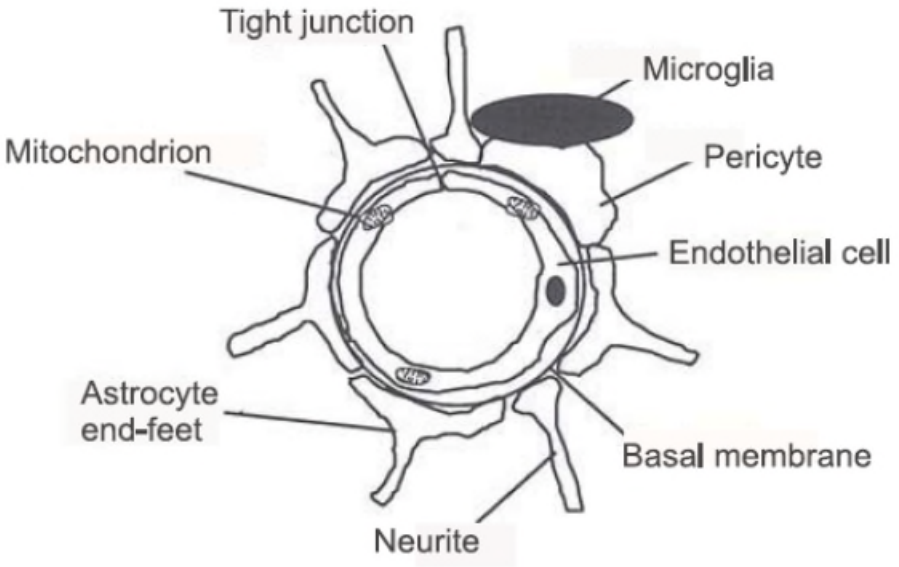


Figure 2: The blood-brain barrier: Morphology of the capillary epithelium in the region of the central nervous system and surrounding tissue.

Source: Bock et al

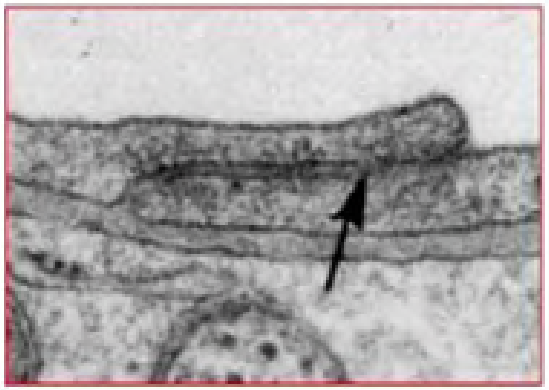
Differences between BMEC and normal endothelial cells

- Structural differences:
  - Absence of fenestrations
  - More extensive tight junctions (TJ)
- Functional differences:
  - Impermeable to most substances
  - Sparse pinocytic vesicular transport
  - Increased expression of transport and carrier proteins: receptor mediated endocytosis
  - No gap junctions, only tight junctions
  - Limited paracellular and transcellular transport

Integrity of BBB

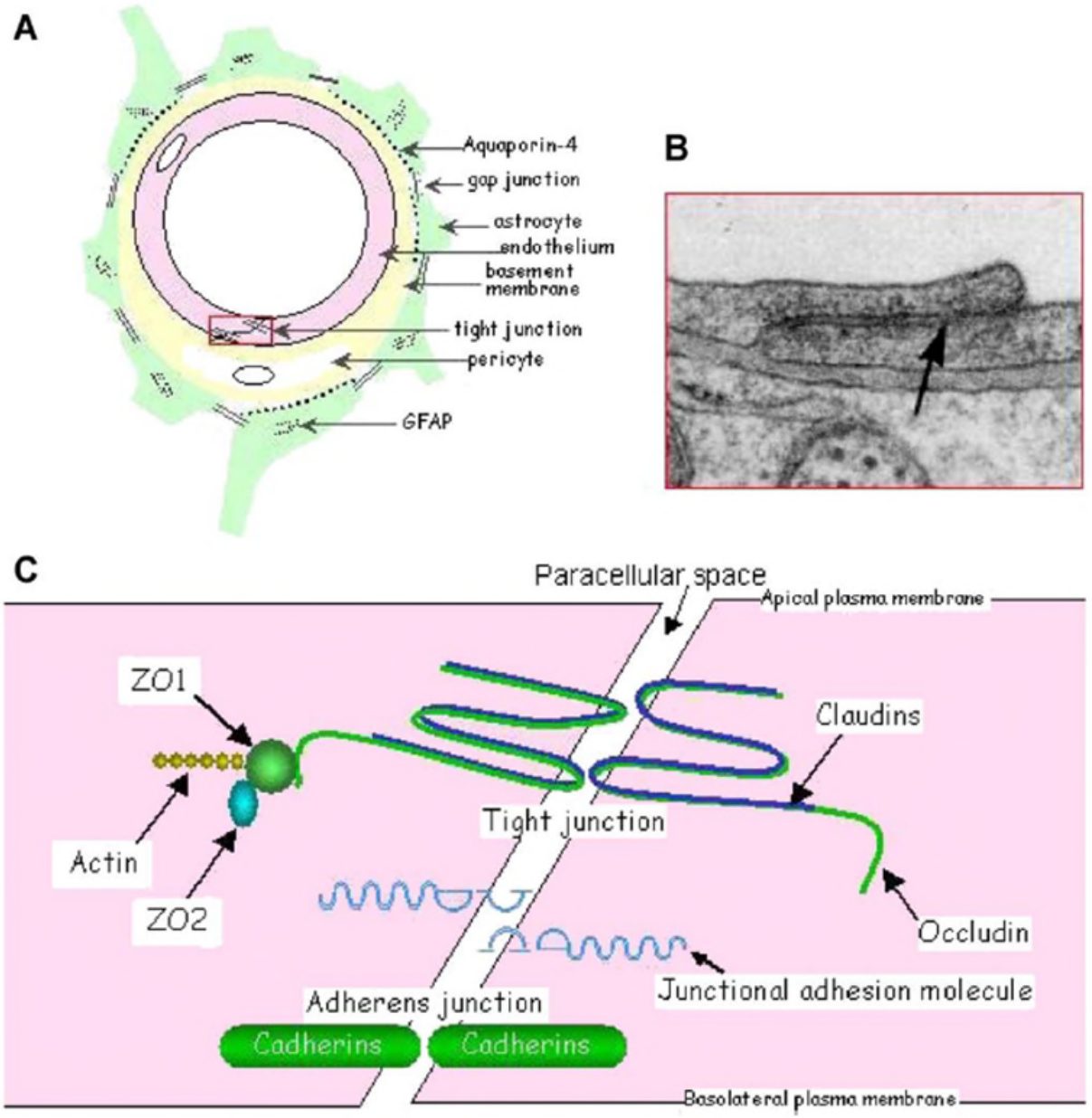
- Tight Junctions
- Adherens Junctions
- Pericytes
- Astrocyte end feet

Tight Junctions between BMEC



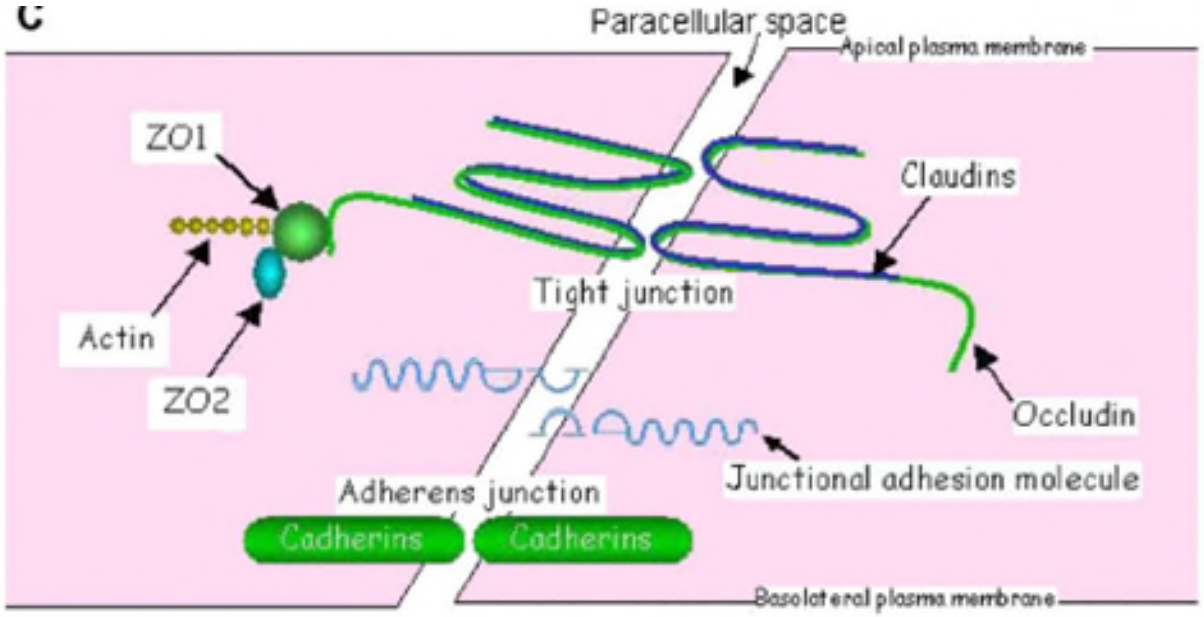
Source: Ballabh et al

- Appear at sites of apparent fusion between outer leaflets of plasma membrane of endothelial cells
- Continuous
- Anastomosing
- Intramenbranous strands or fibrils on P face with complementary groove on E face
- Protein components:
  - Claudin
  - Occludin
  - Junction Adhesion Molecules
  - Accessory proteins


A
Aquaporin-4
gap junction
astrocyte
endothelium
basement membrane
tight junction
pericyte
GFAP
B
C
Paracellular space
Apical plasma membrane
ZO1
Claudins
Tight junction
Actin
ZO2
Occludin
Junctional adhesion molecule
Adherens junction
Cadherins
Cadherins
Basolateral plasma membrane

Claudin

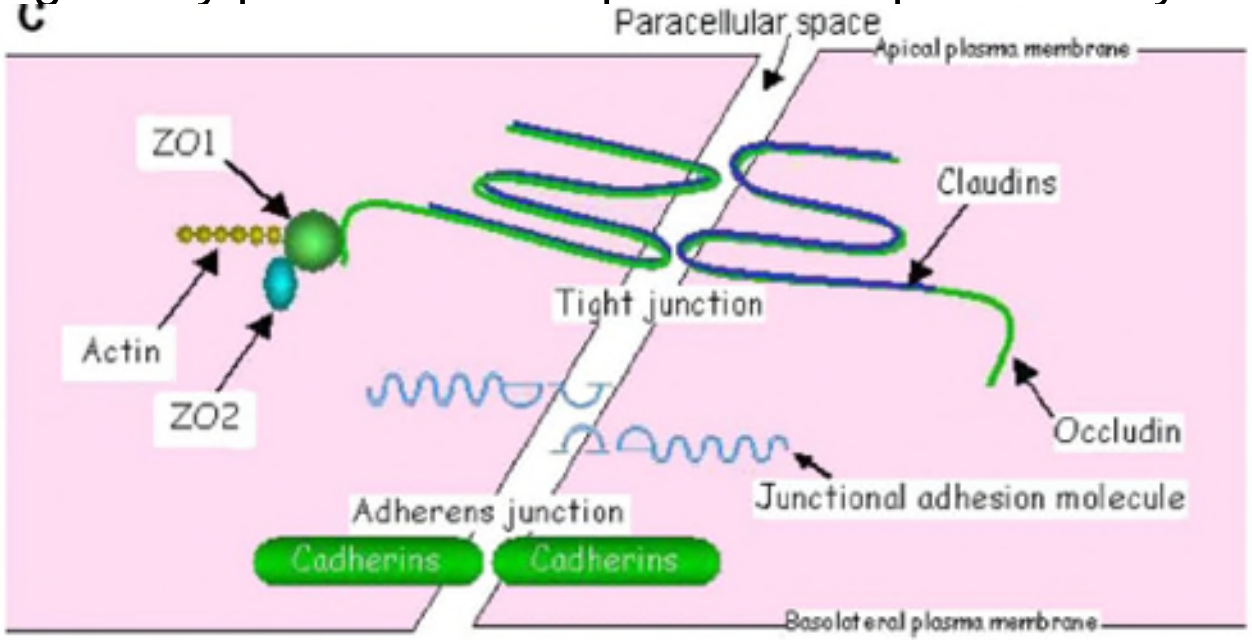
- 22kDa phosphoprotein
- 4 transmembrane domains
- localized in TJ strands



Source: Ballabh et al

Occludin

- 65kDa phosphoprotein,
- 1° structure very different from claudin
- Regulatory proteins: alters paracellular permeability.



Source: Ballabh et al

Barrier Function of Occludin and Claudin

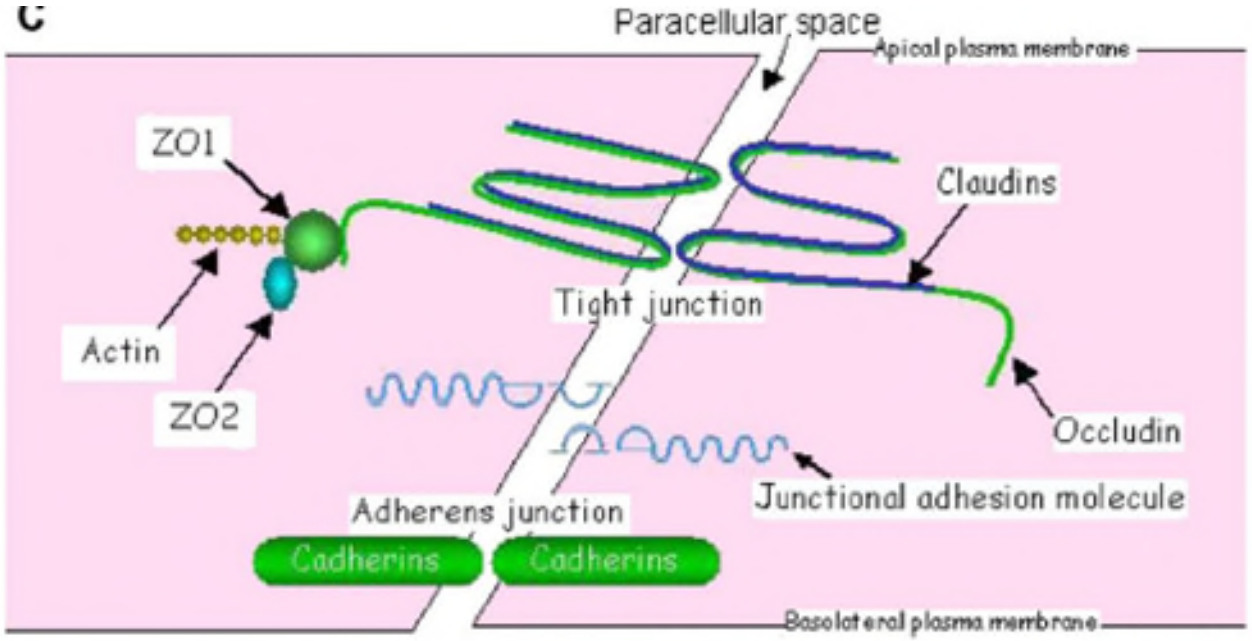
- Assemble into heteropolymers and form intramembranous strands which contain channels allowing selective diffusion of ions and hydrophilic molecules.

- Breakdown of BBB in tissue surrounding brain tumors occurs with concomitant loss of 55kDa occludin expression

Junction Adhesion Molecules:

- 40kDa
- Integral membrane protein, single transmembrane region
- Belongs to immunoglobulin superfamily
- Localizes at tight junctions
- Involved in cell-to-cell adhesion and monocyte transmigration through BBB
- Regulates paracellular permeability and leukocyte migration
- Also found on circulating leukocytes, platelets and lymphoid organs.

BMEC intercellular space



Source: Ballabh et al

Barrier function of JAM

- **Homotypic** binding between JAM molecules on adjacent endothelial cells acts as a barrier for circulating leukocytes
- **Heterotypic** binding of endothelial JAM to leukocyte JAM might guide transmigration of leukocytes across interendothelial junctions
- So factors that decrease leukocyte migration must either strengthen homotypic interactions or weaken heterotypic interactions.

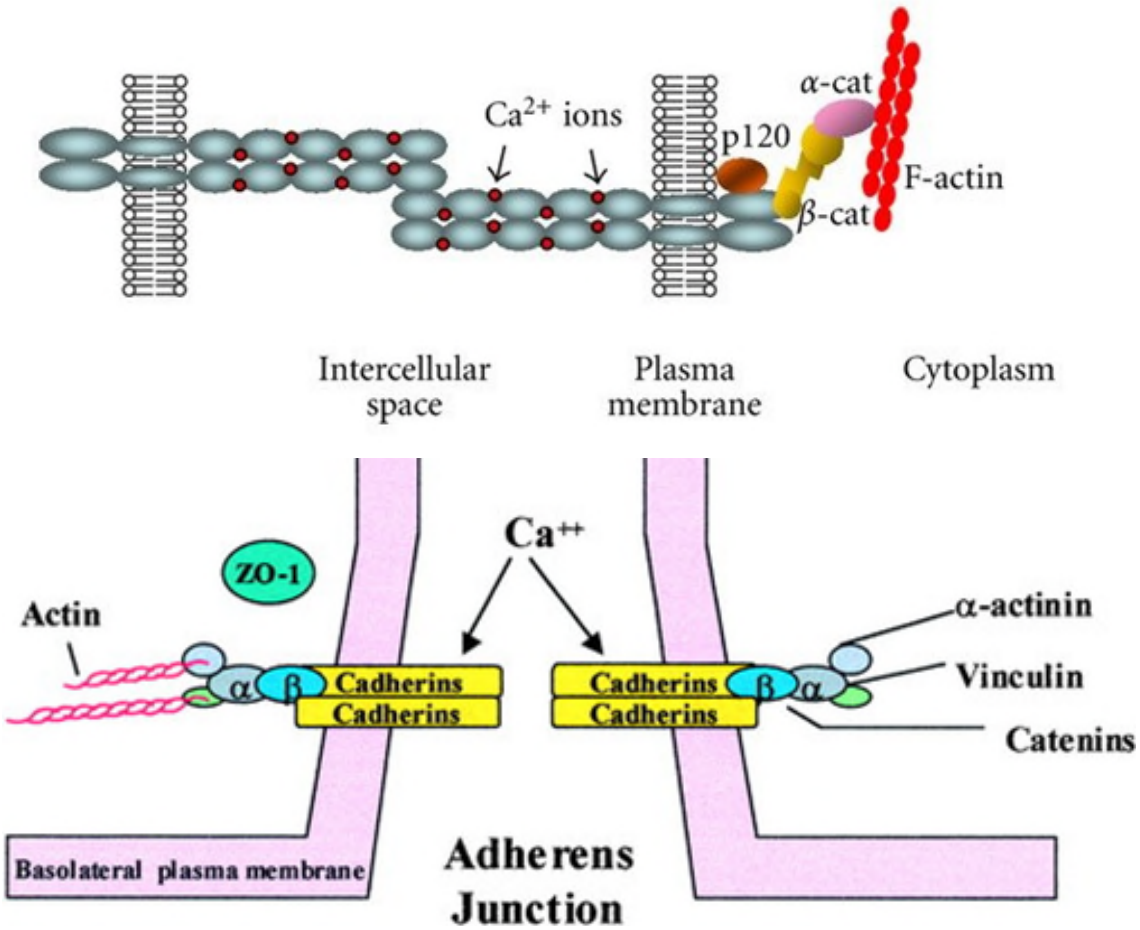
Cytoplasmic accessory proteins

- (ZO-1, ZO-2, ZO-3, cingulin etc)
  - These link membrane proteins to actin
  - maintenance of structural and functional integrity of endothelium
  - crosslink transmembrane proteins.
- Membrane associated guanylate kinase-like proteins (MAGUKS)
  - subunits function as protein binding molecules
  - role in organization the plasma membrane

Adherens Junction

- Complex between membrane protein cadherin and intermediary proteins called catenins
- Cadherin-catenin complex joins to actin cytoskeleton
- Form adhesive contacts between cells.
- Assemble via homophilic interactions between extracellular domains of calcium ion dependent cadherins on surface of adjacent cells

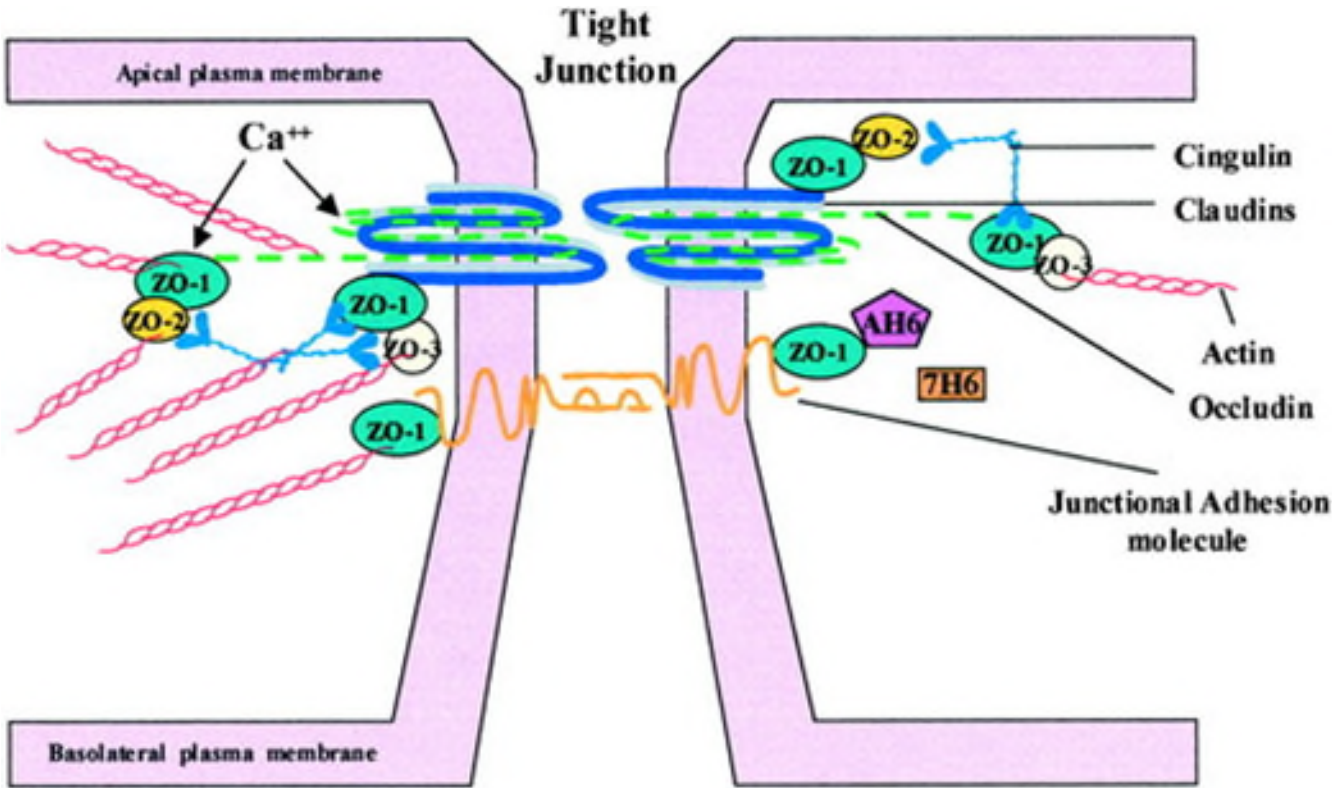
Calcium Modulation of Adherens Junction and Tight Junction



Low-calcium media results in AJ dissociation mediated by protein kinase A (PKA), causing redistribution of E-cadherin and ZO-1.29 Low levels of calcium disrupt AJ in endothelial cells, presumably by removing calcium from binding sites on E-cadherin extracellular domains

Rachel C. Brown. Stroke. Calcium Modulation of Adherens and Tight Junction Function, Volume: 33, Issue: 6, Pages: 1706-1711, DOI: (10.1161/01.STR.0000016405.06729.83)

Calcium Modulation of Adherens Junction and Tight Junction



In calcium-deficient media endothelial cells lose membrane association of ZO-1, ZO-2, and occludin thus, decreasing transepithelial resistance, and increasing permeability. Association of ZO-1, ZO-2, and occludin with actin is not disrupted.

Rachel C. Brown. Stroke. Calcium Modulation of Adherens and Tight Junction Function. Volume: 33. Issue: 6. Pages: 1706-1711. DOI: (10.1161/01.STR.0000016405.06729.83)

Pericytes:

- Cells of microvessels including capillaries, venules, and arterioles that wrap around endothelial cells.
- Provide structural support and vasodynamic capacity to microvasculature.
- Role in structural stability of vessel wall
- Endothelial cells associated with pericytes are more resistant to apoptosis than isolated endothelial cells
  - Indicates role of PC in structural integrity and genesis of the BBB
- Phagocytic activity

Astrocyte end feet

- Star shaped glial cells
- Provides biochemical support for BMEC
- Influence of morphogenesis and organization of vessel wall
- Factors released by astrocytes involved in postnatal maturation of BBB
- Direct contact between endothelial cells and astrocytes necessary to generate the BBB (Rubin et al, 1991)
- Co-regulate function by the secretion of soluble cytokines such as (LIF, leukemia inhibiting factor), Ca^{2+} dependent signals by intracellular IP-3 and gap junction dependent pathways, and second messenger pathways involving extracellular diffusion of purinergic messenger.

Regions of brain not enclosed by BBB

- Circumventricular organs
  - area postrema,
  - median eminence,
  - neurohypophysis,
  - pineal gland,
  - subfornical organ and
  - lamina terminalis

  These are regions which need to respond to factors present in systemic circulation

Normal BBB transport

- Diffusion
- Facilitated transport by carrier systems
- Receptor mediated endocytosis
- Paracellular transfer more common than transcellular transfer

Facilitated transport

- Carrier systems
  - particular essential amino acids, glucose, these are extremely specific
    - transport D-glucose only,
    - large neutral amino acids which act as precursors for neurotransmitters,
    - only which the brain cannot make,
    - glycine: it can block the transmission of nerve signals, hence special carrier which ensures that glycine can be removed from brain
- Receptor mediated endocytosis
  - Leptin, insulin, overlaps with carrier systems

Substances that Cross BBB

- blood gases (O_2, CO_2 , carbon monoxide)
- blood sugars (D-glucose, D-hexose)
- Electrolytes (Na+, K+, Cl-, etc.)
- some amino acids
- small molecule drugs (alcohol, caffeine, nicotine, morphine, heroin, cocaine, etc.)

- *However, large carrier molecules required to deliver medications, cannot cross BBB.*

Solubility BBB & Cell Membranes

Small **fat soluble** molecules more easily cross Phospholipid bi-layers & the Blood-Brain Barrier

- barbiturates
- librium
- valim
- halcion
- xanax
- opium
- heroin
- morphine
- cocaine
- marijuana
- nicotine
- alcohol
- amphetamine
- methamphetamine

Materials that do NOT easily Escape Brain Capillaries and Enter Brain Tissue

- Microorganisms
- Large molecules
- Molecules that are not very lipid soluble
- Molecules with a high electrical charge
- Hormones that work outside the CNS
- T-cells and B-cells of the immune system
- Drugs bound to plasma proteins (99%)

Factors which cause increase in BBB permeability during pathophysiology

- **Factors produced by astrocytes**
  - **Glutamate,**
  - **Aspartate**
  - **Taurine**
  - **ATP**
  - **Endothelin-1**
  - **NO**
  - **MIP-2**
  - **Tumor necrosis factor alpha TNF-α**
  - **Interleukin beta IL-β**
- **Paracrine signals secreted by endothelium cells or nerve terminals of neurons running close to blood vessels**
  - **Bradykin**
  - **5HT**
  - **Histamine**
  - **Thrombin**
  - **UTP**
  - **UMP**
  - **Substance P**
  - **Qionolonic acid**
  - **Platelet activating factor**
- **Free radicals**