ION TRANSPORT MECHANISMS AT THE BLOOD-BRAIN BARRIER AND BLOOD-CSF BARRIERS

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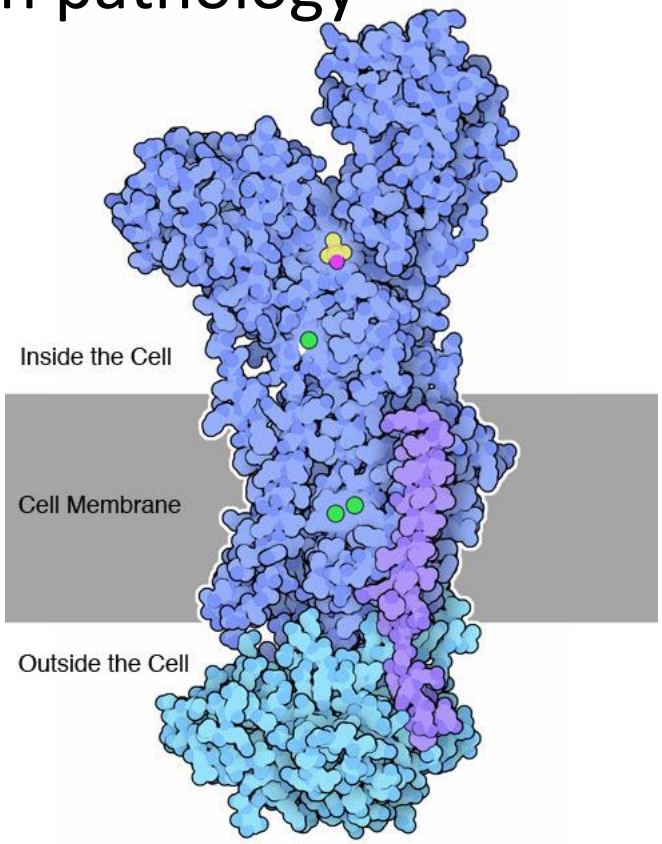
Structure and functions of the brain barriers

Ion transporters at the BBB and BCSFB

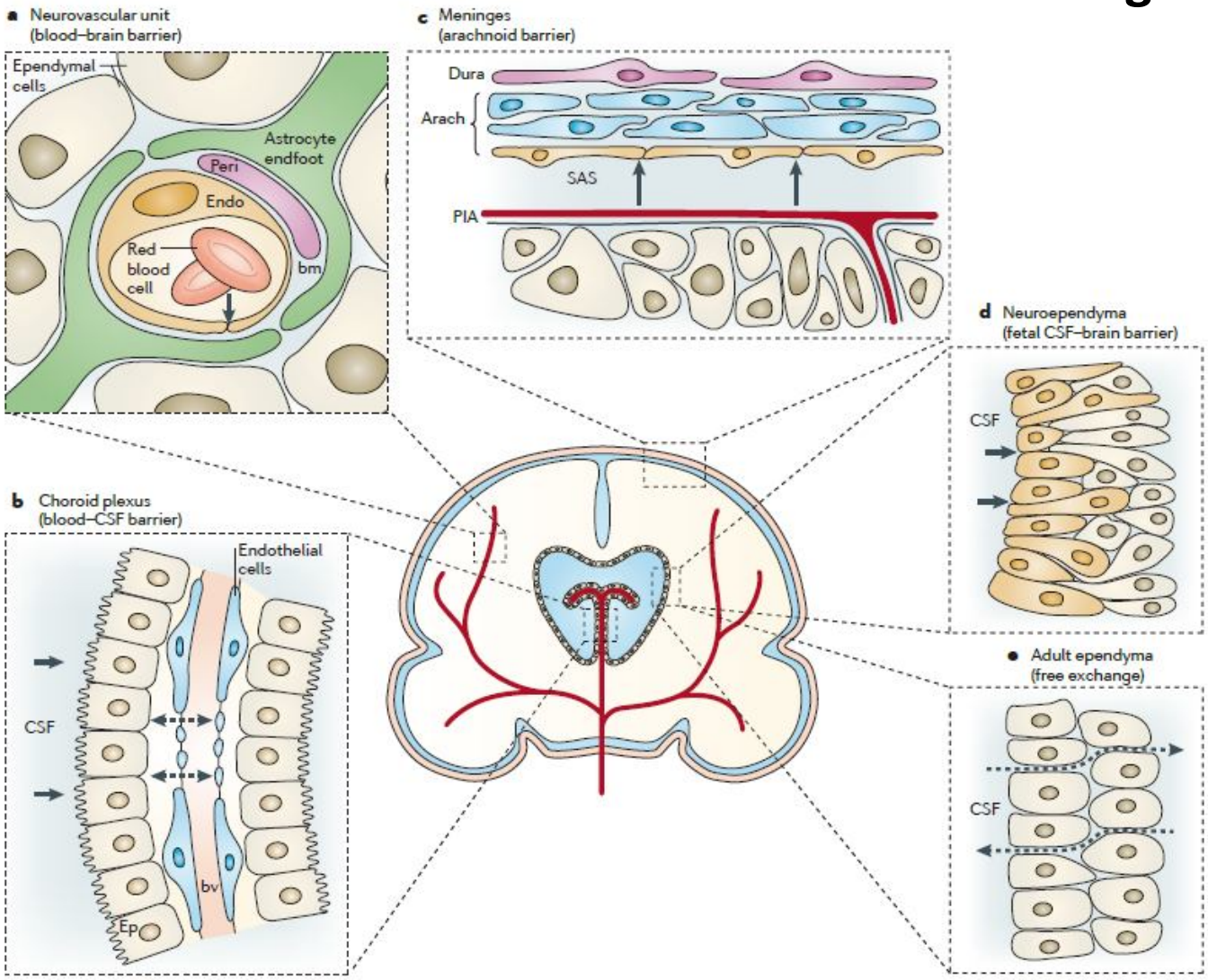
Ion transporters at brain barriers in pathology



BARRIERS OF THE CENTRAL NERVOUS SYSTEM

BBB

Meninges



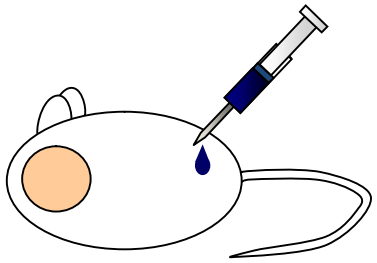
BCSFB/ choroid plexus

Ependyma

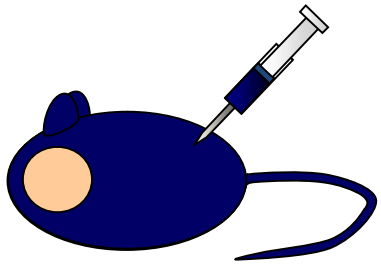
Neuwelt et al., Nature Rev Neurosci, 2011

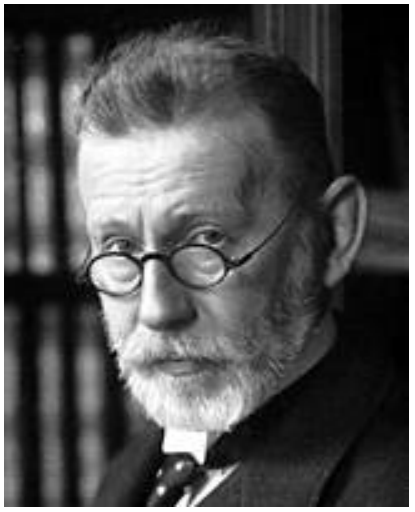
MILESTONES IN BLOOD-BRAIN BARRIER RESEARCH

1885 Paul Ehrlich

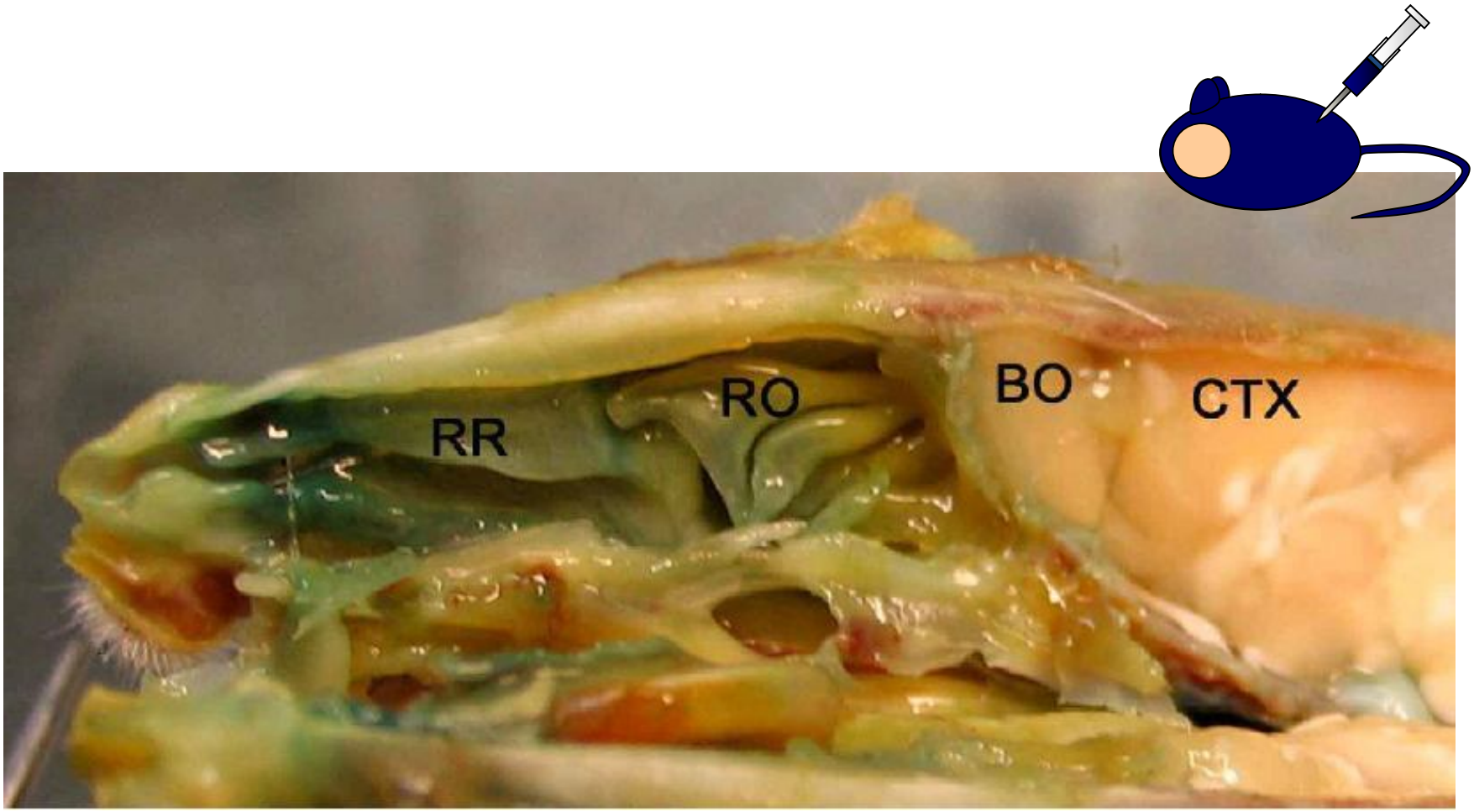


experiments with azo-dyes





PERMEABILITY OF BRAIN AND NASAL BLOOD VESSELS



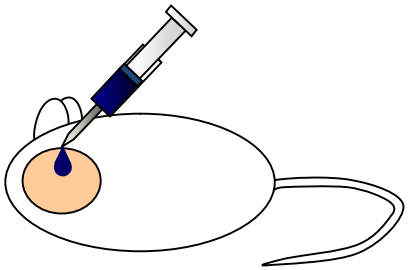
2 % fluorescein + Evans blue dyes injected intravenously
RR: respiratory region, RO: olfactory region, BO: olfactory bulb, CTX: brain cortex

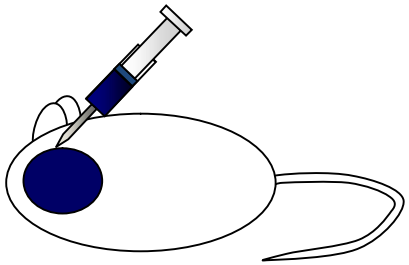
Wolburg et al., Histochem. Cell Biol, 2008

MILESTONES IN BLOOD-BRAIN BARRIER RESEARCH

1913 Edwin Goldmann

dye injected to cerebrospinal fluid
brain capillaries





1967 Reese and Karnovsky

electron microscopy: brain endothelial cells

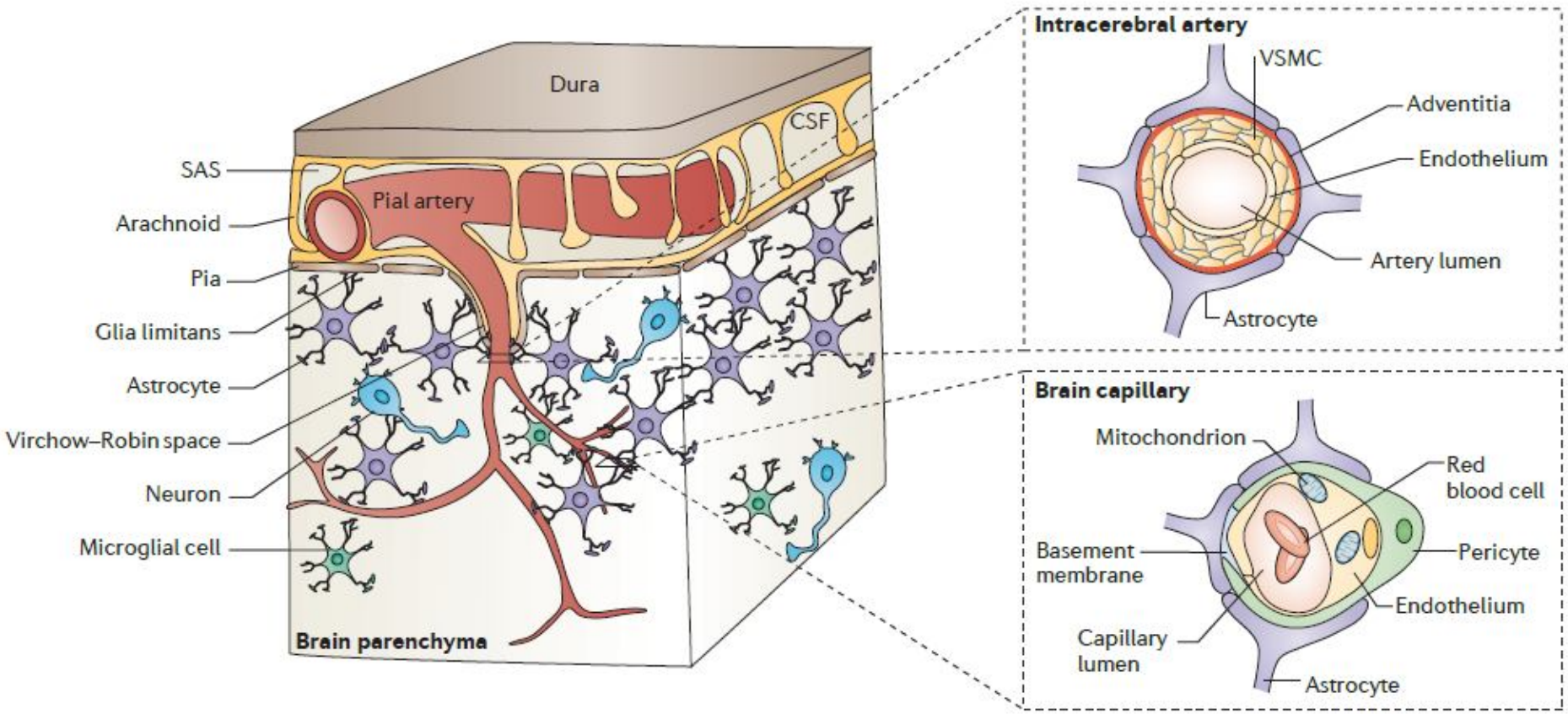
1982 Crone and Olesen

electrical resistance of
brain microvessels

1000-2000 Ω

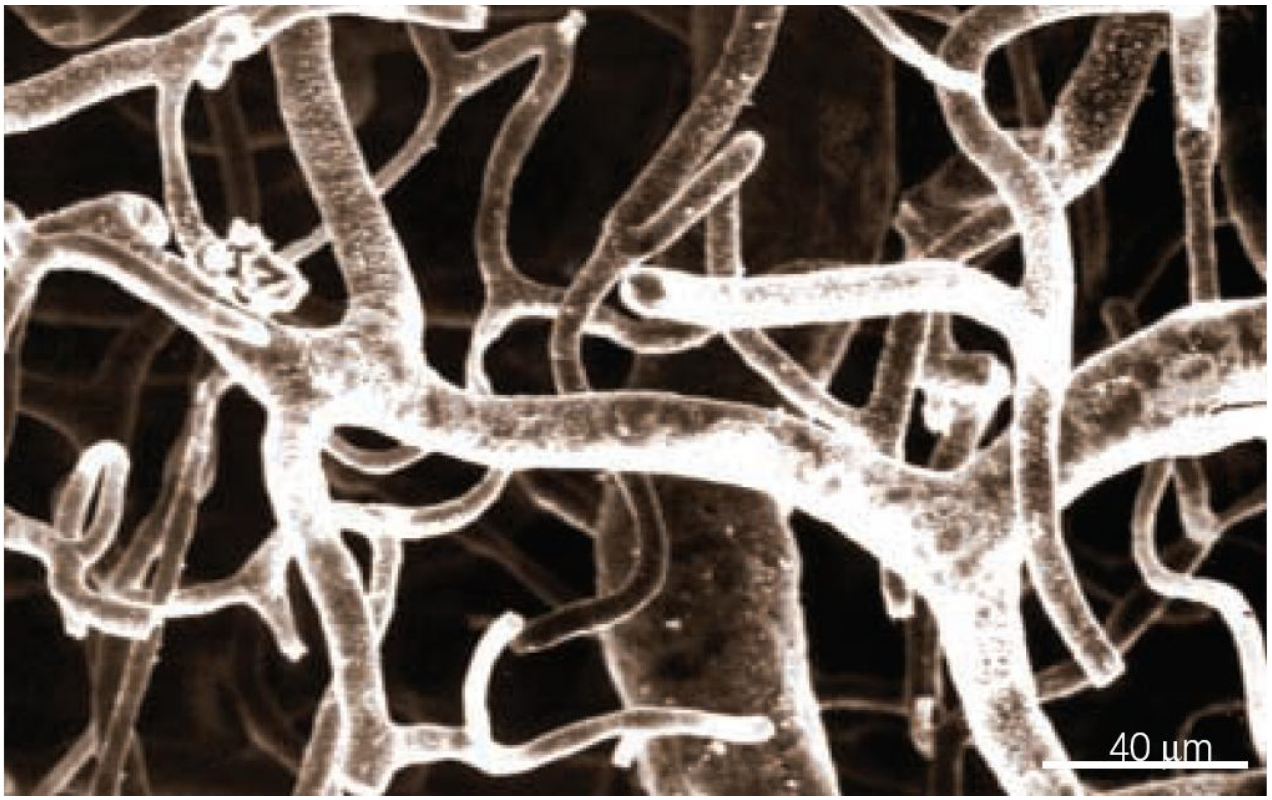
Christian Crone

LOCALIZATION OF BRAIN CAPILLARIES



Zlokovic, Nature Rev Neurosci, 2011

BLOOD-BRAIN BARRIER = BRAIN CAPILLARIES

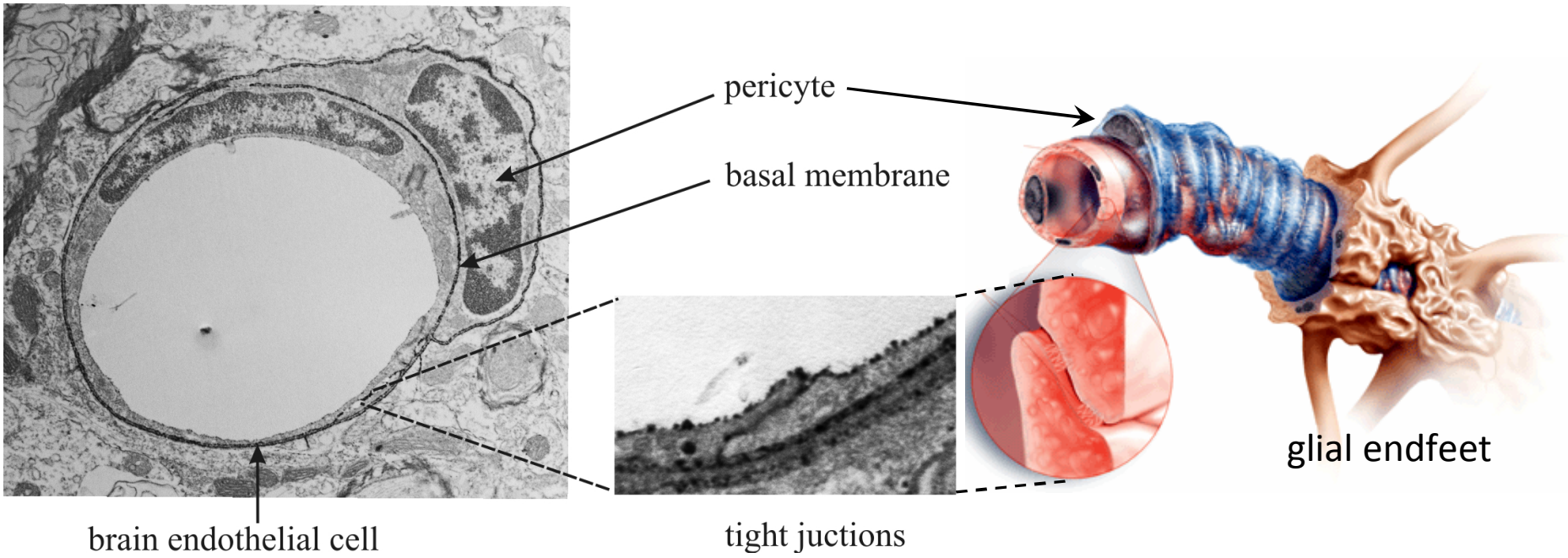


Brain capillaries length: 650 km (Budapest – Adriatic sea)

surface: 10-20 m^2

0.1% of brain volume

BLOOD-BRAIN BARRIER



Dynamic interface between the blood and the central nervous system

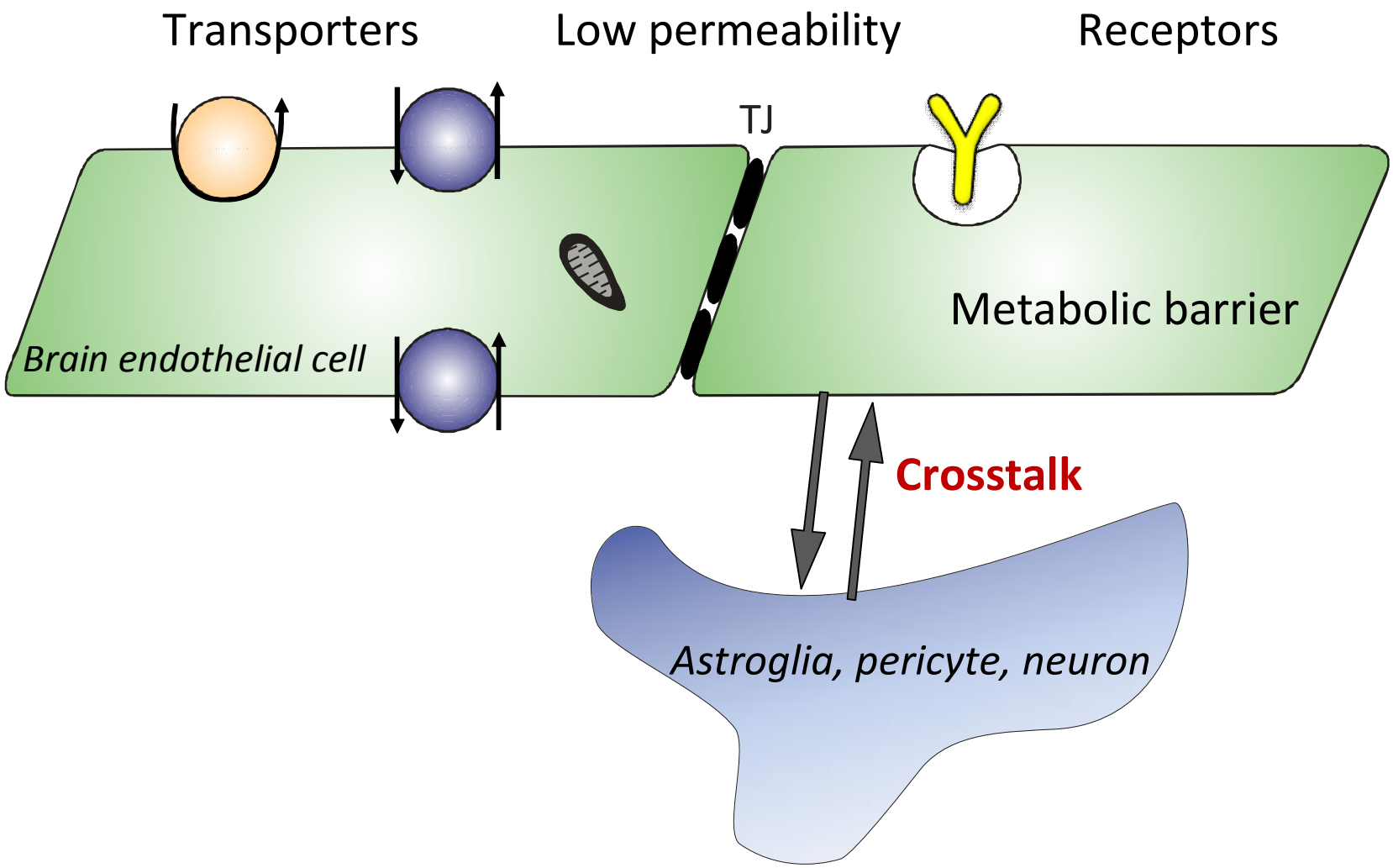
Roles creates – **ionic** - homeostasis for neuronal functions

provides brain with nutrients

defense system against toxic insults

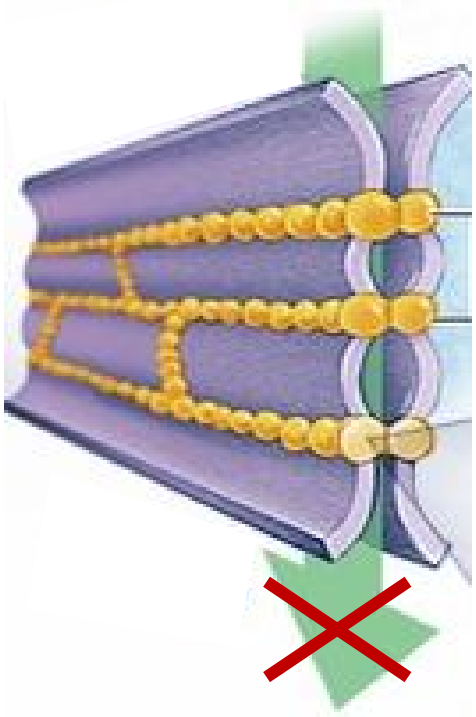
communication between the periphery and the CNS

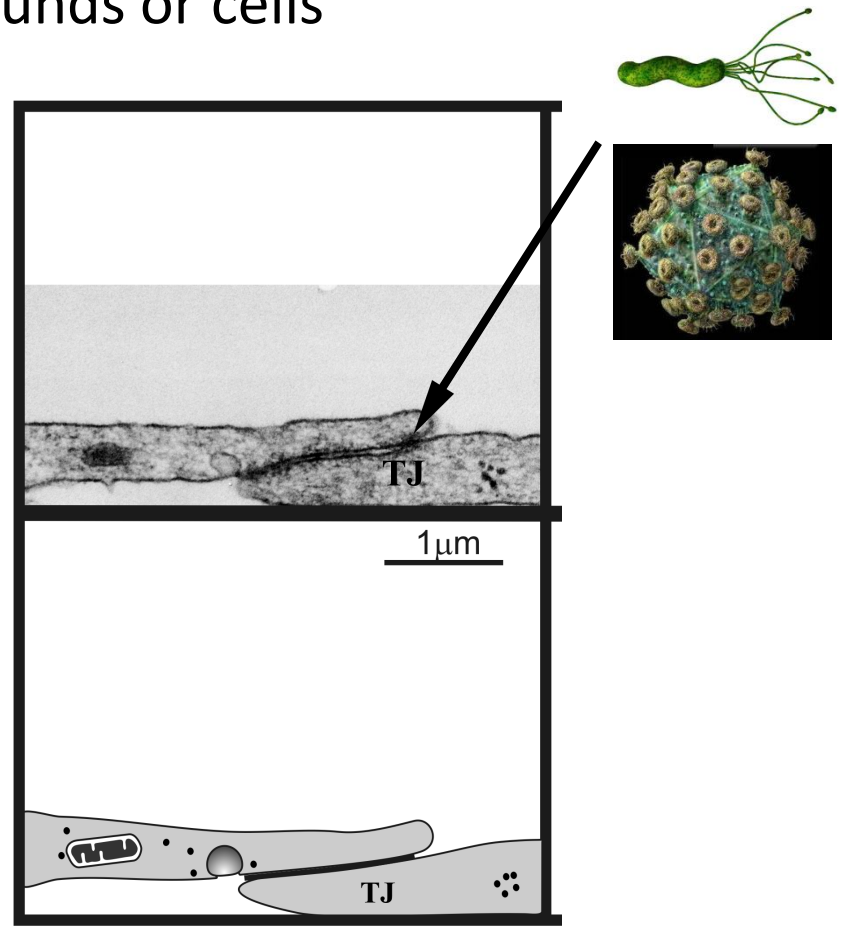
FUNCTIONS AT THE BBB – ORGAN SPECIFIC



BLOOD-BRAIN BARRIER: PROTECTION OF CNS

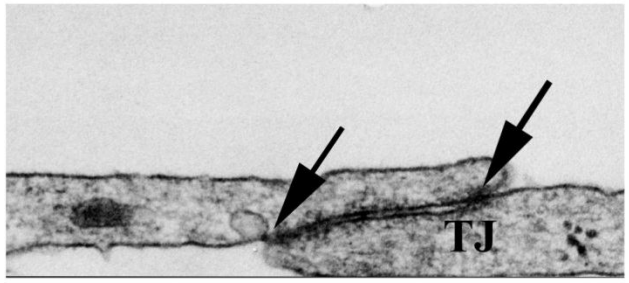
Physical barrier
Protection against toxic compounds or cells



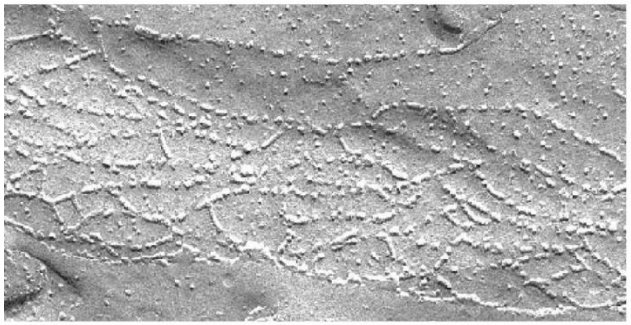


tight junctions (TJ) between brain endothelial cells

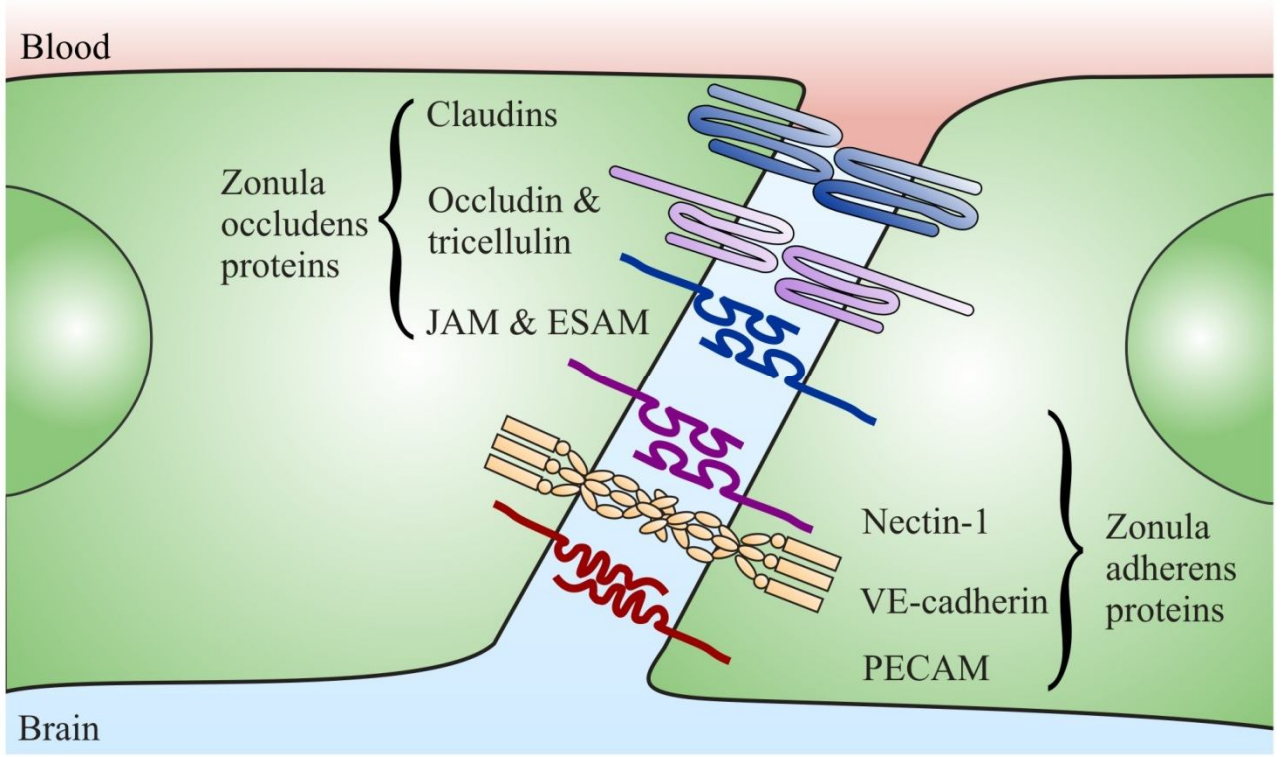
COMPOSITION OF TIGHT JUNCTIONS



Tight junction crossection



Tight junction strands



THE ROLE OF TIGHT JUNCTIONS

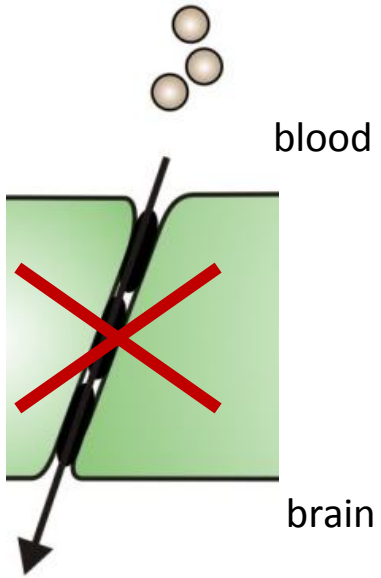
GATE

low paracellular permeability

cells, macromolecules, water, **ions**

high transendotelial electrical resistance

in vivo: 2000 Ω



FENCE

polarity of the cells

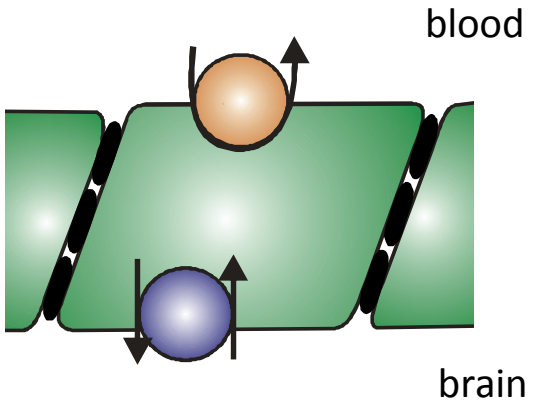
separates membranes

luminal (blood) & abluminal (brain)

transporter, receptor, enzyme expression

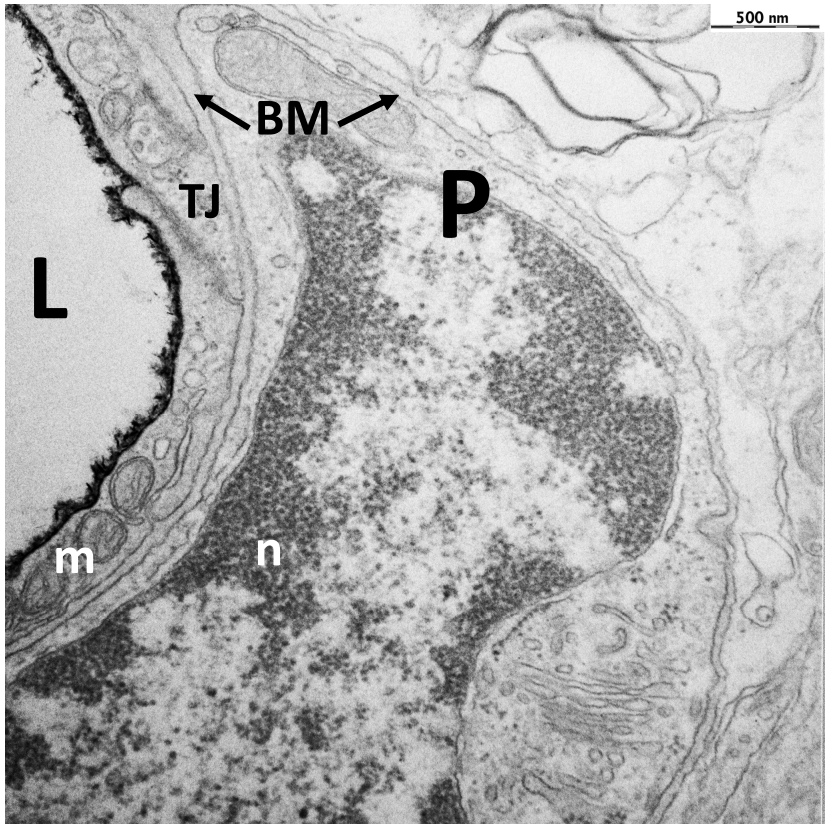
luminal: P-glycoprotein

abluminal: **Na^+/K^+ATPase**



GLYCOCALYX AT THE BBB

Rat brain capillary



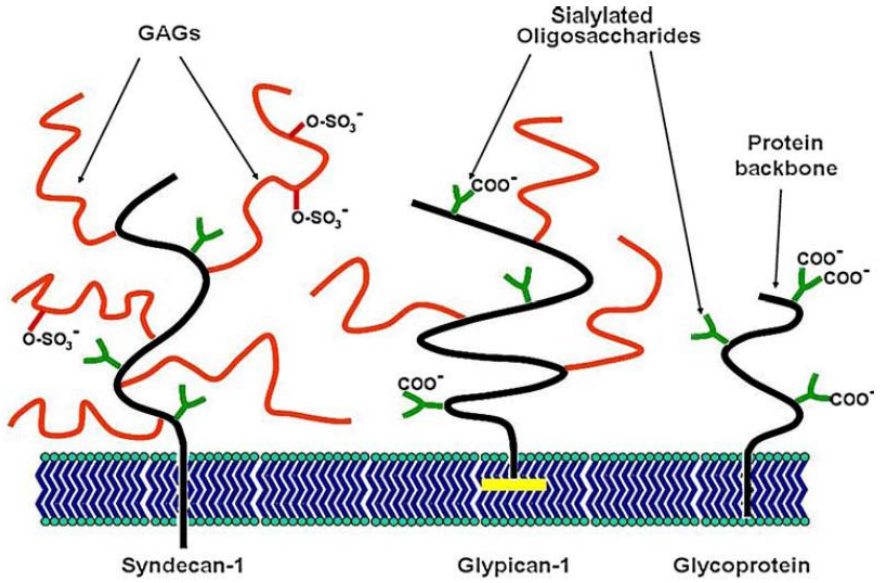
Alcian blue staining (cationic dye containing copper)
Deli MA, Kittel Á, et al.

Negative charge barrier

Luminal and abluminal

Heparan sulfate proteoglycans: syndecan-1, -2, -4, glypican-1, perlecan

Sialoglycoproteins



Hervé et al. AAPSJ 2008

METABOLIC BARRIER

Protection from toxic compounds & metabolites

Purine & pyrimidine metabolizing enzymes

Adenosine deaminase (ADA) ➔ anti-HIV dideoxynucleotides

Drug metabolizing enzymes

Monoamine oxidase MAO

Dopamine and drug metabolism

Cytochrome P450 isoforms

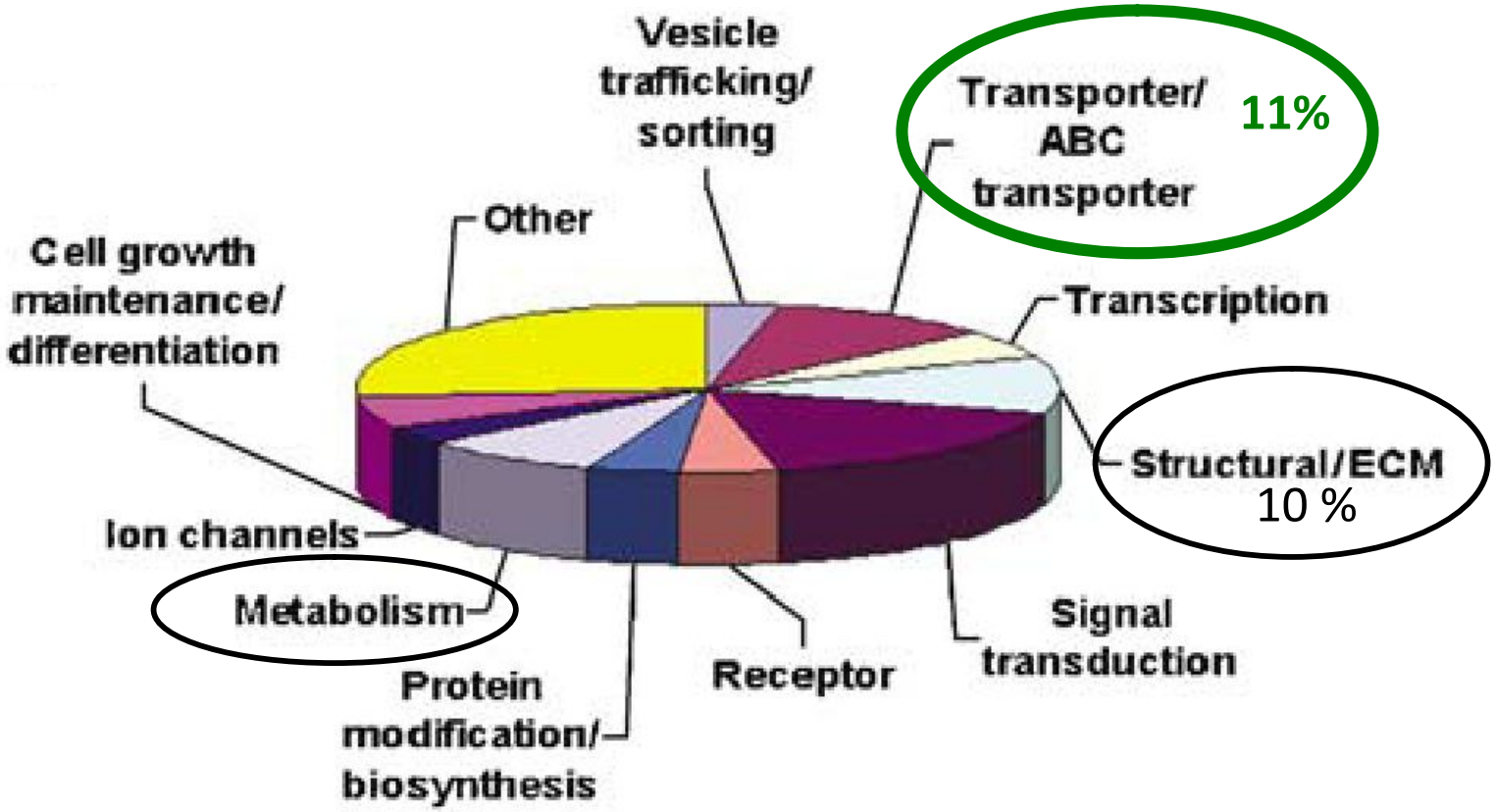
Glutathione S-transferase (GSTπ, GSTα)

Sulfotransferase (SULT1)

Alcohol metabolizing enzymes

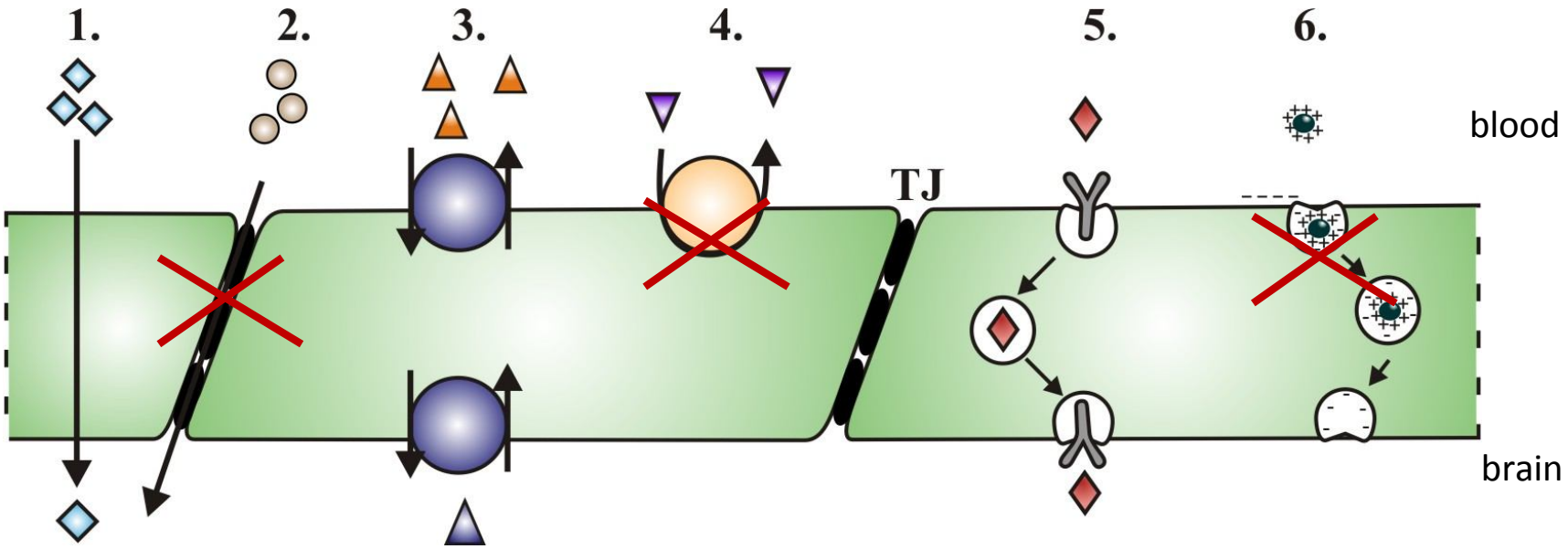
CYP2E1	EtOH ➔ ROS + acetaldehyde
Alcohol dehydrogenase	EtOH ➔ acetaldehyde

GENE EXPRESSION PROFILE IN BRAIN CAPILLARIES



Enerson and Drewes, JCBFM 2005

TRANSPORT PATHWAYS AT THE BLOOD-BRAIN BARRIER



Open pathways

1. Lipid-mediated free diffusion (lipophilic molecules)
3. Solute carrier (nutrients)
5. Receptor-mediated transcytosis (peptides, proteins)

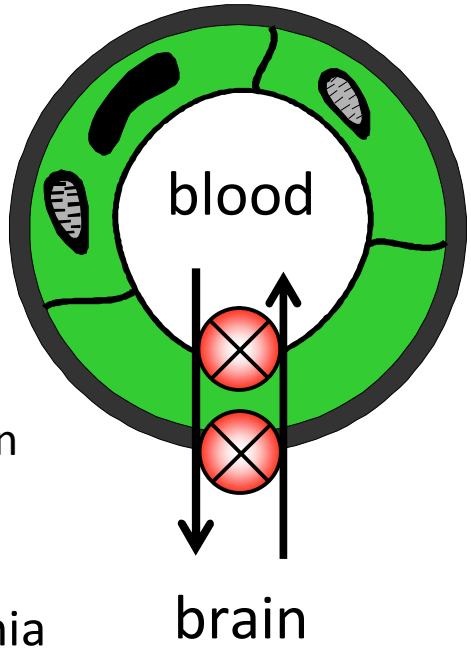
Blocked pathways

2. Paracellular traffic by tight junctions (hydrophilic molecules, cells)
4. Efflux transporters (xenobiotics, drugs)
6. Non-specific vesicular transcytosis (plasma proteins)

Abbott et al. 2006

CARRIER MEDIATED TRANSPORT AT THE BBB

Solute carrier family (>40 members at the BBB)
Bidirectional
Small molecule polar nutrients



Glucose transporter 1 (GLUT1, SLC2A1)

Primary transporter of glucose

1965 C Crone : Facilitated transfer of glucose from blood to brain

GLUT1 deficiency syndrome (GLUT1DS)

hypoglycorrhachia (low glucose in CSF) with normoglycemia

acquired microcephaly, dementia, spasticity, ataxia, retarded development

epilepsy refractory to standard antiepileptic drugs

ketogenic diet

Monocarboxylic acid transporters ()

MCT1, SLC16A1: lactate, acetate

MCT8, SLC16A2: thyroid hormones

CARRIER MEDIATED TRANSPORT AT THE BBB

Amino acids

Cationic amino acid transporter CAT-1/SLC7A1 (Arg, Lys)

Neutral amino acid transporter LAT-1/SLC7A5 (Phe, Leu, Ile)

Amino acid transporter SN1/SLC38A3 (Asp, His, Gln)

Fatty acids

Fatty acid transporter FATP1/SLC27A1 (long chain FA)

Creatine CRT1/SLC6A8

Choline CTL1 /SLC44A1, organic cation transporter OCT2

Nucleosides CNTs/SLC28A

Vitamines

Biotin, panthotenate: SMVT/SLC5A6

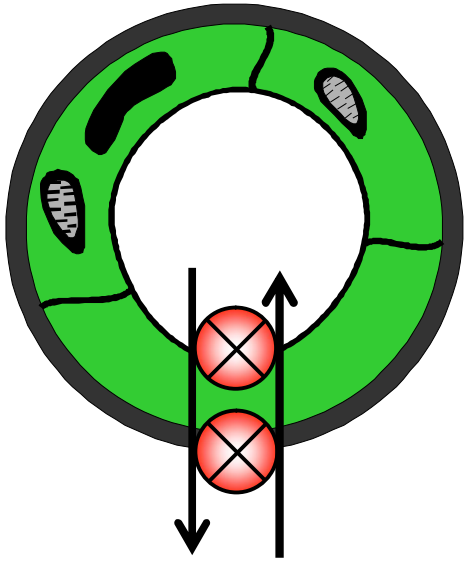
Folate: RFC/SLC19A1

(vitamin C: choroid plexus!)

Metal ions

DMT1/SLC11A2, IREG1/SLC40A1

Peptides PHT2/SLC15A3



Campos-Bedolla et al. 2014

EFFLUX TRANSPORT AT THE BBB - SLC TRANSPORTERS

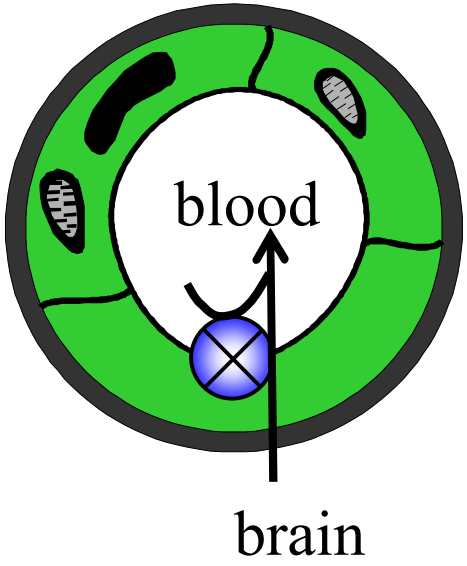
Excitatory amino acid transporters
EAAT1-3/SLC1A
glutamate, aspartate

Organic anion transporting polypeptides
OATPB/Oatp2b1 (Slco2b1)
bile acids, opiates, drugs

Organic anion transporters (Oat1, Oat3)
antibiotics, cimetidine

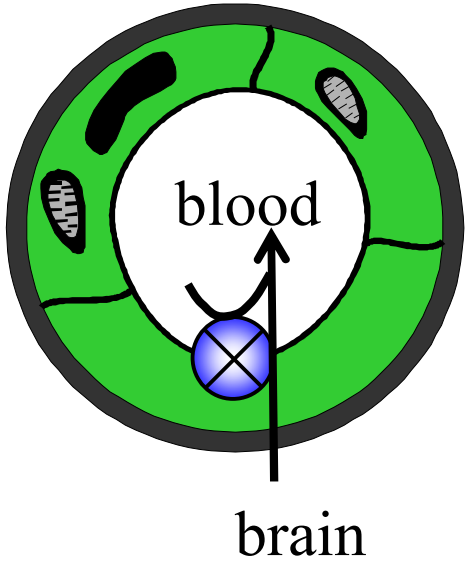
Plasma membrane monoamine transporter
PMAT/SLC29A4
monoamines, cationic neurotoxins

Multidrug and toxic compound extrusion protein-1
MATE1/SLC47A1
organic cations and cationic drugs



EFFLUX TRANSPORT AT THE BBB - ABC TRANSPORTERS

Limits the influx : drugs & xenobiotics
Efflux: CNS metabolic by-products



P-glycoprotein (ABCB1)

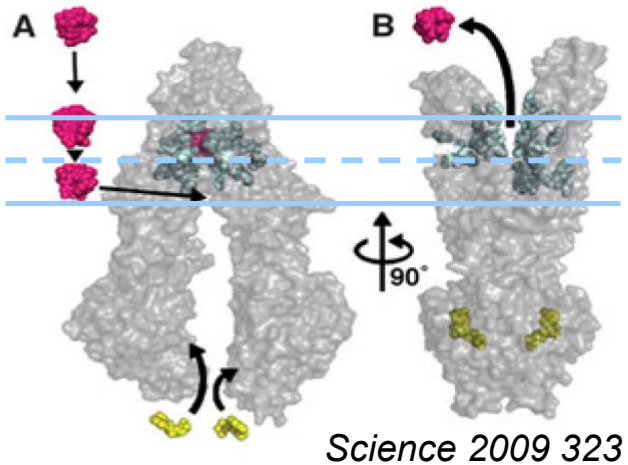
drugs, xenobiotics: ivermectin,
anthracyclins, Vinca alkaloids, taxol
aldosterone, glucocorticoids
cyclosporin A, HIV-protease inhibitors
antiepileptics: phenytoin, phenobarbital, valproic acid
plant polyphenols, nutraceuticals

Brain multidrug resistance protein

ABCG2/BCRP
drugs, xenobiotics

Multidrug resistance-assoc. proteins 1-6

ABCC1-6
glucuronide and glutathione conjugates



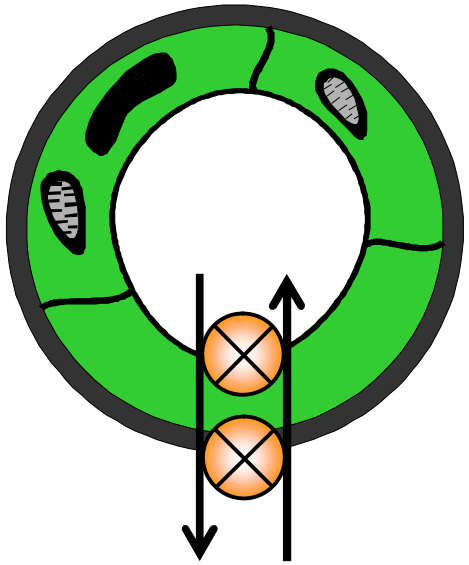
Science 2009 323 27

RECEPTOR MEDIATED TRANSCYTOSIS AT THE BBB

Receptors for peptides or lipoproteins

Bidirectional system

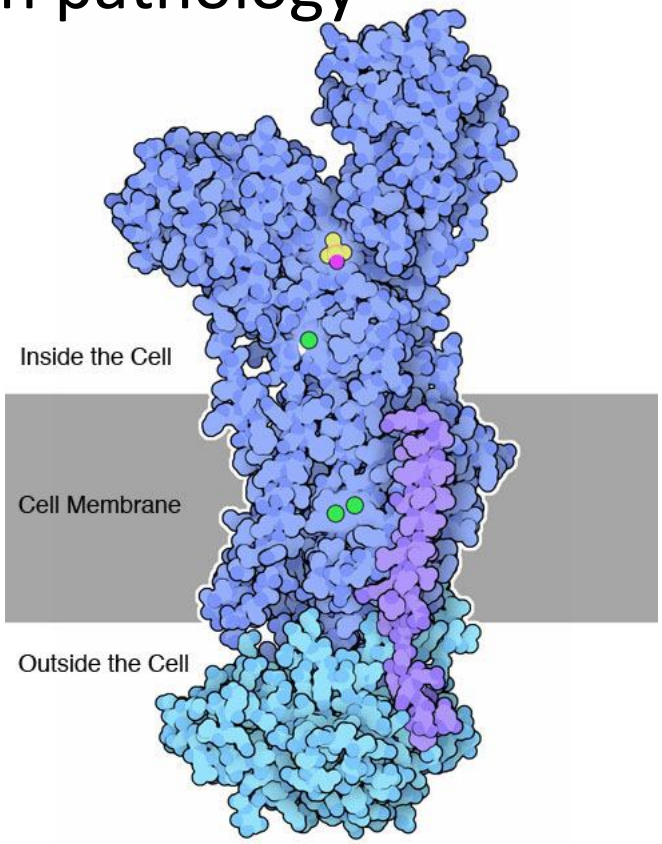
- Insulin receptor
- Insulin-like growth factor
- Transferrin receptor
- LDL receptor
- LRP-1 és 2
- Diphteria toxin/HB-EGF receptor
- Leptin and ghrelin receptors
- RAGE



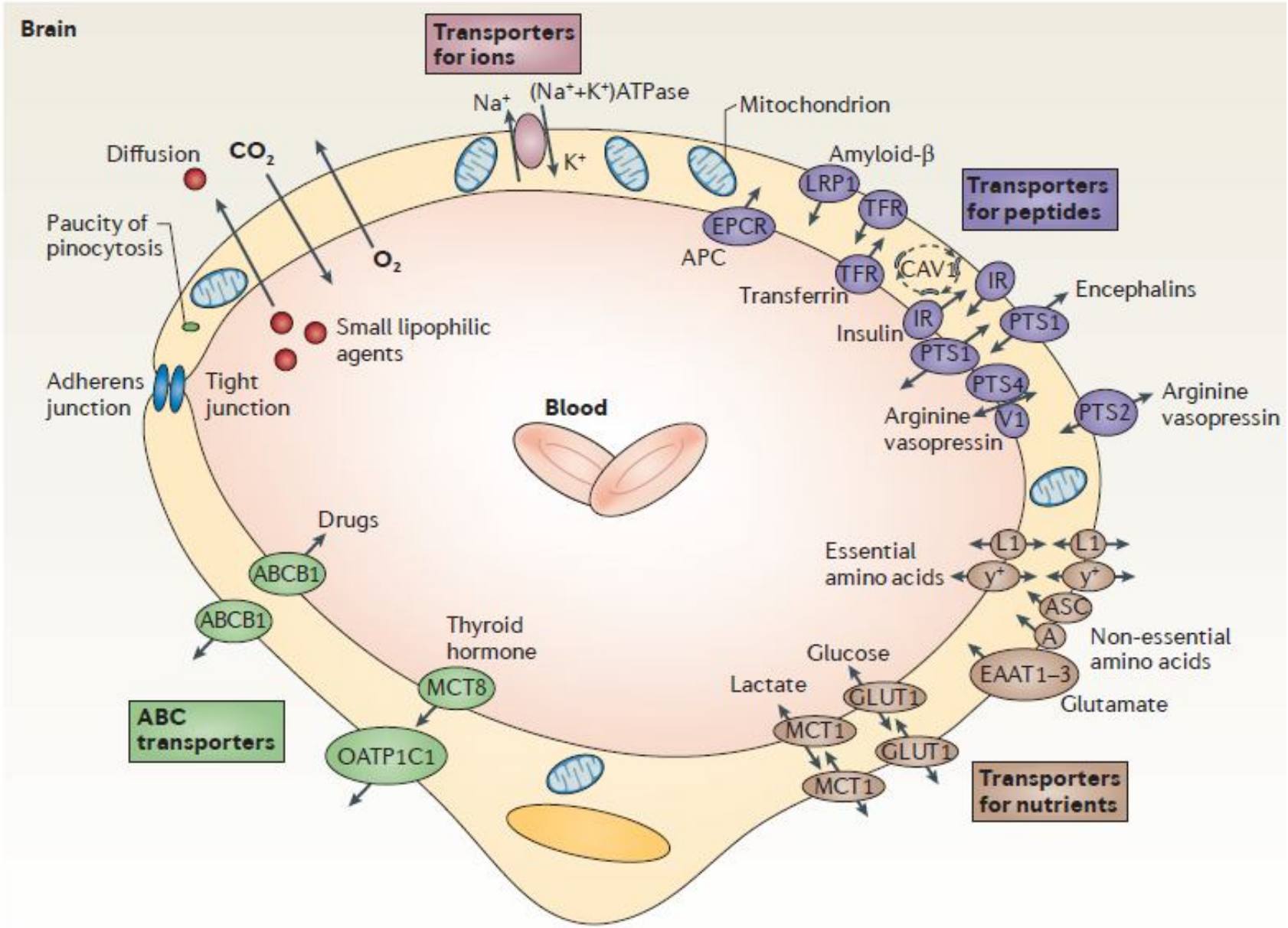
Structure and functions of the brain barriers

Ion transporters at the BBB and BCSFB

Ion transporters at brain barriers in pathology



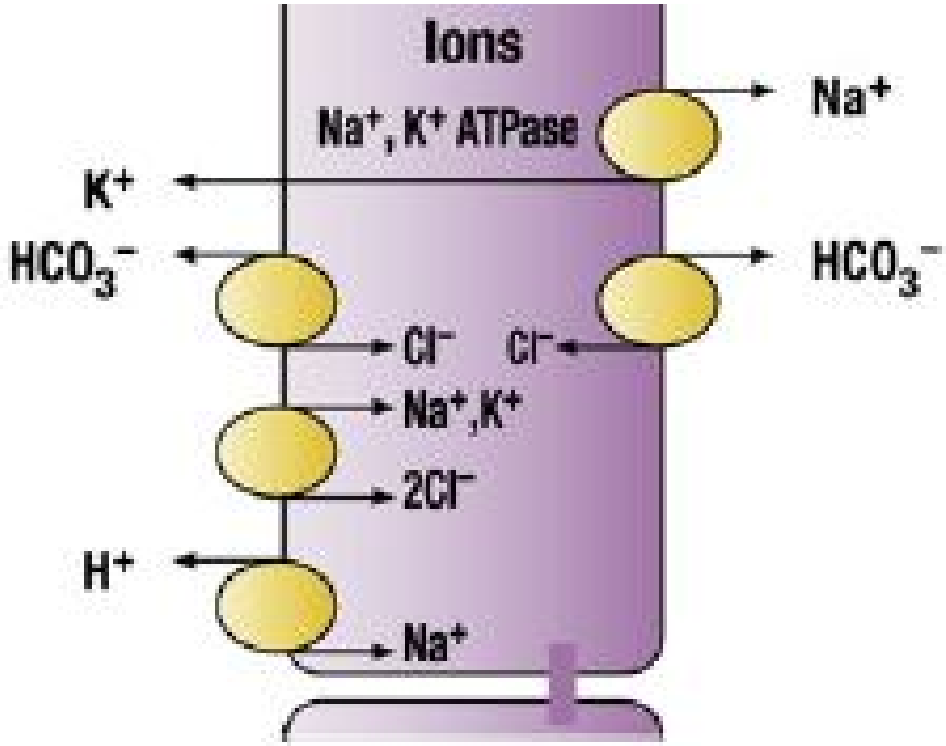
ION TRANSPORTERS AT THE BBB



Zlokovic, Nature Rev Neurosci, 2011

ION TRANSPORTERS AT THE BBB

blood



brain

Sodium pump / Na^+, K^+-ATPase

Chloride-bicarbonate exchanger

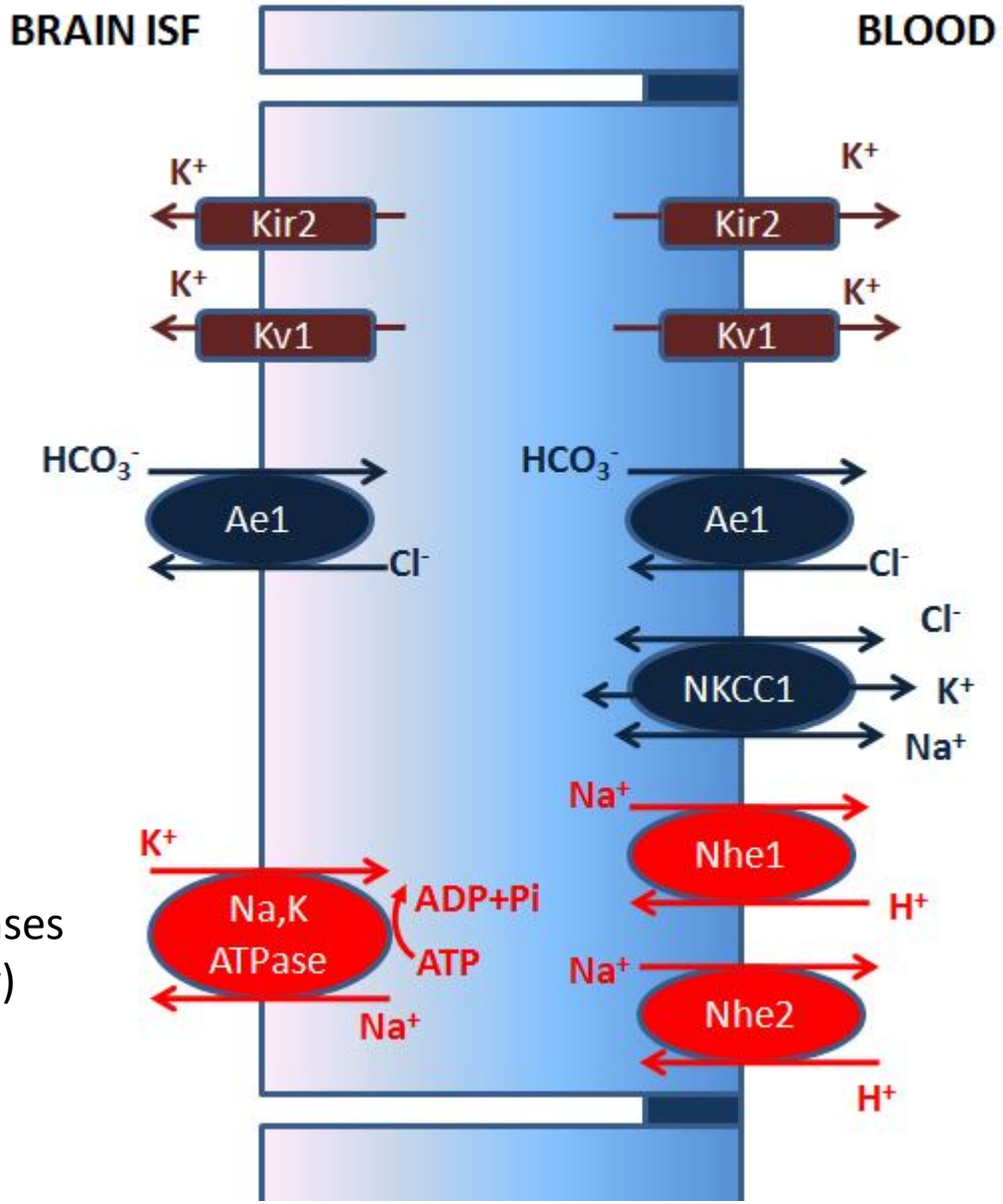
Na^+-K^+-$2Cl^-$ cotransporter

Sodium-hydrogen exchanger

Zlokovic, Neuron, 2008

ION TRANSPORTERS AT THE BBB

Potassium channels



Chloride-bicarbonate exchanger

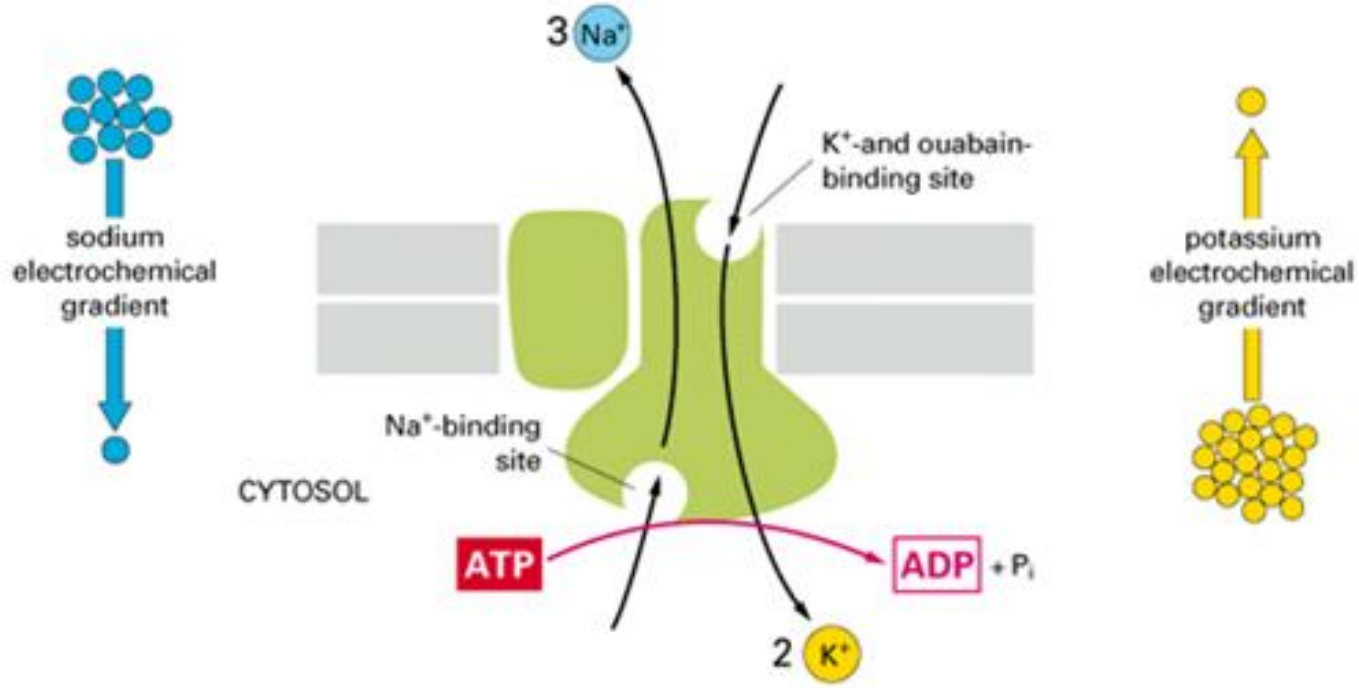
Na^+, K^+, 2Cl cotransporter (SLC12 family)

Na^+, K^+-ATPases (ATP1 family)

Ion exchangers (SLC)

Redzic, FBCNS, 2011

SODIUM PUMP / Na^+, K^+-ATPase (NKAT)



Jens Christian Skou, 1957; Nobel prize 1997

Moves 3 sodium ions out and 2 potassium ions / 1 ATP

Generates ion gradient - heart signals, cell volume regulation

Blocking the sodium-potassium pump

plant (digitalis, ouabain) or animal (poisonous toads) toxins, drugs

pumping of ions ↓, level of Na^+↑ inside the cell

sodium-calcium exchanger ↓, level of Ca^{2+}↑, force of heart contractions ↑

PROPAGATION OF ACTION POTENTIAL IN NEURONAL AXONS

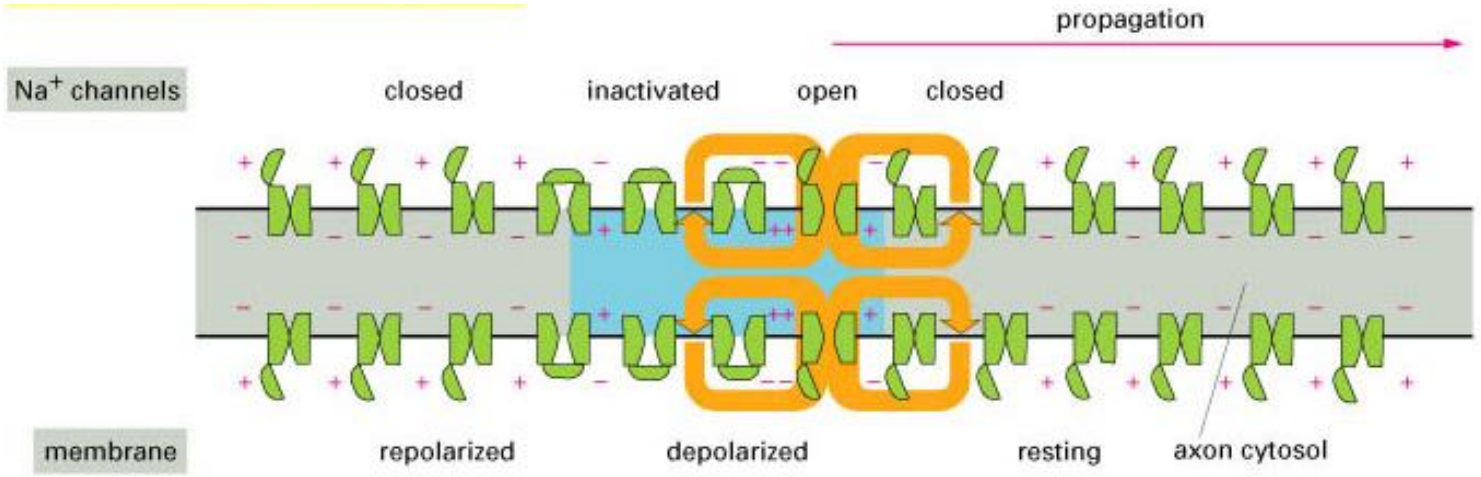
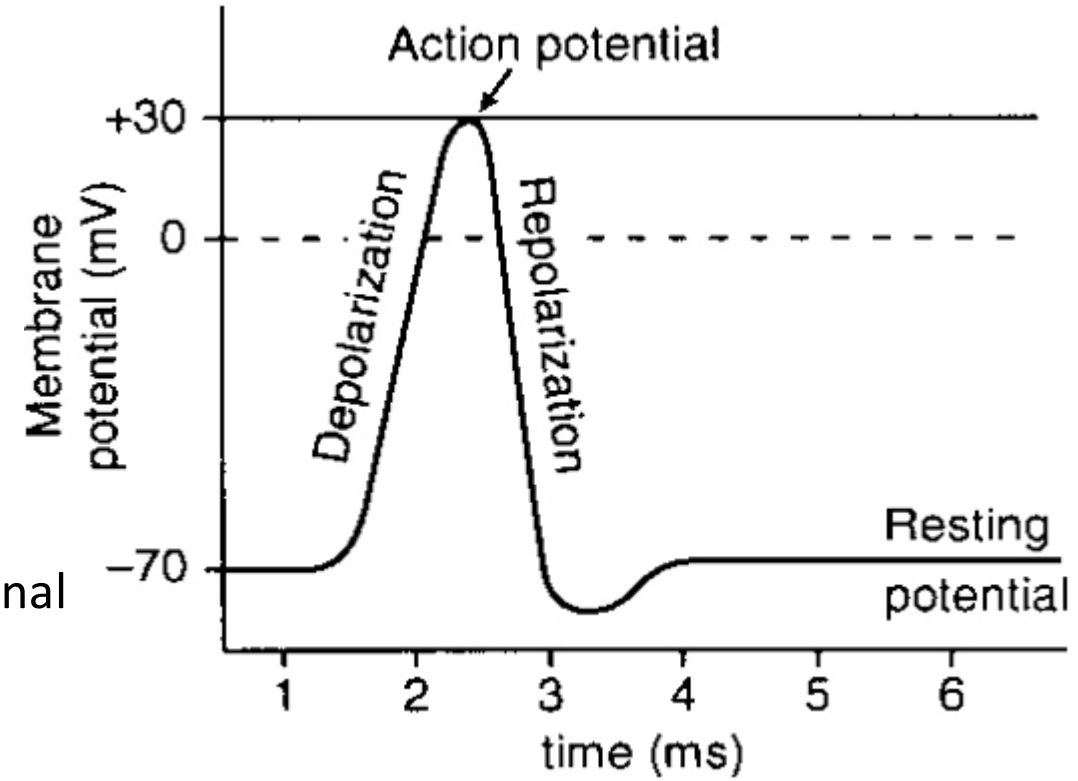


Figure 12-38 Essential Cell Biology, 2/e. (© 2004 Garland Science)

nerve axons Na^+ ↓

voltage-gated Na^+ channels: Na^+ ↑
action potential

Na^+, K^+-ATPase
keeps the resting potential
axons ready for the next signal



SODIUM PUMP / Na^+, K^+-ATPase AT THE BBB

Localized on the abluminal membrane of brain endothelium

Subunit isoforms

3 α - ATP1A1-3

2 β - ATP1B1-2

Driving force for

ion and water movement

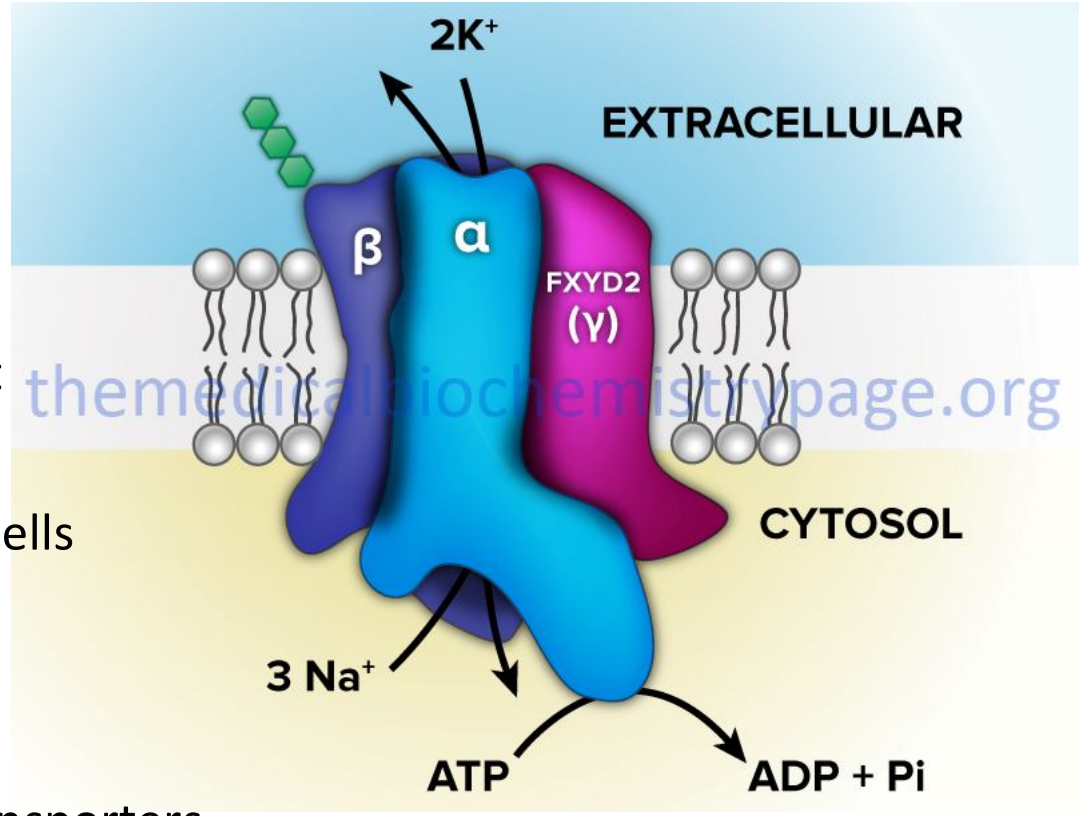
Controls

osmotic pressure inside cells

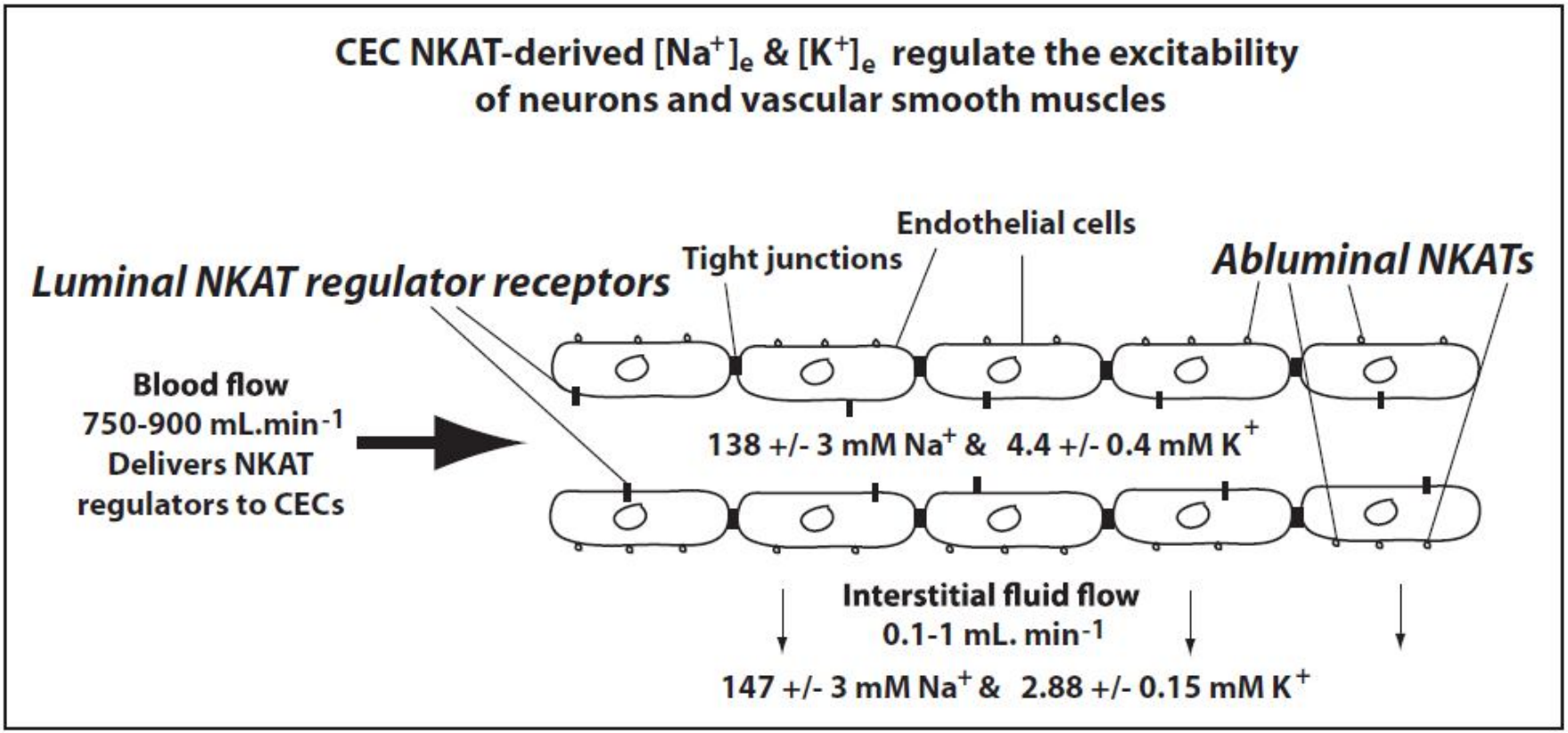
Powers other pumps

Na^+ dependent

eg. Ca^{2+} or glucose co-transporters



BBB NKAT REGULATES ION COMPOSITION IN BRAIN FLUIDS



In brain ISF and CSF: Na^+ ↑ and K^+ ↓ compared with blood

Brain endothelial cells 500-fold more NKAT than peripheral endothelium

NKAT –at BBB - main source of Na^+ in brain tissue

Harrington et al, Headache, 2009

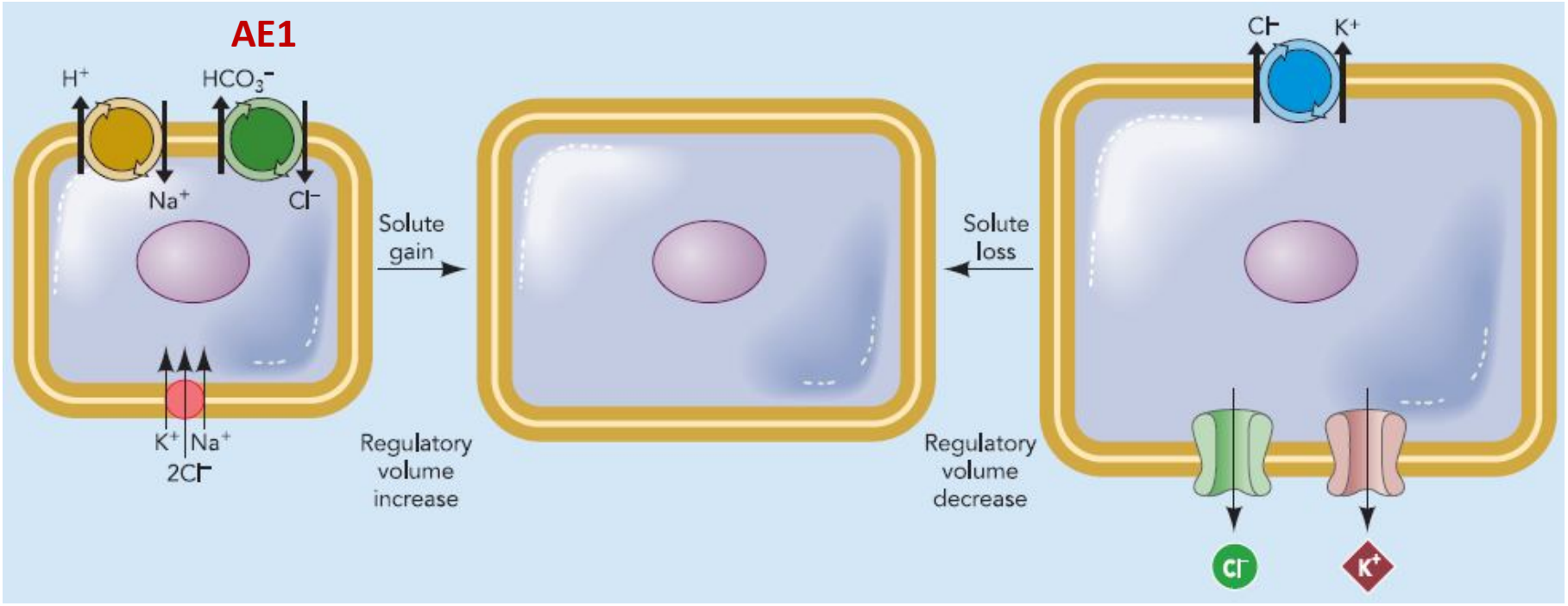
CHLORIDE-BICARBONATE EXCHANGER

Anion exchanger 1 (AE-1) - **SLC4A1** - Band 3 anion transport protein

Major role in kidney and erythrocytes

Present on the BBB

- Both membrane domains
- Electroneutral chloride and bicarbonate exchange
- Regulation of endothelial cell pH
- Links plasma membrane to membrane skeleton (ankyrin and protein 4.2)



SODIUM-POTASSIUM-CHLORIDE COTRANSPORTER

Na^+, K^+, 2Cl cotransporter-1 (NKCC1) – **SLC12A2**

Major role in fluid secreting organs (kidney, cochlea) and exocrine glands

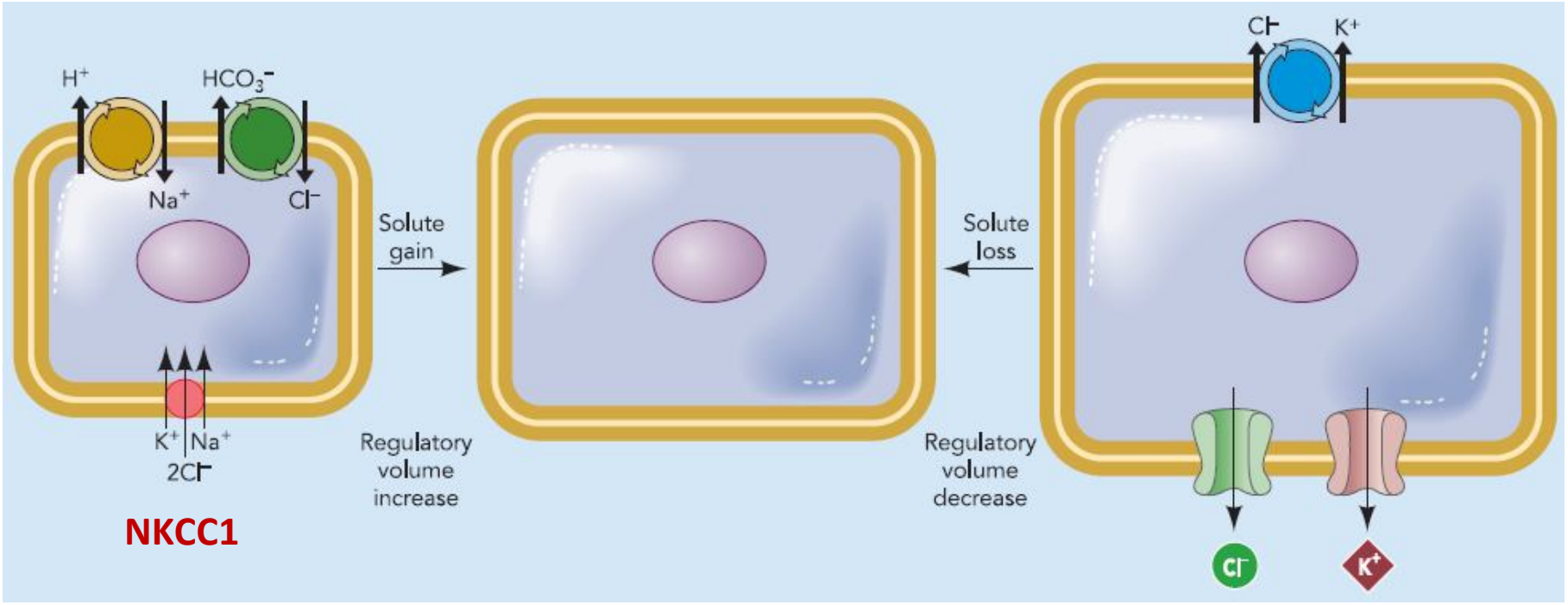
Brain: regulation of chloride concentration in neurons

Present on the BBB

only on luminal membrane

symporter: moves all three ions in the same direction

active pump, needs high levels of ATP



SODIUM-HYDROGEN ANTIPORTERS

Na^+ / hydrogen exchanger-1 (NHE-1) – **SLC9A1**

Na^+ / hydrogen exchanger-2 (NHE-2) – **SLC9A2**

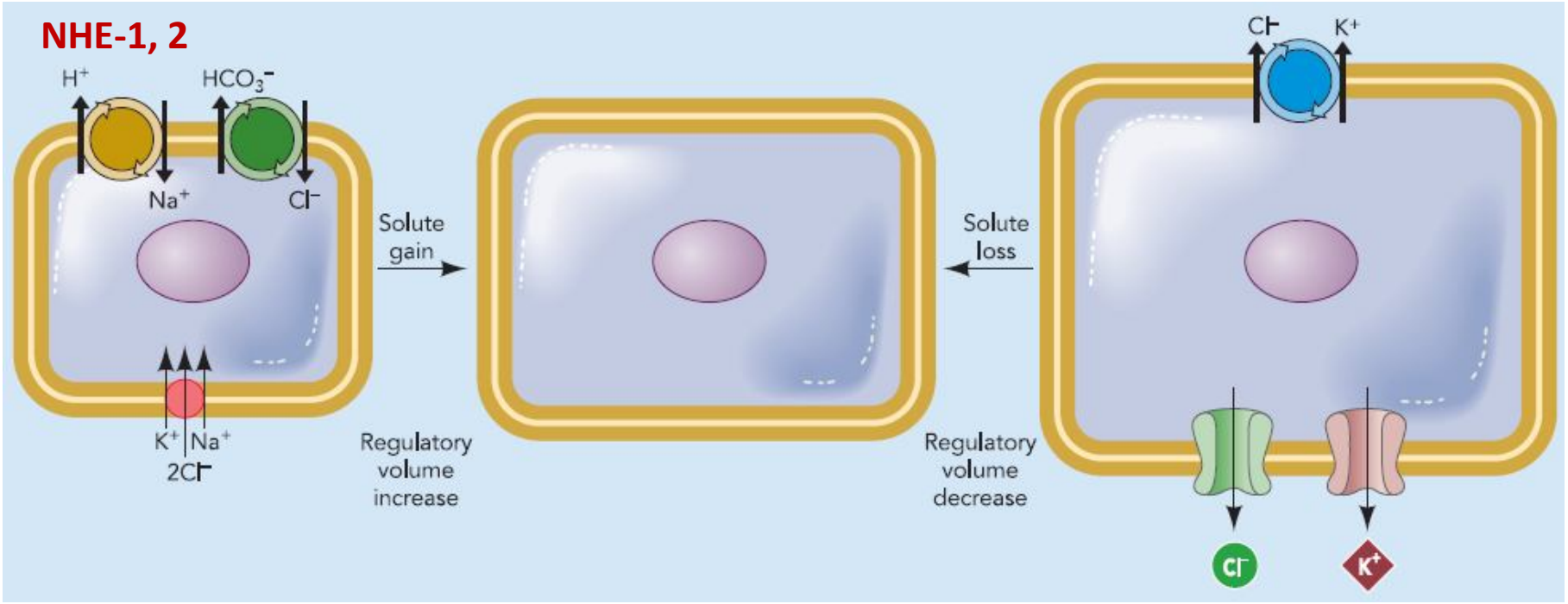
Major role in kidney

Volume and pH regulation in cells; inhibited by amiloride

Present on the BBB

only on luminal membrane

antiporter: moves Na^+ in H^+ out of cells



ROLE OF POTASSIUM CHANNELS IN BRAIN ENDOTHELIAL TURNOVER

Stimulation of metabotropic ATP receptors (P2Y)

↑ intracellular Ca2 concentration ([Ca2]i)

activation of SK2 (apamin-sensitive small-conductance Ca^2-activated K channels)

membrane hyperpolarization

↑ cell proliferation

in response to ATP

anomalous membrane hyperpolarization

Kir2.1 inward rectifier K channel was extensively expressed

↓ the cell number

P2Y stimulation in brain endothelial cells

↑ cell proliferation by SK2 activation

triggers cell death in cells expressing Kir2.1

Dual action of P2Y stimulation: facilitate endothelial turnover

Yamazaki et al, AmJPhysiol, 2011

ION TRANSPORTERS AT THE BCSFB

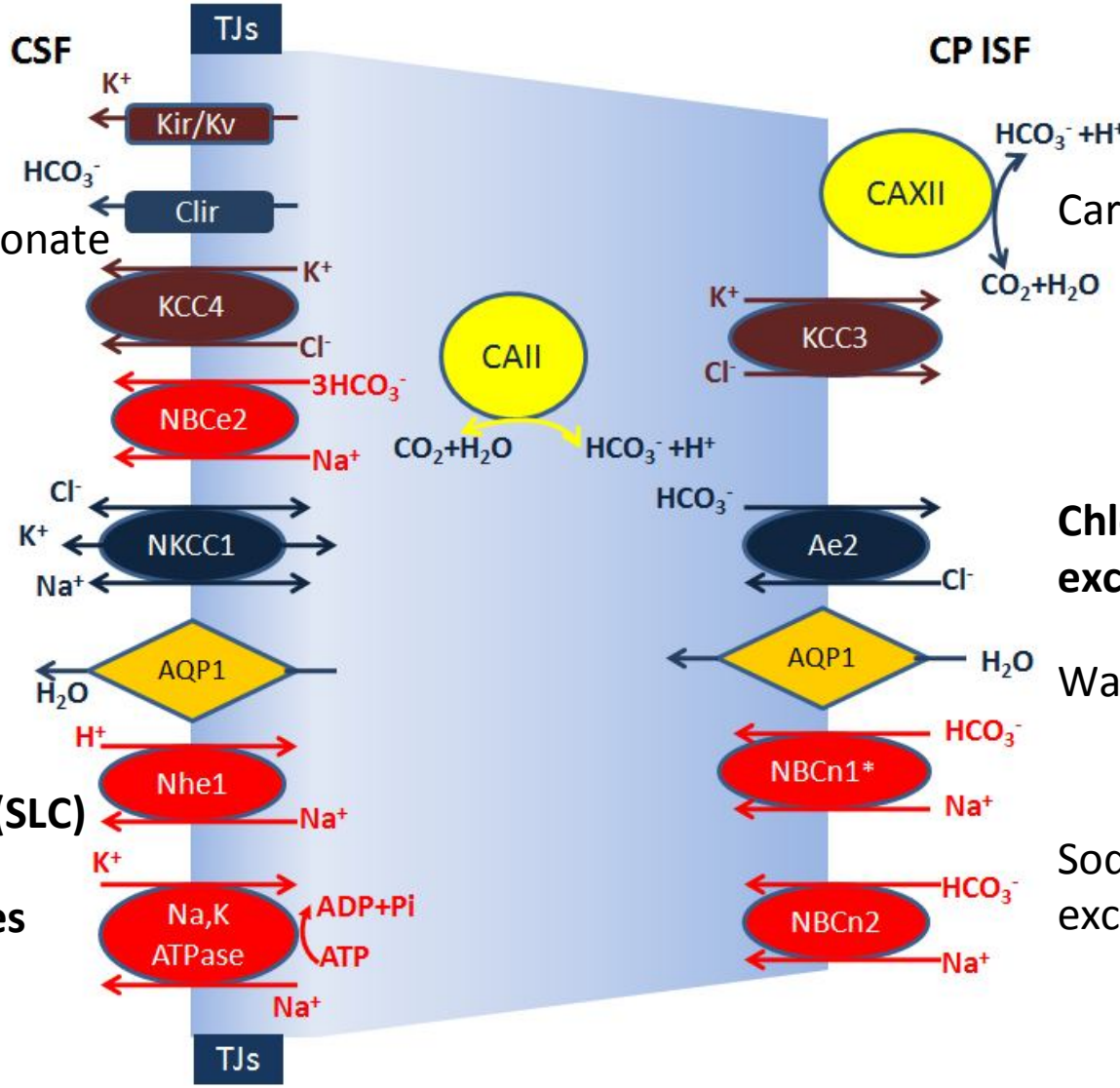
Potassium channels

Chloride/bicarbonate channel

Na+, K+, 2Cl cotransporter (SLC12 family)

Ion exchangers (SLC)

Na+, K+-ATPases (ATP1 family)



Carbonic anhydrase

Chloride-bicarbonate exchanger

Water channel

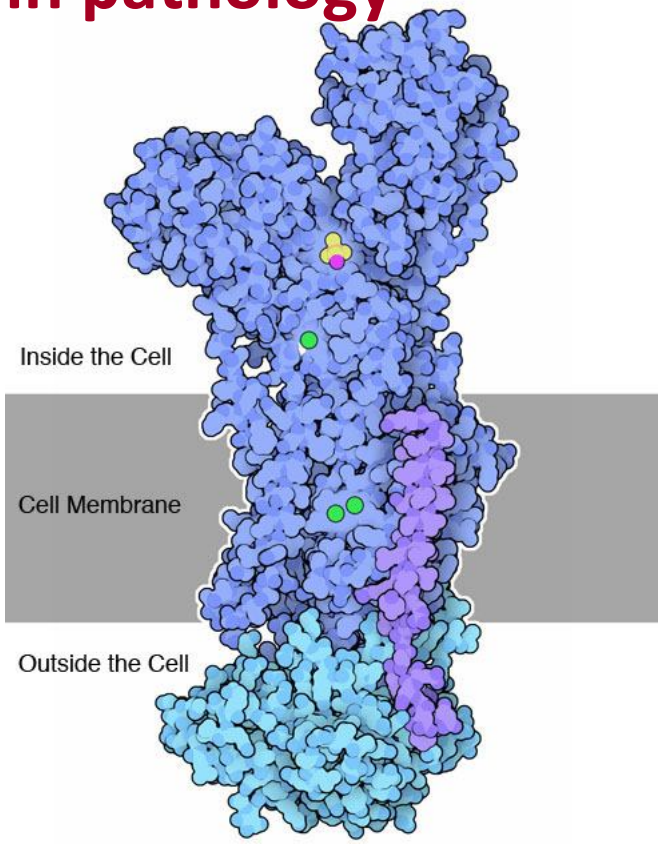
Sodium-bicarbonate exchangers

Redzic, FBCNS, 2011

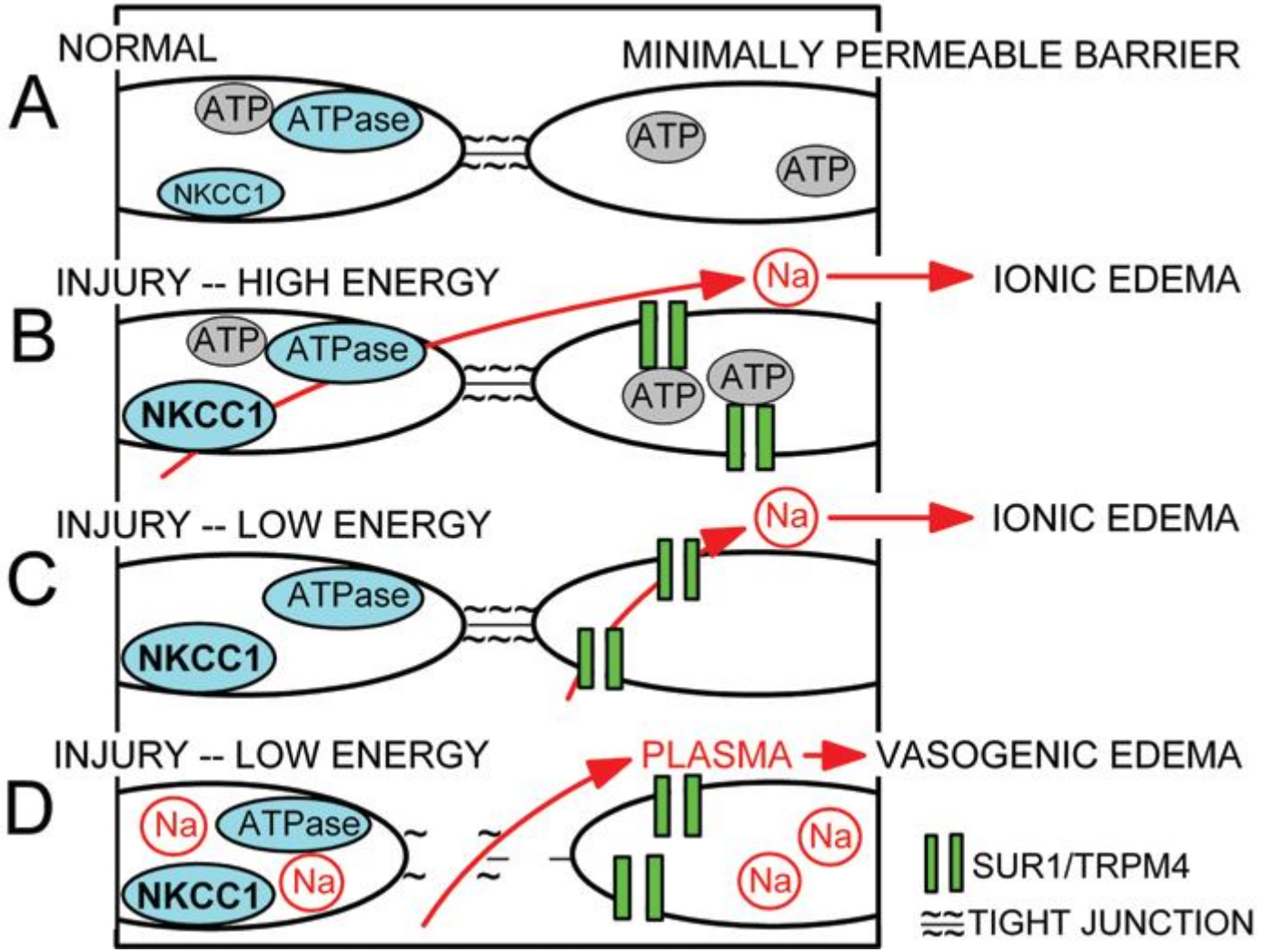
Structure and functions of the brain barriers

Ion transporters at the BBB and BCSFB

Ion transporters at brain barriers in pathology



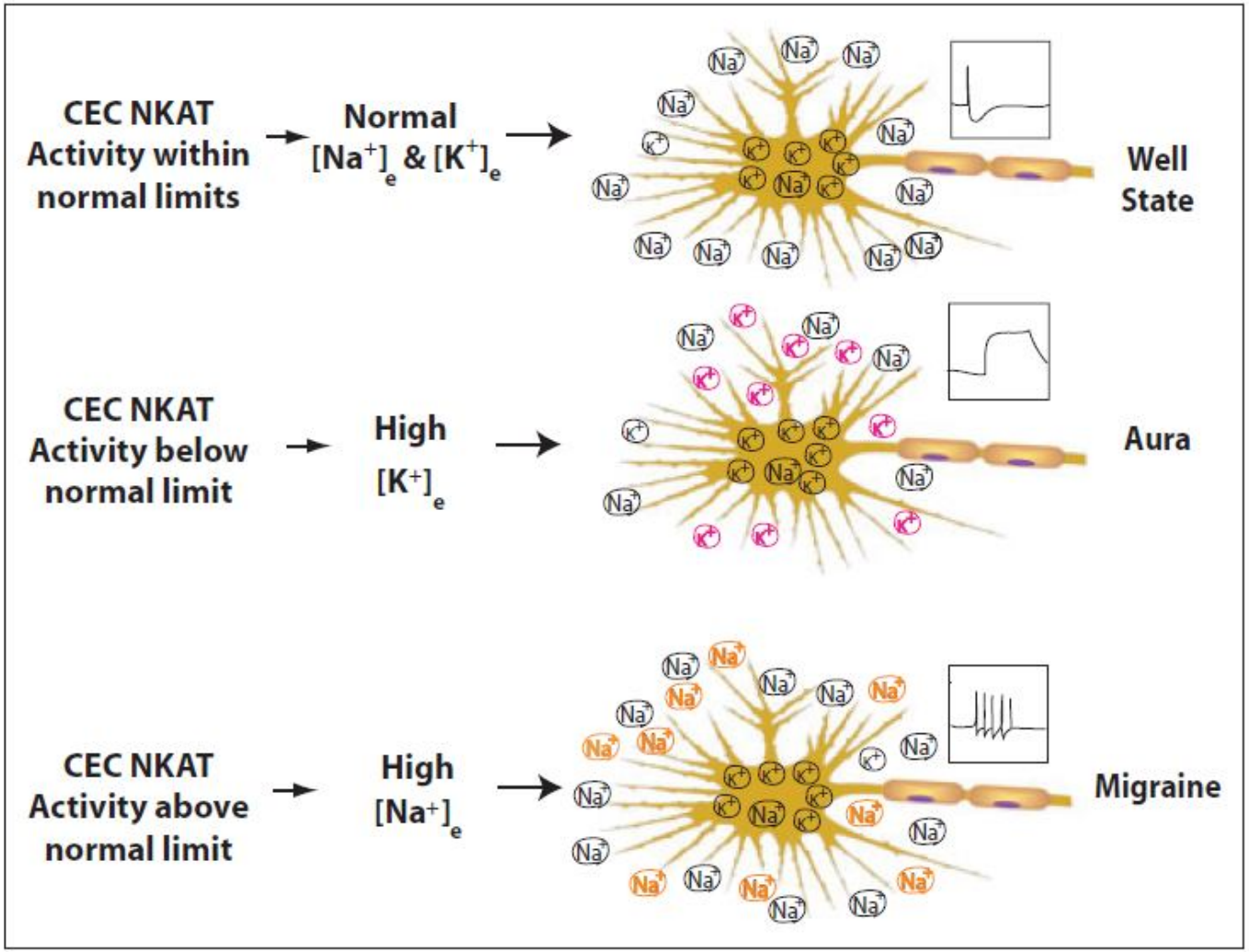
ION TRANSPORTERS AT BBB – ROLE IN BRAIN EDEMA



trauma/ischemia-induced SUR1-regulated NCCa-ATP (SUR1/TRPM4) channel

Simard et al, J Neurosurg, 2010

NKAT AT BBB – ROLE IN MIGRAINE



Migraine inducers which are regulators of NKAT

CGRP, estrogens, glutamate, cannabinoids, NO, NA, 5HT, DA, caffeine

BBB: serotonin, estrogen, and phospholipid receptors, and GPCRs

Harrington et al, Headache, 2009

SUMMARY

Ion transporters and channels

present at the blood-brain and blood-CSF barriers

Regulate

ion composition of the brain fluids

brain endothelial cell volume and pH

Participate in diseases

stroke, migraine