Multiple Brain Barriers

1. **Blood-Brain Barrier**: **capillary endothelium** (between blood and brain interstitial fluid)

2. **CSF-Meninges: arachnoid epithelium** (between subarachnoid CSF and dura mater/blood)

3. **Blood-CSF Barrier**: **choroid plexus epithelium** (between blood and and ventricular CSF)

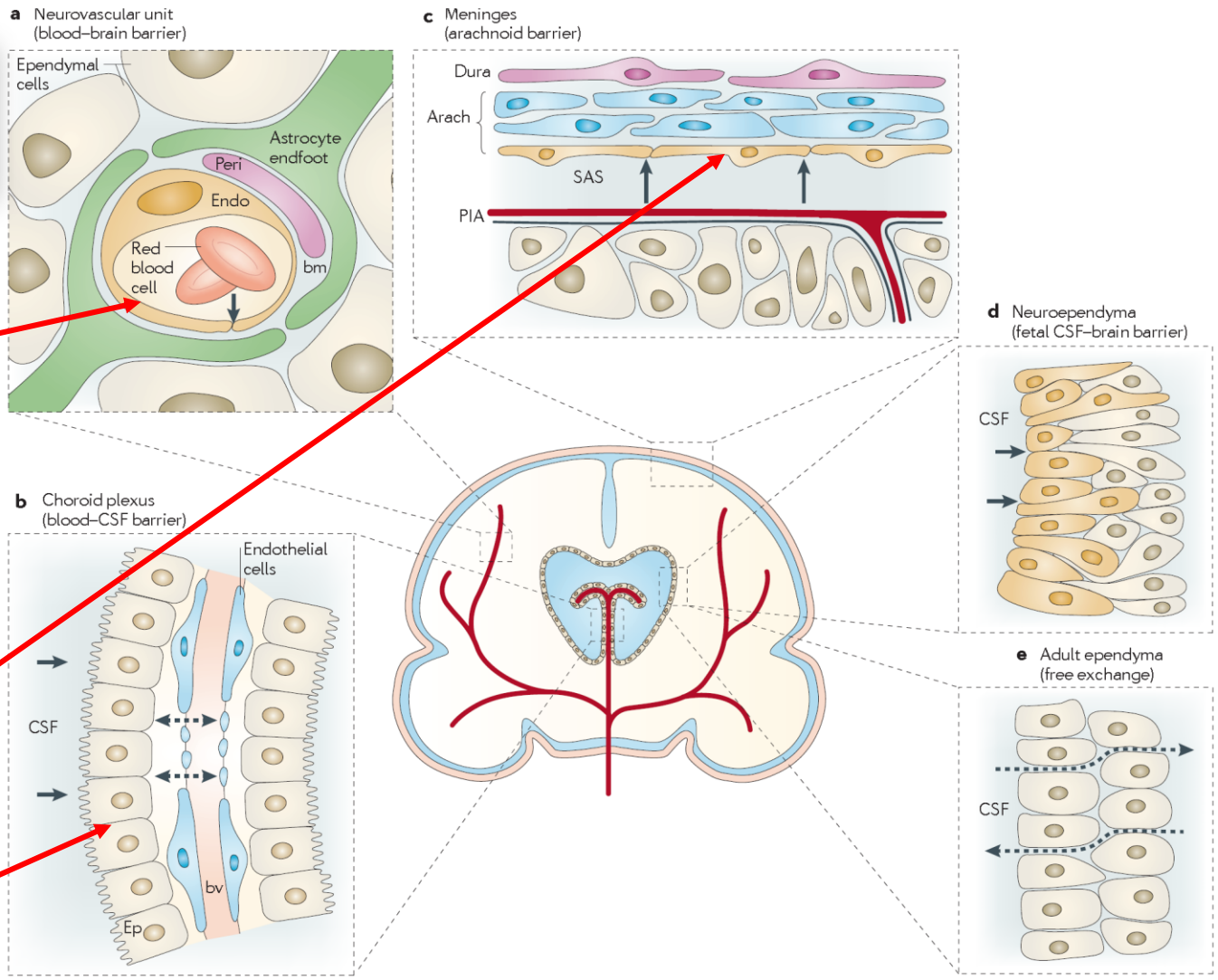


Figure 3 | **Barrier interfaces. a** | Endothelial cells (Endo) in the neurovascular unit have luminal tight junctions (shown by the arrow) that form the physical barrier of the interendothelial cleft. Outside the endothelial cell is a basement membrane (bm) which also surrounds the pericytes (Peri). Around all of these structures are the astrocyctic endfeet processes from nearby astrocytes. **b** | The endothelial cells of choroid plexus blood vessels are fenestrated and form a non-restrictive barrier (shown by dashed arrows) between the cerebrospinal fluid (CSF) and blood vessel (bv). The epithelial cells (Ep) have apical tight junctions (shown by arrows) that restrict intercellular passage of molecules. **c** | In the meninges, the blood vessels of the dura are fenestrated and provide little barrier function (not shown). However, the outer cells of the arachnoid membrane (Arach) have tight junctions (shown by arrows) and this cell layer forms the physical barrier between the CSF-filled subarachnoid space (SAS) and overlying structures. The blood vessels between the arachnoid membrane and the pial surface (PIA) have tight junctions (not shown). **d** | In early development the neuroependymal cells are connected to each other by strap junctions (shown by arrows) that are believed to form the physical barrier restricting the passage of larger molecules, such as proteins, but not smaller molecules, such as sucrose. **e** | The mature adult ventricular ependyma does not restrict the exchange of molecules (shown by dotted arrows). The neurovascular unit (**a**), blood–CSF barrier (**b**) and arachnoid barrier (**c**) are common between developing and adult brain, whereas fetal neuroependyma (**d**) differs from adult ependyma (**e**). Figure is reproduced, with permission, from REF. 162 © (2008) Cell Press.

Neuwelt et al. 2011
Nature Reviews Neuroscience,
doi:10.1038/nrn2995

The CSF is mainly secreted by **choroid plexus epithelial cells**

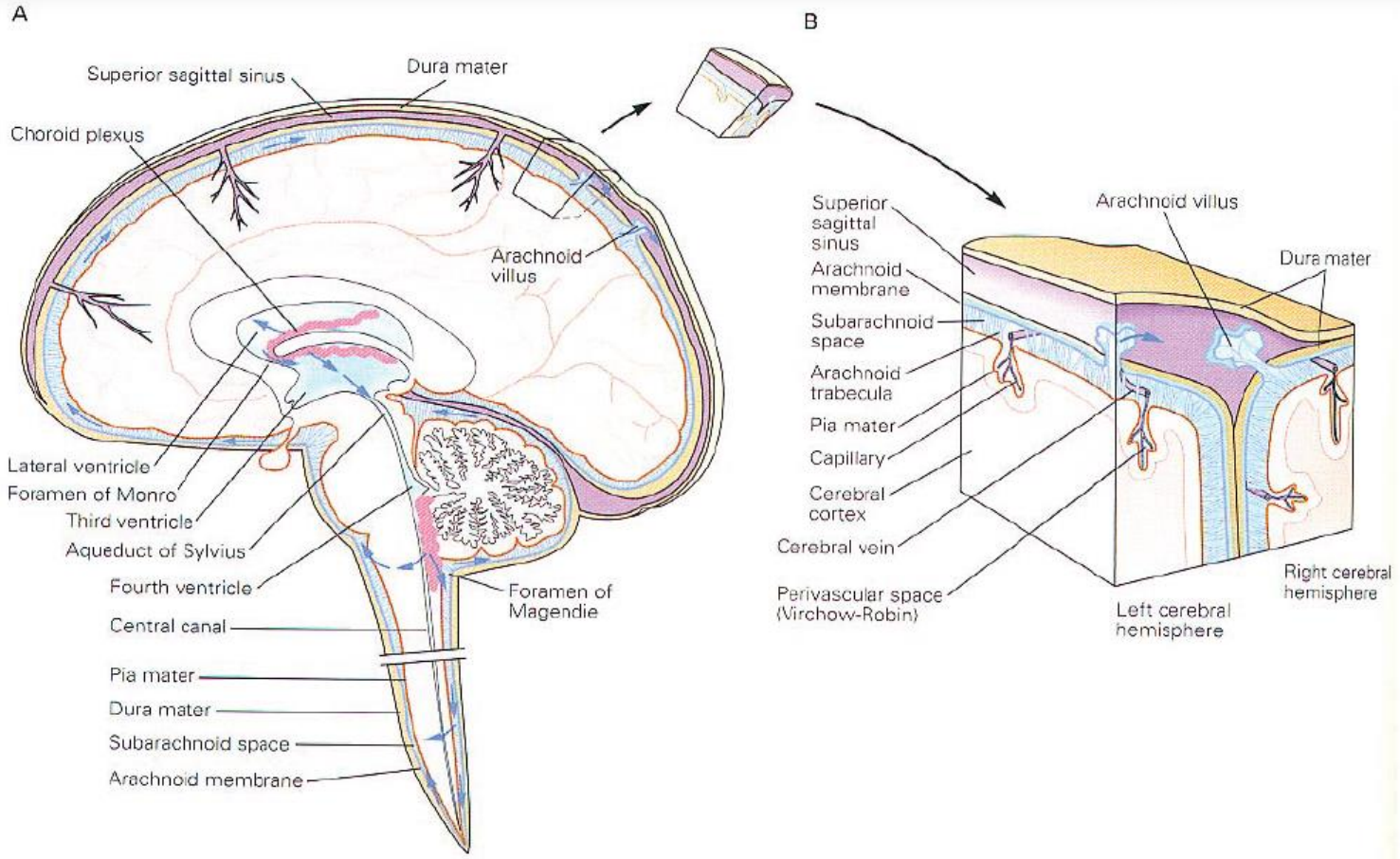


Figure B-6 Distribution of CSF. (Adapted from Carpenter 1978 and Fishman 1992.)

A. Sites of formation, circulation, and absorption of CSF. All spaces containing CSF communicate with each other. Choroidal and extrachoroidal sources of the fluid exist within the ventricular system. CSF circulates to the subarachnoid space and is absorbed into the venous system via the arachnoid villi. The presence of arachnoid villi adjacent to the spinal roots supplements the absorption into the intracranial venous sinuses. (Adapted from Fishman 1992.)

B. The subarachnoid space is bounded externally by the arachnoid membrane and internally by the pia mater, which extends along blood vessels that penetrate the surface of the brain. (Adapted from Carpenter 1978.)

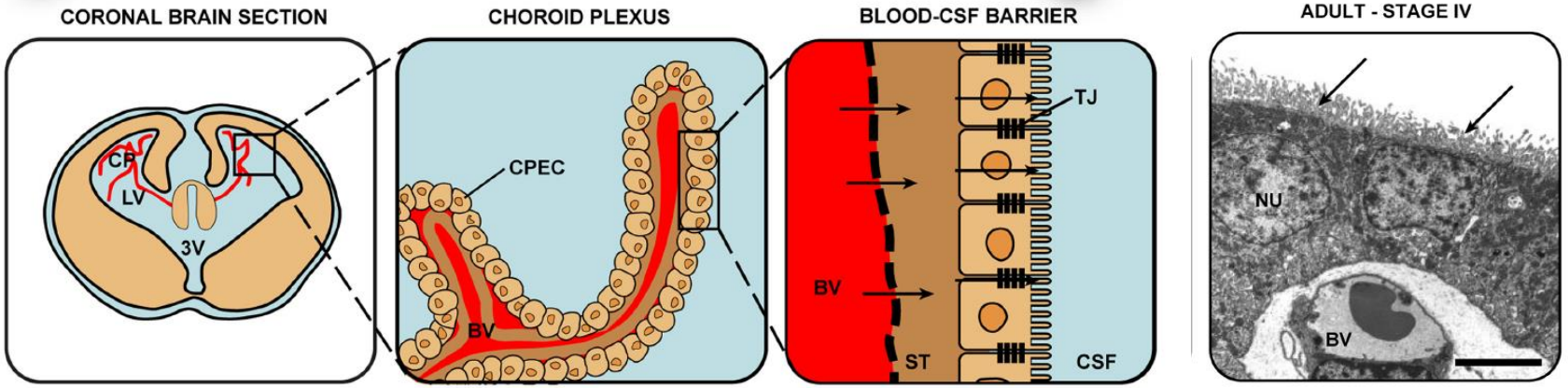
Blood–CSF barrier: **choroid plexus epithelium**

The choroid plexuses, found in the lateral, third and fourth ventricles of the brain, are epithelial tissue masses highly vascularized with fenestrated blood vessels. These structures constitute a transfer interface between blood and CSF in the ventricles of the brain. Approximately two thirds of this CSF is produced and secreted by the choroid plexus.

The choroid plexus provides:

a) a physical barrier to impede entrance of toxic metabolites to the brain
b) a “biochemical” barrier that facilitates removal of moieties that circumvent this physical barrier
c) buoyant physical protection by CSF itself

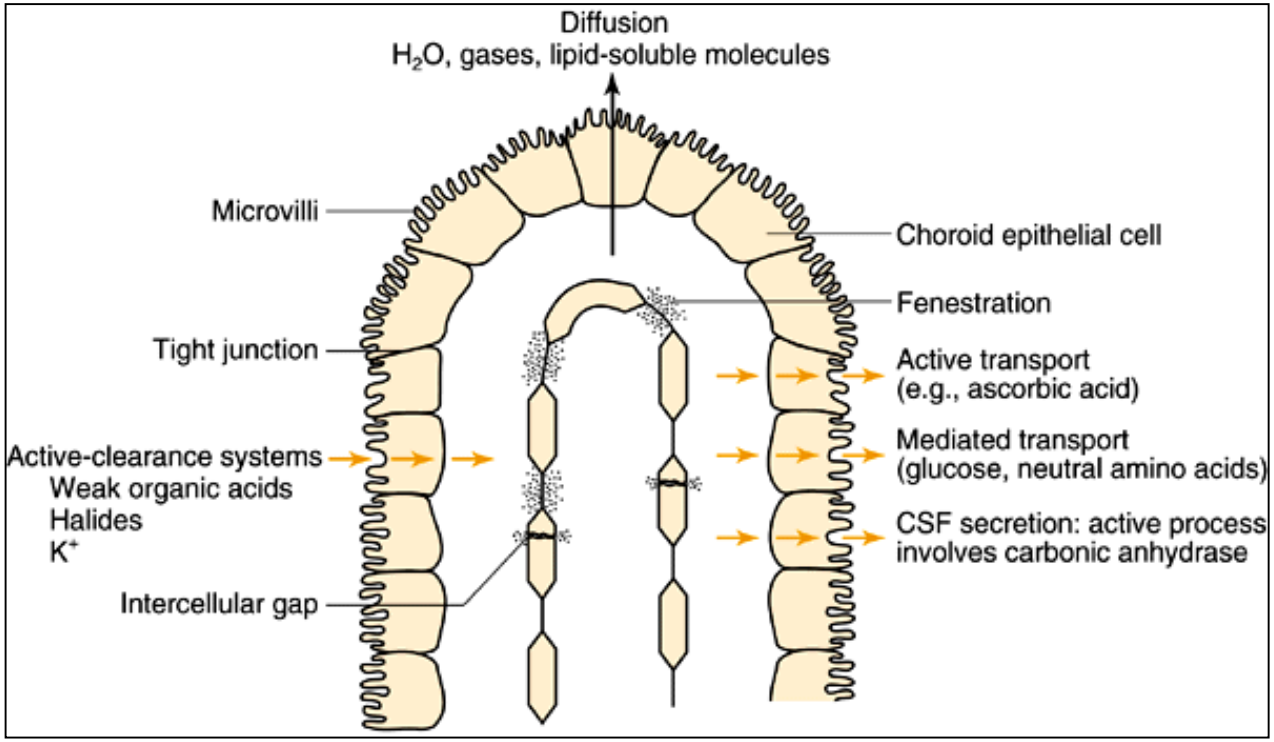
Mechanisms involved combine structural diffusion restraint between plexus epithelial cells (tight junctions physical barrier) and specific exchange mechanisms across the interface (enzymatic barrier).



Shane A. Liddelow[1,2]*

Blood–CSF barrier: **choroid plexus epithelium**

The **capillaries** in the choroid plexus differ from those of the brain in that there is free movement of molecules across the endothelial cell through **fenestrations** and intercellular gaps. **The blood–CSF barrier is at the choroid plexus epithelial cells**, which are joined together by **tight junctions**. **Microvilli** are present on the CSF-facing surface. These greatly increase the surface area of the apical membrane and may aid in fluid secretion. Diffusion, facilitated diffusion and active transport into CSF, as well as active transport of metabolites from CSF to blood, have been demonstrated in the choroid plexus.



Discovery of the **Blood Brain Barrier (BBB)**

Already in the 19th century it was observed that molecules injected in the peripheral circulation do not enter the brain parenchima….. leading to the concept of the Blood Brain Barrier (BBB)

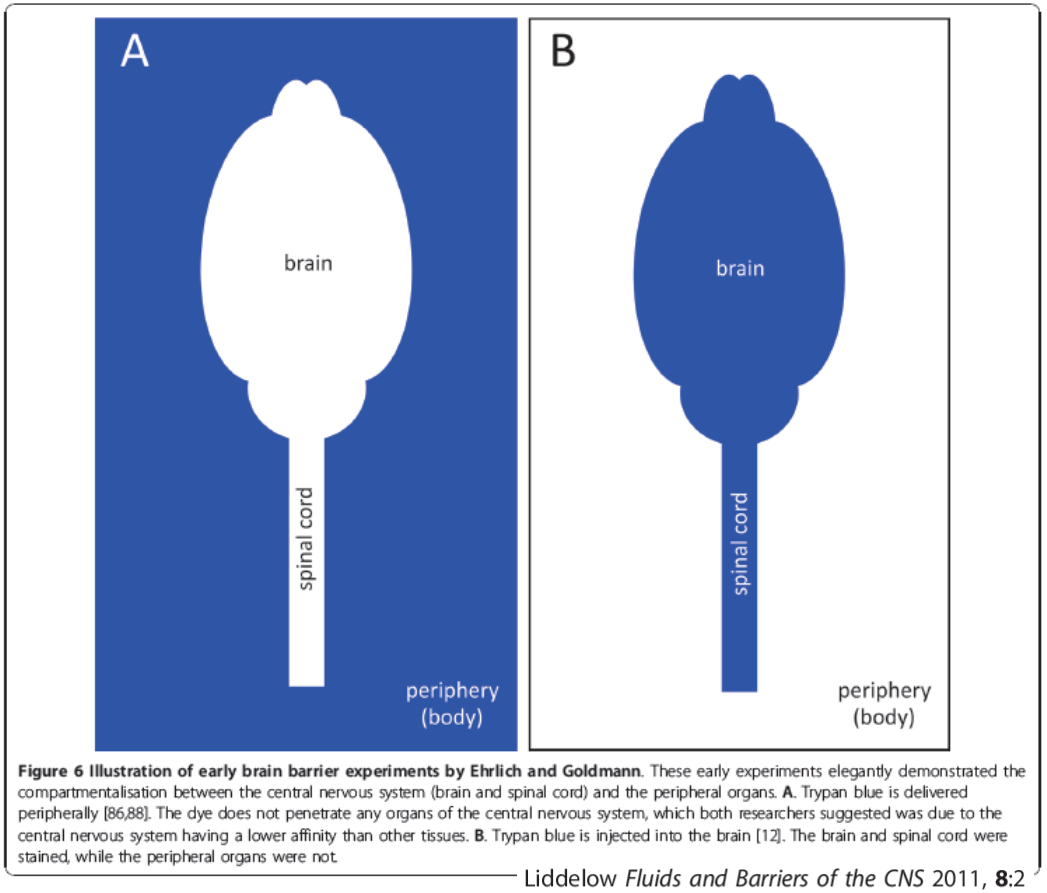
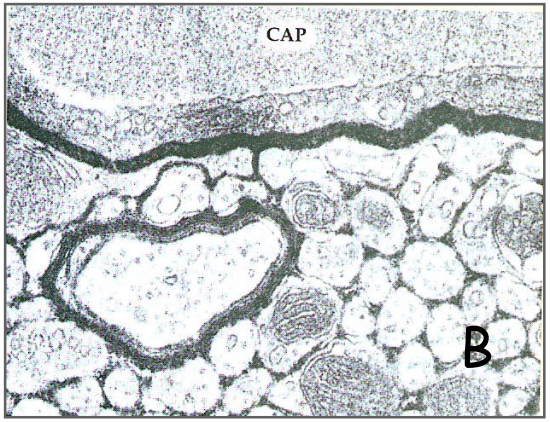
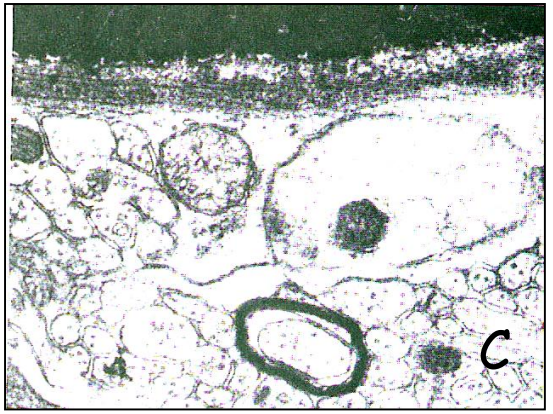


Figure 6 Illustration of early brain barrier experiments by Ehrlich and Goldmann. These early experiments elegantly demonstrated the compartmentalisation between the central nervous system (brain and spinal cord) and the peripheral organs. **A.** Trypan blue is delivered peripherally [86,88]. The dye does not penetrate any organs of the central nervous system, which both researchers suggested was due to the central nervous system having a lower affinity than other tissues. **B.** Trypan blue is injected into the brain [12]. The brain and spinal cord were stained, while the peripheral organs were not.

Liddelow *Fluids and Barriers of the CNS* 2011, **8**:2
http://www.fluidsbarrierscns.com/content/8/1/2

(B) Demonstration in the mouse that the enzyme microperoxidase diffuses freely from cerebrospinal fluid into the intercellular spaces of the brain, which are filled with the dark reaction product. No enzyme is seen in the capillary (CAP). (C) When injected into the circulation, the enzyme fills the capillary but is prevented by the capillary endothelium from escaping into the intercellular spaces. (B and C from Brightman, Reese, and Feder, 1970.)





AREAS OF BRAIN WITHOUT A BLOOD-BRAIN BARRIER

Pituitary gland

Median eminence

Area postrema

Preoptic recess

Paraphysis

Pineal gland

Endothelium of the choroid plexus

Neuropeptides secretion to blood, chemosensitivity to monitor blood composition

Presence of TJ between specialized ependymal cells in CVOs and astrocytic processes that isolate the CVOs from brain parenchyma

Circumventricular organs have **no BBB**

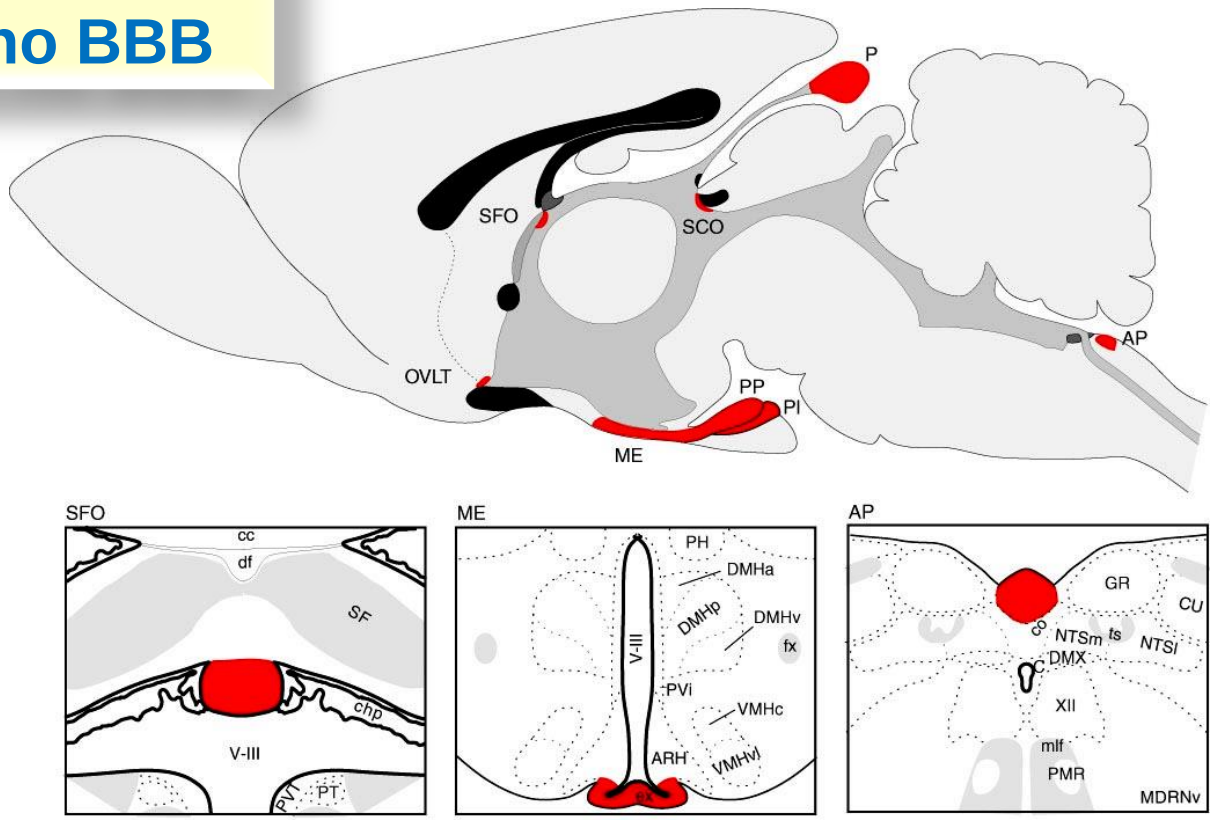
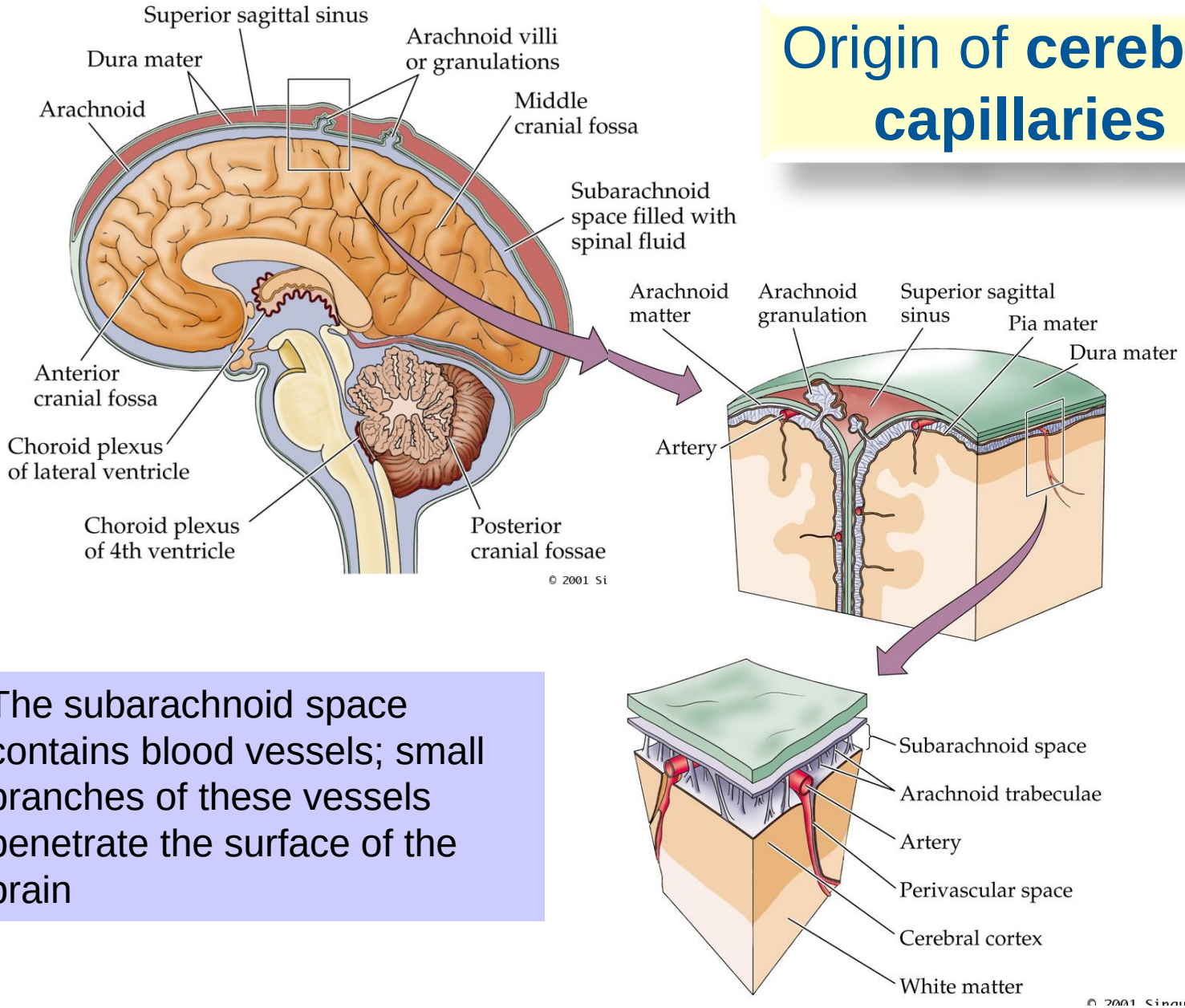


FIGURE 3 Location of the six circumventricular organs (shown in red) in the rat brain (midsaggital section). Three regions that have an intimate functional association with the hypothalamus are also illustrated in transverse section in the lower figures. AP, area postrema; ARH, arcuate nucleus; cc, corpus callosum; CU, cuneate nucleus; df, dorsal fornix; DMHa, anterior portion of the dorsomedial nucleus; DMHp, posterior portion of dorsomedial nucleus; DMHv, ventral portion of dorsomedial nucleus; DMX, dorsal motor vagal nucleus; GR, gracile nucleus; ME, median eminence; mlf, medial longitudinal fasiculus; co, commissural portion of the nucleus of the solitary tract; NTSl, lateral portion of the nucleus of the solitary tract; NTSm, medial portion of the nucleus of the solitary tract; OVLT, vascular organ of the lamina terminalis; PH, posterior hypothalamus; P, pineal gland; PMR, paramedian reticular nucleus; PVi, intermediate part of periventricular nucleus; SCO, subcommissural organ; SF, septofimbrial nucleus; SFO, subfornical organ; ts, tractus solitarius; V-III, third ventricle; VMHc, central part of ventromedial nucleus; VMHvl, ventrolateral part of ventromedial nucleus; XII, hypoglossal nucleus.

Origin of **cerebral capillaries**

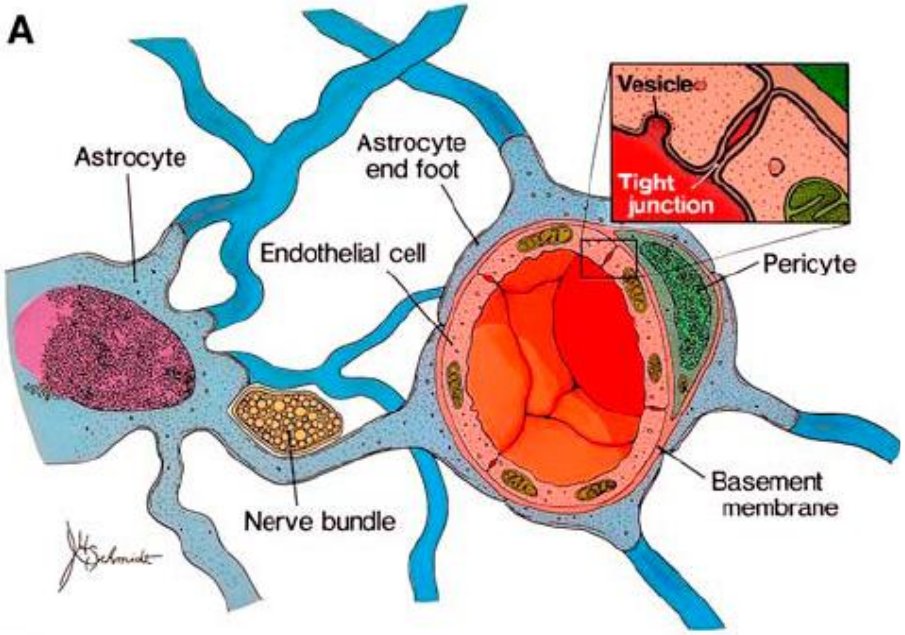


The subarachnoid space contains blood vessels; small branches of these vessels penetrate the surface of the brain

The Blood-Brain Barrier
capillary endothelium

- increased mitocondrial content
- lack of fenestrations
- minimal pinocytotic activity
- presence of Tight Junctions (TJ)

• no paracellular transport
• controlled transcellular transport



Hawkins et al., 2006
J. Nutr. 136: 218S–226S

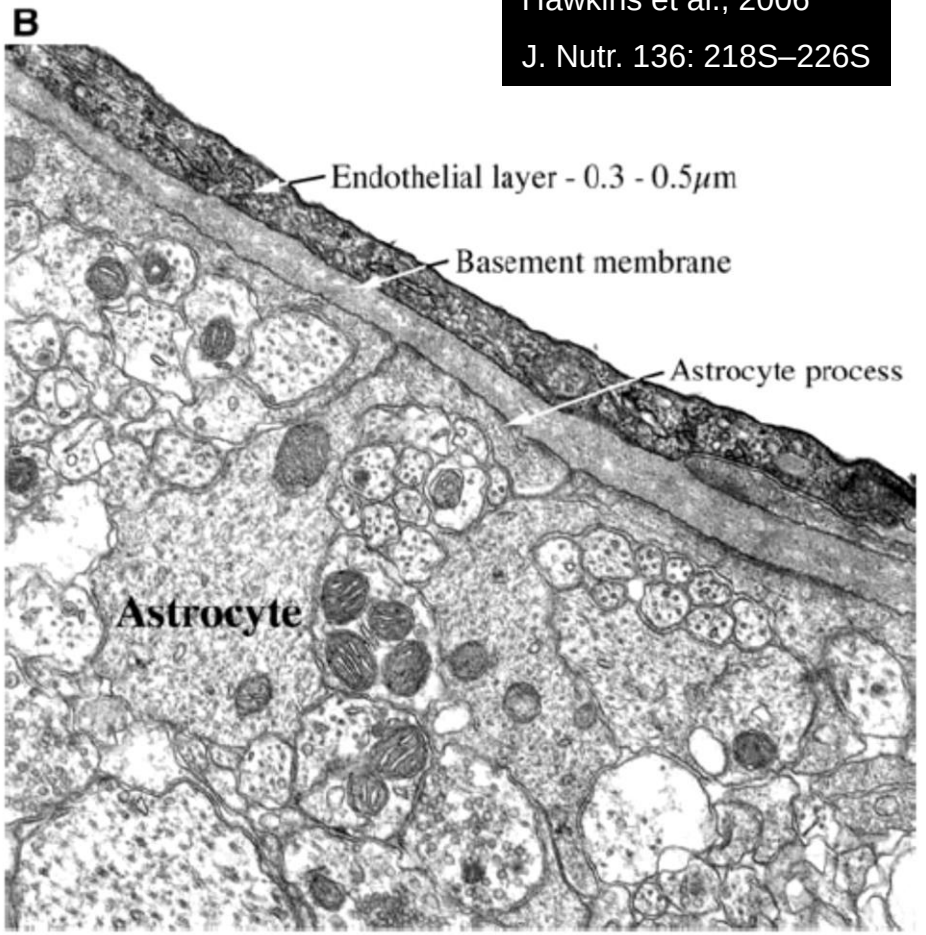
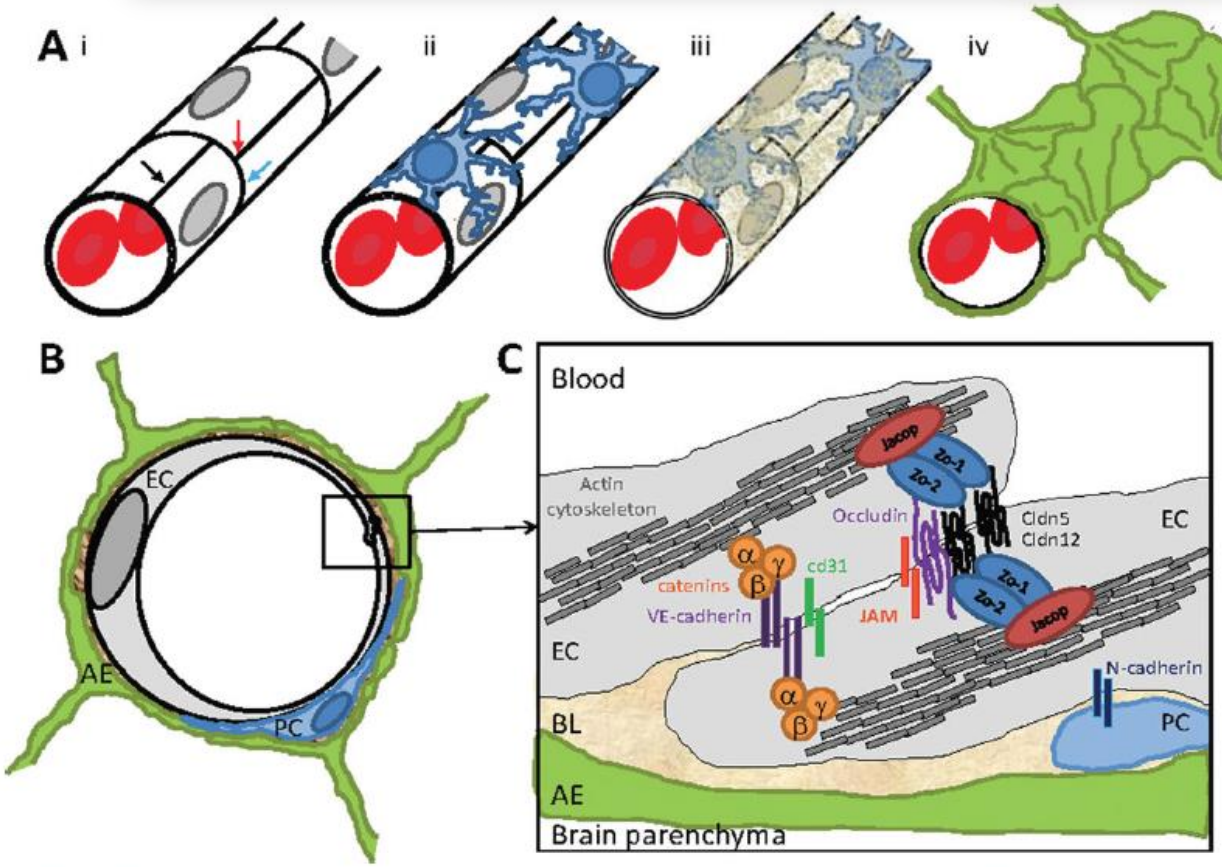


FIGURE 2 (A) The BBB exists at the level of the endothelial cells of cerebral capillaries. The endothelial cells are joined together by an extensive network of tight junctions and surrounded by a basement membrane, within which pericytes reside. Astrocytic processes (so-called end-feet) surround cerebral capillaries (previously published in IUBMB Life). (B) Right, an electron micrograph of a cerebral capillary shows the basic elements. The electron micrograph was provided through the courtesy of Robert Page, MD; Professor, Neurosurgery and Anatomy, Pennsylvania State University College of Medicine.

Cellular and molecular organization of the BBB



BBB is a physical barrier: molecular composition of endothelial tight junctions

FIGURE 1: Schematic representation of the blood–brain barrier. (A) Overlay schematic representation of the major cell types of capillaries that form the blood–brain barrier. (i) Endothelial cells form a tube that allows blood to flow through. The endothelial cells fold on themselves to form intracellular tight junctions (*black arrow*), and adhere to adjacent endothelial cells through intercellular tight junctions (*blue arrow*), and the point at which the intra- and intercellular junctions meet are tricellular adhesions (*red arrow*). (ii) Pericytes (blue) adhere to the abluminal surface of the endothelial cells. (iii) The vascular tube of endothelial cells and pericytes is surrounded by a layer of basal lamina made up of extracellular matrix proteins secreted by vascular and neural cells. (iv) Endfeet of astrocyte processes (green) ensheathe the blood vessels. (B) Cross-sectional schematic representation of the major cell types of capillaries that form the blood–brain barrier, including endothelial cells (EC; gray), pericytes (PC; blue), basal lamina (BL; brown), and astrocyte endfeet (AE; green). (C) Schematic representation of the tight junctions that hold together capillary endothelial cells of the central nervous system. The tight junction strands between cells are formed by adhesions of transmembrane proteins including claudins, occludin, and junctional adhesion molecules (JAMs), which are linked to the actin cytoskeleton and cadherin/catenin-based adherens junctions by adaptor proteins including ZO-1, ZO-2, and Jacop.

Daneman et al., 2012

ANN NEUROL 72:648–672

Pericytes

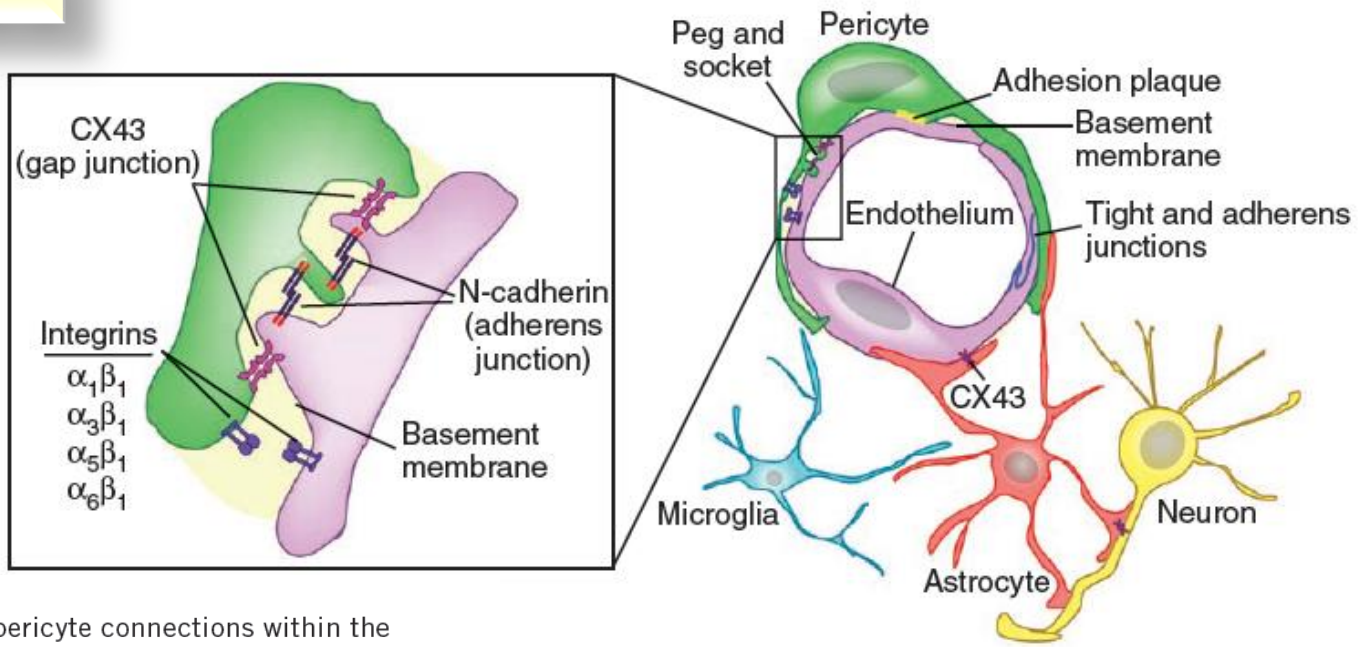


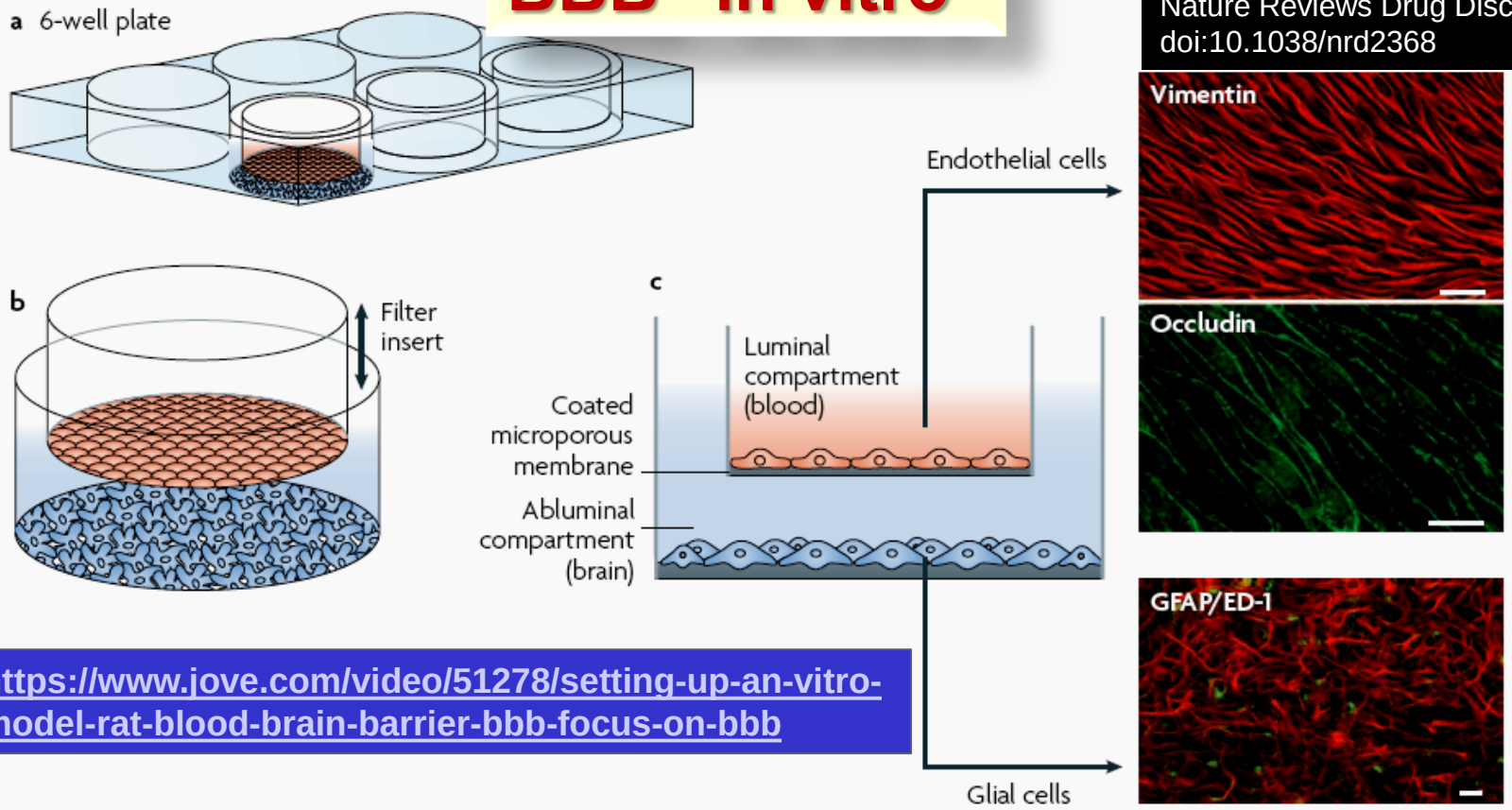
Figure 1 Structural and molecular pericyte connections within the neurovascular unit. Right: pericytes (green) and endothelial cells (purple) are connected to a shared basement membrane (yellow) by several types of integrin molecule. In areas lacking the basement membrane, interdigitations of pericyte and endothelial cell membranes, called peg and socket contacts, form direct connections and contain several different transmembrane junctional proteins (inset). N-cadherin is the key adherens junction protein between pericytes and endothelium. Pairs of connexin 43 (CX43) hemichannels expressed respectively in pericytes and endothelium form gap junctions that allow transfer of molecules between pericytes and endothelial cells. Adhesion plaques similar to desmosomes contain fibronectin deposits in the intercellular spaces between pericytes and endothelial cells. CX43 is also abundant at astrocyte–endothelial cell and astrocyte-neuron interfaces. Different types of tight junction proteins, tight junction adaptor proteins and adhesion junctions regulate direct endothelial cell–endothelial cell contacts forming the anatomical blood-brain barrier.

Winkler et al., 2011
Nature Neuroscience
doi:10.1038/nn.2946

BBB “in vitro”

Cecchelli et al., 2007

Nature Reviews Drug Discovery
doi:10.1038/nrd2368



https://www.jove.com/video/51278/setting-up-an-vitro-model-rat-blood-brain-barrier-bbb-focus-on-bbb

Figure 2 | **Modelling the blood–brain barrier *in vitro*. a** | Brain endothelial cells are grown on filter inserts together with glial cells at the bottom of 6-, 12- or 24-well culture plates. **b** | Glial soluble factors secreted in the culture medium induce the blood-brain barrier (BBB) phenotype in the capillary endothelium. This experimental design can be used for compound screening in the drug discovery process in the pharmaceutical industry but is also well suited for studying mechanistic aspects of BBB transport as well as other biological and pathological processes. **c** | Illustration of a typical experimental design which allows a co-culture of brain endothelial cells and glial cells[74]. Vimentin immunostaining shows a confluent brain endothelial cell monolayer with non-overlapping morphology and typical spindle shaped cells (top right panel). The continuous marginal localization of the tight junction protein occludin reflects the tightness of the barrier and the cerebral origin of the capillary endothelial cells (middle panel). In the bottom right panel, staining for glial fibrillary acidic protein (GFAP) (red) shows astrocytes within the glial cell population and ED-1 staining (green) highlights the presence of microglia. Scale bar represents 25 μm.

BBB “in vitro”

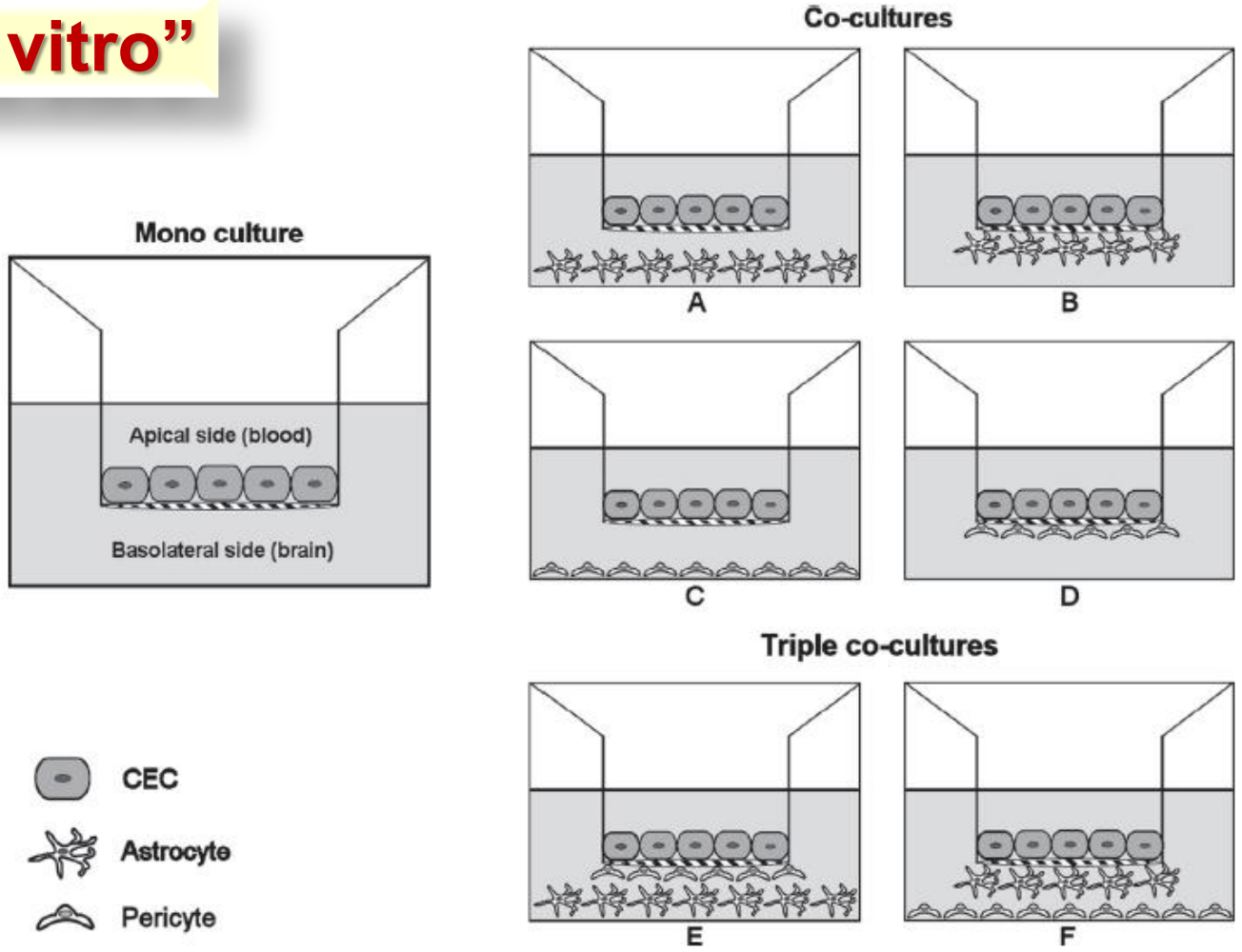


Fig. 2. *In vitro* cell-based blood–brain barrier (BBB) models. Monocultures of cerebral endothelial cells (CECs) are being replaced by co-culture or triple co-culture systems, in which CECs are seeded with other elements of the neurovascular unit, such as astrocytes, pericytes or neurons, in a non-contact (A,C) or contact format (B,D). In triple co-culture systems, more than one cell type is seeded with CECs (E,F).

Bicker et al., 2014
European Journal of Pharmaceutics and Biopharmaceutics 87:409–432

BBB “in vitro”

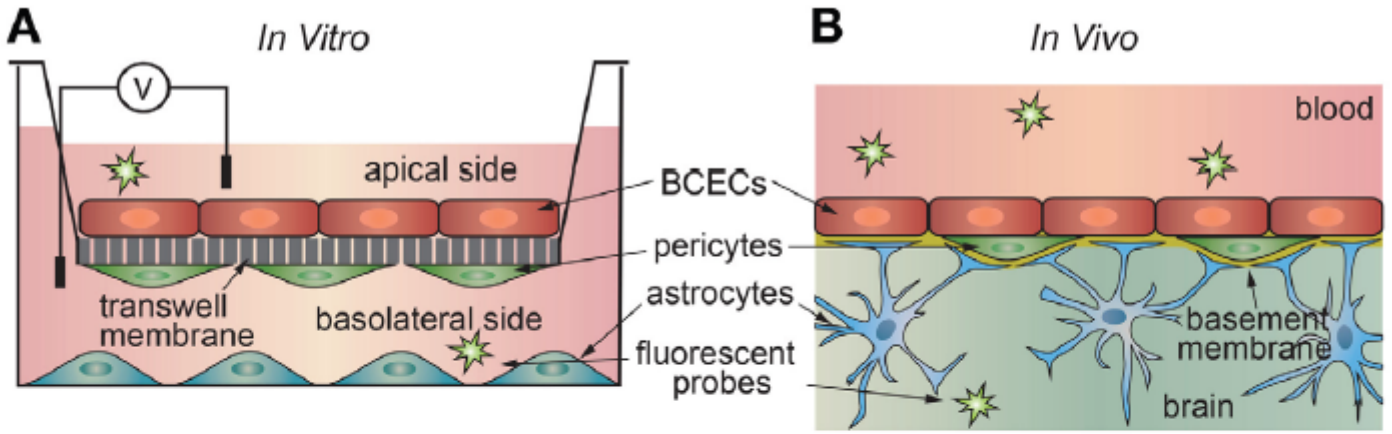


FIGURE 4 | Schematic illustration of (a) *in vitro* and (b) *in vivo* transport measurements. (A) In the 2D transwell assay, a monolayer of cells is formed on a porous membrane separating two compartments. Astrocytes and/or pericytes may be seed on the opposite side of the membrane or in the output chamber. **(B)** *In vivo* studies, a solute is injected into the blood of an animal model, and the penetration into the brain measured using a suitable chemical detection assay or imaging technique.

Wong et al., 2013
Frontiers in Neuroengineering
doi: 10.3389/fneng.2013.00007

In vitro BBB models: good or bad?

Wong et al., 2013
Frontiers in Neuroengineering
doi: 10.3389/fneng.2013.00007

Table 2
Advantages and disadvantages of the use of primary cultures and immortalized cell lines of CECs as *in vitro* models of the BBB.

	Sources	Advantages	Disadvantages		Refs.
Primary cultures	Bovine Porcine Rat	Allow the isolation of a large amount of cells from a single brain Availability of BBB permeation data from *in vivo* pharmacokinetic studies	Difficult to establish *in vitro–in vivo* correlations Low yield; Require the sacrifice of multiple animals; Ethical and economic constraints	Complex, time consuming and labor intensive cell obtaining processes; Inconvenient for routine industrial use; High irreproducibility of the final characteristics of the cell population; Challenging to eliminate non-endothelial cells (e.g. pericytes, smooth muscle cells); Rapid *in vitro* de-differentiation or loss of phenotype	Smith [143] Patabendige [4]; Perrière [98]; Zhang [123]
	Mouse				Steiner [197]
	Human	Important tool for the study of the BBB at a cellular and molecular level, including BBB dysfunction	Low yield; High costs; Difficult to obtain reliable sources of healthy tissue; Batch-to-batch variability		Reichel [54]; Smith [143]; Vu [198]
Immortalized cell lines	Bovine: *t-BBEC-117*	Exhibits the formation of TJ-like structures; Expresses endothelial markers (acLDL uptake), influx (GLUT-1) and efflux transporters (P-gp) and functional endothelial-specific enzymes (AP)	Leaky; Not restrictive enough for permeability studies (i.e. low TEER values and high permeability of paracellular tracers); Difficult maintenance of transporter function and enzymatic activity; Require further characterization		Sobue [142]
	Porcine: *PBMEC/C1-2*	Expresses endothelial markers (vWF), influx (GLUT-1) and functional efflux transporters (P-gp) and functional endothelial-specific enzymes (AP, γ-GTP)			Neuhaus [140]; Lauer [141]; Teifel [199]; Neuhaus [200]; Gomes [201]
	Rat: *RBE4*, *RBE4.B*	Express TJ proteins (e.g. occludin), influx (GLUT-1, LAT-1) and functional efflux transporters (P-gp) and functional endothelial-specific enzymes (AP, γ-GTP)			Cestelli [51]; Bendayan [86]; Roux [125]; Garberg [161]; Gomes [201]
	Mouse: *b.End3*, *b.End5*	Express endothelial markers (vWF), TJ proteins (e.g. occludin, claudins-1,-5, ZO-1) influx (e.g. GLUT-1, LAT-1) and functional efflux transporters (P-gp) and functional endothelial-specific enzymes (AP); Commercially available			Yang [122]; Li [135]; Brown [145]; Omidi [151]; Guo [202]; Burek [203]; Watanabe [204]
	Human: *hCMEC/D3*, *BB19*, *NKIM-6*, *TY08*, *HBMEC/ciβ*	*hCMEC/D3:* Exhibits a non-transformed phenotype over several passages; Expresses endothelial markers, TJ proteins (e.g. claudin-5, ZO-1), functional efflux transporters (e.g. ABCG2, P-gp) and CYP genes *BB19, NKIM-6, TY08, HBMEC/ciβ:* Express endothelial markers (vWF), TJ proteins (e.g. ZO-1) and efflux transporters (ABCG2, P-gp)	*NKIM-6*: low expression of occludin and does not express claudin-5		Urich [124]; Lu [126]; Vu [198]; Weskler [205]; Poller [206]; Tai [207]; Dauchy [208]; Schrade [209]; Weskler [210] Kusch-Poddar [106]; Ketabi-Kiyanvash [211]; Sano [212]; Kamiichi [213]

ABCG2, Breast cancer resistance-associated protein; acLDL, acetylated low density lipoproteins; AP, alkaline phosphatase; BBB, blood–brain barrier; CECs, cerebral endothelial cells; CYP, cytochrome P450; GLUT-1, glucose transporter-1; γ-GTP, γ-glutamyltranspeptidase; LAT-1, large neutral aminoacid transporter; P-gp, P-glycoprotein; TEER, transendothelial electrical resistance; TJ, tight junction; vWF, von Willebrand factor VIII related antigen; ZO, zonula-occludens.
The common disadvantages of primary cultures and immortalized cell lines were adapted from Refs. [50,51,62,89,98,102,125,214].

Box 1: Functions of the BBB.

The blood–brain barrier:

1. Controls molecular traffic, keeps out toxins (minimises neuronal cell death, preserves neural connectivity)
2. Contributes to ion homeostasis for optimal neural signalling
3. Maintains low protein environment in CNS, limits proliferation, preserves neural connectivity
4. Separates central and peripheral neurotransmitter pools, reduces cross-talk, allows non-synaptic signalling in CNS
5. Allows immune surveillance and response with minimal inflammation and cell damage

BBB is a **selective** transport barrier

Brain uptake of blood-circulating molecules

Mikitsh and Chacko
PERSPECTIVES IN MEDICINAL CHEMISTRY 2014:6

A

	Opiate		
	Morphine	Codeine	Heroin
R_1	OH	OCH_3	
R_2	OH	OH	
Log *P*	0.99	1.2	2.3

B

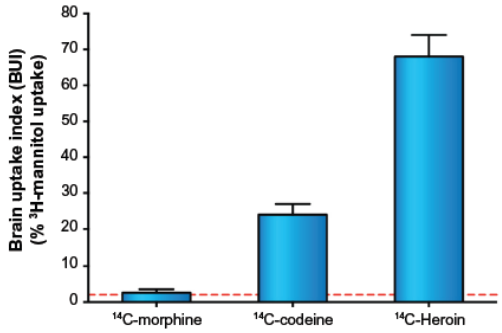


Figure 3. (**A**) Chemical structures of morphine, codeine, and heroin with their respective log *P*. (**B**) Relative rat brain uptake index (BUI) of ^{14}C-morphine, ^{14}C-codeine, and ^{14}C-heroin in rats following a single brain passage after carotid injection. The greater uptake of codeine and heroin relative to morphine can be explained on the basis of their greater lipid solubility (as reflected by log *P*) relative to morphine. ^{3}H-mannitol was used as a reference ligand for its poor BBB permeability. For each mean and standard deviation, $n = 6$.[59]

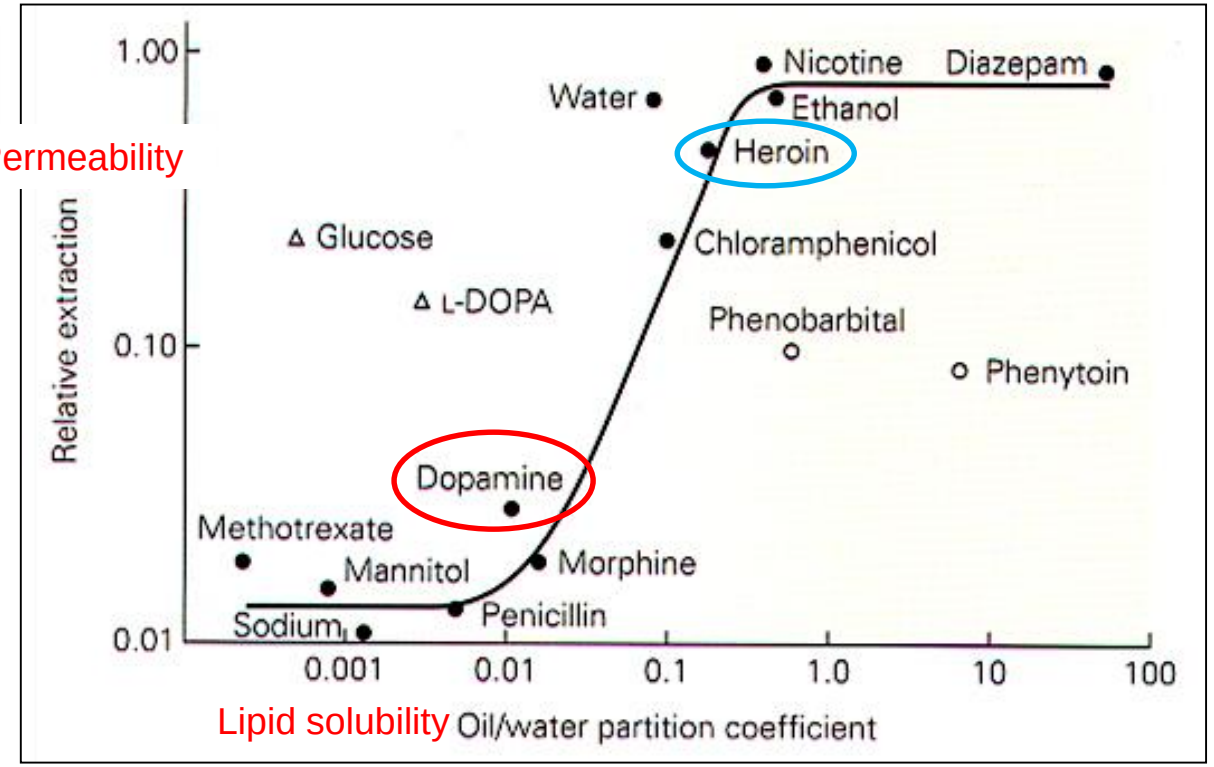
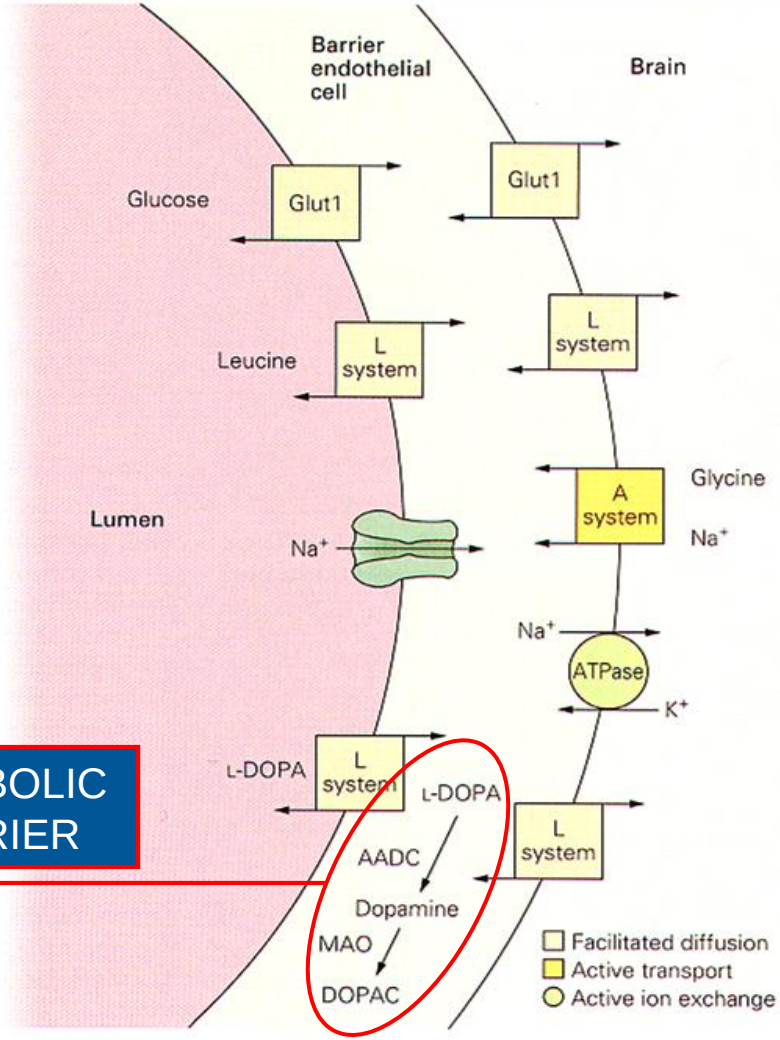


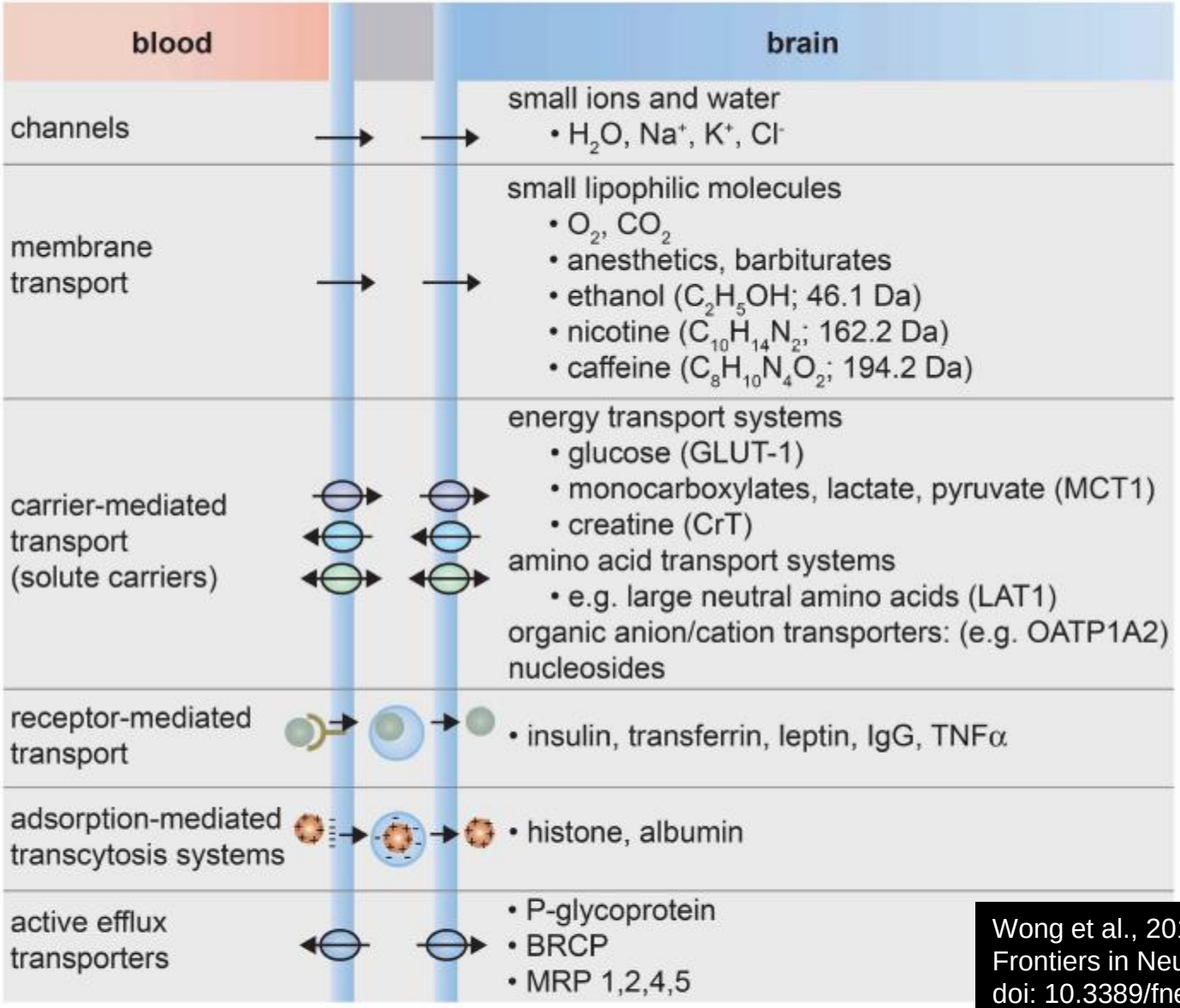
Figure B-4 The oil-water partition coefficient indicates the relationship between lipid solubility and brain uptake of selected compounds. The distribution into olive oil relative to water for each test substance serves as a measure of its lipid solubility. The brain uptake is determined by comparing the extraction of each test substance relative to a highly permeable tracer during a single passage through the cerebral circulation. In general, compounds with higher oil-water partition coefficients show increased entry into brain. Uptake of the anticonvulsants phenobarbital and phenytoin is lower than predicted from their lipid solubility partly because of their binding to plasma proteins. This explains the slower onset of anticonvulsant activity of these agents compared to diazepam. Uptake of glucose and L-DOPA is greater than predicted by their lipid solubility because specific carriers facilitate their transport into the brain capillary. (From Goldstein and Betz 1986.)



METABOLIC BARRIER

Luminal and abluminal membranes of endothelial cells express a number of **transporters** and **channels**

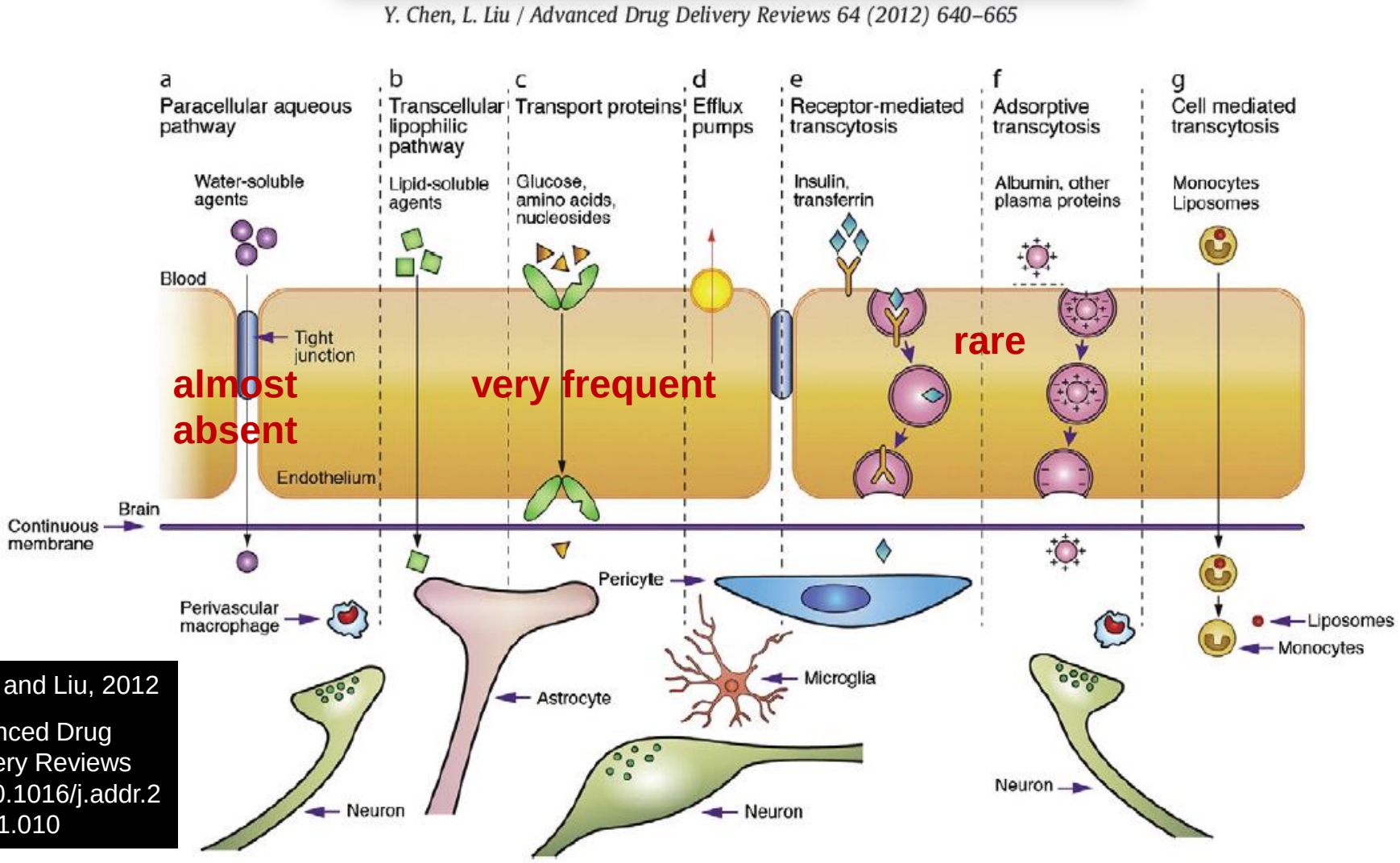
Figure B-5 A complex system of polarized transporter proteins and ionic channels determine the specific movement of water-soluble compounds and ions across barrier endothelial cells. Some transporters (eg, Glut1 and L system) facilitate the movement of substrates down concentration gradients, and others (eg, A system and Na^+-K^+-ATPase) actively transport substrates via energy-dependent mechanisms. Enzyme systems such as amino acid decarboxylase (AADC) and monoamine oxidase (MAO) function as a metabolic barrier by converting within the barrier endothelial cells substances such as L-DOPA to 3,4-dihydroxyphenylacetic acid.



Wong et al., 2013
Frontiers in Neuroengineering
doi: 10.3389/fneng.2013.00007

FIGURE 3 | Transport systems at the blood-brain barrier. (1) Small ions and water molecules can cross the blood-brain barrier through ion channels. (2) Small lipophilic molecules that are soluble in the hydrophobic core of the cell membrane can be transported passively across the cell. (3) Essential polar molecules that cannot diffuse through the cell membrane are shuttled across the cell membranes by carrier-mediated transport. These solute carriers may be directional, in or out of the cell, or bidirectional. Other molecules can be actively transported across endothelial cell membranes by carrier-mediated transporters, receptor-mediated transporters, adsorption-mediated transcytosis, or efflux pumps.

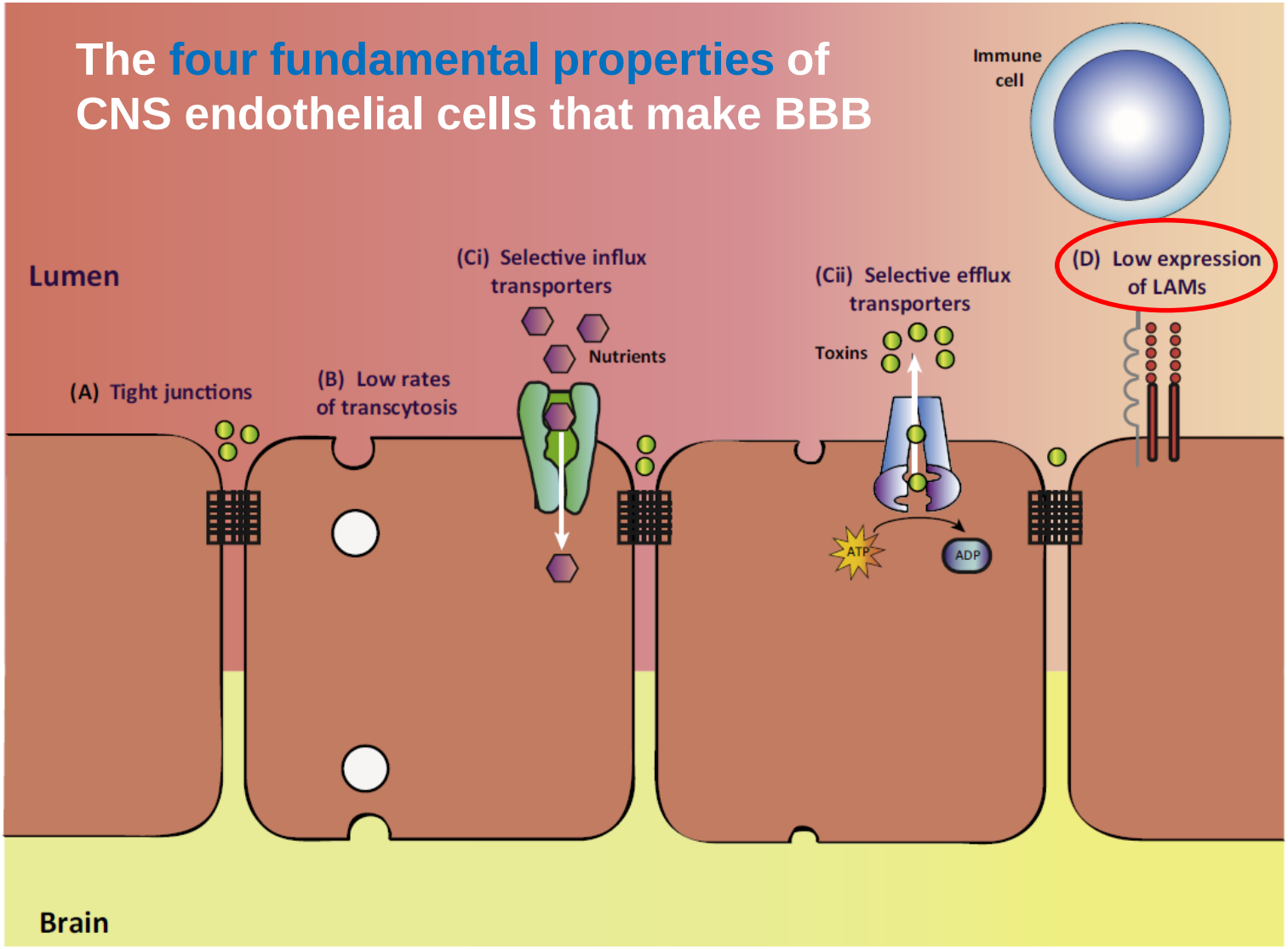
Pathways across the BBB



Chen and Liu, 2012

Advanced Drug Delivery Reviews
doi:10.1016/j.addr.2011.11.010

Transport routes across the blood–brain barrier. Pathways "a" to "f" are common for solute molecules; the route "g" involves monocytes, macrophages and other immune cells and can be used for any drugs or drugs incorporated liposomes or nanoparticles.



CNS endothelial cells lack expression of leukocyte adhesion molecules (LAMs) such as E-selectin and Icam1. The lack of these luminal surface molecules prevents the entry of immune cells from the blood into the parenchyma, resulting in a paucity of immune cells in the brain microenvironment. As a result, the **healthy brain is 'immune privileged',** whereby introduced antigens do not elicit the development of adaptive immune responses

Figure 2. The Four Fundamental Molecular Properties of Central Nervous System (CNS) Endothelial Cells that Contribute to Blood–Brain Barrier (BBB) Integrity and Function. (A) Specialized tight junction complexes between endothelial cells prevent paracellular flux. (B) CNS endothelial cells have low rates of transcytosis, limiting transcellular flux. CNS endothelial cells mediate (Ci) the selective uptake of nutrients and molecules from the blood using selective influx transporters and (Cii) efflux of toxins against their concentration gradient with ATP-dependent selective efflux transporters. (D) The low expression of leukocyte adhesion molecules (LAMs) contributes to the low level of immune surveillance in the CNS.

Chow & Chenghua 2015
Trends in Neurosciences
http://dx.doi.org/10.1016/j.tins.2015.08.003

Normal BBB

Pathological BBB

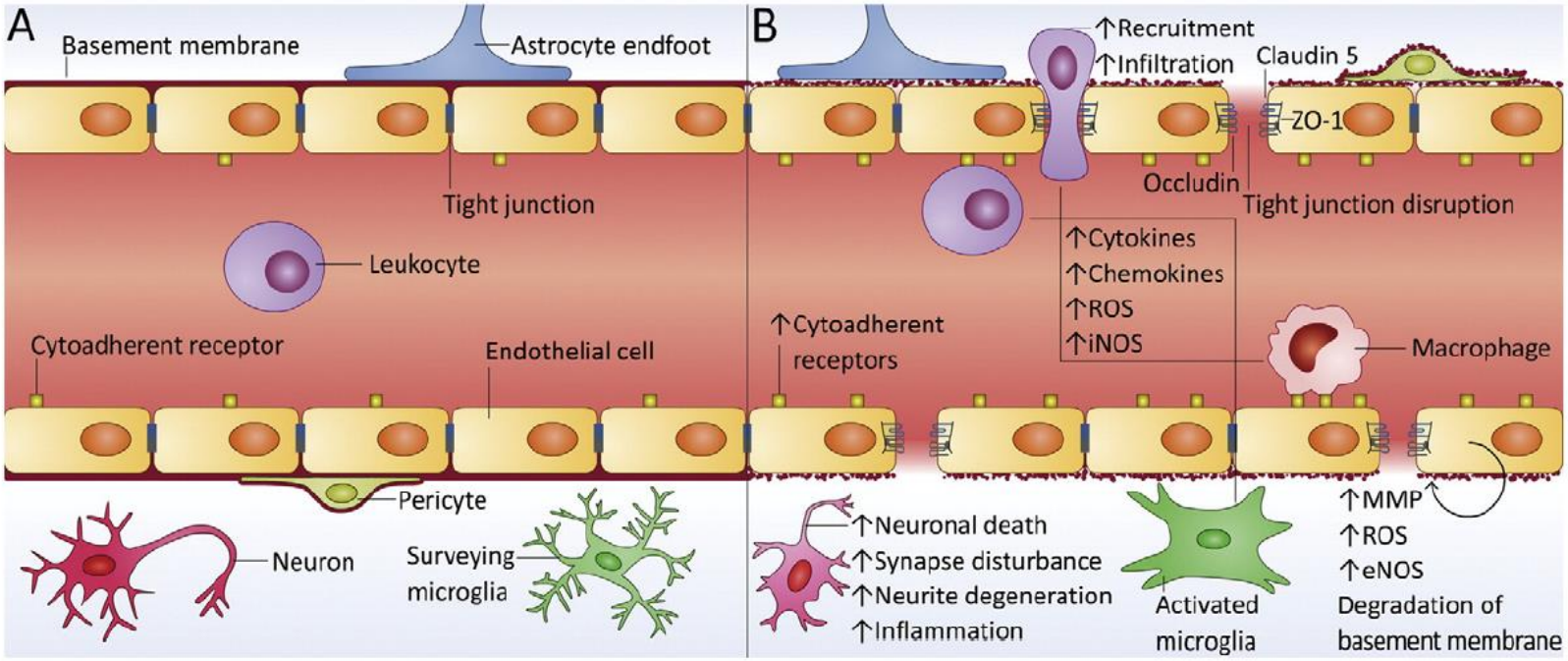


Fig. 1. Blood–brain barrier (BBB) composition and main alterations found in pathological conditions. A) The BBB is mainly composed of vascular endothelial cells, highly connected by adherens and tight junctions (TJs), and a sparse layer of pericytes. A basement membrane and a layer of astrocyte end-foot processes surround the endothelium. Neurons and surveying microglia are also important mediators of BBB integrity in physiological conditions. B) In pathological conditions several BBB alterations occur culminating in increased permeability. Increased matrix metalloproteinase (MMP) activity, higher reactive oxygen species (ROS) and nitric oxide levels (derived from endothelial cells — via endothelial nitric oxide synthase (eNOS) or from microglia/macrophage cells — via inducible NOS (iNOS)) along with release of cytokines and chemokines by activated microglia/macrophages lead to basement membrane degradation, TJ disruption (namely in occludin, zonula occludens (ZO)-1 and claudin 5 integrity) and an inflammatory response. Altogether these events culminate in neuroinflammation, leukocyte recruitment and brain parenchyma invasion, neuronal dysfunction and neurodegeneration.

Saraiva et al, 2016

J Controlled Release
http://dx.doi.org/10.1016/j.jconrel.2016.05.044

BBB brakedown in Multiple Sclerosis

Alvarez et al.: GLIA 2013;61:1939–1958

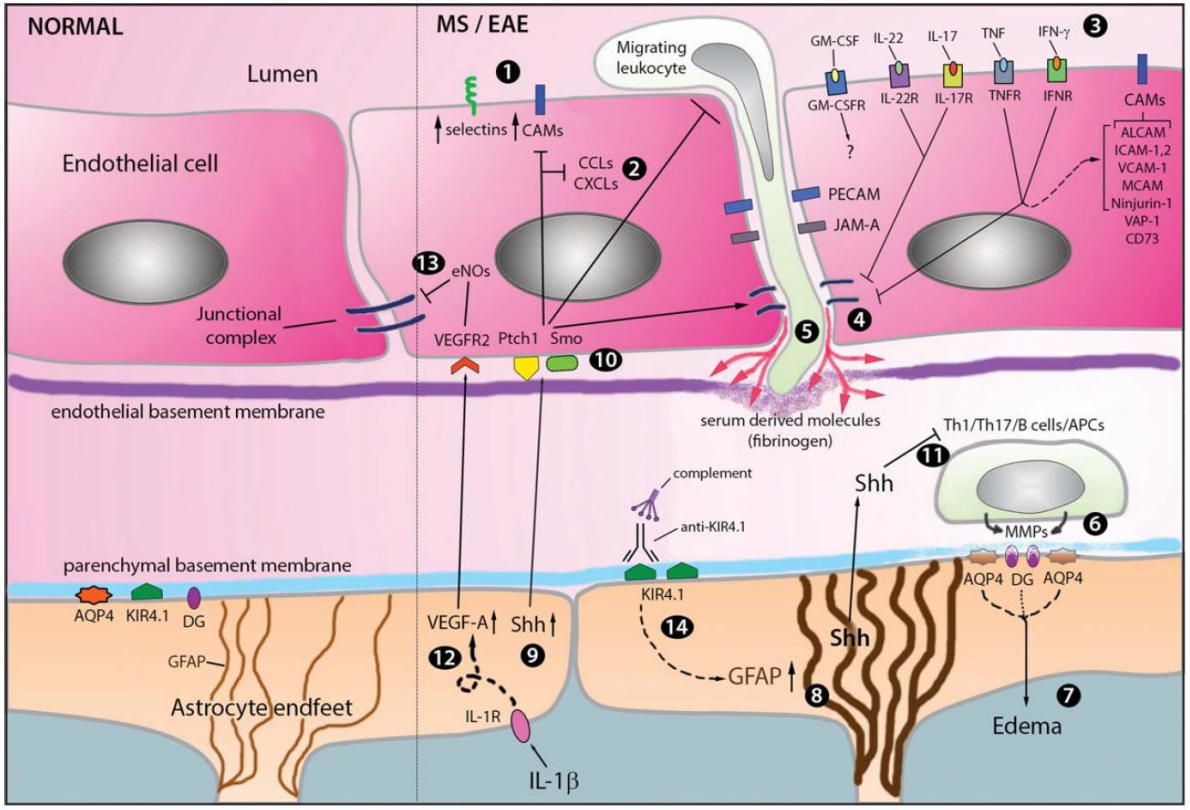


FIGURE 5: BBB changes during MS/EAE. During neuroinflammation, early BBB disturbances are associated with EC activation that is characterized by increased expression of E- and P-selectins, cell adhesion molecules (CAMs) including ALCAM, ICAM-1, ICAM-2, VCAM-1, MCAM, Ninjurin-1, VAP-1 and CD73 [1], chemokines (CCLs-CXCLs) [2] and cytokine receptors such as IFNR, TNFR, IL-17R, IL-22R, and GM-CSFR [3]. Such activation compromises the BBB phenotype by affecting junctional proteins [4] and enhances the expression of additional factors supporting the infiltration of T helper (Th) 1, Th17, and CD8 T cells as well as antigen presenting cells (APCs) [5] that accumulate in the perivascular space in a multifocal pattern. To gain access into the CNS, the infiltrating leukocytes secrete Matrix Metalloproteinases (MMPs) which target components of the astroglial basement membrane and dystroglycan (DG) at the astrocyte endfeet [6], during this process the polarity of the water channel AQP4 is perturbed leading to edema [7]. The endfeet depolarization activates the reactive gliotic program which results in increase expression of the intermediate filament GFAP [8]. Astrocytes also increase the expression of Shh [9] and ECs upregulate the Hh receptors Patched 1 (Ptch1) and Smoothened (Smo) [10] to repair the BBB and downregulate EC activation and leukocyte migration. Shh also modulates the phenotype of pathogenic Th cells by downregulating their expression of inflammatory cytokines and CAMs [11]. In the parenchymal milieu, microglia secrete IL-1β and activate the production of VEGF on astrocytes [12], which induces endothelial nitric oxidase synthase (eNOs) production and promotes junctional protein damage [13]. Finally, 50% of MS patients have autoantibodies against KIR4.1 that fix complement and induce the reactive gliosis program [14].

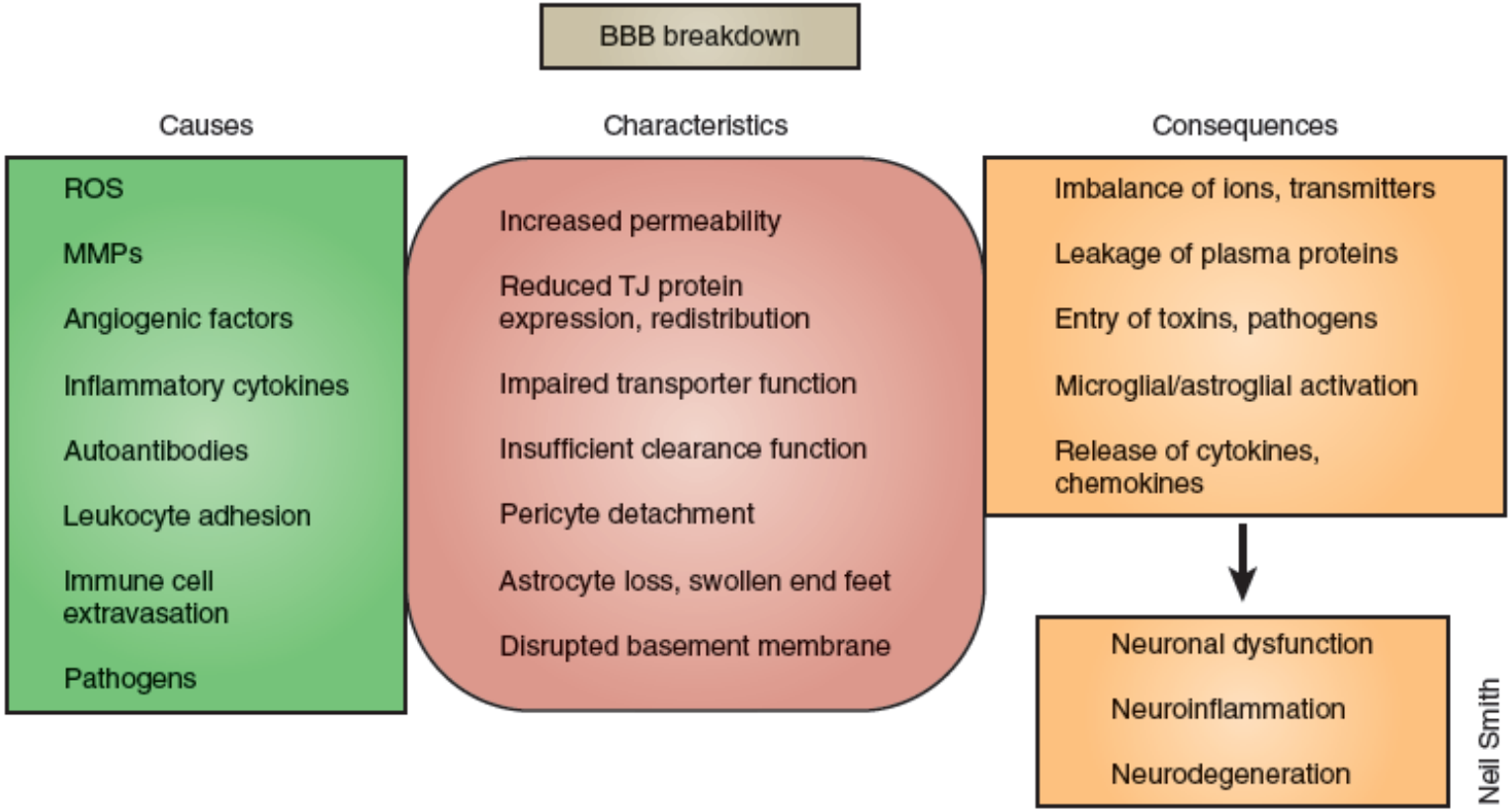


Figure 4 Causes, characteristics and consequences of BBB breakdown. Factors that can disrupt the BBB are varied, ranging from secreted elements to immune cells and pathogens. Compromised BBB integrity manifests mainly as increased barrier permeability. In addition to direct effects on endothelial cells, BBB breakdown can affect other members of the neurovascular unit, that is, pericytes, astrocytes and basement membrane, which in turn aggravate impairment of BBB functions. Consequences vary from dysregulated molecular and ionic flux across the damaged BBB to the initiation of a central inflammatory response. Despite manifold causes, characteristics and consequences, BBB breakdown generally culminates in neuronal dysfunction, neuroinflammation and neurodegeneration. Downstream pathological outcomes and potential for recovery are diverse.

Table 2 Diseases linked to BBB dysfunction

Disease	Level of BBB effect[a]	Comment	Refs.
Stroke	Primary	Microvascular injury induced by oxidative stress during ischemia-reperfusion	160
Epilepsy	Primary	Systemic inflammation can disturb brain homeostasis by allowing entry of ions and epileptogenic substances across the BBB	161,162
	Secondary	Seizures reduce BBB integrity, which enables entry of plasma proteins into the brain that sustain the epileptogenic state	
AD	Primary	BBB dysfunction, including defective amyloid-β clearance from brain and congophilic angiopathy	163,164
Familial ALS	Primary	Loss of BBB integrity at an ultrastructural level associated with expression of mutant SOD1 in brain capillary endothelial cells	164,165
PD	Secondary	Increased BBB permeability and decreased transport activity across the BBB, including inefficient efflux of toxic molecules via P-glycoprotein	166,167
MS	Secondary	Extravasation of autoreactive T cells and monocytes across a compromised BBB	168
Natalizumab-PML with IRIS	Secondary	Infiltration of T cells in perivascular space and parenchyma after discontinuation of natalizumab in context of PML	169
NMO	Primary	BBB breakdown including loss of AQP4 and of astrocytes caused by AQP4-specific IgG	170
Primary CNS vasculitis	Primary	Inflammation of cerebral vessels without systemic disorder	171,172
Secondary CNS vasculitis	Primary	Inflammation of cerebral vessels associated with systemic inflammatory illness	171
VZV vasculopathy	Primary	Viral infection (primary or upon reactivation) of cerebral arteries	173
Cerebral malaria	Primary	Sequestration of parasitized red blood cells in lumen of cerebral microvasculature	174
Primary CNS lymphoma	Secondary	Leaky angiogenic vessels in malignant tissue	175
Glioblastoma	Secondary	Leaky neoangiogenic vessels and loss of BBB integrity in preexisting vessels (by subcellular mislocalization of astroglial AQP4) in malignant tissue	176
PRES	Primary	Vascular injury by systemic influence, such as disorders of clotting or bleeding, and chemotherapy agents (particularly those which inhibit VEGFR kinase)	177
TBI	Secondary	Mechanical disruption of BBB followed by post-traumatic BBB dysfunction	178
Migraine	Secondary	Cortical spreading depression with subsequent vascular reaction	179
Diabetes	Secondary	Increased BBB permeability, possibly leading to cognitive impairment	180

[a]Primary level of BBB effect indicates that the cerebrovasculature is probably compromised upstream from CNS pathogenesis, whereas secondary level of BBB effect is interpreted as happening downstream from the initial insult and aggravating disease. AD, Alzheimer's disease; PD, Parkinson's disease; PML, progressive multifocal leukoencephalopathy; IRIS, immune reconstitution inflammatory syndrome; VZV, varicella zoster virus; PRES, posterior reversible encephalophathy syndrome; TBI, traumatic brain injury.

Non-invasive techniques to deliver drugs to the brain

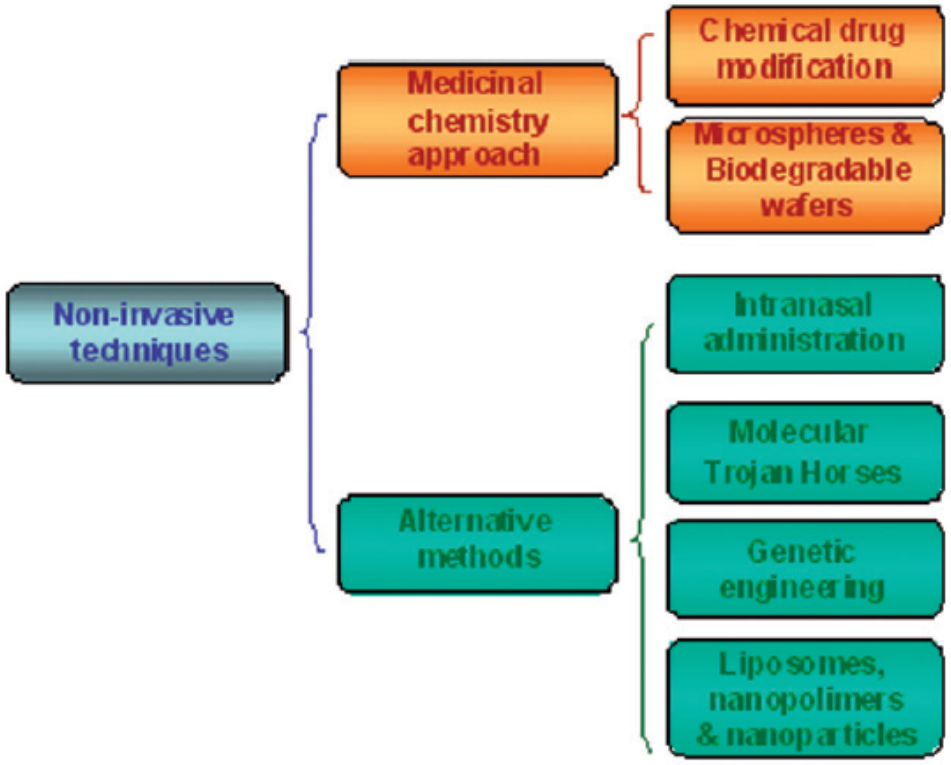


Figure 5. A schematic representation of current strategies to deliver drugs to the brain by non-invasive techniques. Non-invasive techniques include drug modification by medicinal chemistry approaches and drug encapsulation through nanotechnological carriers.

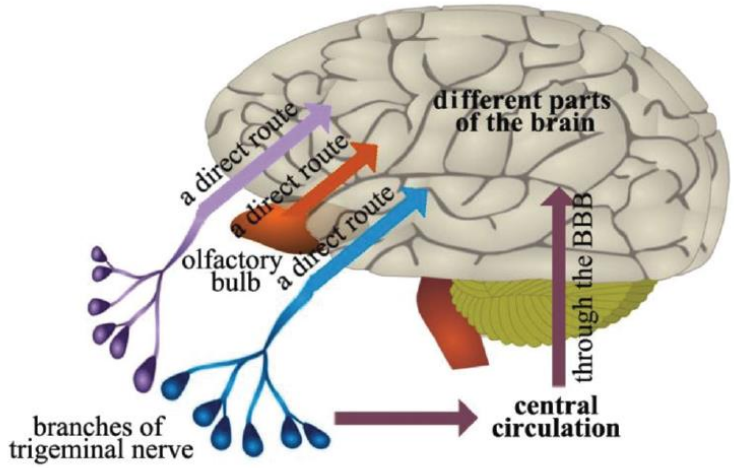


Fig. 3 Schematic of the pathways of drug delivery to the brain from the nasal cavity.

Zhang et al, 2016, Biomater. Sci., 2016, 4, 219

Nanoparticles in drug delivery

Multiple structures have been proposed for drug delivery in recent years. In general, there are two large families of transporters, ie, **reversible and irreversible nanoparticles**. **Reversible nanoparticles** are supramolecular complexes generated on the basis of noncovalent intermolecular interactions, ie, Van der Waals forces or lipophilic interactions. Liposomes and micelles are the most well known examples of these types of nanoparticles (Figure 2). These are molecules formed by noncovalent binding of their components which can self-assemble spontaneously and reversibly into organized structures under specific environmental conditions, eg, temperature, pH, and polarity of the medium.

Conversely, the broad family of **nonreversible nanoparticles** (including dendrimers, nanocapsules, nanospheres, nanocages, and nanotubes, Figure 2) comprises molecules with strong molecular interactions, eg, covalent or metallic bonds, which confer a high degree of stability, thereby facilitating their manufacturing for commercial purposes, but are more rigid in their synthesis and handling.

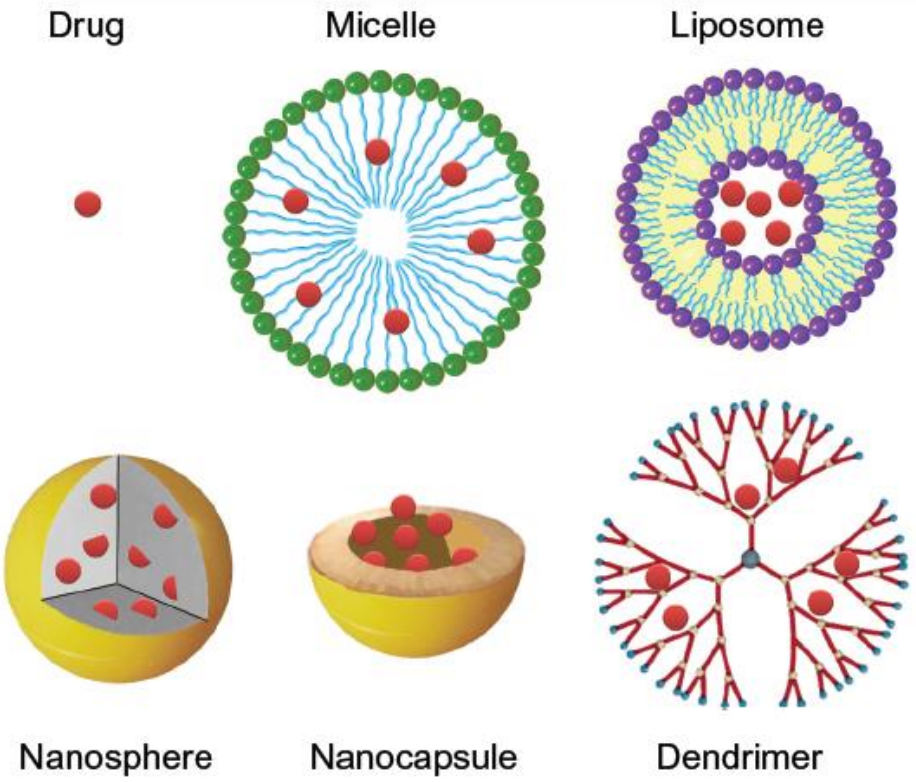


Figure 2 Schematic representation of different particulate systems for drug transport and delivery.

Notes: Some of these systems include self-assembling molecules, such as liposomes and micelles, while others are based on nonreversible organic or inorganic structures, such as nanospheres, nanocapsules, and dendrimers.

Ramos-Cabrer and Campos
International Journal of Nanomedicine 2013:8

BBB transport mechanisms for **brain delivery of nanoparticles (NPs)**

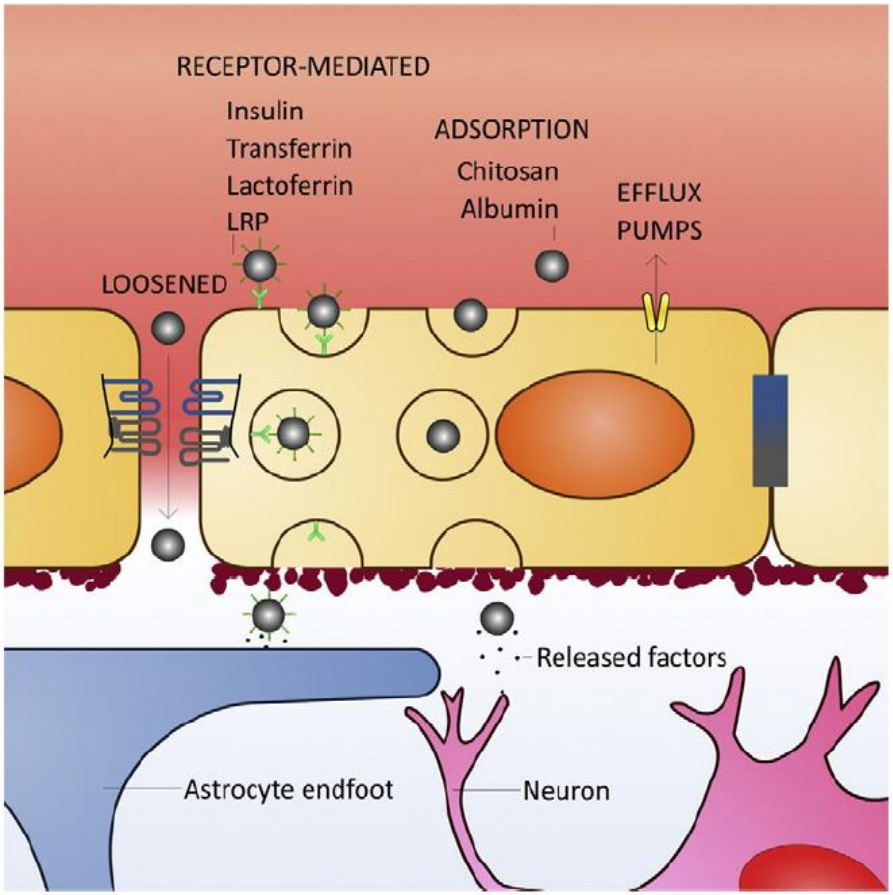
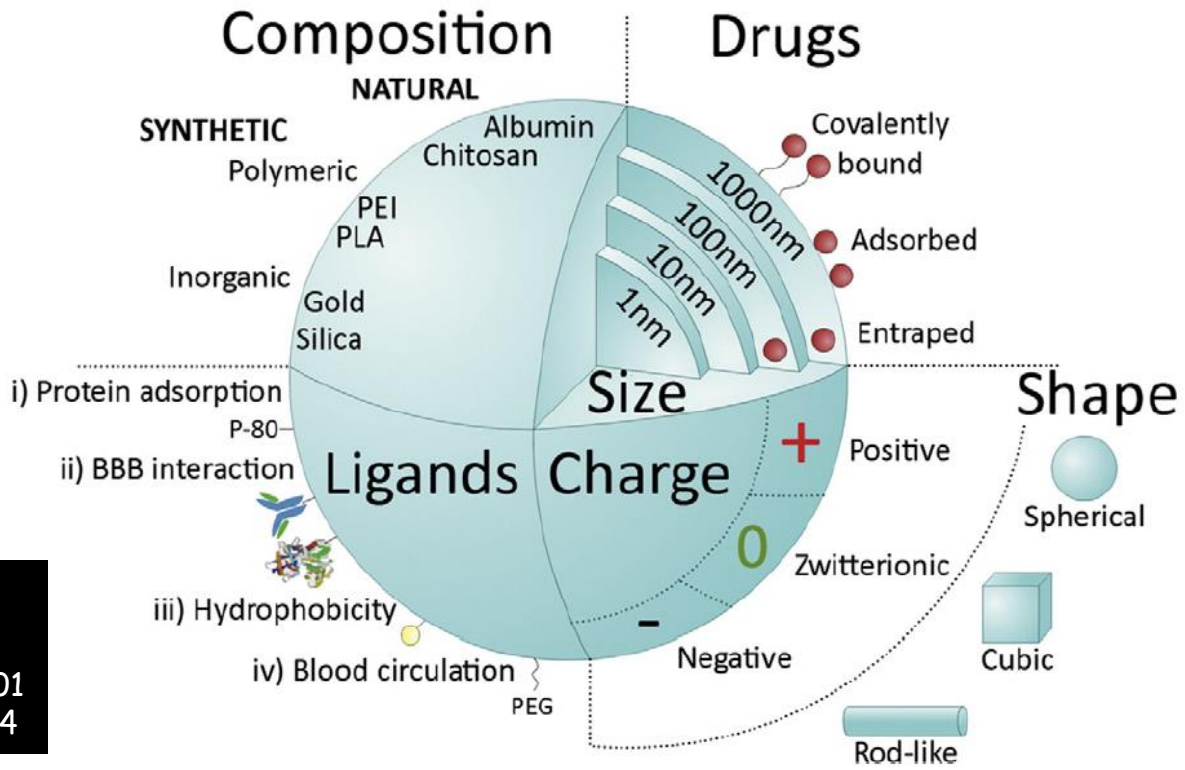


Fig. 2. Blood–brain barrier (BBB) transport mechanisms for brain delivery of nanoparticles (NPs). The BBB is highly selective and has specific transport mechanisms allowing a close control of molecules/cells that enter the brain parenchyma. Loosened tight junctions (TJs) allow the cross of NPs through the BBB, either by the presence of a surfactant in NPs able to disrupt the TJs or by BBB impairment due to pathological conditions. Receptor-mediated transcytosis is the most common type of transport for NP entry into the brain. NPs can be functionalized with different types of ligands (such as insulin, transferrin, lactoferrin or antibodies against some endothelial receptors), or surfactants like polysorbate 80 (that adsorbs plasma proteins, namely apolipoprotein E enabling their binding to the lipoprotein receptor-related proteins (LRPs)). The interaction between NP ligands and respective receptors in the endothelial cell (luminal side) surface triggers plasma membrane invaginations followed by pinch free forming vesicles, which facilitates the release of the NPs in the opposite site of the membrane (parenchymal side). NPs coated with molecules such as albumin or chitosan can cross the BBB by adsorptive transcytosis. Efflux pumps may reduce the amount of NPs retained in brain parenchyma.

Saraiva et al, 2016

J Controlled Release
http://dx.doi.org/10.1016/j.jconrel.2016.05.044

Nanoparticle features influencing BBB passage



Saraiva et al, 2016

J Controlled Release
http://dx.doi.org/10.1016/j.jconrel.2016.05.044

Fig. 3. Main nanoparticle (NP) features influencing systemic delivery and blood brain barrier (BBB) passage. NPs can be classified into natural, when molecules such as proteins (albumin), polysaccharides, chitosan, among others are used, or synthetic. Synthetic NPs can be made of very common polymers such as poly(lactic-*co*-glycolic acid) (PLGA), poly(ethylenimine) (PEI), polyesters (poly(lactic acid) (PLA), or from inorganic agents like gold, silica or alumina. NPs can vary in their size (1–1000 nm) and are able to deliver drugs into cells by entrapping, adsorbing or covalently bounding them. NPs can assume different shapes (spherical, cubic, and rod-like) and charges (negative, zwitterionic, and positive); negatively charged spheres are widely used in intravenous applications. Another important feature of NPs is the possibility of functionalization with different types of ligands. Ligands are distributed into four major categories: i) capable of mediating protein adsorption (e.g. poly(sorbate) 80 (P-80)); ii) able to interact directly with the BBB (e.g. transferrin proteins, antibody or peptides); iii) capable of increasing hydrophobicity (e.g. amphiphilic peptides); and iv) able to improve blood circulation (e.g. poly(ethylene glycol) (PEG)).

Delivering therapeutic molecules through Cell-Penetrating Peptides (CPP)

Guidotti et al, 2017

Trends in Pharmac Sci
http://dx.doi.org/10.1016/j.tips.2017.01.003

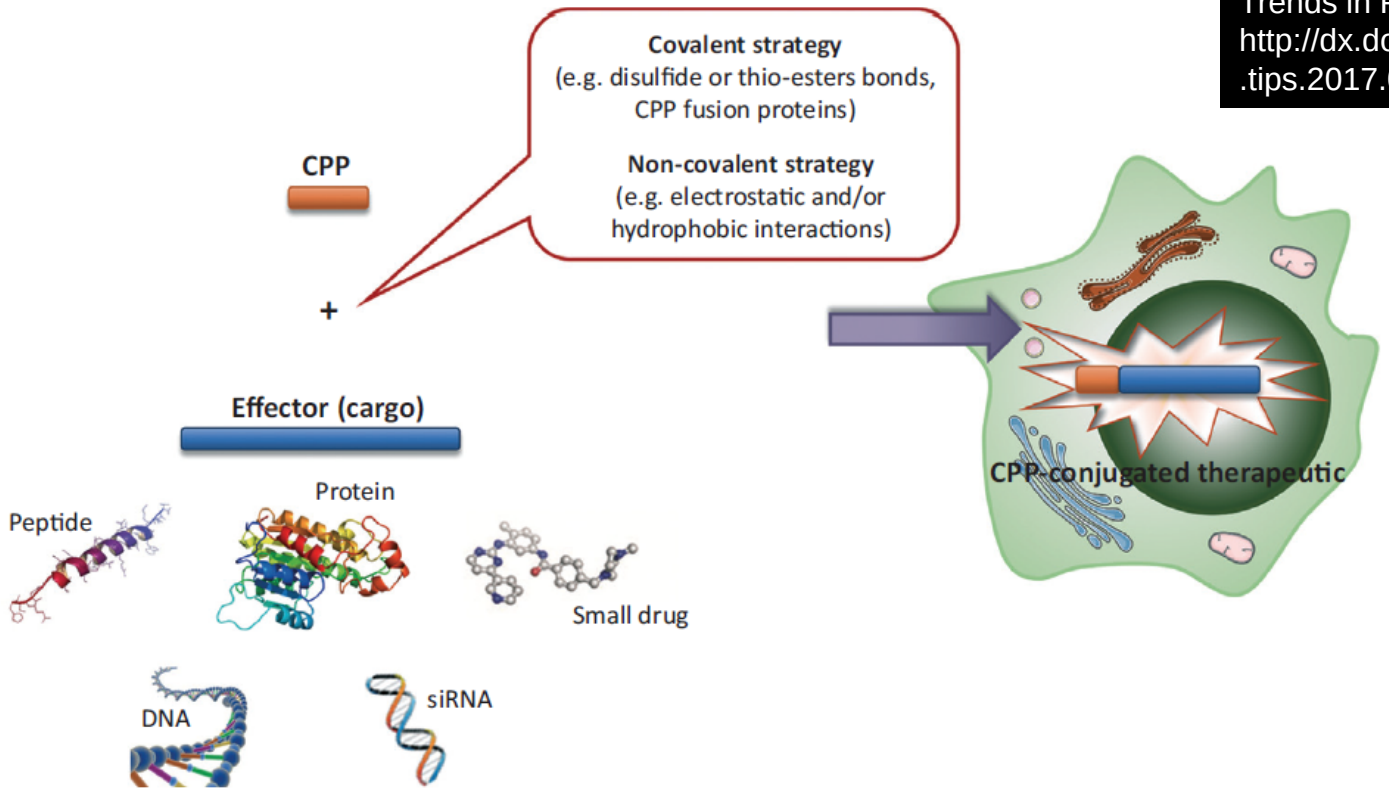


Figure 1. Schematic Drawings Representing Cell-Penetrating Peptide (CPP)-Based Technologies. The hydrophilic nature of effectors/cargoes (blue), such as peptides, proteins, nucleic acids, or small drugs, can prevent their cellular uptake and hamper their access to intracellular targets. Conjugating the effector (cargo) to a CPP (orange) by covalent bonds or noncovalent complex formation enables the CPP–effector conjugate (CPP–conjugated therapeutic) to cross the cell membrane and reach intracellular areas that are difficult to access, thereby enhancing the therapeutic effectiveness.

Proposed mechanisms for CPP Internalization

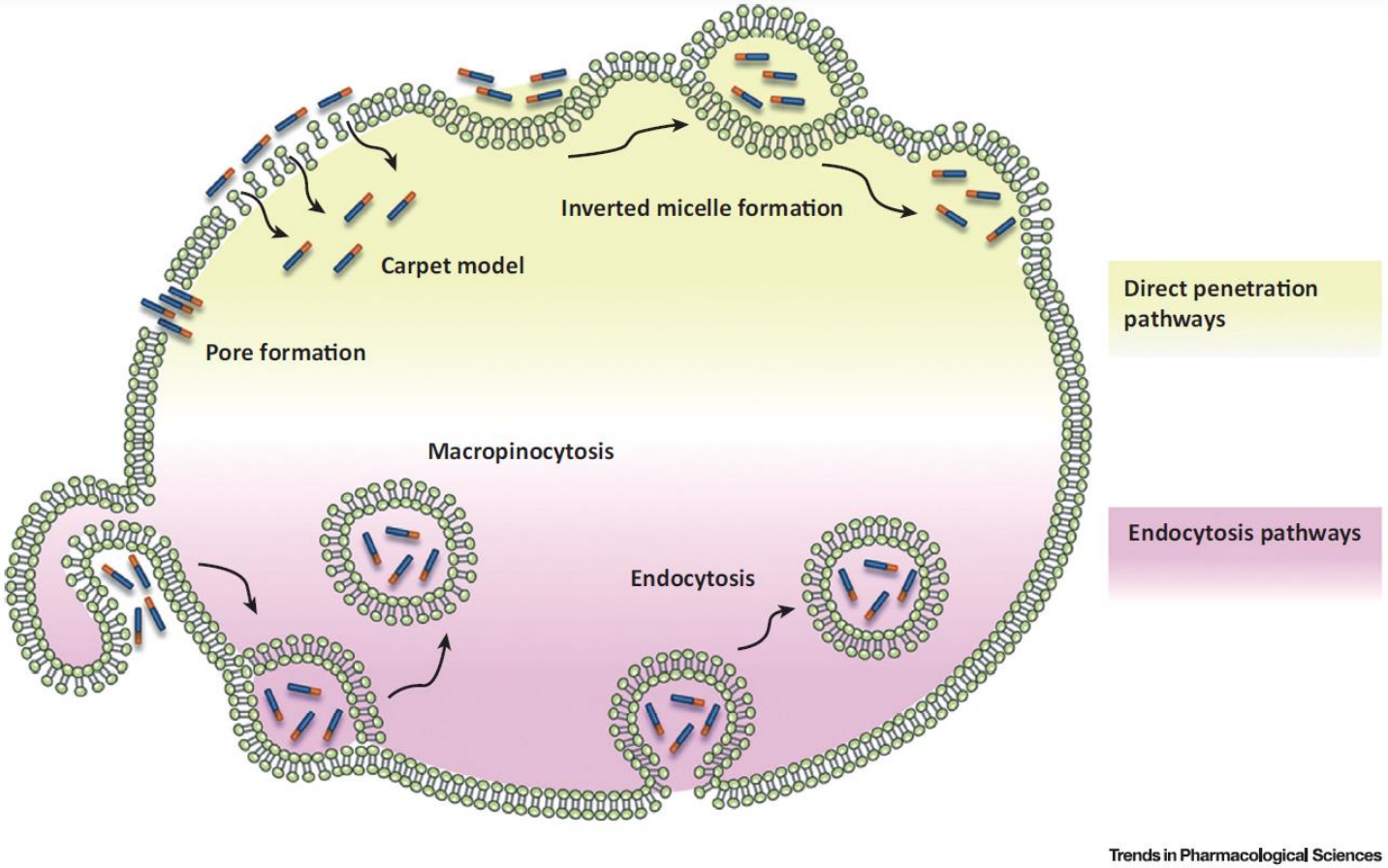


Figure 2. Schematic Representation of Proposed Mechanisms for Cell-Penetrating Peptide (CPP) Internalization. The diagram illustrates that the involved pathways can be divided into two groups: direct penetration of plasma membrane (yellow) and endocytic pathways (purple). The first type of process involves several energy-independent models including membrane insertion of CPPs through pore formation and membrane destabilization through the carpet-like model or inverted micelle formation. Endocytic internalization of CPPs is an energy-dependent process that comprises macropinocytosis and endocytosis.

Guidotti et al, 2017

Trends in Pharmac Sci
http://dx.doi.org/10.1016/j.tips.2017.01.003

The marriage between drug delivery and molecular imaging disciplines has resulted in a relatively new discipline, known as **theranostics**, which represents the basis of the concept of personalized medicine. Involves use of **nanotechnology** to assemble molecular platforms that **simultaneously perform a therapeutic and diagnostic function.**

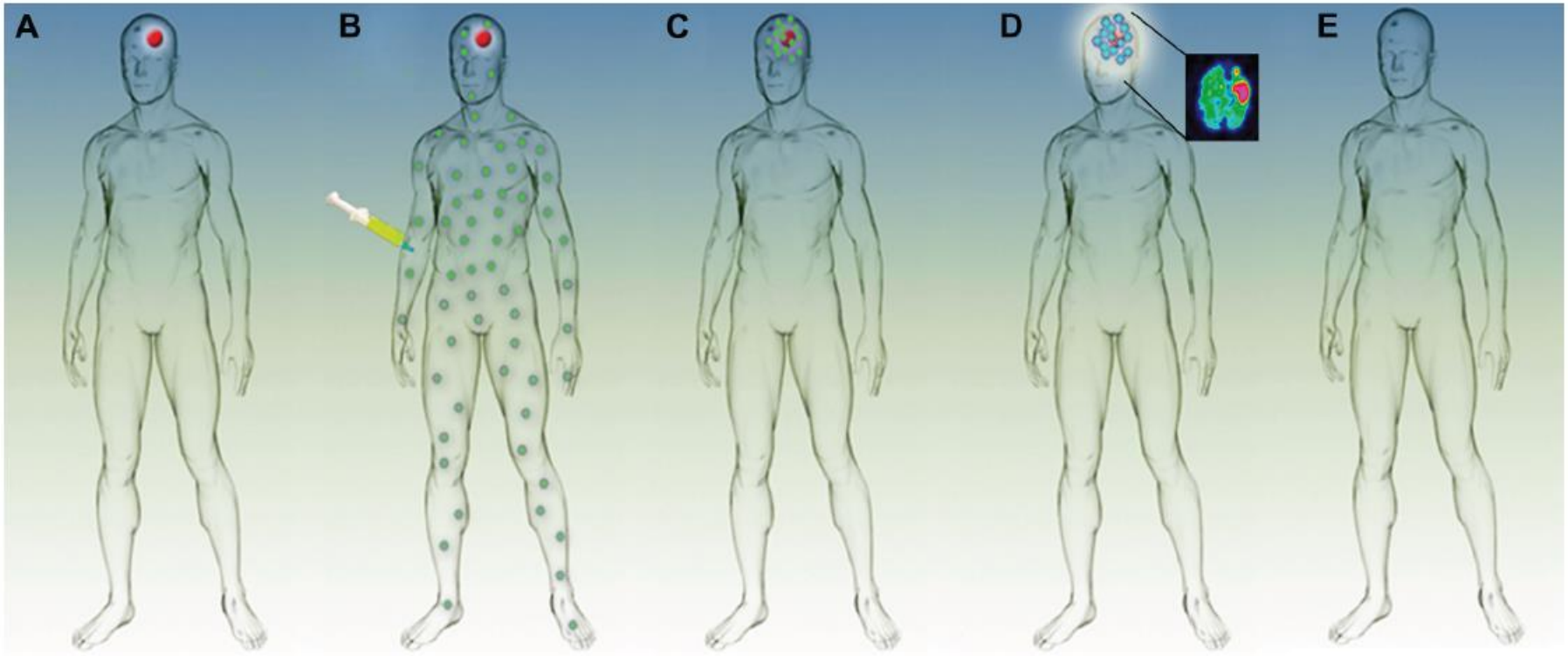


Figure 3 Concept of theranostics. (**A**) A pathological process localized to the brain. (**B**) Systemic administration of a therapeutic agent distributes the theranostic throughout the entire body. (**C**) Nanotechnology enables concentration of the agent in the targeted area. (**D**) Inclusion of imaging probes within the agent enables monitoring of the process in vivo. (**E**) By focusing the action of the therapeutic agent in the targeted area, the treatment becomes more effective.

it is common for theranostic agents to contain iron oxide particles for their in vivo detection using **magnetic resonance imaging**, along with radioactive isotopes for detection using **positron emission tomography** or **single photon emission computed tomography**, and fluorescence probes, quantum dots, or bioluminescent probes for detection using **fluorescence or optical imaging techniques**

Ramos-Cabrer and Campos International Journal of Nanomedicine 2013:8

Targeting of nanoparticles: new **cell-surface biomarkers** are needed!

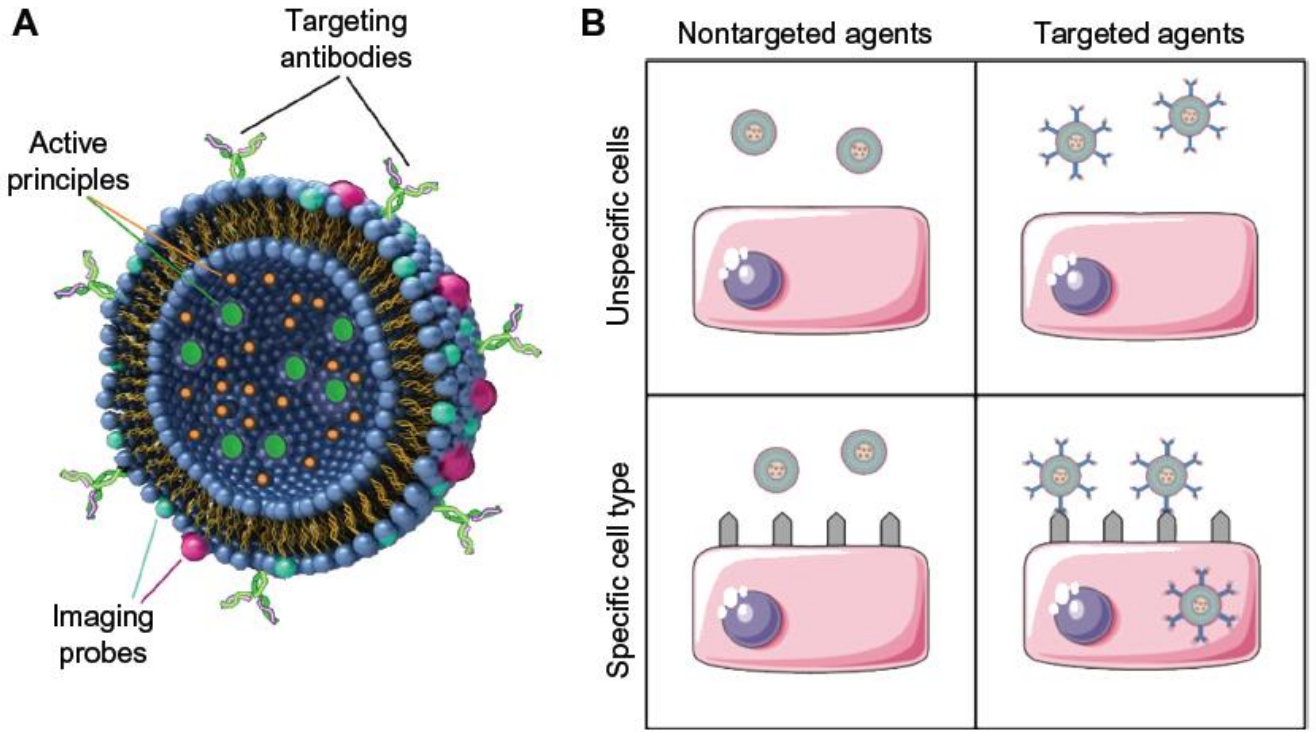


Figure 4 Theranostic agents in molecular recognition processes. (**A**) A liposomal theranostic agent includes surface antibodies that participate in the molecular recognition process with targeted cells, imaging probes (for diagnostic purposes), and active principles of treatment. (**B**) Targeting of specific cells occurs via expression of specific surface receptors against which theranostic agents are "immunized".
Note: Both immunized agents and expression of cell biomarkers (low-right corner) are required for the molecular recognition process.