# 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)

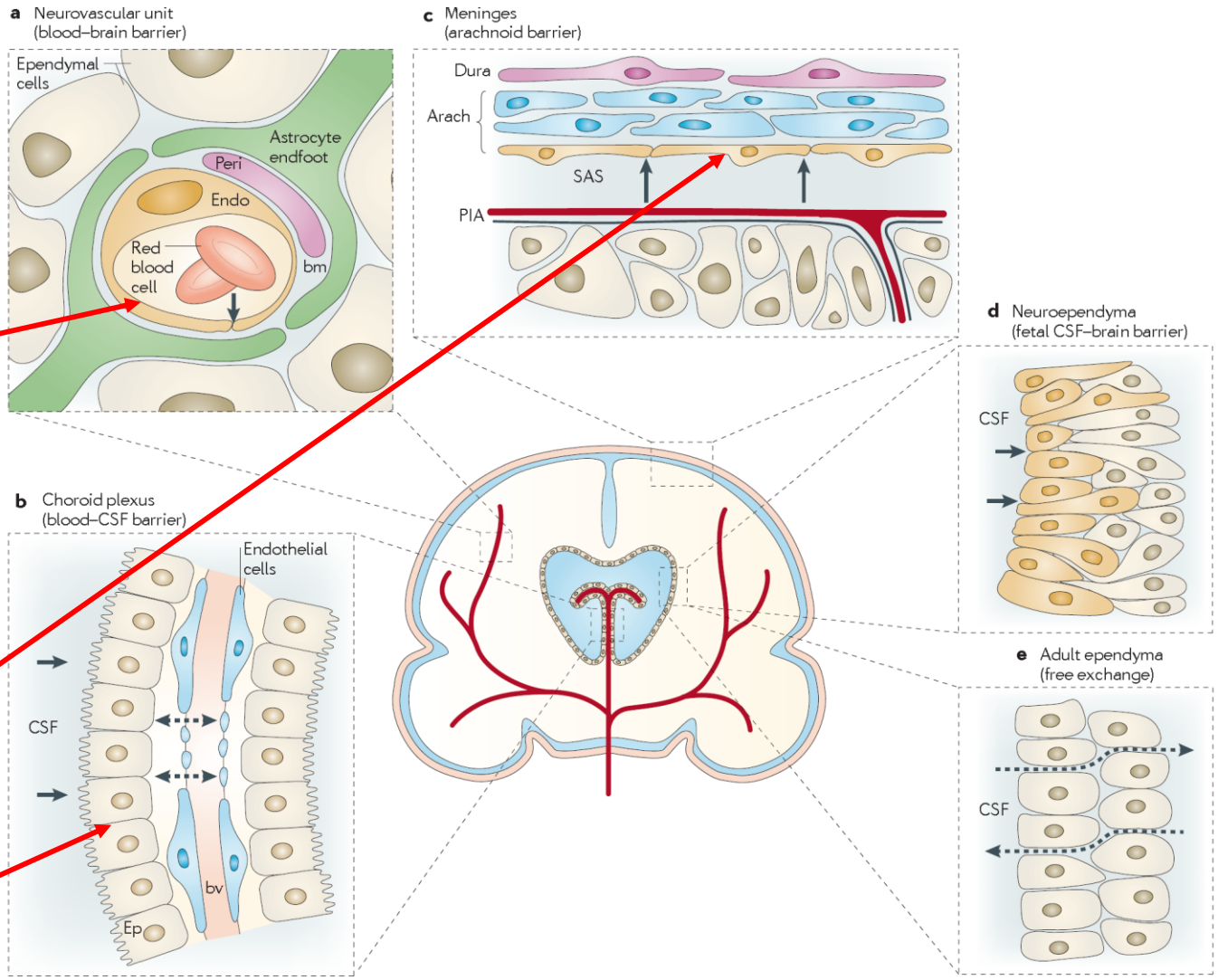



Figure 3 | **Barrier interfaces. a** | Endothelial cells (Endo) in the neurovascular unit have luminal tight junctions (shown by the arrow) that form the physical barrier of the interendothelial cleft. Outside the endothelial cell is a basement membrane (bm) which also surrounds the pericytes (Peri). 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.

Saunders et al. 2008

*Trends in Neurosciences* Vol.31 No.6

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

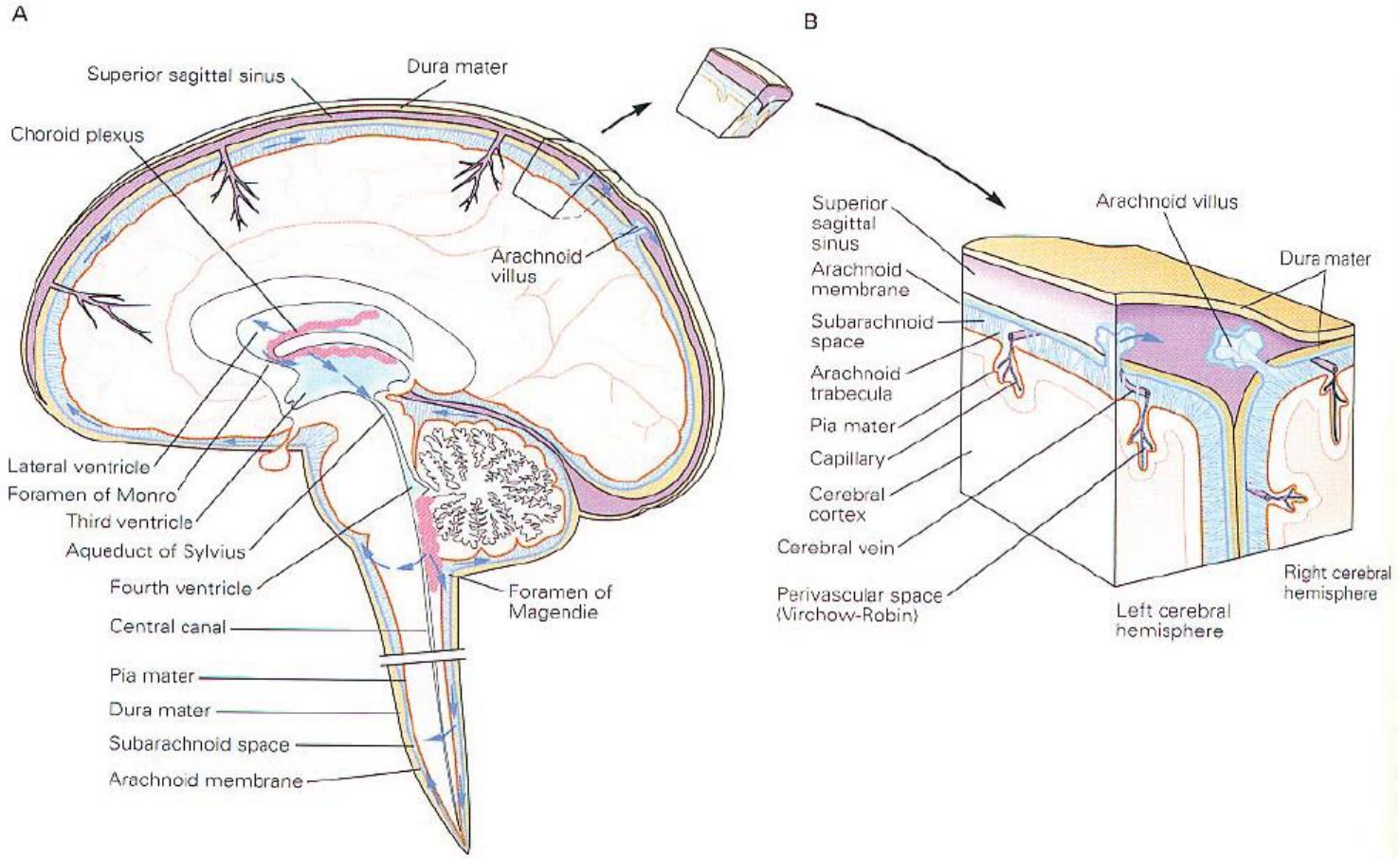



**Figure B-6 Distribution of CSF.** (Adapted from Carpenter 1978 and Fishman 1992.)

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.)

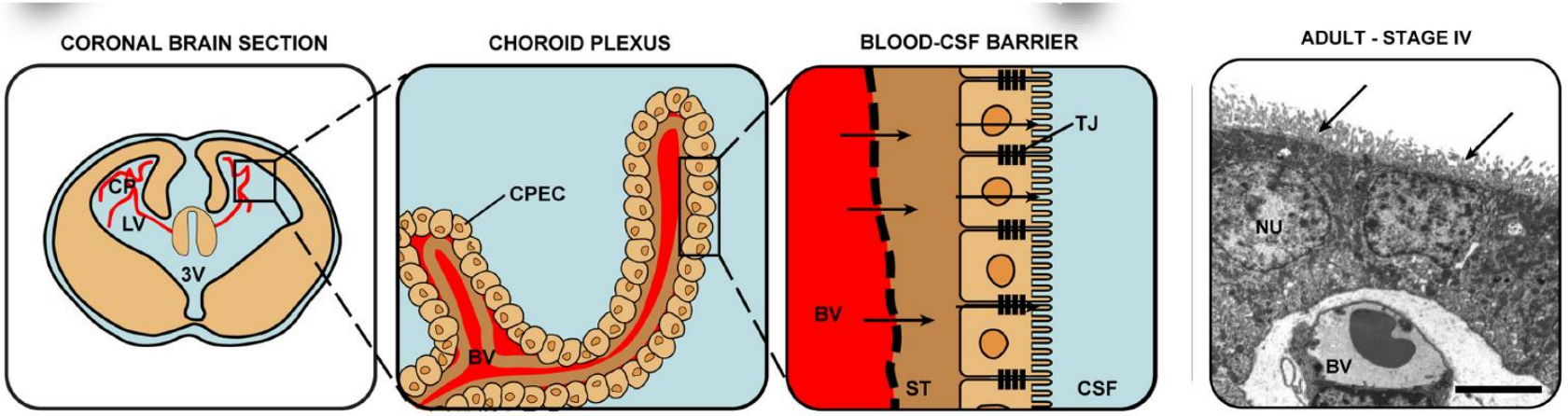
# Blood-CSF barrier: **choroid plexus epithelium**

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

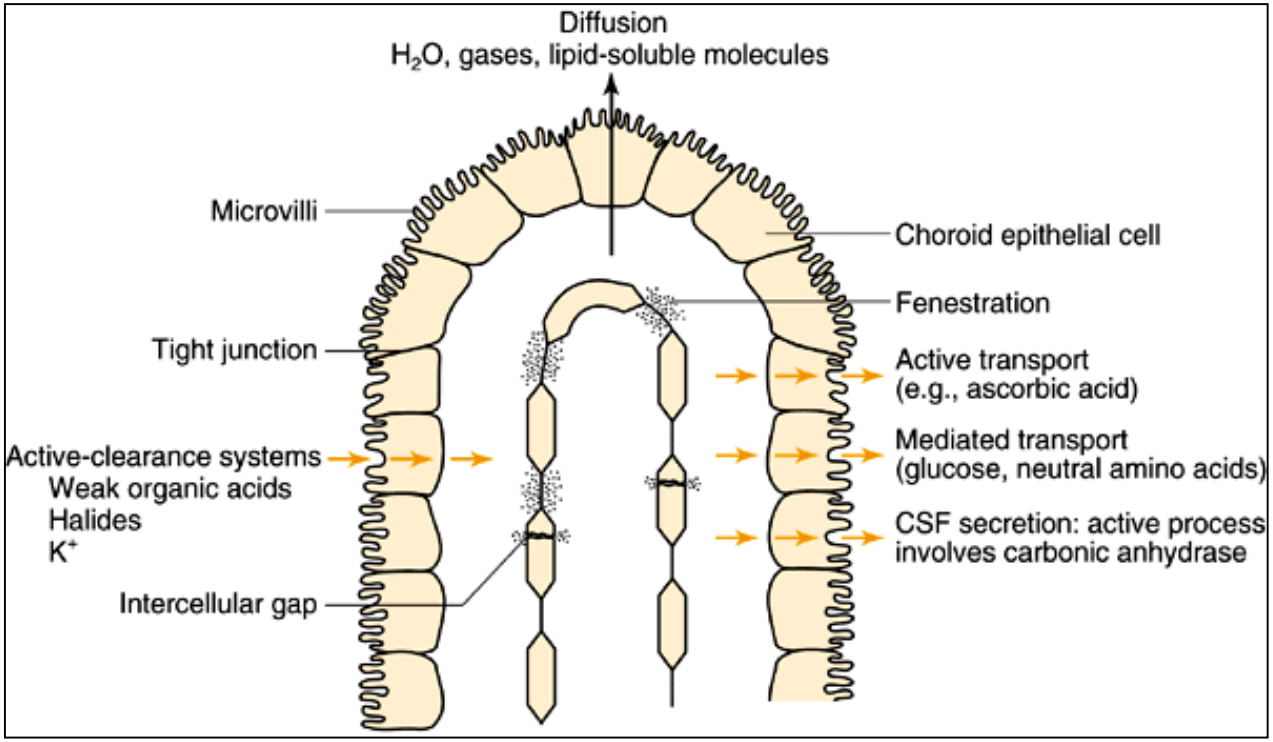
Mechanisms involved combine structural diffusion restraint between plexus epithelial cells (tight junctions physical barrier) and specific exchange mechanisms across the interface (enzymatic barrier).




*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)

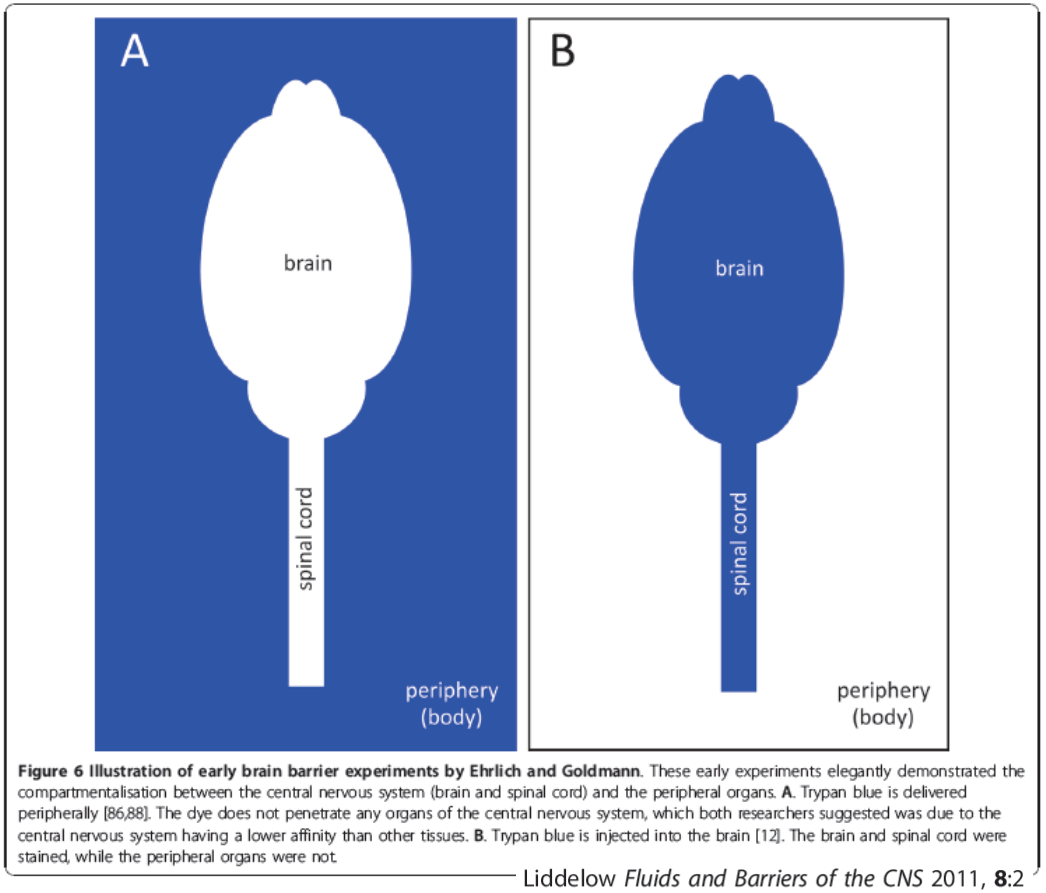
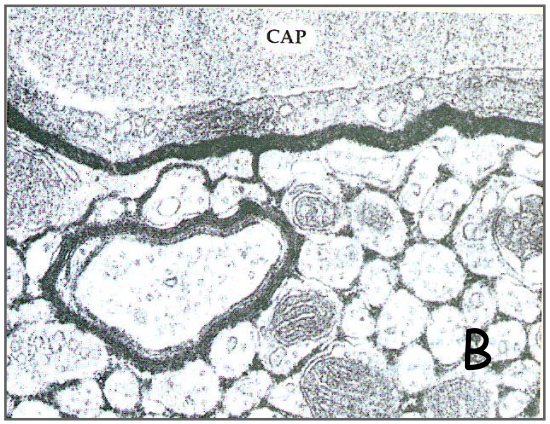
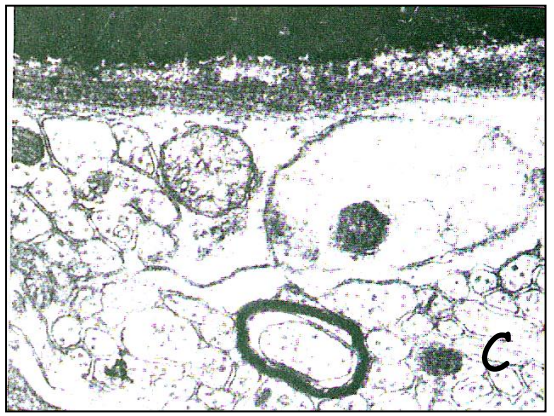



**Figure 6 Illustration of early brain barrier experiments by Ehrlich and Goldmann.** These early experiments elegantly demonstrated the compartmentalisation between the central nervous system (brain and spinal cord) and the peripheral organs. **A**. Trypan blue is delivered peripherally [86,88]. The 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

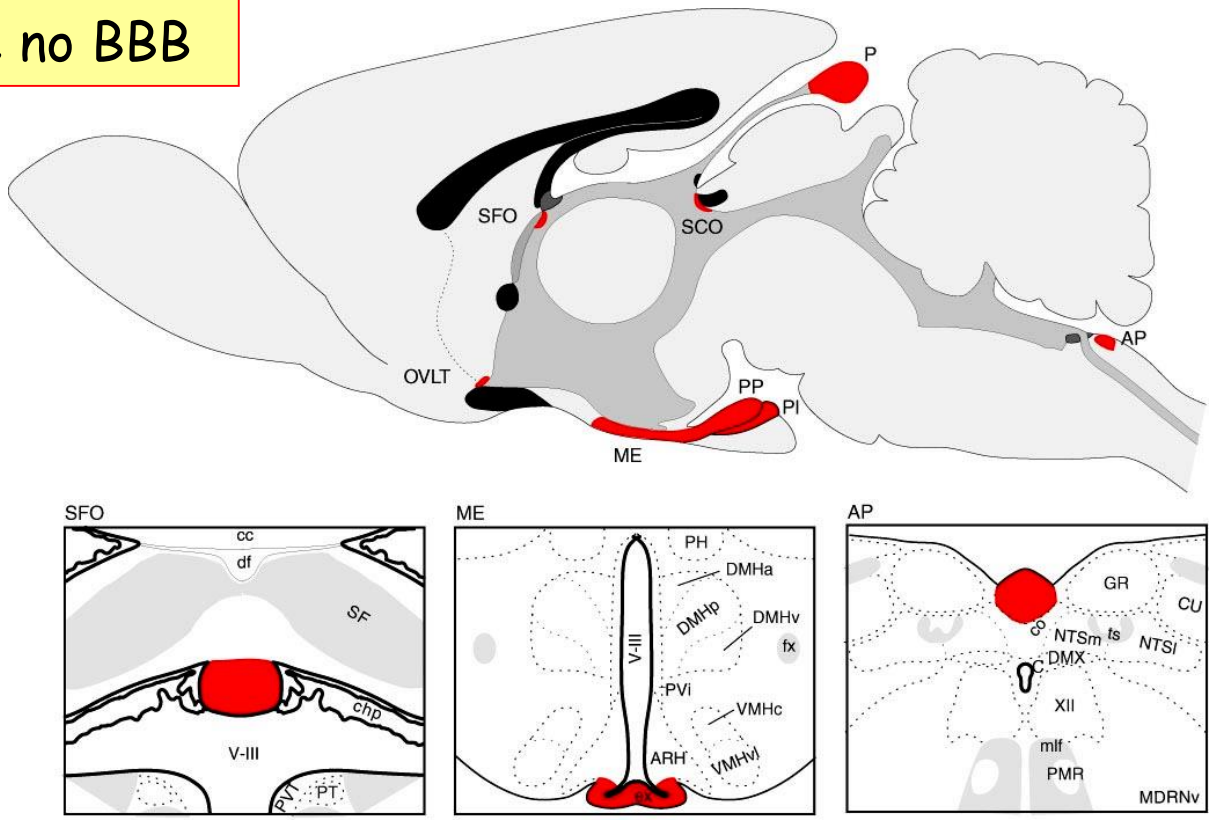
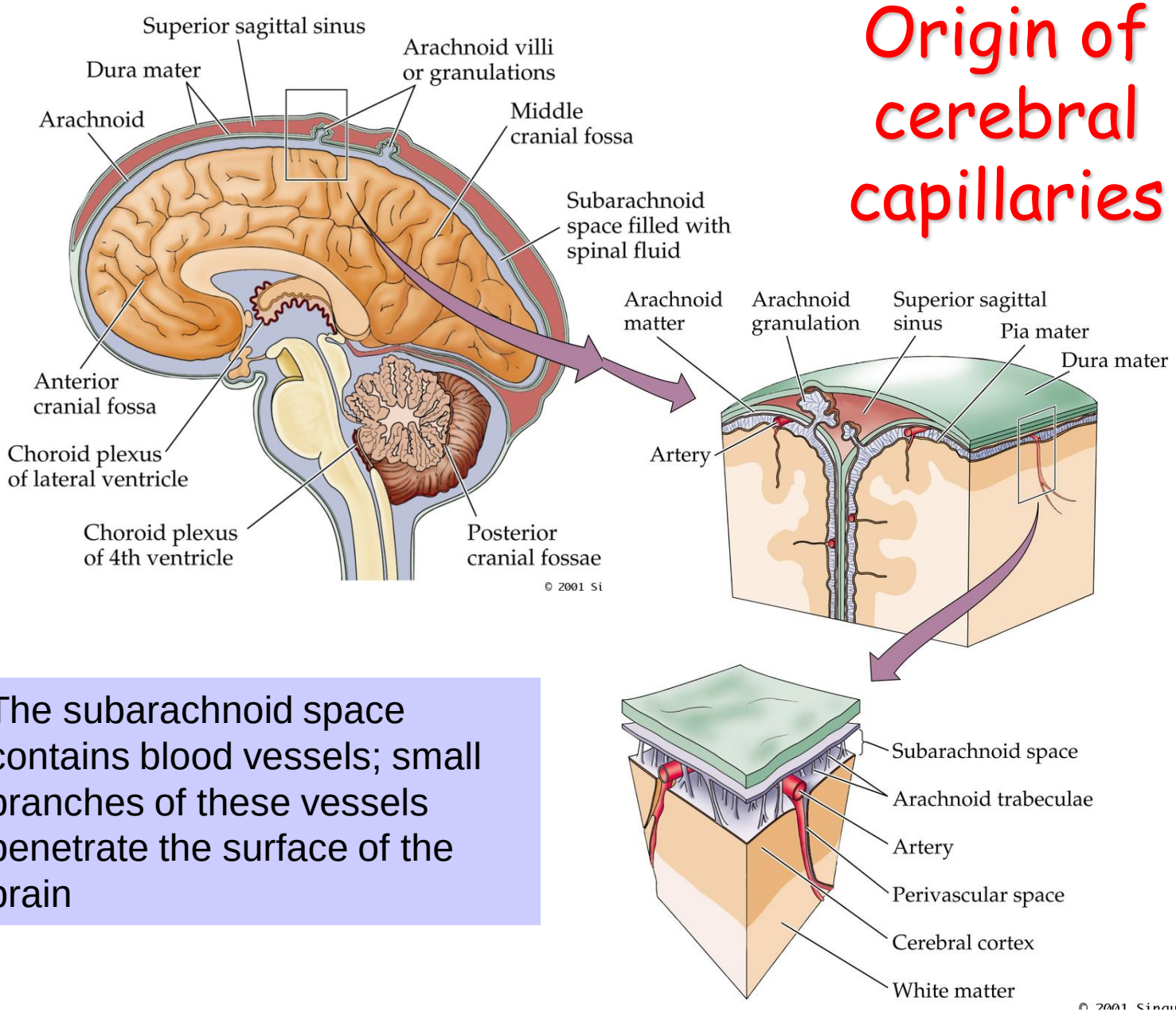



**FIGURE 3** Location of the six circumventricular organs (shown in red) in the rat brain (midsaggital section). Three regions that have an intimate functional association with the hypothalamus are also illustrated in transverse section in the lower figures. AP, area postrema; ARH, arcuate nucleus; cc, 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

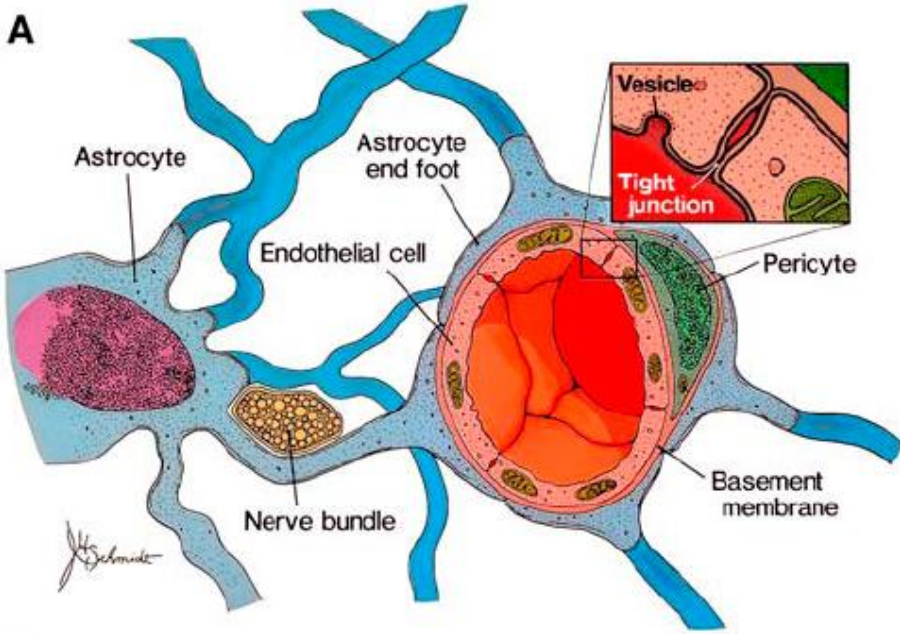



The subarachnoid space contains blood vessels; small branches of these vessels penetrate the surface of the brain

# 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

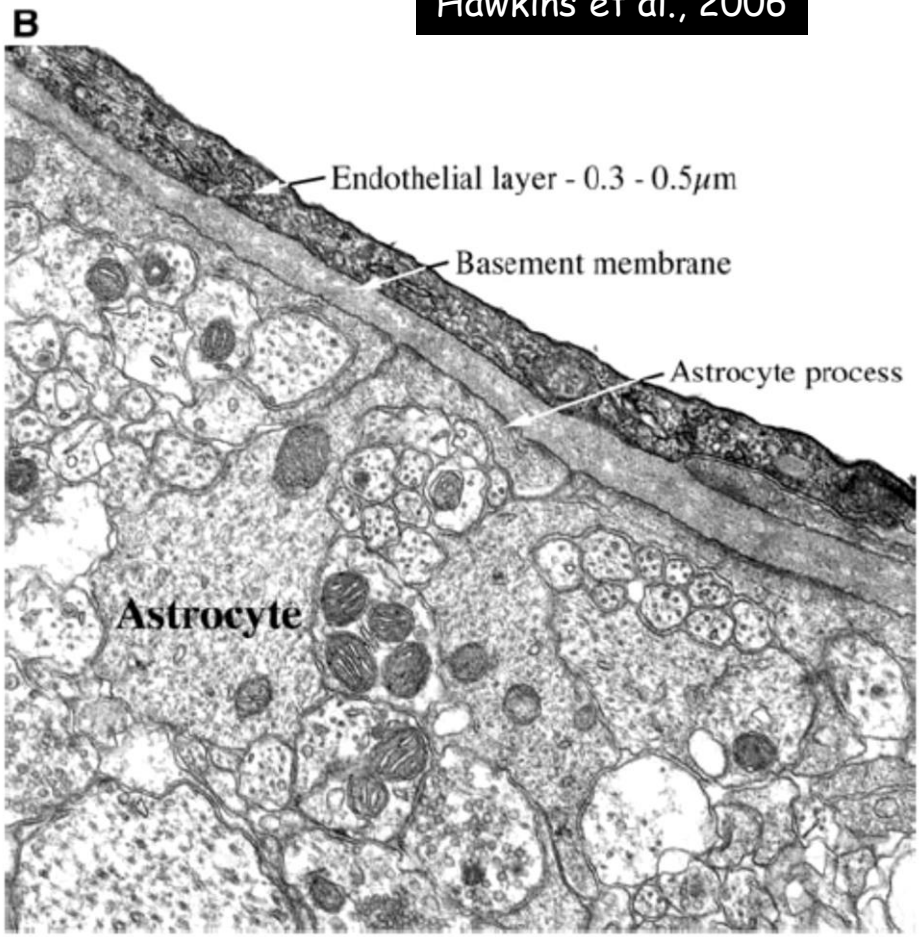
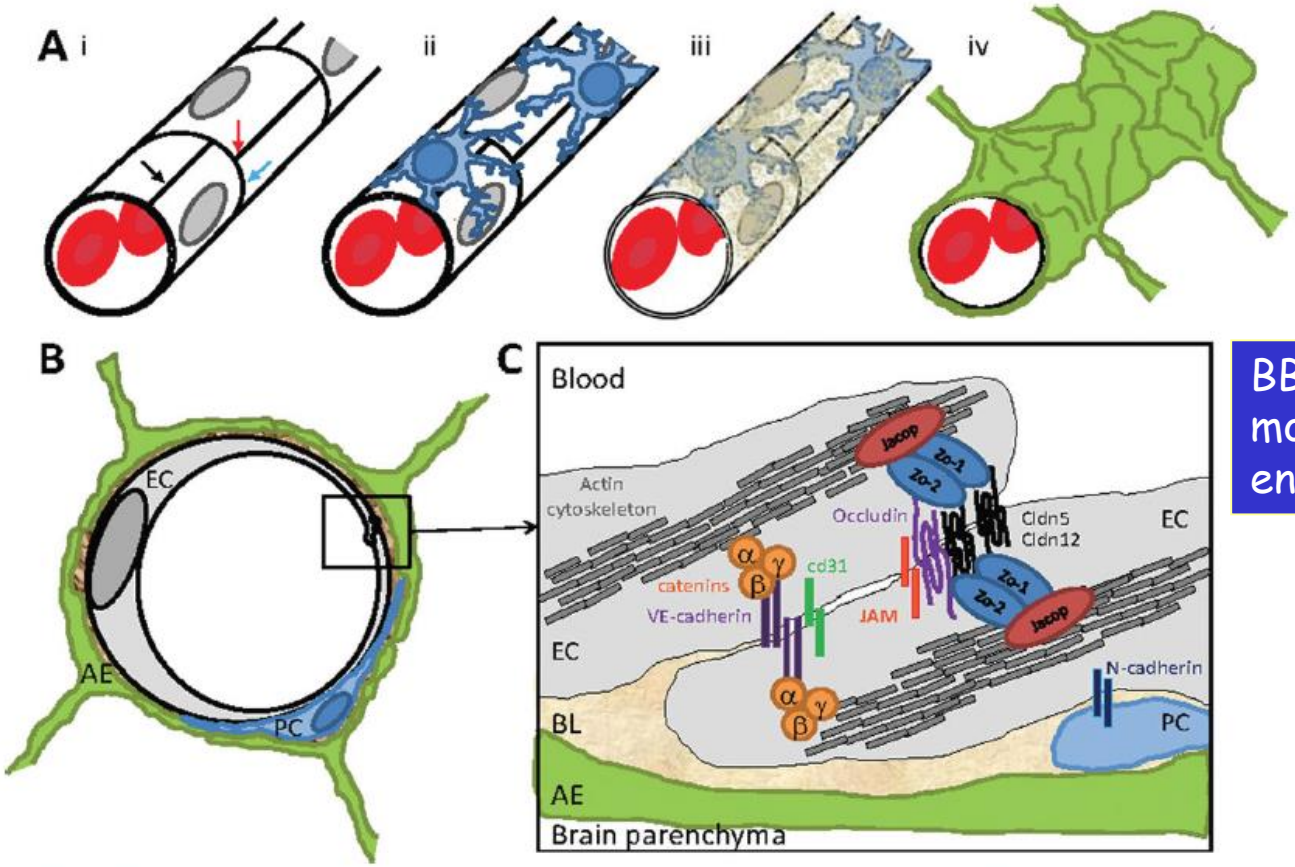



**FIGURE 2** (A) The BBB exists at the level of the endothelial cells of cerebral capillaries. The endothelial cells are joined together by an extensive network of tight junctions and surrounded by a basement membrane, within which pericytes reside. Astrocytic processes (so-called end-feet) surround 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

# Pericytes

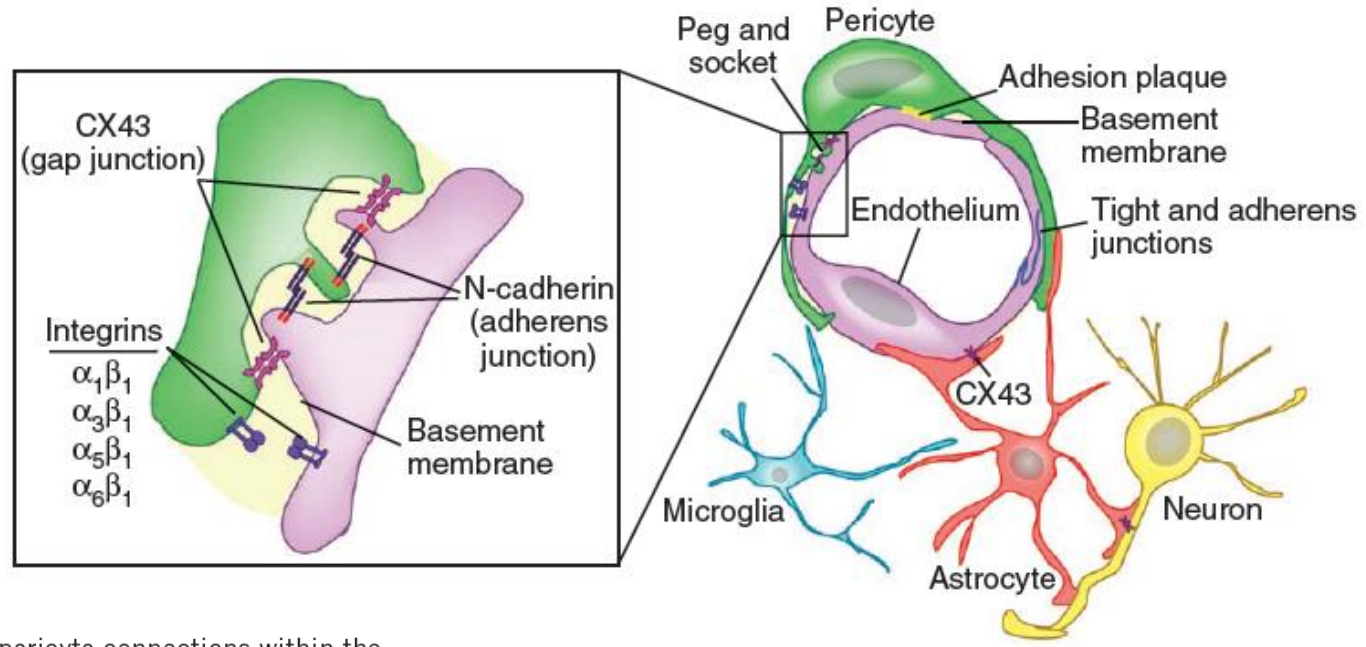



**Figure 1** Structural and molecular pericyte connections within the neurovascular unit. Right: pericytes (green) and endothelial cells (purple) are connected to a shared basement membrane (yellow) by several types of integrin molecule. In areas lacking the basement membrane, interdigitations of pericyte 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.

## 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

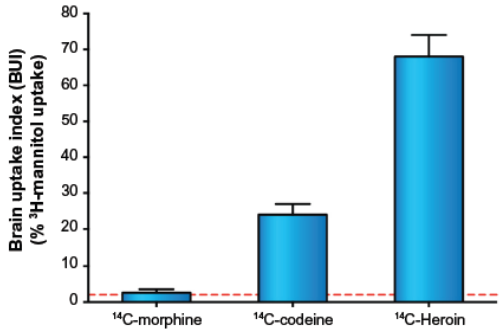



**Figure 3.** (**A**) Chemical structures of morphine, codeine, and heroin with their respective log *P*. (**B**) Relative rat brain uptake index (BUI) of $^{14}$C-morphine, $^{14}$C-codeine, and $^{14}$C-heroin in rats following a single brain passage after carotid injection. The greater uptake of codeine 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]

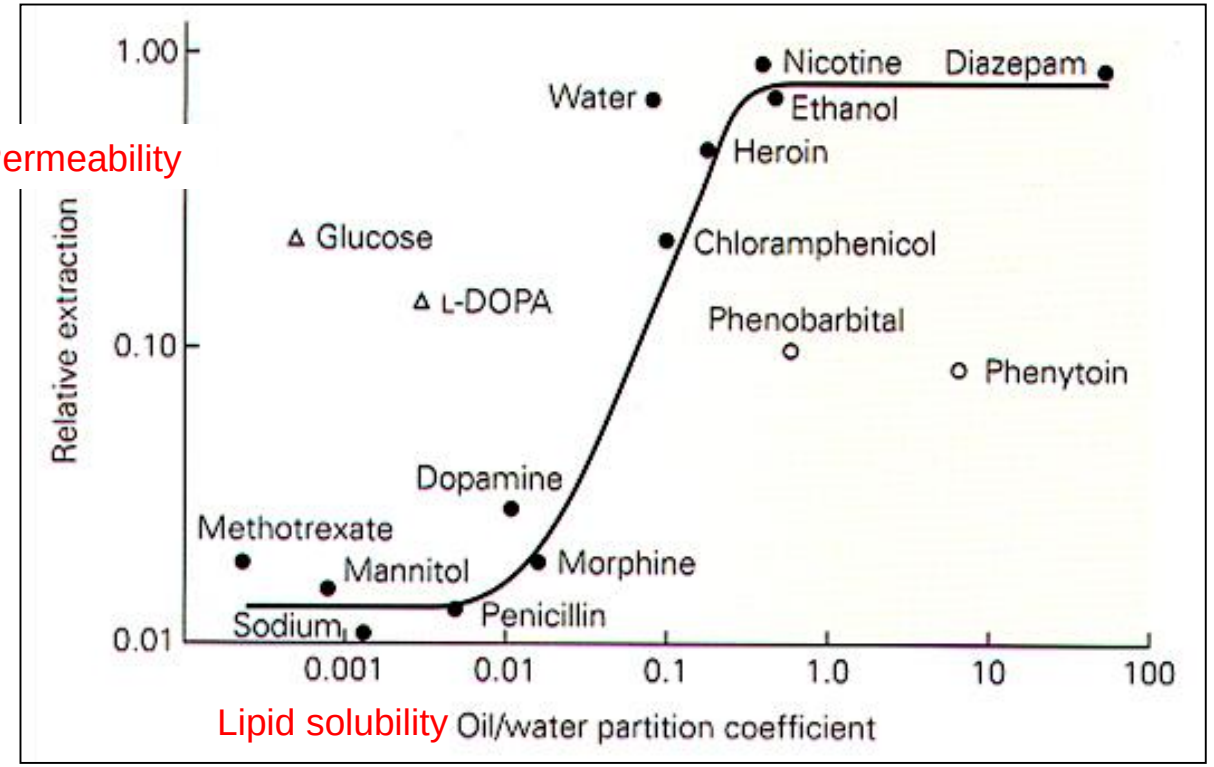
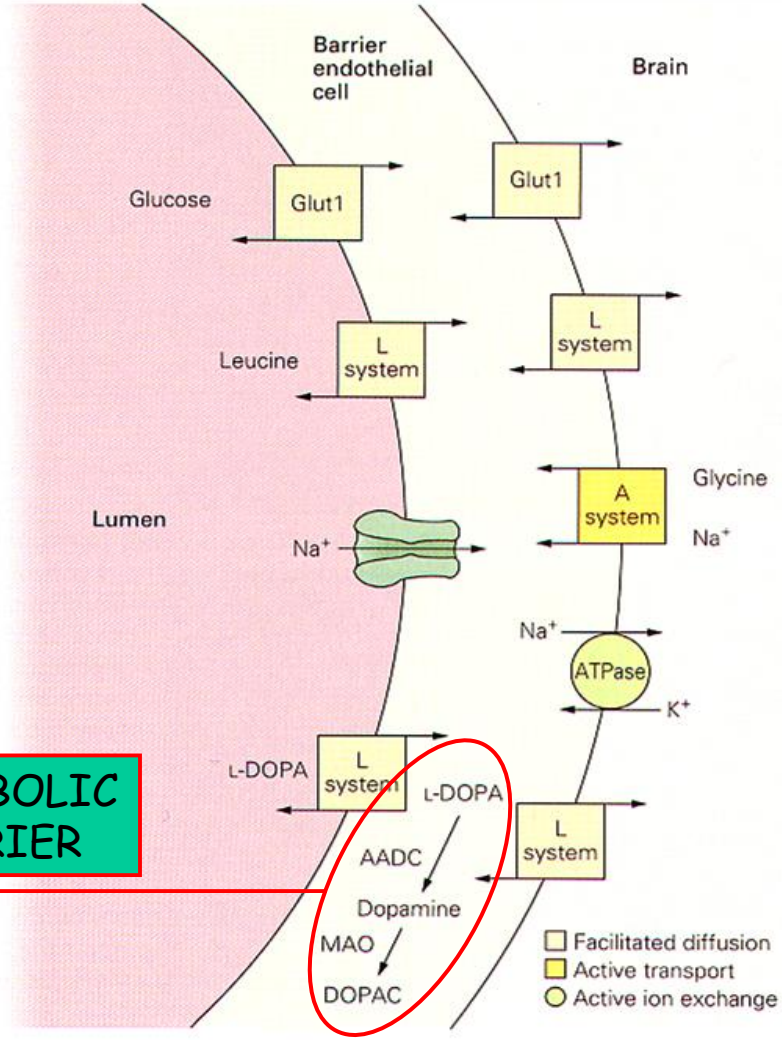


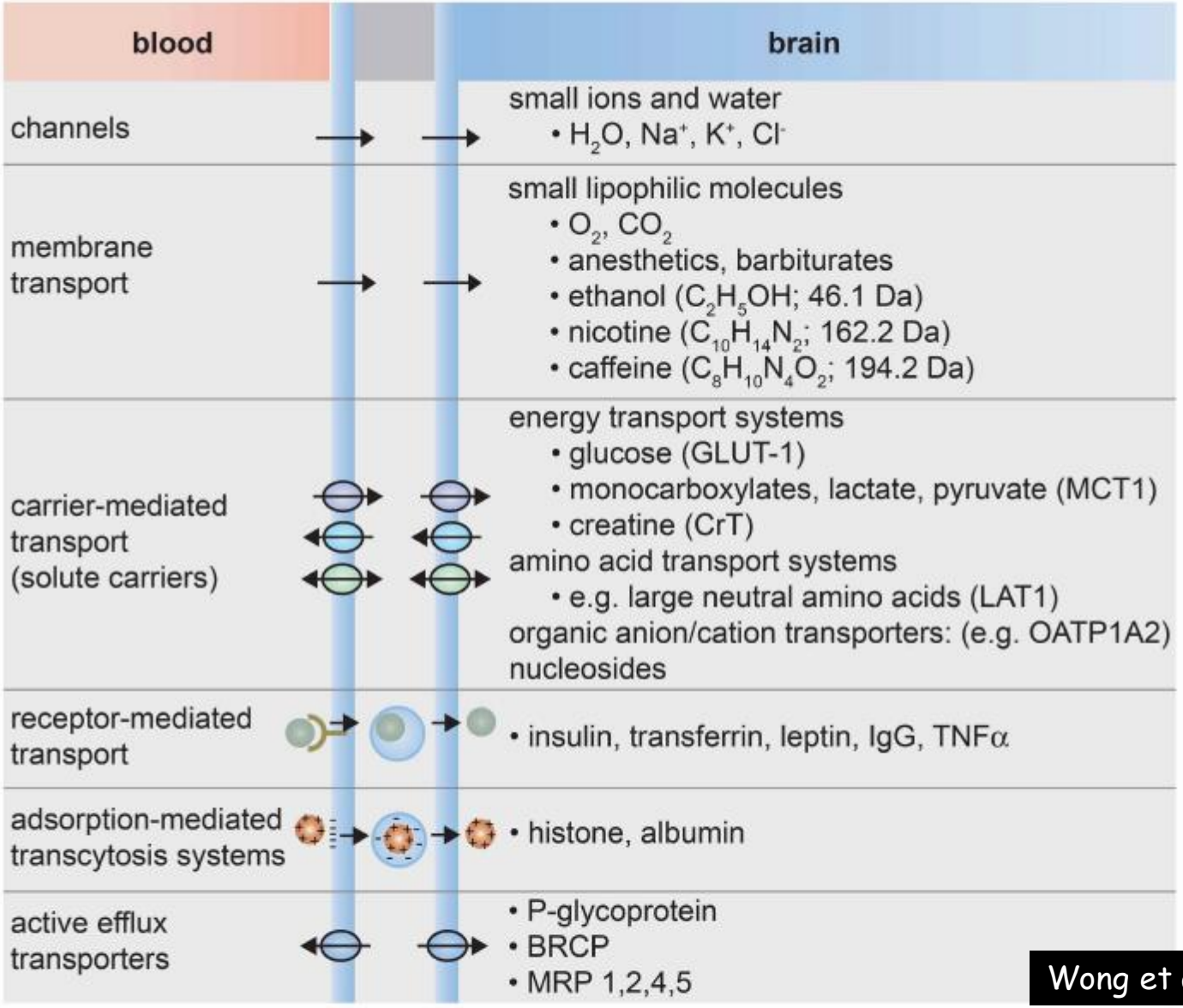

**Figure B-4** The oil-water partition coefficient indicates the relationship between lipid solubility and brain uptake of selected compounds. The distribution into olive oil relative to water for each test substance serves as a measure of its lipid solubility. The brain uptake is determined by comparing 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 receptors

**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

**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.

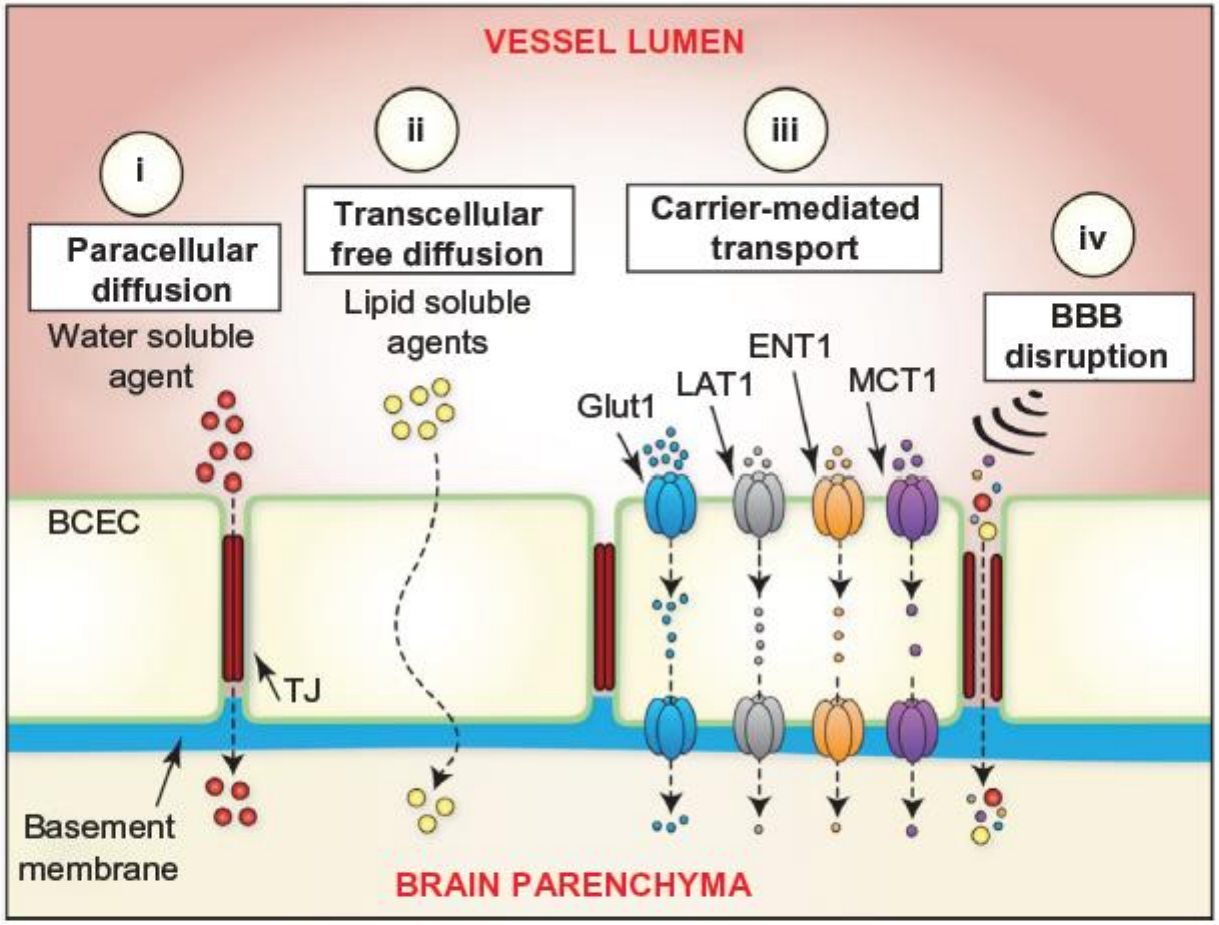
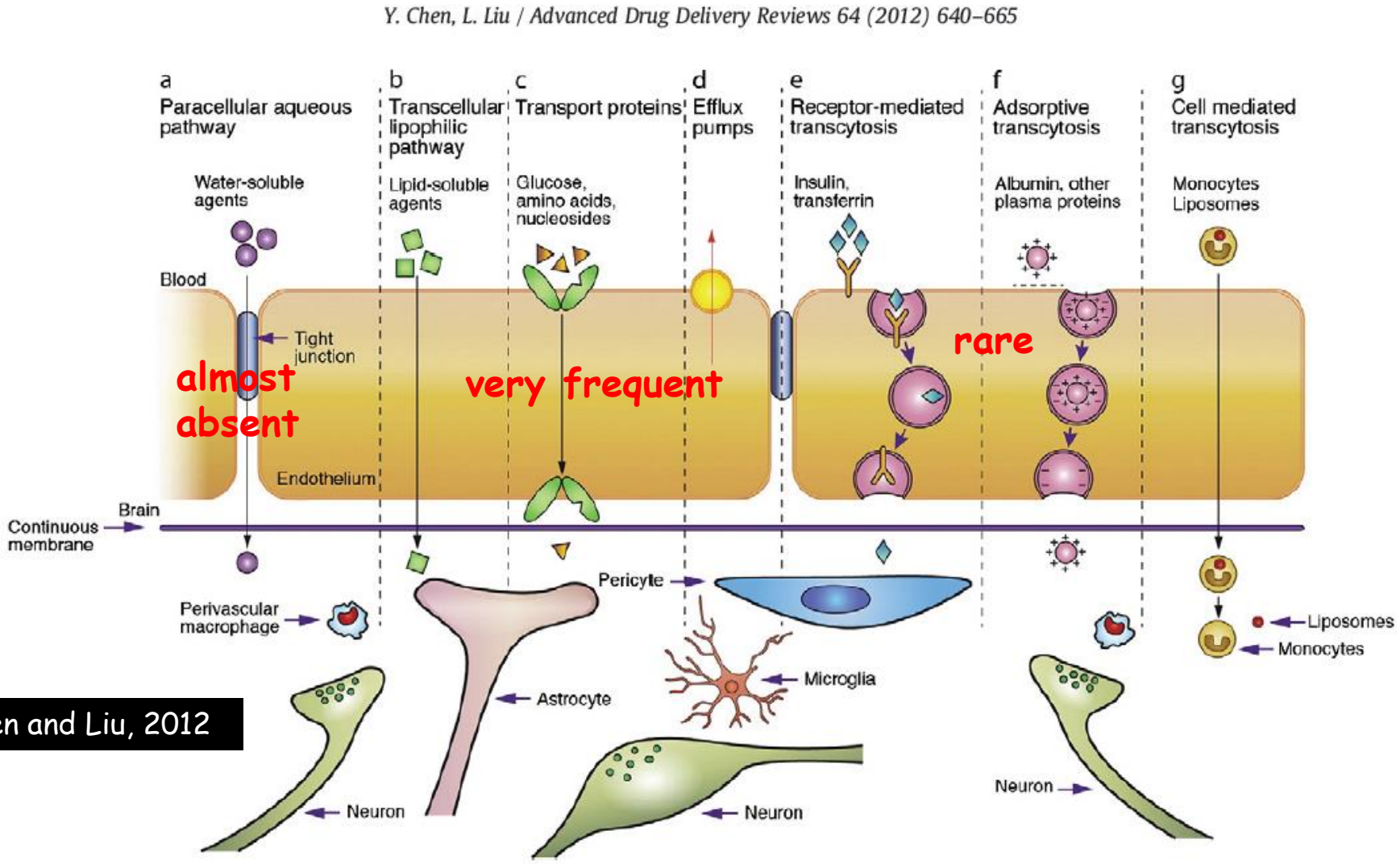



**Figure 2.** Schematic depicting various methods for small molecules to cross the BBB. There are four routes for small molecules to cross the BBB and reach the parenchyma. **(i)** Paracellular diffusion of agents is primarily impacted by size and requires small molecules to pass through TJs between BCECs. **(ii)** Transcellular free diffusion of small molecules is governed by specific physiochemical parameters that favor diffusion across the phospholipid membrane of BCECs. Parameters to consider include size, lipophilicity, polar surface area, hydrogen bonding potential, and molecular charge. **(iii)** Carrier-mediated transport is an energy-independent process involving small molecules crossing the BBB along their concentration gradient with the assistance of suitable transporters. Each transporter class (Glut1, LAT1, ENT1, and MCT1) exhibits substrate specificity and saturable transport capacity (see Table 2). **(iv)** BBB disruption of the BCEC TJs due to pharmacological/physical/pathophysiological mechanisms causes an increase in BBB permeability by permitting otherwise limited paracellular diffusion. Small molecules not capable of diffusing through the phospholipid membrane of BCECs can easily pass from vessel lumen to brain parenchyma through these disrupted TJs.

# Pathways across the BBB

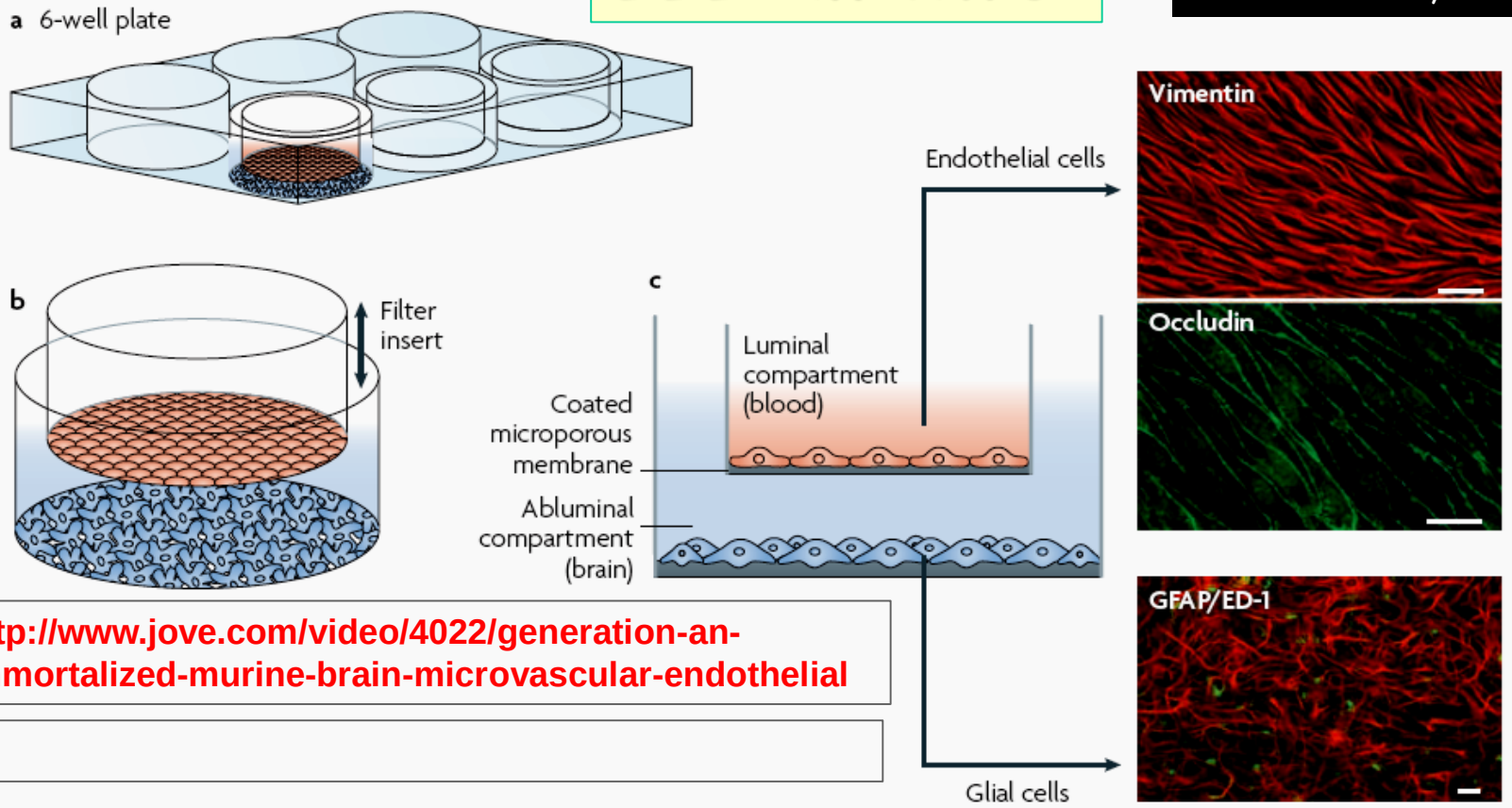



Chen and Liu, 2012

**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.**

# BBB "in vitro"

Cecchelli et al., 2007




http://www.jove.com/video/4022/generation-an-immortalized-murine-brain-microvascular-endothelial

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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.

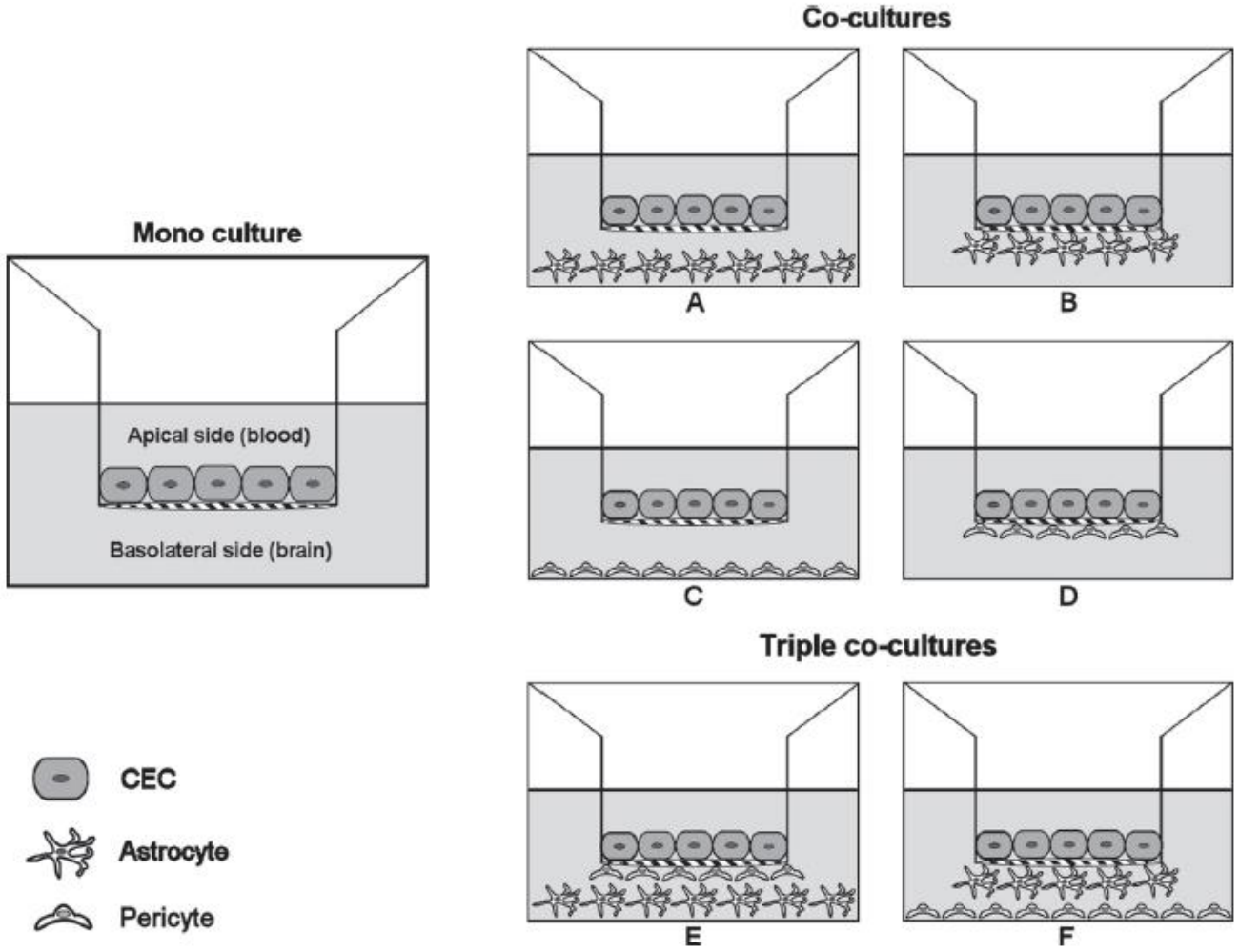



**Fig. 2.** *In vitro* cell-based blood–brain barrier (BBB) models. Monocultures of cerebral endothelial cells (CECs) are being replaced by co-culture or triple co-culture systems, in which CECs are seeded with other elements of the neurovascular unit, such as astrocytes, pericytes or neurons, in a non-contact (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 (2014) 409–432

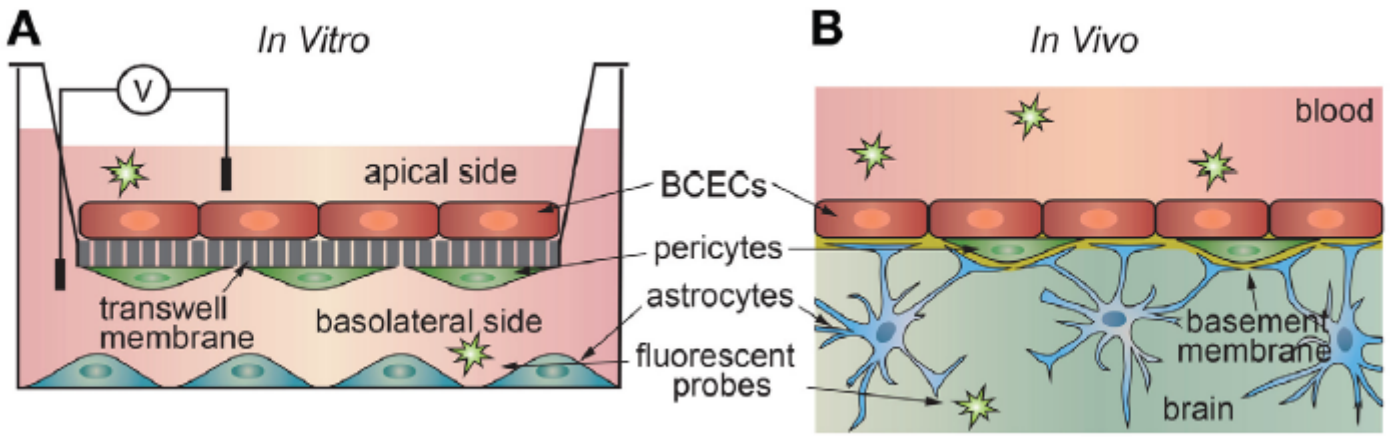



**FIGURE 4 | Schematic illustration of (a) *in vitro* and (b) *in vivo* transport measurements. (A)** In the 2D transwell assay, a monolayer of cells is formed on a porous membrane separating two compartments. Astrocytes and/or pericytes may be seed on the opposite side of the membrane or in the output 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

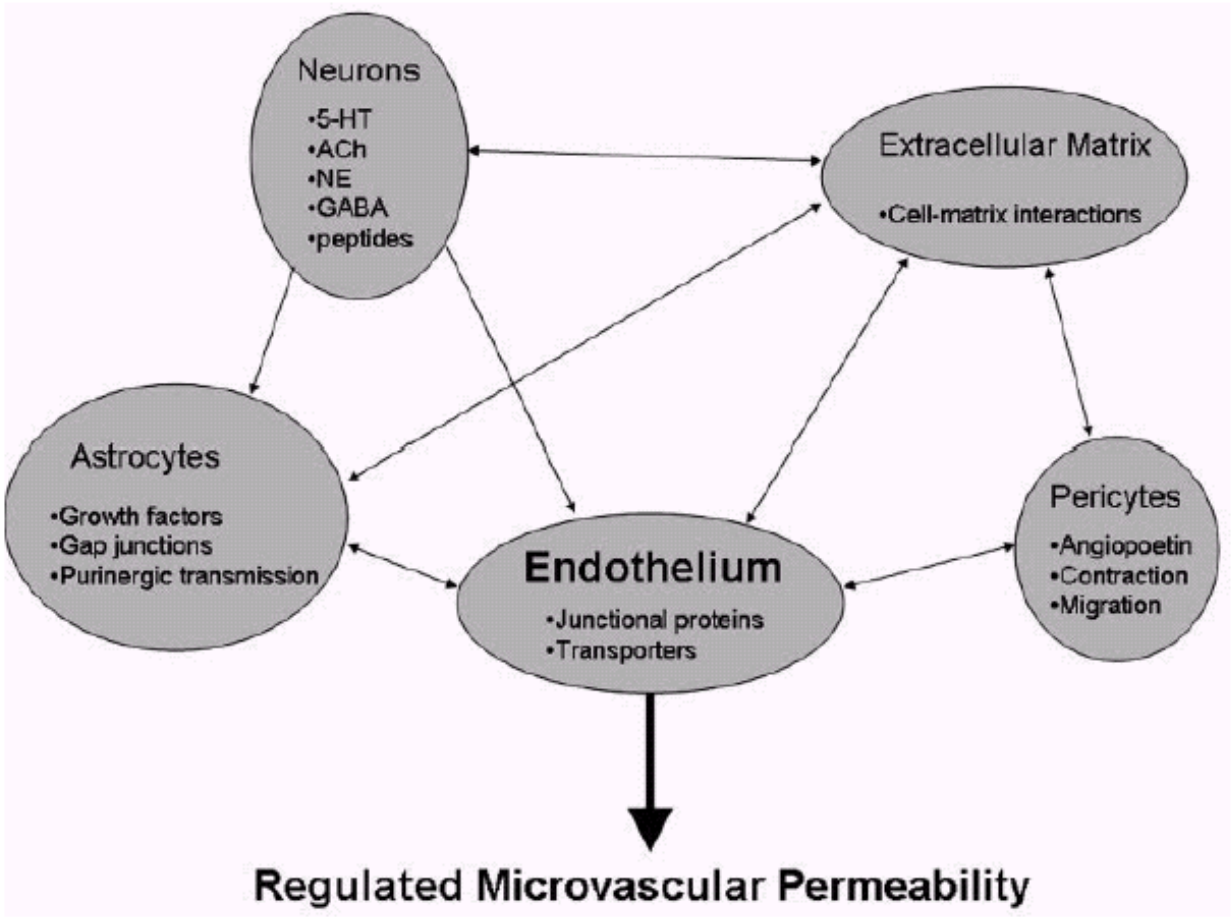
# In vitro BBB models: good or bad?

**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<br>Porcine | Allow the isolation of a large amount of cells from a single brain | Difficult to establish *in vitro–in vivo* correlations | Complex, time consuming and labor intensive cell obtaining | Smith [143] |
| | Rat | Availability of BBB permeation data from *in vivo* pharmacokinetic studies | Low yield; Require the sacrifice of multiple animals; Ethical and economic constraints | processes; Inconvenient for routine industrial use; High irreproducibility of the final characteristics of the cell | 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 | population; Challenging to eliminate non-endothelial cells (e.g. pericytes, smooth muscle cells); Rapid *in vitro* de-differentiation or loss of phenotype | 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 | | | Urich [124]; Lu [126]; Vu [198]; Weskler [205]; Poller [206]; Tai [207]; Dauchy [208]; Schrade [209]; Weskler [210] |
| | | *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 | | 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].

# The ‘neurovascular unit’: microvascular endothelium, astrocytes, pericytes, neurons, and extracellular matrix




BBB is a dynamic structure

Hawkins & Davis, 2005

# Interaction between endothelial cells and nearby cells can contribute to the properties of the BBB

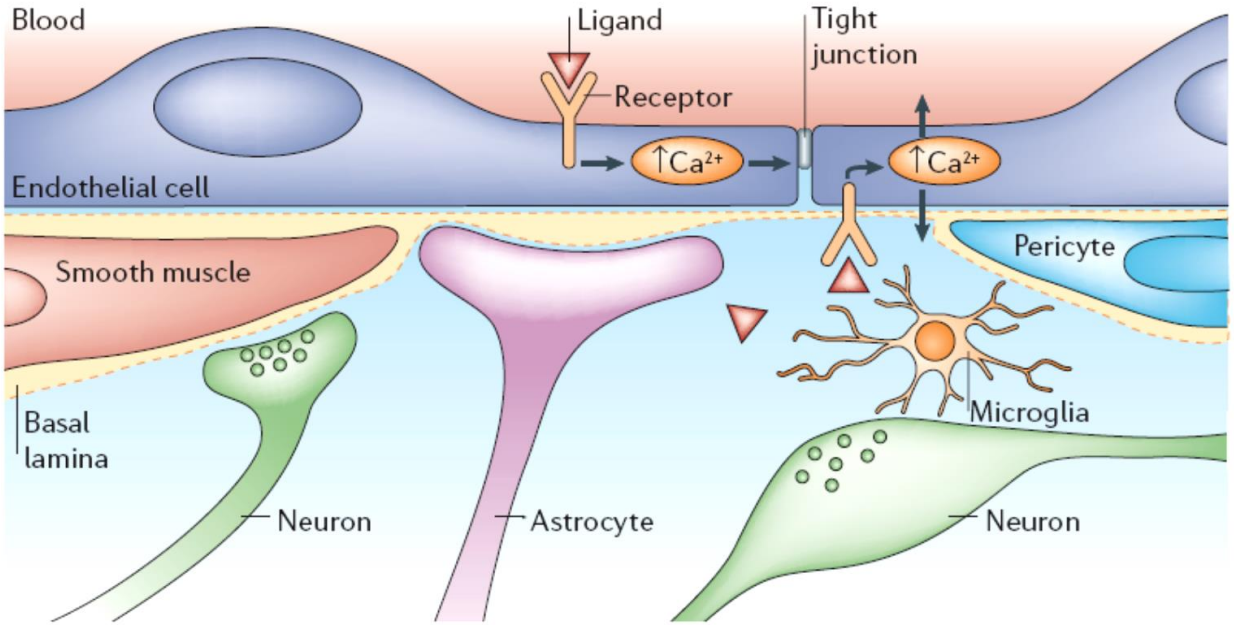



Figure 5 | **Complex cell–cell signalling at the blood–brain barrier.** A portion of a brain capillary wall, showing the main cell types present with the potential to signal to each other. Pericytes are enclosed within the endothelial basal lamina and form the closest associations with endothelium. The endfeet of astrocytic glial cells are apposed to the outer surface of the basal lamina. In the perivascular space are found microglia, the synaptic terminals and boutons of nerve fibres, and (in arterioles) smooth muscle cells. In the larger vessels, cells of the meninges form a perivascular cuff or sheath that projects down from the brain surface and demarcates the Virchow–Robin space (not shown). Agents such as ATP and histamine can influence endothelial function by ligand–receptor interaction, from the blood or the brain side. Some receptors are coupled to increases in the concentration of intracellular $Ca^{2+}$. The arrows indicate the ability of the endothelium to release substances to the blood or brain side after receptor activation, as part of their 'effector' function. Modified, with permission, from REF. 16 © (2005) Springer.

Abbott et al., 2006

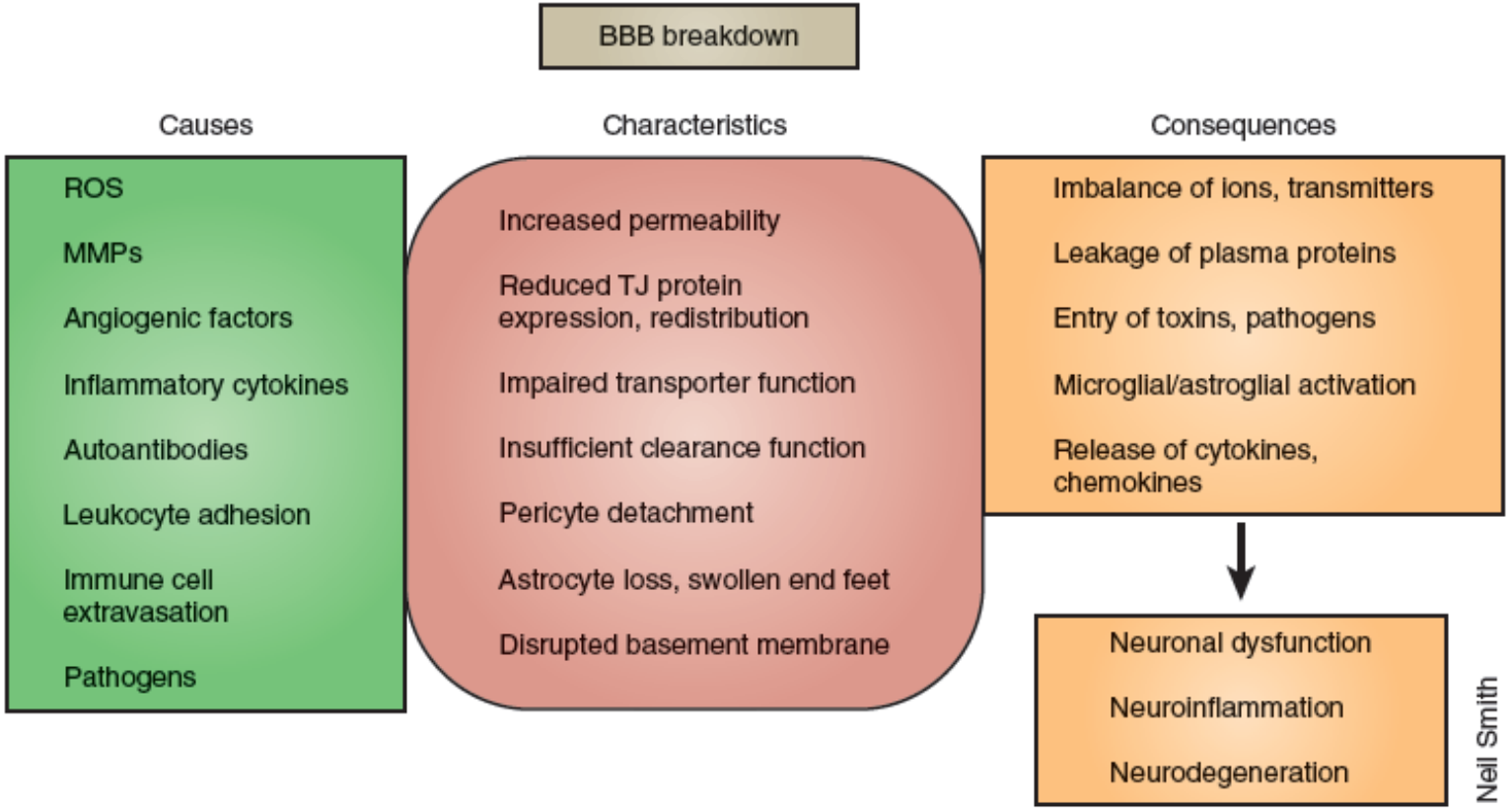



**Figure 4** Causes, characteristics and consequences of BBB breakdown. Factors that can disrupt the BBB are varied, ranging from secreted elements to immune cells and pathogens. Compromised BBB integrity manifests mainly as increased barrier permeability. In addition to direct effects on endothelial 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.

# BBB changes in pathological conditions are mediated by several signalling molecules

Disruption in BBB integrity and concomitant increase in BBB permeability can be due to exposure of BBB to pro-inflammatory cytokines TNF-α and IL-1β, which induces expression of matrix metalloproteases (MMPs). Upregulation and activation of MMPs degrade endothelium basement membranes, disrupting BBB stability. Pro-inflammatory cytokines can induce JAM-A shedding from BCECs, although this does not necessarily correlate with a loss in BBB function alone. CNS tumors associated with angiogenesis can also disrupt the interaction between astrocytes and BCECs and destabilize the BBB to enhance permeability.

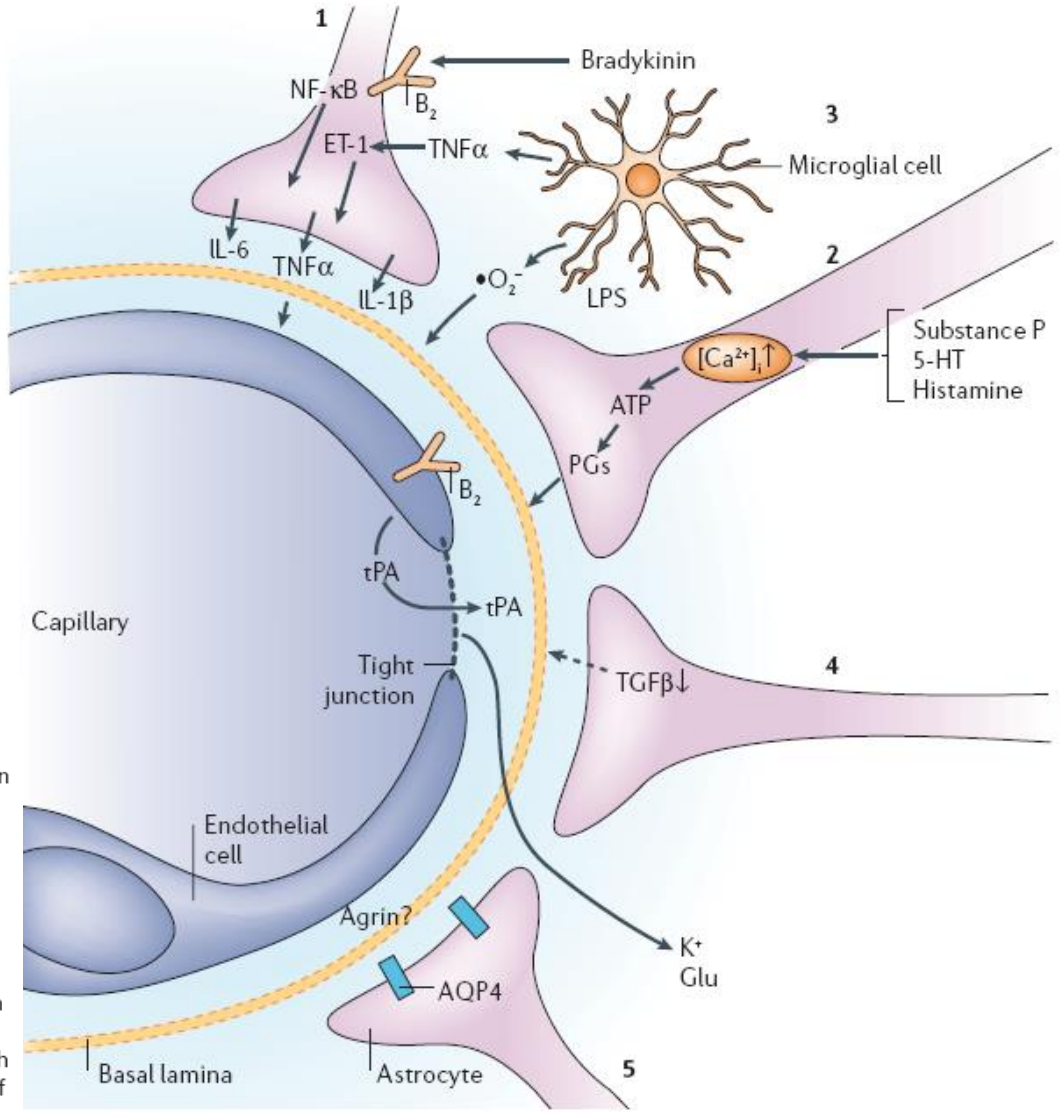



Figure 6 | **Astroglial–endothelial signalling under pathological conditions.** Examples of astroglial–endothelial signalling in infection or inflammation, stroke or trauma, leading to opening of the blood–brain barrier (BBB) and disturbance of brain function. bradykinin, produced during inflammation in stroke or brain trauma, acts on endothelial and astroglial bradykinin $B_2$ receptors, leading to an increase in the concentration of intracellular $Ca^{2+}$. In astrocytes, this can trigger the production of interleukin-6 (IL-6) through activation of nuclear factor-κB (NF-κB) (**1**). Bradykinin, substance P, 5-hydroxytryptamine (5-HT, serotonin) and histamine acting on astrocytes can lead to the formation of ATP and prostaglandins (PGs), with effects on vascular tone and endothelial permeability (**2**) by mechanisms that are known to involve endothelium. Lipopolysaccharide (LPS), formed in infections, leads to the release from microglia of tumour necrosis factor-α (TNFα), IL-1β and reactive oxygen species (including $O_2^{•-}$), all of which have the ability to open the BBB (**3**). Astrocytes downregulate tissue plasminogen activator (tPA) production via transforming growth factor-β (TGFβ), but there is still sufficient tPA to open the BBB, leading to an influx of tPA from the blood (**4**). Following disruption of the BBB involving a decrease in agrin expression, $K^+$ and glutamate (Glu) from the blood can reach the brain extracellular space. Aquaporin 4 (AQP4) is upregulated on the astroglial endfeet, leading to astroglial swelling (**5**). ET1, endothelin 1.

Abbott et al., 2006

# BBB brakedown in Multiple Sclerosis

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

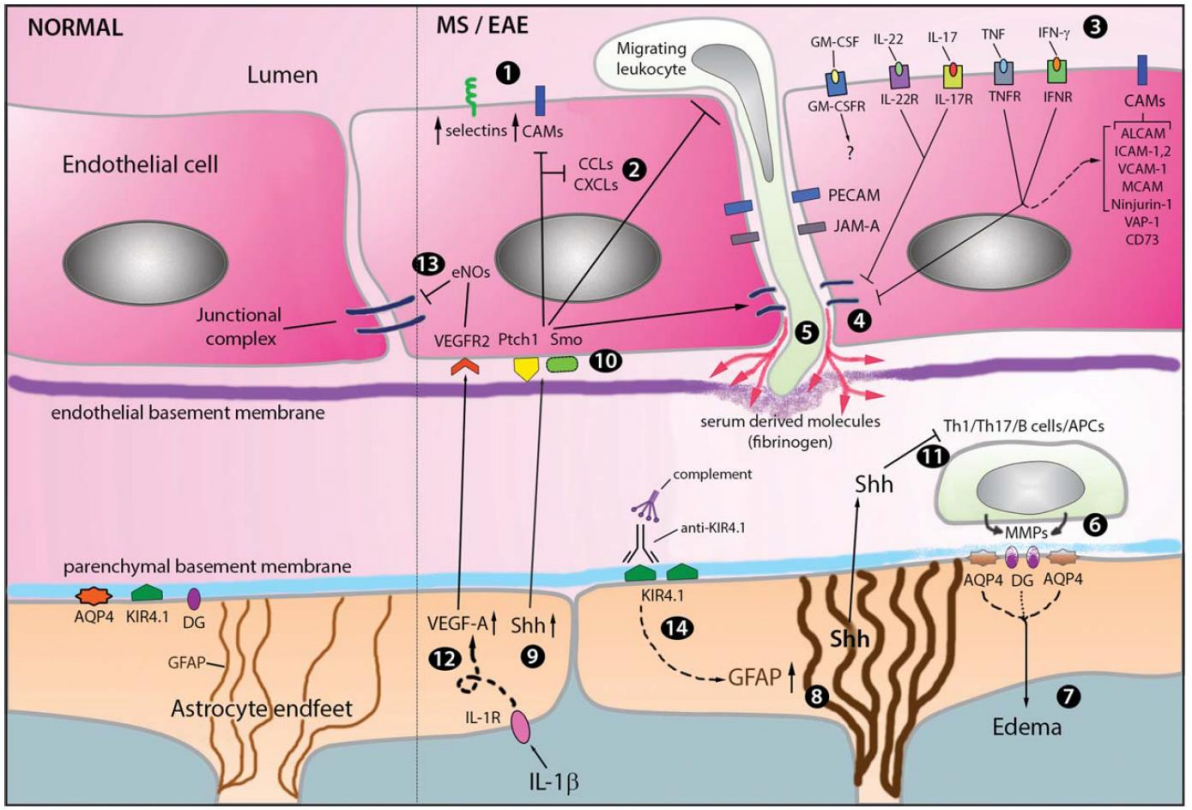



FIGURE 5: BBB changes during MS/EAE. During neuroinflammation, early BBB disturbances are associated with EC activation that is characterized by increased expression of E- and P-selectins, cell adhesion molecules (CAMs) including ALCAM, ICAM-1, ICAM-2, VCAM-1, MCAM, Ninjurin-1, VAP-1 and CD73 [1], chemokines (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-1$\beta$ 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].

# Non-invasive techniques to deliver drugs to the brain



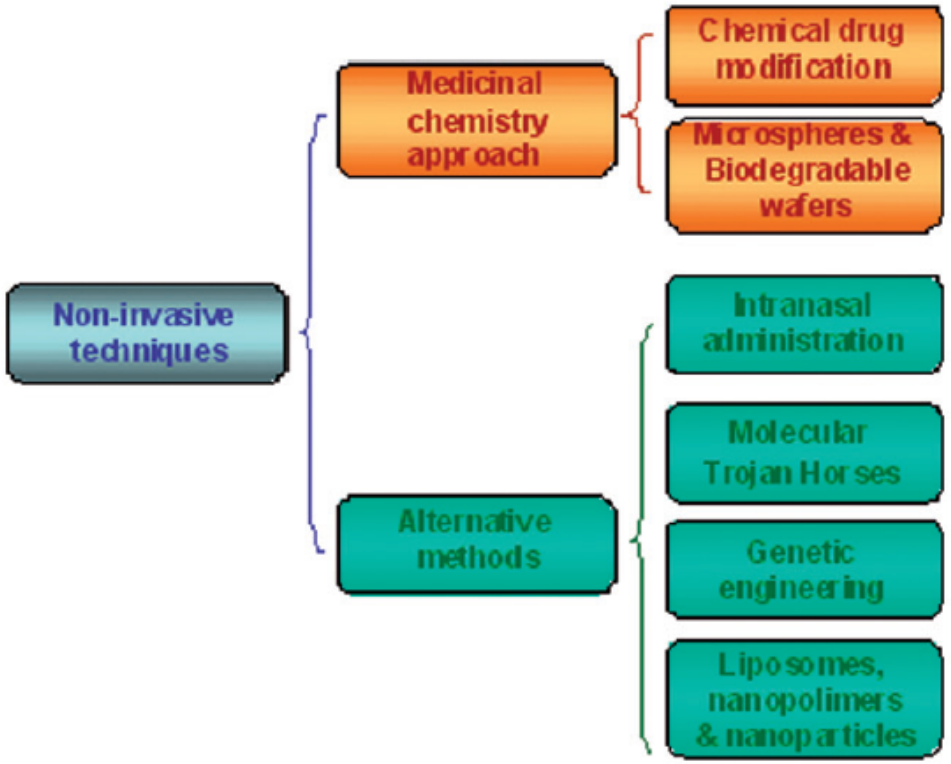



Figure 5. A schematic representation of current strategies to deliver drugs to the brain by non-invasive techniques. Non-invasive techniques include drug modification by medicinal chemistry approaches and drug encapsulation through nanotechnological carriers.

# 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.

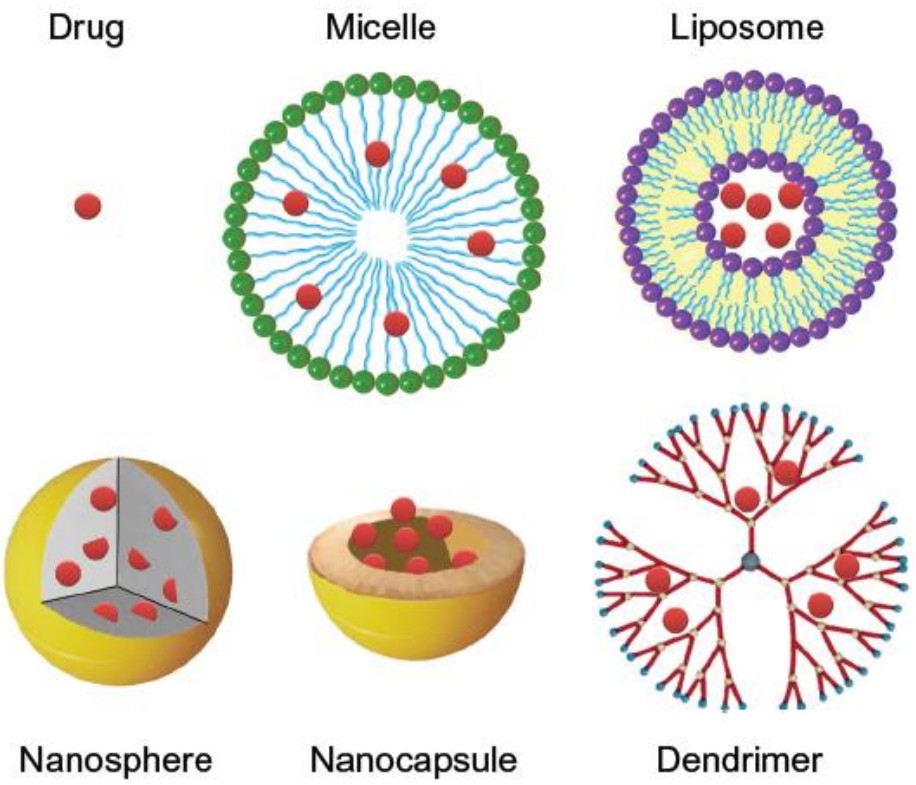



**Figure 2** Schematic representation of different particulate systems for drug transport and delivery.

**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
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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.**

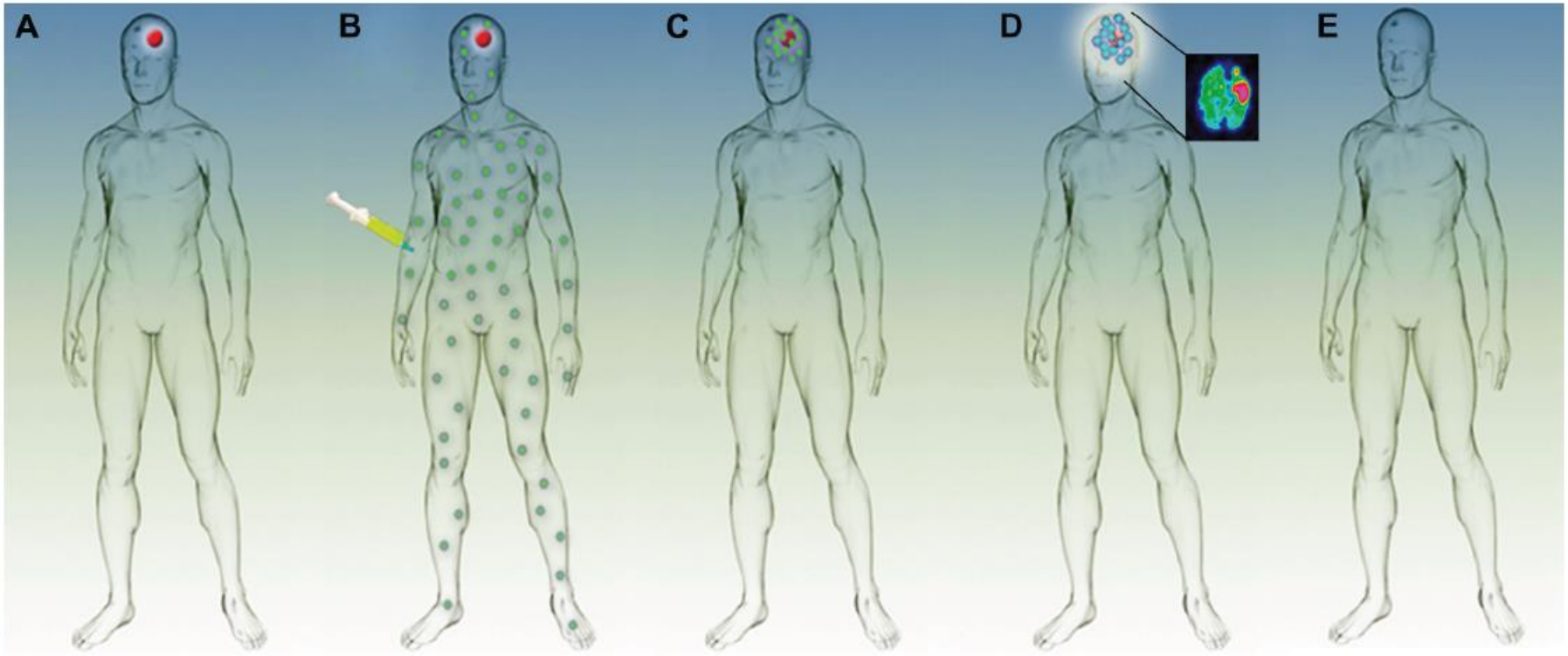


**Figure 3** Concept of theranostics. (**A**) A pathological process localized to the brain. (**B**) Systemic administration of a therapeutic agent distributes the theranostic throughout the entire body. (**C**) Nanotechnology enables concentration of the agent in the targeted area. (**D**) Inclusion 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**

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

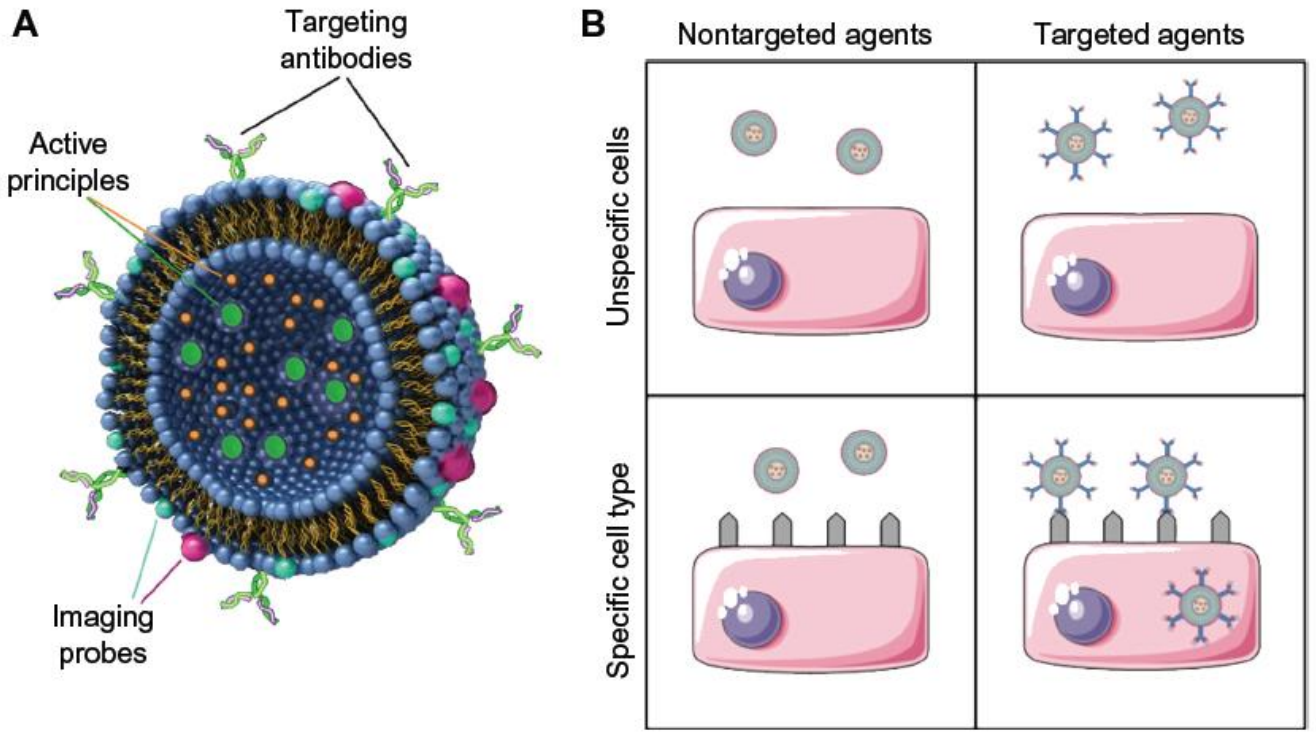


**Figure 4** Theranostic agents in molecular recognition processes. (**A**) A liposomal theranostic agent includes surface antibodies that participate in the molecular recognition process with targeted cells, imaging probes (for diagnostic purposes), and active principles of treatment. (**B**) Targeting 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.