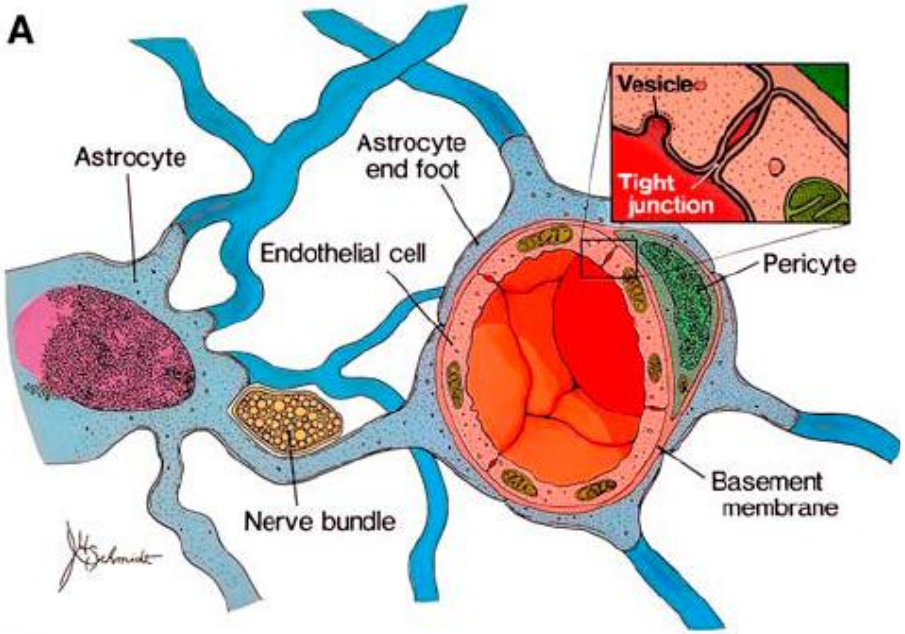
The Blood-Brain Barrier
capillary endothelium

- 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

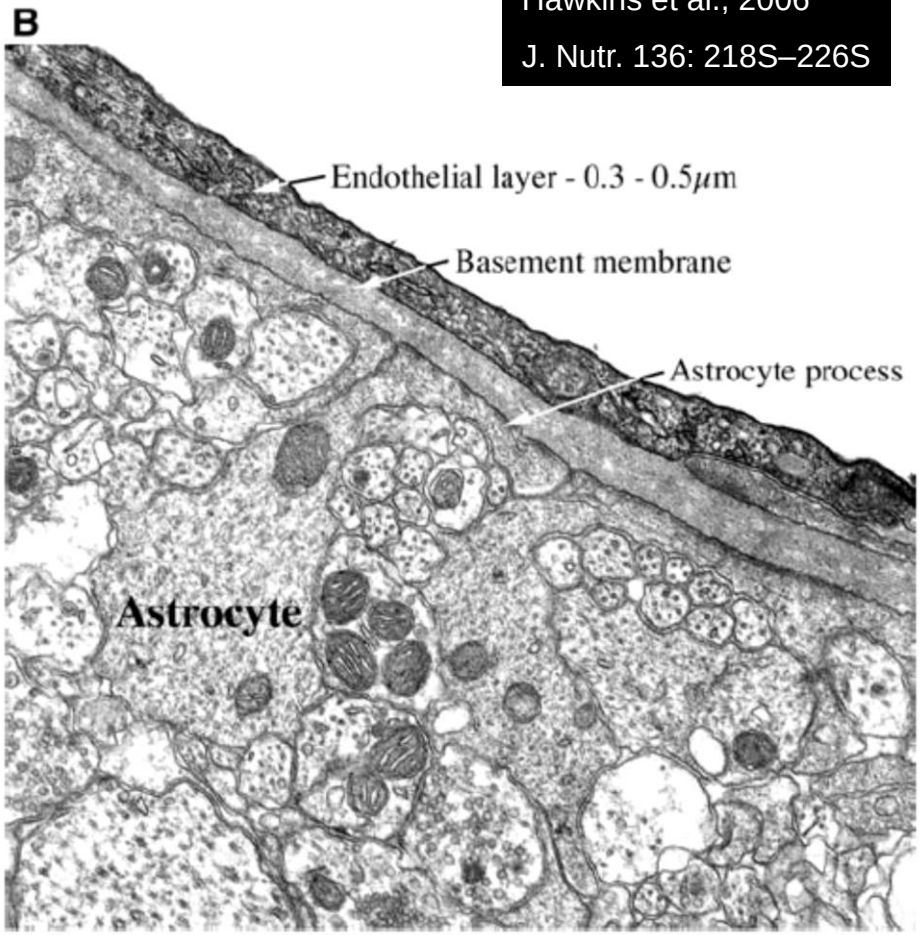
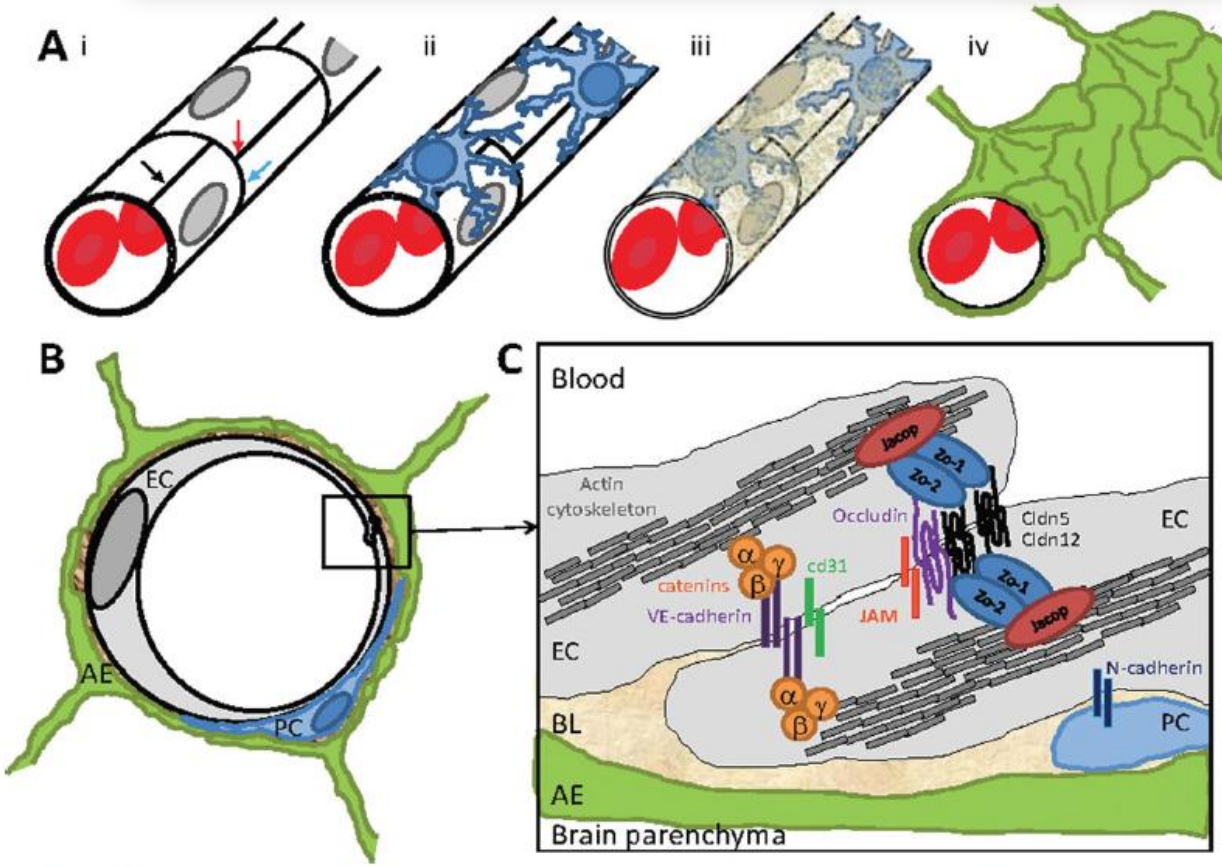


FIGURE 2 (A) The BBB exists at the level of the endothelial cells of cerebral capillaries. The endothelial cells are joined together by an extensive network of tight junctions and surrounded by a basement membrane, within which pericytes reside. Astrocytic processes (so-called end-feet) surround cerebral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

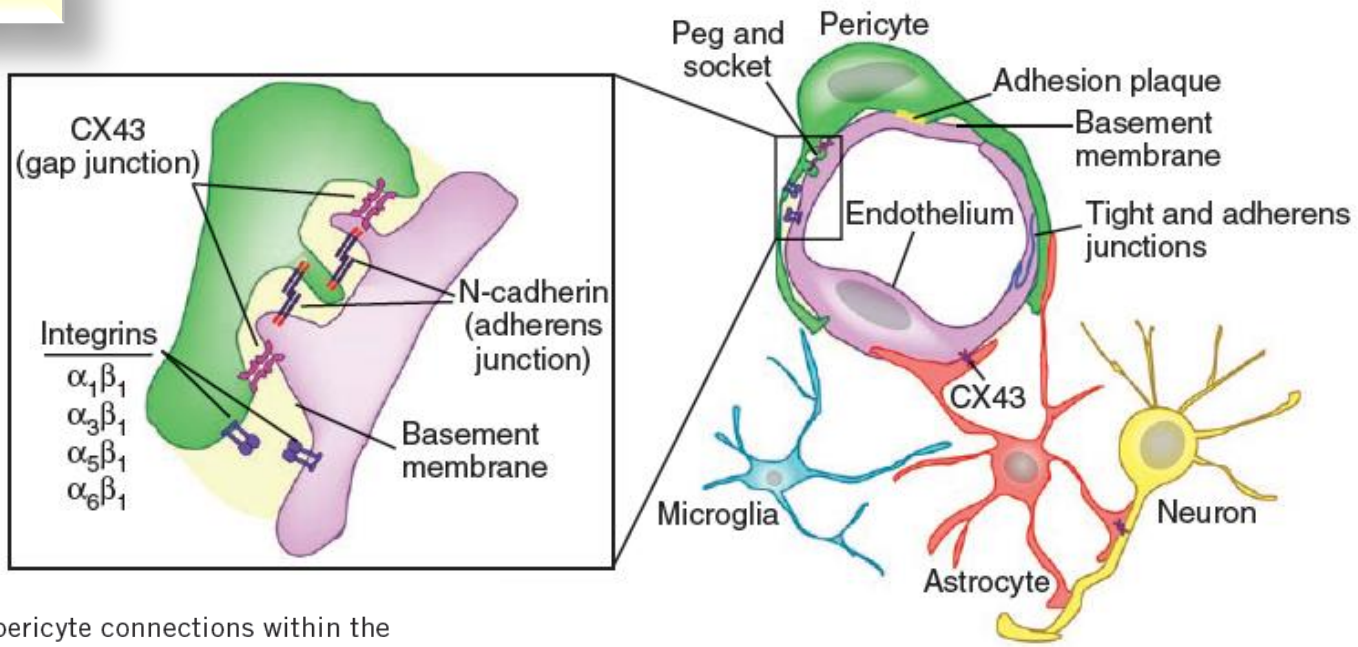


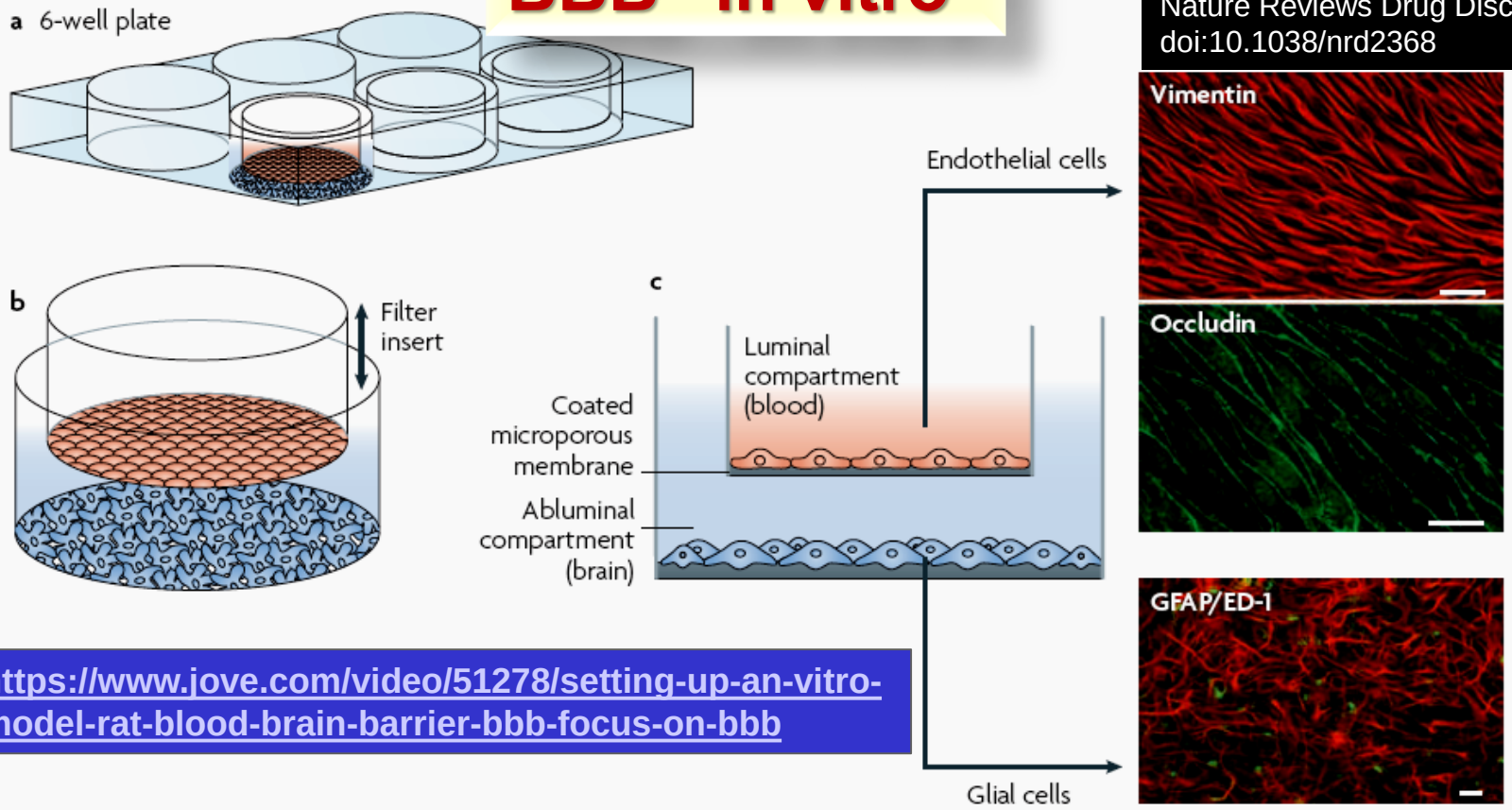
Figure 1 Structural and molecular pericyte connections within the neurovascular unit. Right: pericytes (green) and endothelial cells (purple) are connected to a shared basement membrane (yellow) by several types of integrin molecule. In areas lacking the basement membrane, interdigitations of pericyte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

“in vitro” BBB models

Lauschker et al., 2017
STEM CELLS AND DEVELOPMENT
DOI: 10.1089/scd.2017.0003

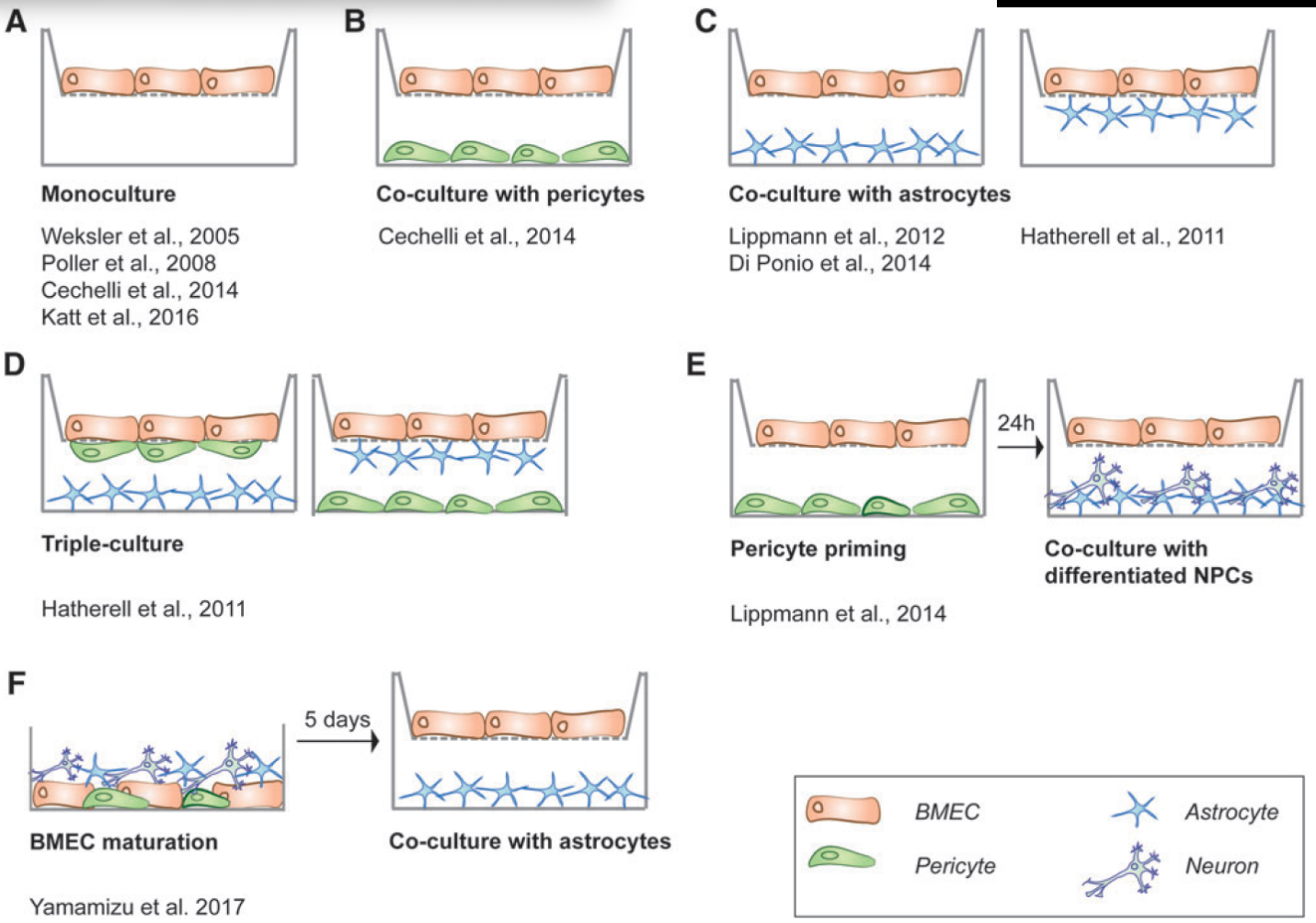


FIG. 3. In vitro models of the BBB based on human BMECs. In the transwell system, several settings and combinations of the cell types of the NVU have been published: **(A)** Monoculture of BMECs grown on the upper side of the transwell filter. **(B)** Co-culture of BMECs grown on the upper side of the filter with pericytes on the bottom of the well. **(C)** Co-culture of BMECs grown on the upper side of the filter with astrocytes either on the bottom of the well or on the lower side of the transwell filter. **(D)** In the triple culture setting, BMECs are cultured with pericytes and astrocytes on the bottom of the transwell or the lower side of the filter. **(E)** BMECs on the upper side of the filter are first cultured with pericytes on the bottom of the well. After 24 h, BMECs are cultured together with astrocytes and neurons, derived from NPCs, on the bottom of the well. **(F)** Co-culture of BMECs with PSC-derived pericytes, astrocytes, and neurons for 5 days, followed by BMEC maintenance on the upper side of the filter and astrocyte growth on the bottom of the transwell. NPC, neural progenitor cells.

Kaisar et al., 2017

EXPERT OPINION ON DRUG DISCOVERY
http://dx.doi.org/10.1080/17460441.2017.1253676

TEER is a quantitative measure of barrier integrity performed by applying a voltage across the cell monolayer, measuring resulting current, and calculating resistance using Ohm's Law.

Table 2. BBB models based on primary BMECs.

Origin	Cell	Culture	Model design	Approx. TEER (ohm.cm^2)
Mouse	Mouse primary BMEC	Monoculture	EC on top of Transwell insert	~50 ~150
		Coculture with murine pericyte	EC on top of Transwell insert and pericyte on bottom well	~150
		Coculture with mouse astrocyte cell lines	EC on top & astrocytes on the bottom of Transwell insert	80
		Coculture with rat astrocyte	EC on top of Transwell insert and astrocyte on bottom well	~200
Rat	Rat primary BMEC	Coculture with rat astrocyte	EC on top of Transwell insert and astrocyte on bottom well	300 >600
		Triple culture with rat astrocytes and rat pericytes	EC on top, pericyte on bottom of Transwell insert and astrocyte on bottom well	400
Bovine	Bovine primary BMEC	Coculture with rat astrocytes		600–1800
Porcine	Porcine primary BMEC	Coculture with rat astrocytes	EC on top of Transwell insert and astrocyte on bottom well	800
		Triple culture with rat/porcine astrocyte and pericyte	EC on top, pericyte on bottom of Transwell insert and astrocyte on bottom well	>1000

EC: endothelial cell.

TEER = trans-endothelial electrical resistance, measured in Ohms (Ω) × cm^2.

Modeling Neurovascular Disorders with Human-Induced Pluripotent Stem Cells

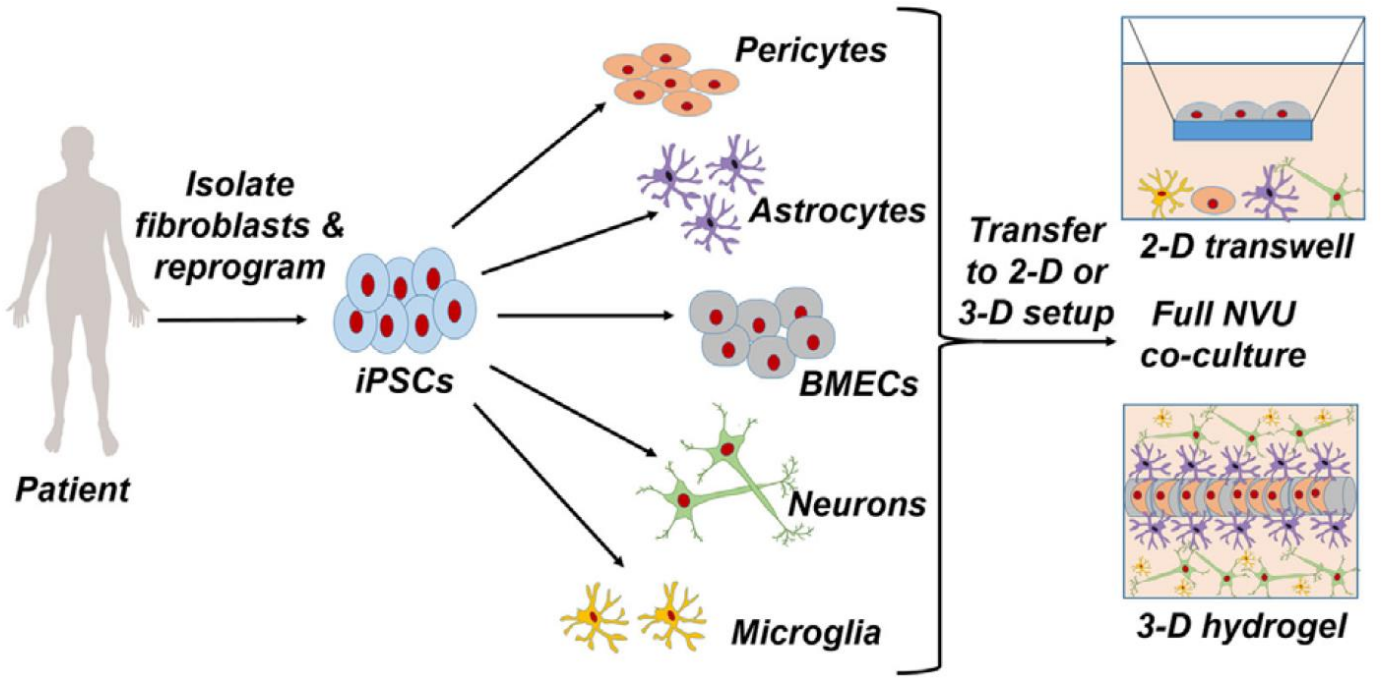


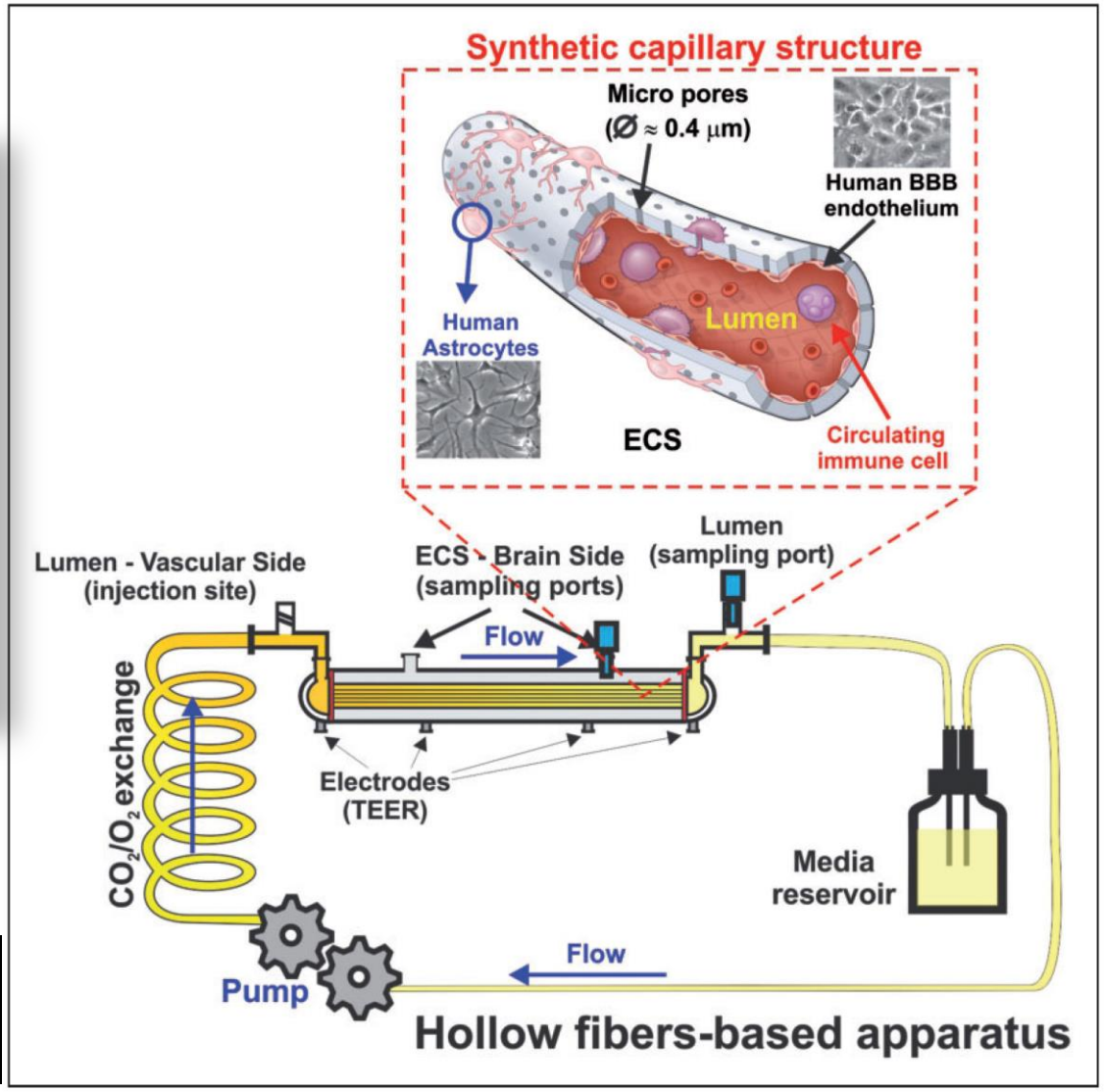
FIGURE 1 | Patient-specific fibroblasts are isolated and reprogrammed to a pluripotent state, from which pericytes, astrocytes, brain microvascular endothelial cells, neurons, and microglia are differentiated. Full coculture models of the neurovascular unit are achieved through a two-dimensional Transwell setup or a three-dimensional hydrogel setup.

Bosworth et al., 2018

Frontiers in Bioengineering and Biotechnology
doi: 10.3389/fbioe.2017.00087

Dynamic in vitro BBB model

This model allows the use of co-cultures and creates intraluminal flow through artificial capillary-like structural supports



Sivandzade & Cucullo, 2018

Journal of Cerebral Blood Flow & Metabolism
DOI: 10.1177/0271678X18788769

Figure 3. Schematic illustration of the DIV-BBB model. In this system, BBB endothelial cells are cultured inside hollow fiber structures (lumen) coated with fibronectin or ECM matrices. Astrocytes can be seeded on the abluminal surface of the same hollow fibers are in juxtaposition to ECs once the abluminal surface is properly coated. The bundle of hollow fibers is suspended inside a sealed chamber and in continuity with gas-permeable silicon tubing circulating media throughout the system. Access to the luminal (vascular) and abluminal (parenchymal) compartments is granted through inlet and outlet ports positioned on the opposite sides of the module and two additional ports on top of the longitudinal section, respectively. TEER is measured in real time through a set of electrodes embedded in the module's scaffold. The electrodes are in contact with either the luminal or the abluminal chambers.

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

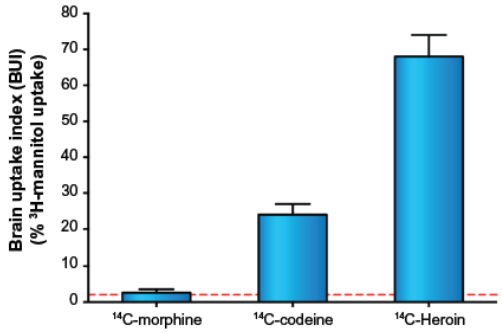


Figure 3. (**A**) Chemical structures of morphine, codeine, and heroin with their respective log *P*. (**B**) Relative rat brain uptake index (BUI) of ^{14}C-morphine, ^{14}C-codeine, and ^{14}C-heroin in rats following a single brain passage after carotid injection. The greater uptake of codeine and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

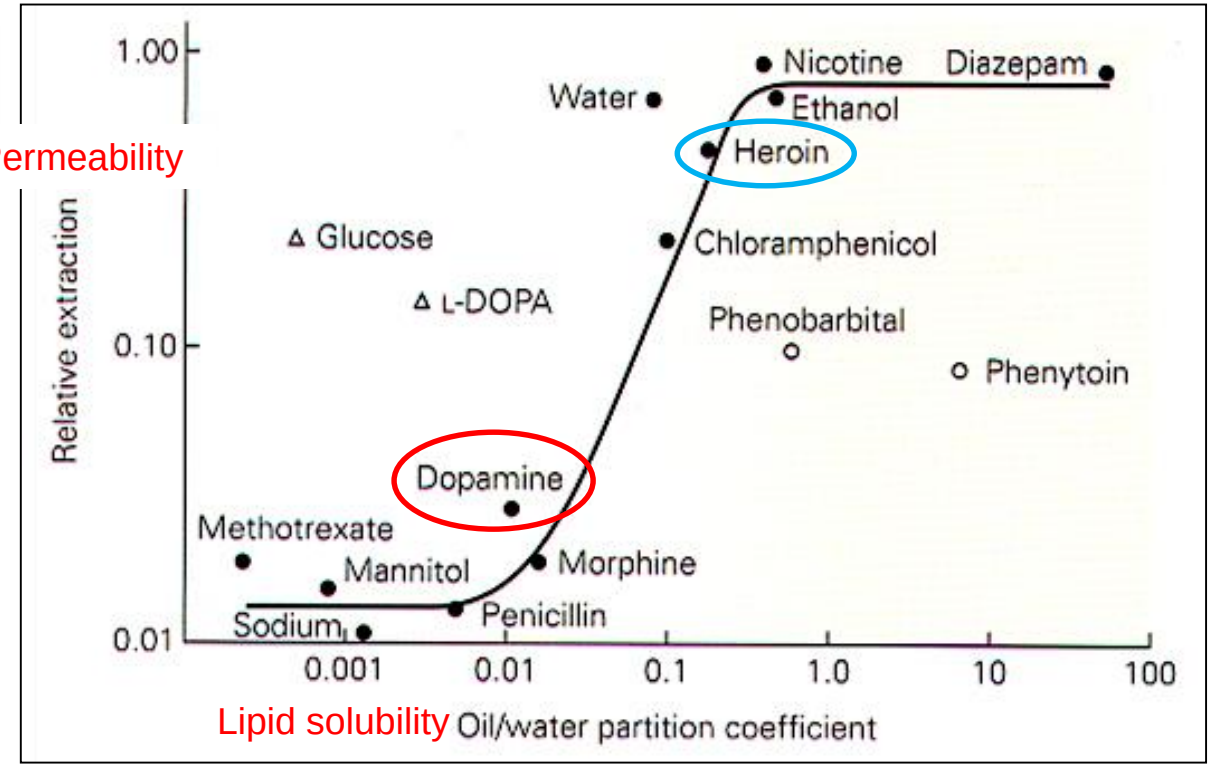
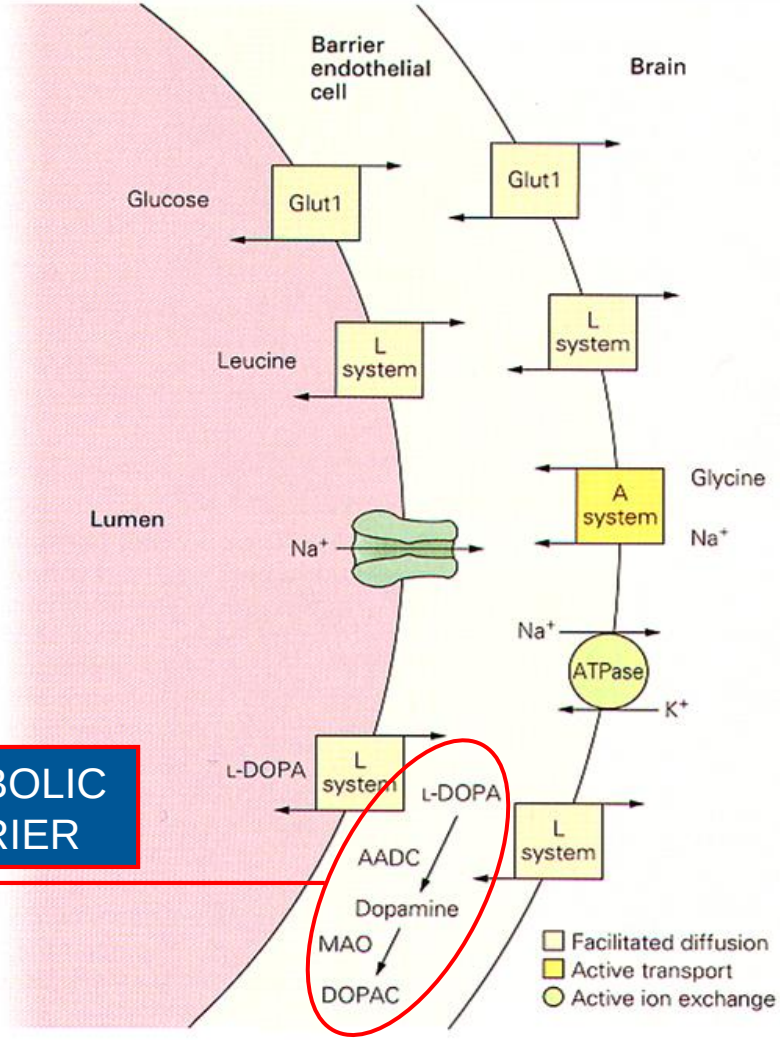


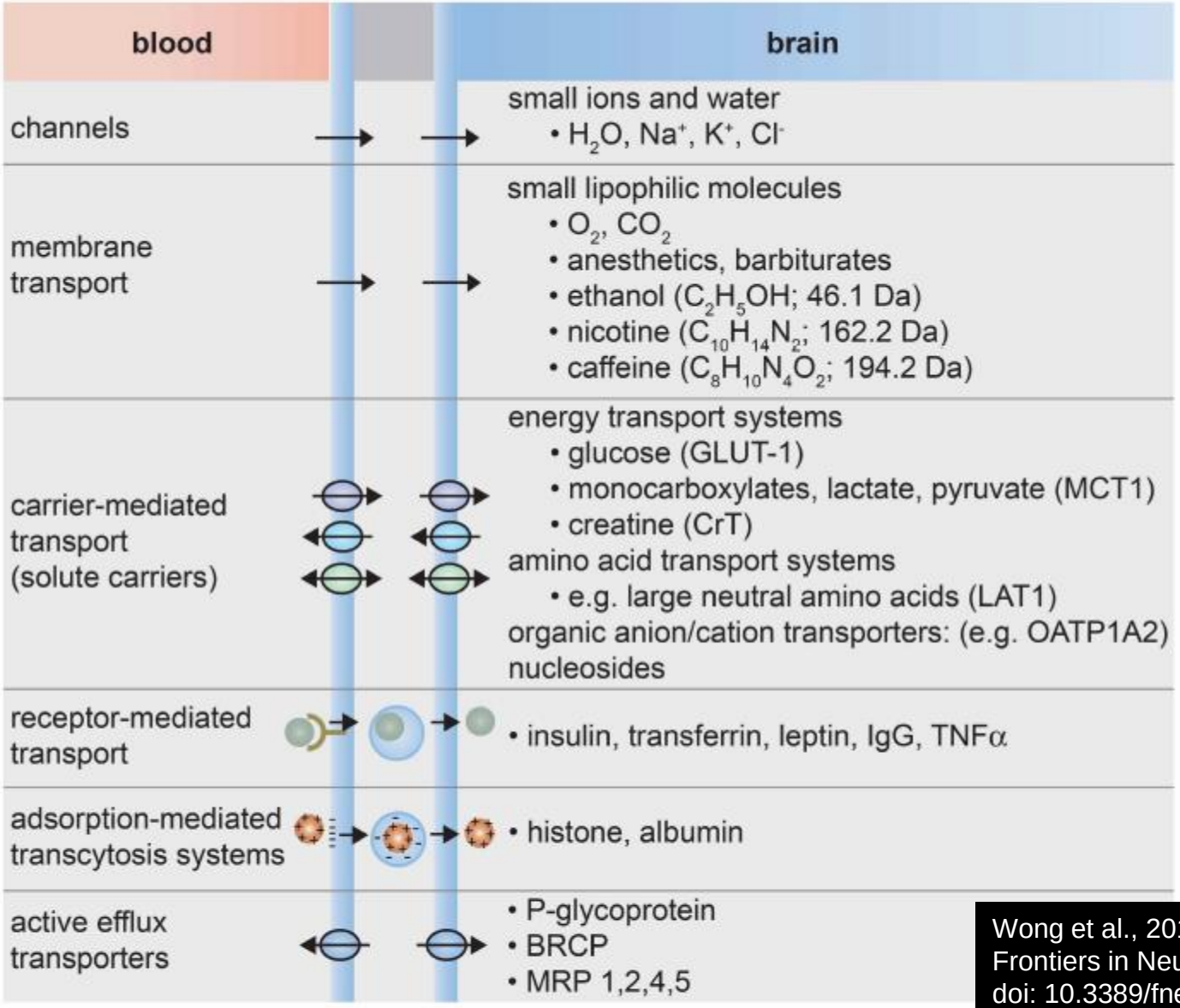
Figure B-4 The oil-water partition coefficient indicates the relationship between lipid solubility and brain uptake of selected compounds. The distribution into olive oil relative to water for each test substance serves as a measure of its lipid solubility. The brain uptake is determined by comparing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

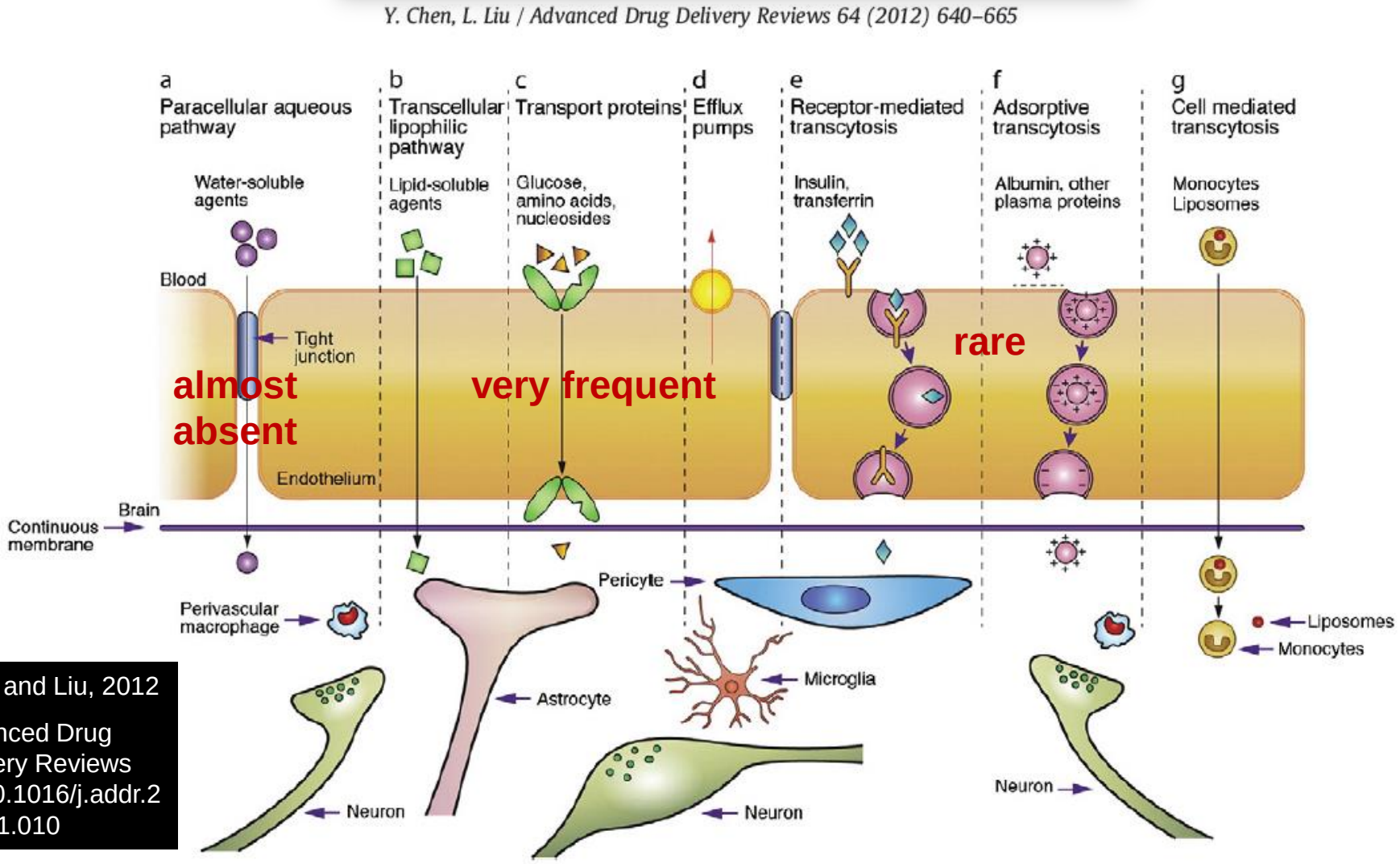
Figure B-5 A complex system of polarized transporter proteins and ionic channels determine the specific movement of water-soluble compounds and ions across barrier endothelial cells. Some transporters (eg, Glut1 and L system) facilitate the movement of substrates down concentration gradients, and others (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.

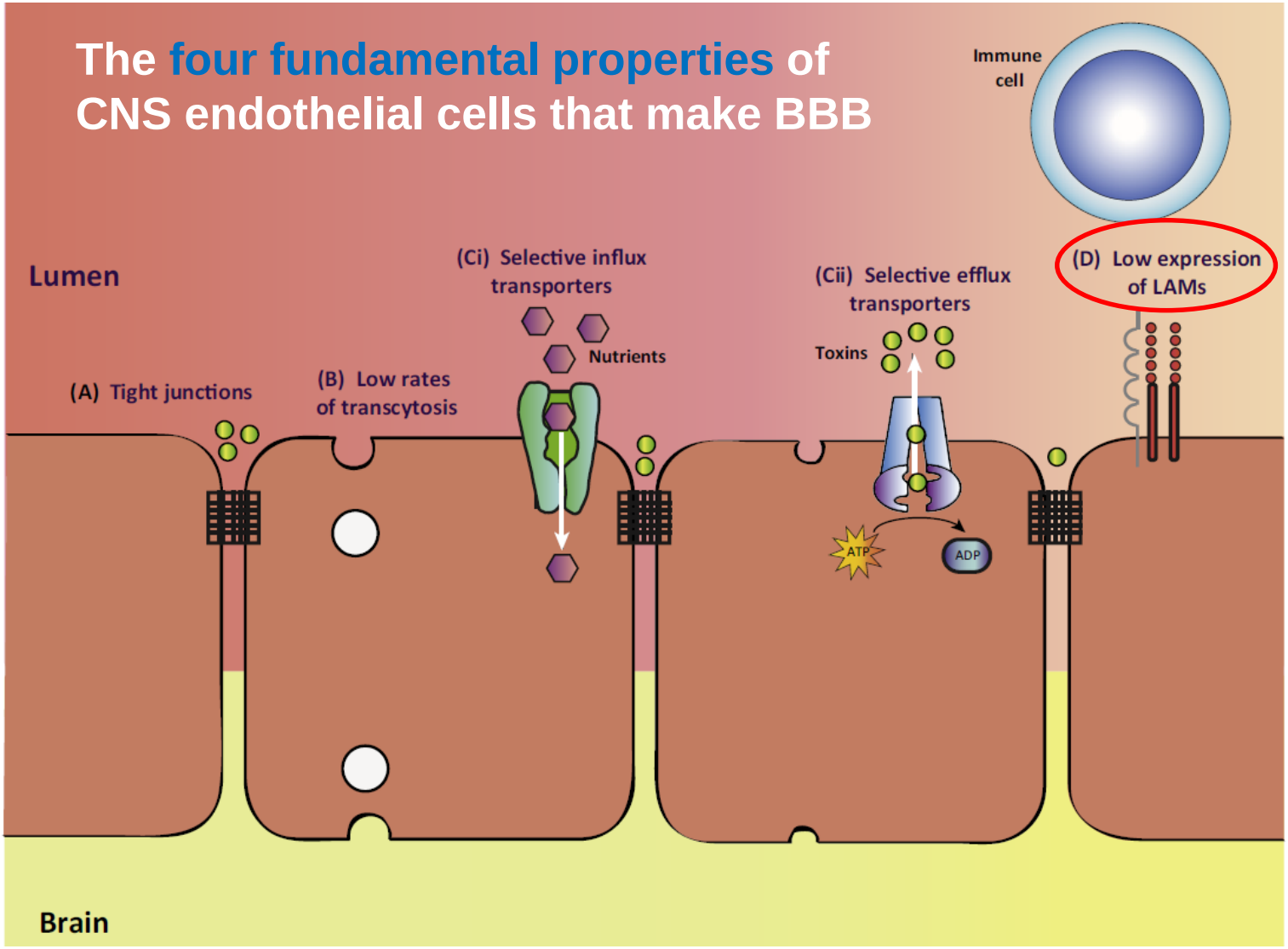
Pathways across the BBB



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

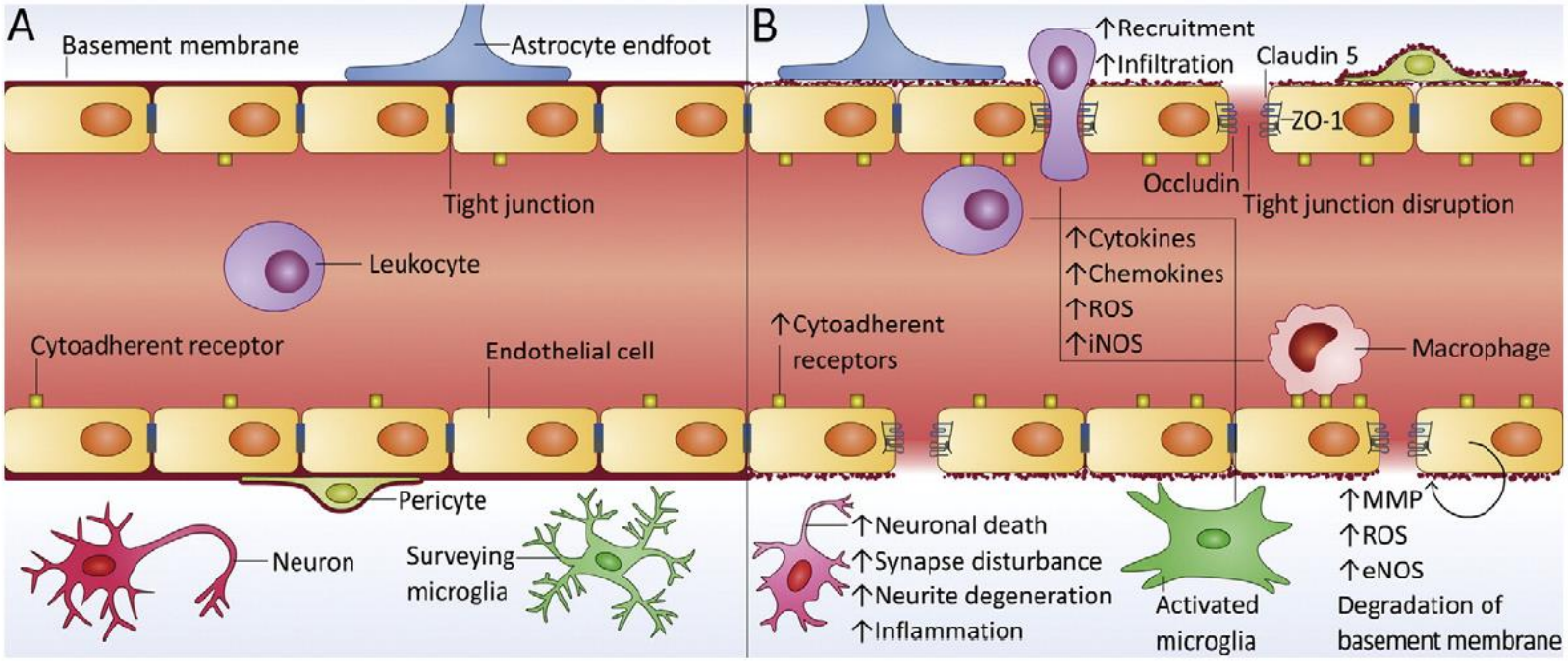


Fig. 1. Blood–brain barrier (BBB) composition and main alterations found in pathological conditions. A) The BBB is mainly composed of vascular endothelial cells, highly connected by adherens and tight junctions (TJs), and a sparse layer of pericytes. A basement membrane and a layer of astrocyte end-foot 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

1016/j.jconrel.2J Controlled Release
http://dx.doi.org/10.016.05.044

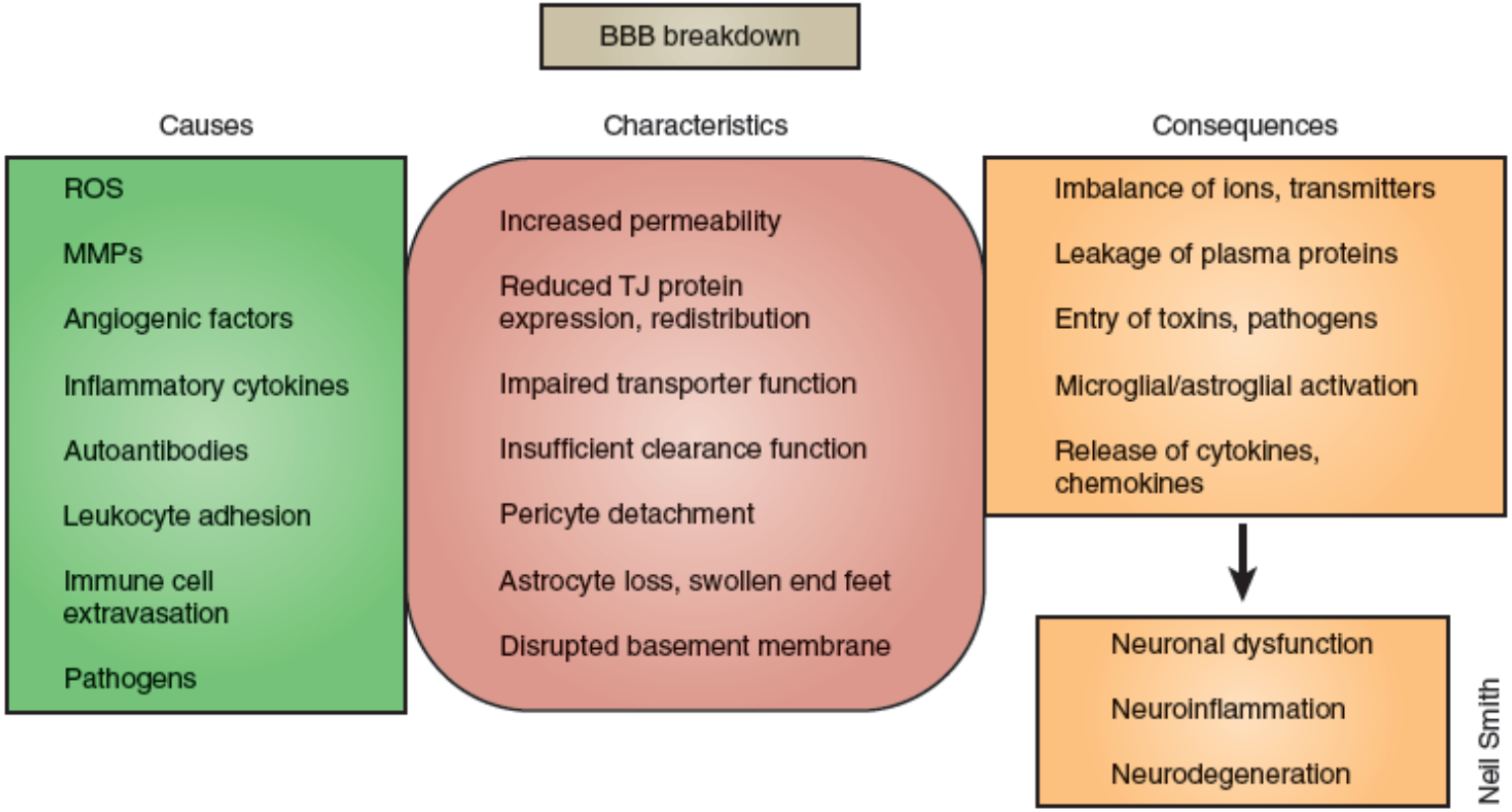


Figure 4 Causes, characteristics and consequences of BBB breakdown. Factors that can disrupt the BBB are varied, ranging from secreted elements to immune cells and pathogens. Compromised BBB integrity manifests mainly as increased barrier permeability. In addition to direct effects on endothelial cells,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

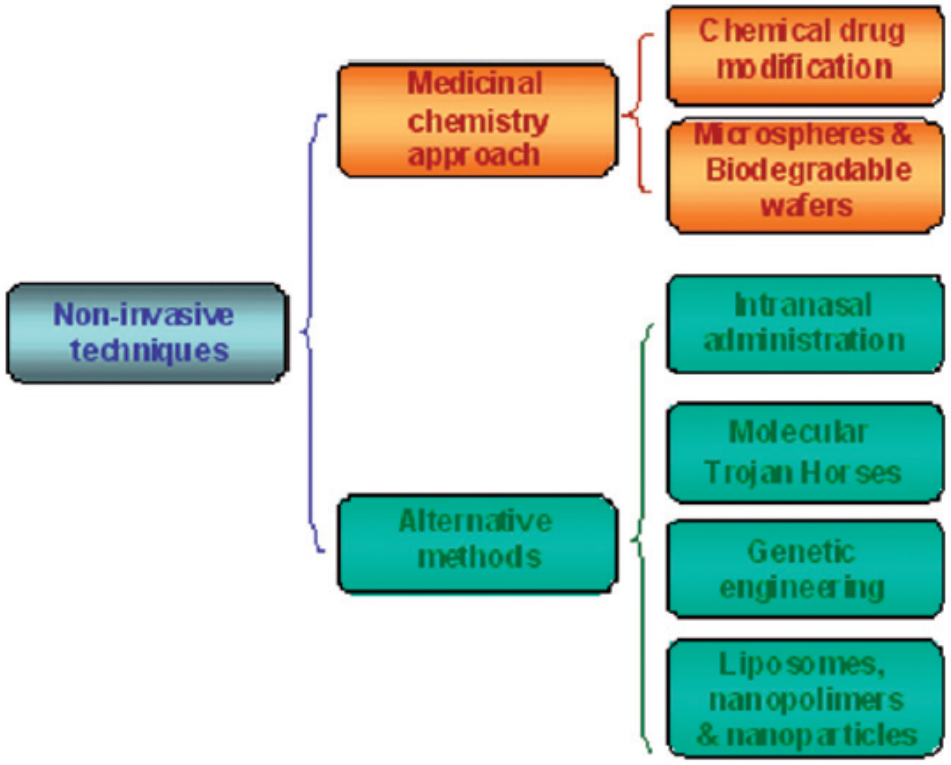


Figure 5. A schematic representation of current strategies to deliver drugs to the brain by non-invasive techniques. Non-invasive techniques include drug modification by medicinal chemistry approaches and drug encapsulation through nanotechnological carriers.

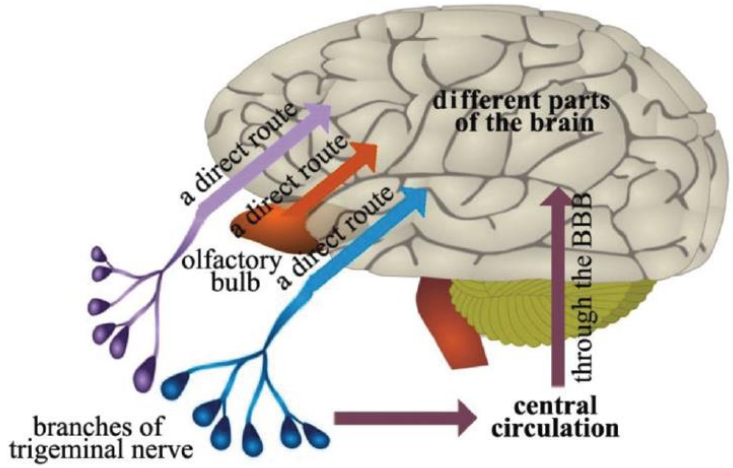


Fig. 3 Schematic of the pathways of drug delivery to the brain from the nasal cavity.

Zhang et al, 2016

Biomater. Sci., 2016, 4, 219

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

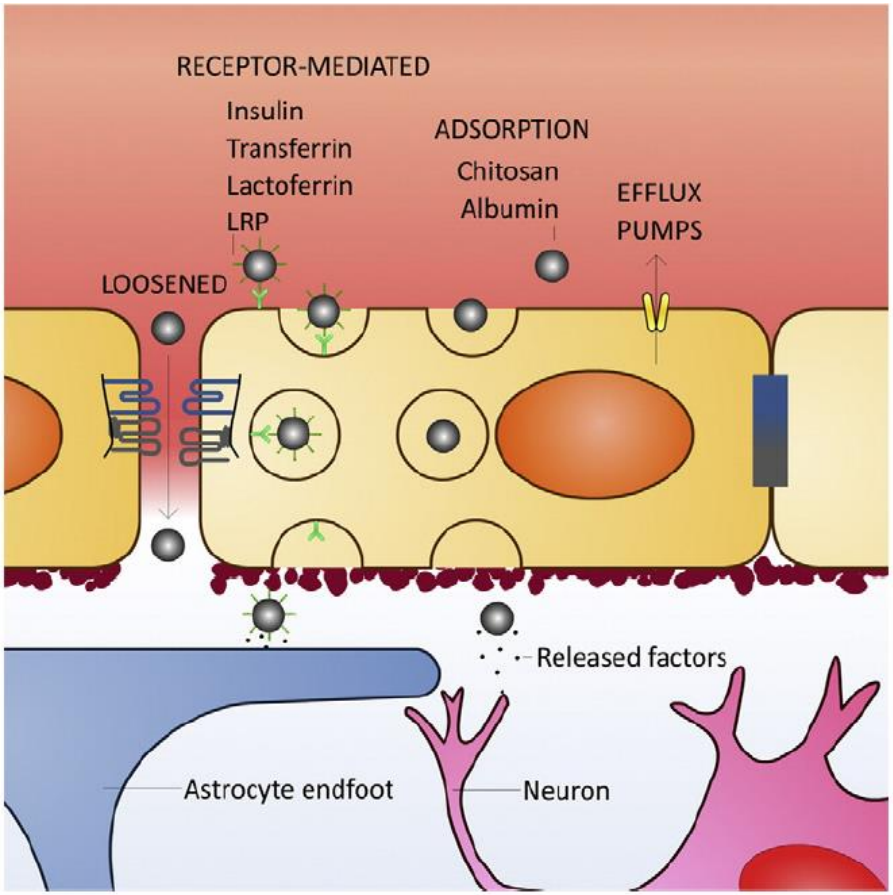


Fig. 2. Blood–brain barrier (BBB) transport mechanisms for brain delivery of nanoparticles (NPs). The BBB is highly selective and has specific transport mechanisms allowing a close control of molecules/cells that enter the brain parenchyma. Loosened tight junctions (TJs) allow the cross of NPs through 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

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.**

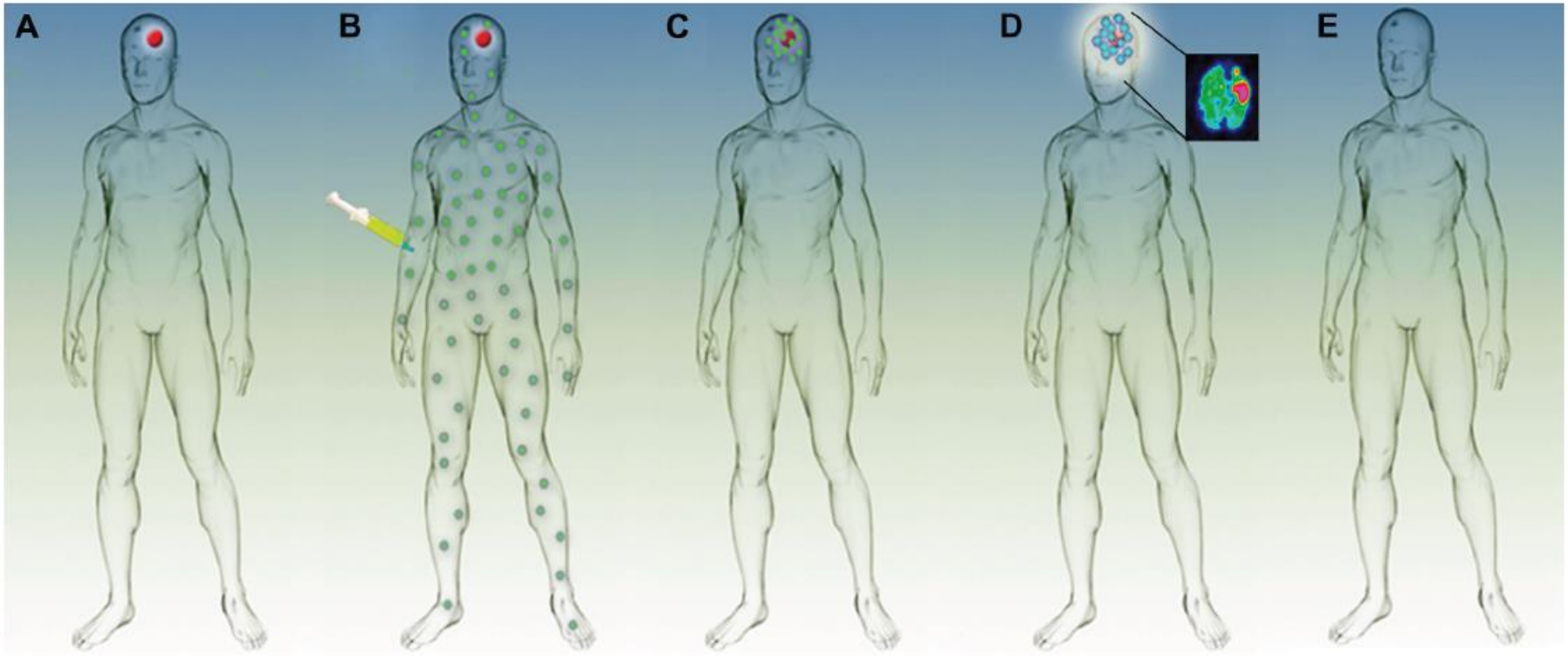


Figure 3 Concept of theranostics. (**A**) A pathological process localized to the brain. (**B**) Systemic administration of a therapeutic agent distributes the theranostic throughout the entire body. (**C**) Nanotechnology enables concentration of the agent in the targeted area. (**D**) Inclusion of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

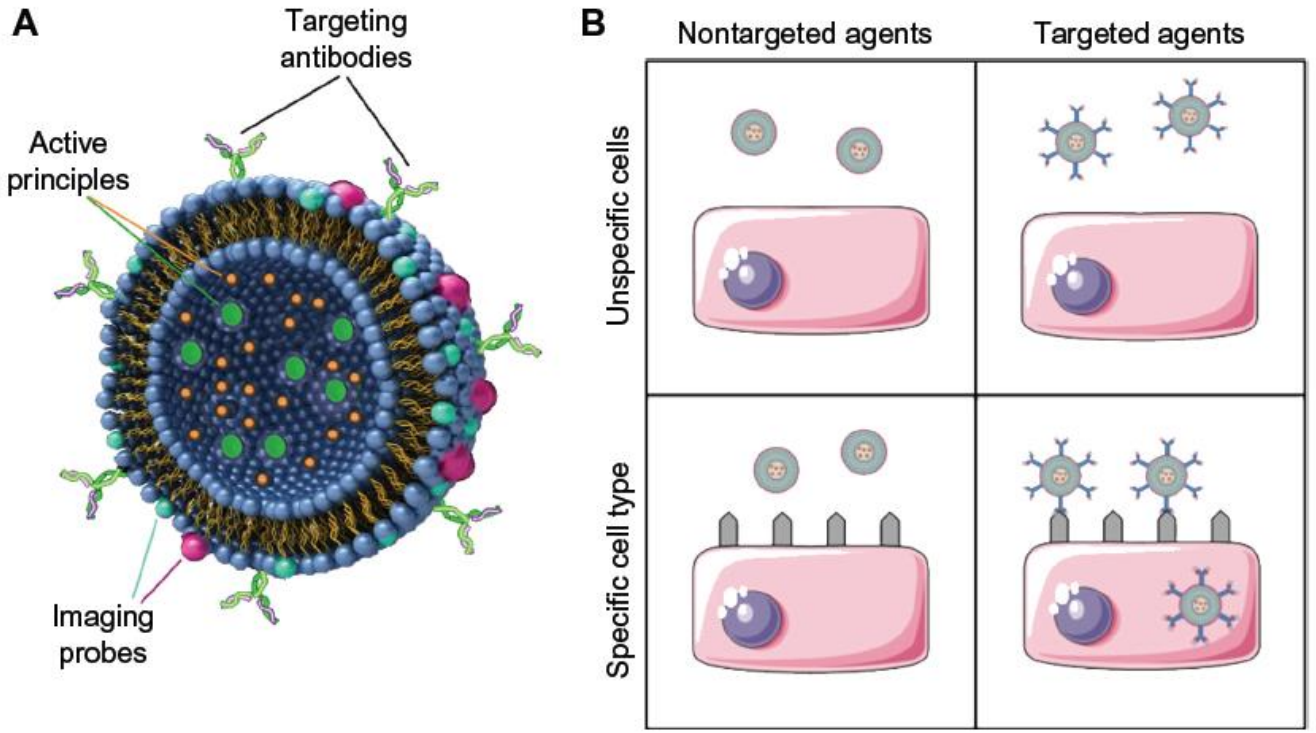


Figure 4 Theranostic agents in molecular recognition processes. (**A**) A liposomal theranostic agent includes surface antibodies that participate in the molecular recognition process with targeted cells, imaging probes (for diagnostic purposes), and active principles of treatment. (**B**) Targeting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