# CNS barriers: critical interfaces for CNS entry of paraquat

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***BBB Group, King's College London***

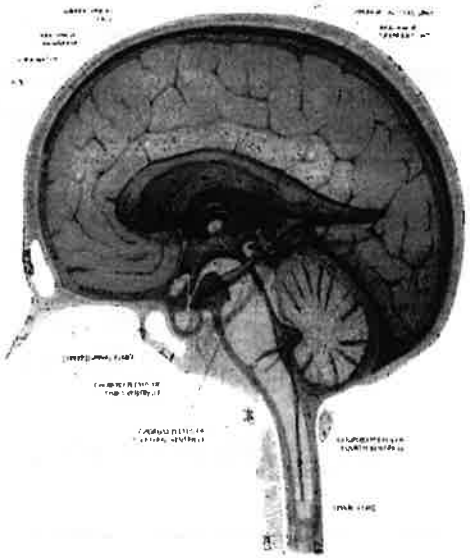

*Syngenta Discussion*
*Marlow*
*20 April 2009*

Botham, Philip
Exhibit_98
6/19/2020



## BRAIN FLUID COMPARTMENTS

- Blood
- Cerebrospinal fluid (CSF)
- Interstitial fluid (ISF = ECF)



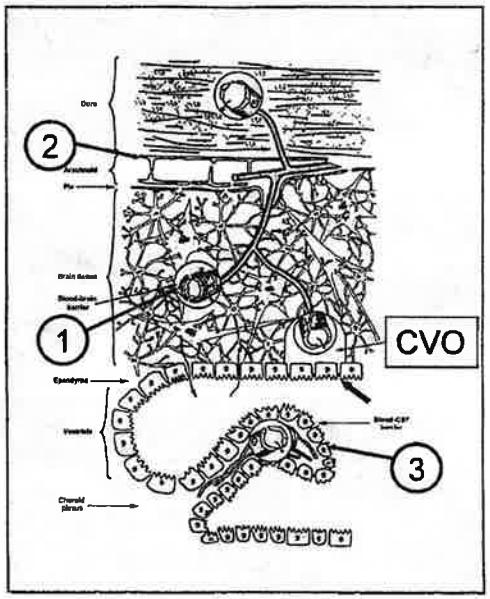
*Spector & Johnson 1989, Scientific American*



## TOPICS

- CNS barriers, BBB, BSCB
- Neurovascular unit and BBB
- CNS barrier development
- Routes across brain endothelium
- CNS drug metabolism
- Regional and species differences
- Paraquat and CNS barriers
- Future perspectives





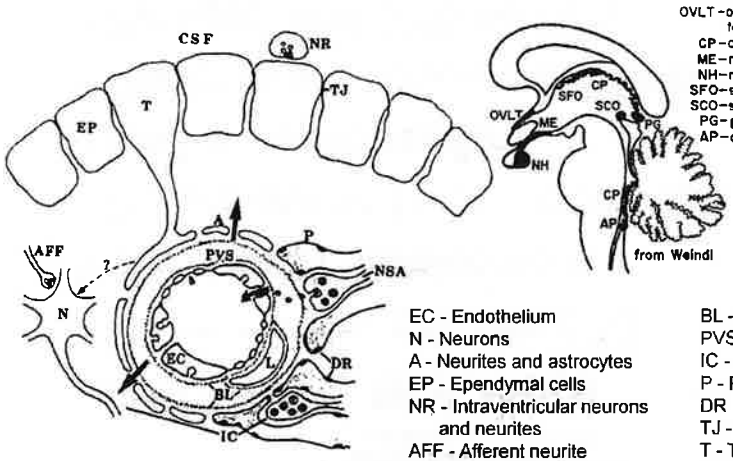

## SITES OF CNS BARRIERS

1. Brain parenchymal capillary endothelium
2. Arachnoid epithelium
3. Choroid plexus epithelium

CVO : circumventricular organs

*Segal and Zlokovic 1990, modified by A Reichel*

# CIRCUMVENTRICULAR ORGANS (CVO)





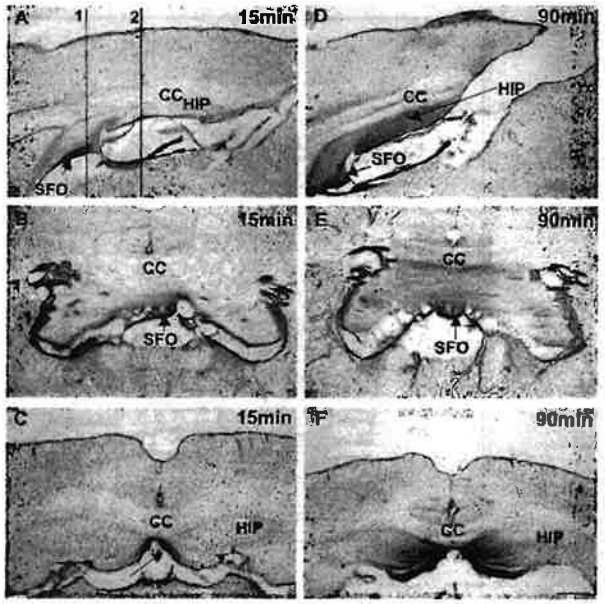


OVLT - organum vasculosum lamina terminalis
CP - choroid plexus
ME - median eminence
NH - neurohypophysis
SFO - subfornical organ
SCO - subcommissural organ
PG - pineal gland
AP - area postrema

EC - Endothelium
N - Neurons
A - Neurites and astrocytes
EP - Ependymal cells
NR - Intraventricular neurons and neurites
AFF - Afferent neurite
NSA - Neurosecretory axon
BL - Basal lamina
PVS - Perivascular space
IC - Interstitial compartment
P - Pituicyte
DR - Dynorphin receptor
TJ - Tight junction
T - Tanycyte
L - Leptomeningeal cell

*Prescott & Brightman, 1998. In: Introduction to the BBB, Ed WM Pardridge, CUP pp270-289*






HRP tracer distribution in brain after iv injection

..often in regions adjacent to CVOs

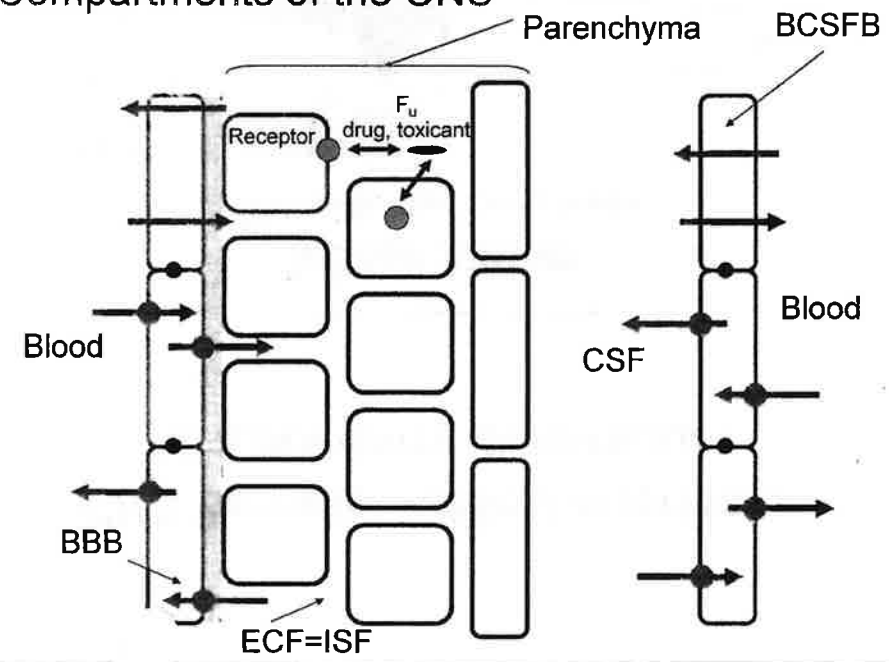
Mouse
SFO: subfornical organ (CVO)

CC: corpus callosum
HIP: hippocampus

*Ueno 2007 Curr Med Chem 14: 1199*

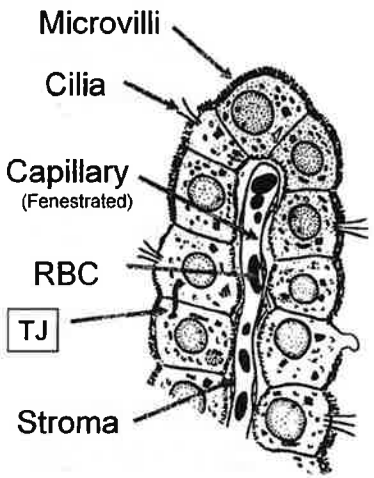


# Compartments of the CNS






# CHOROID PLEXUS EPITHELIUM

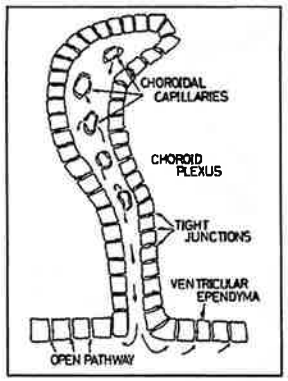



Anatomical barrier site:

- Leaky endothelium
- Moderately tight epithelium
- Microvilli and basal infoldings increase surface area
- Apical:basal specializations

Functions: CSF secretion, ionic regulation, xenobiotic exclusion, secretion of peptides

## Plasma and CSF solutes




The functional leak or extracellular pathway.
Possible sites:
- CP stroma
- pial vessels

**Typical plasma and cerebrospinal fluid concentrations for some selected solutes**

| Solute | Units | Plasma | CSF | Ratio |
|---|---|---|---|---|
| $Na^+$ | mM | 140 | 141 | ~1 |
| $K^+$ | mM | 4.6 | 2.9 | 0.63 |
| $Ca^{++}$ | mM | 5.0 | 2.5 | 0.5 |
| $Mg^{++}$ | mM | 1.7 | 2.4 | 1.4 |
| $Cl^-$ | mM | 101 | 124 | 1.23 |
| $HCO_3^-$ | mM | 23 | 21 | 0.91 |
| Osmolarity | mOsmol | 305.2 | 298.5 | ~1 |
| pH | | 7.4 | 7.3 | |
| | | | | |
| Glucose | mM | 5.0 | 3.0 | 0.6 |
| | | | | |
| **Total amino acid** | µM | 2890 | 890 | 0.31 |
| Leucine | µM | 109 | 10.1-14.9 | 0.10-0.14 |
| Arginine | µM | 80 | 14.2-21.6 | 0.18-0.27 |
| Glycine | µM | 249 | 4.7-8.5 | 0.012-0.034 |
| Alanine | µM | 330 | 23.2-32.7 | 0.07-0.1 |
| Serine | µM | 149 | 23.5-37.8 | 0.16-0.25 |
| Glutamic acid | µM | 83 | 1.79-14.7 | 0.02-0.18 |
| Taurine | µM | 78 | 5.3-6.8 | 0.07-0.09 |
| | | | | |
| **Total Protein** | mg/ml | 70 | 0.433 | 0.006 |
| **Albumin** | mg/ml | 42 | 0.192 | 0.005 |
| Immunoglobulin G (IgG) | mg/ml | 9.87 | 0.012 | 0.001 |
| Transferrin | mg/ml | 2.6 | 0.014 | 0.005 |
| Plasminogen | mg/ml | 0.7 | 0.000025 | 0.00004 |
| Fibrinogen | mg/ml | 325 | 0.00275 | 0.000008 |
| α 2-macroglobulin | mg/ml | 3 | 0.0046 | 0.0015 |
| Cystatin C | mg/ml | 0.001 | 0.004 | 4.0 |

Compiled from various sources, values mostly human.

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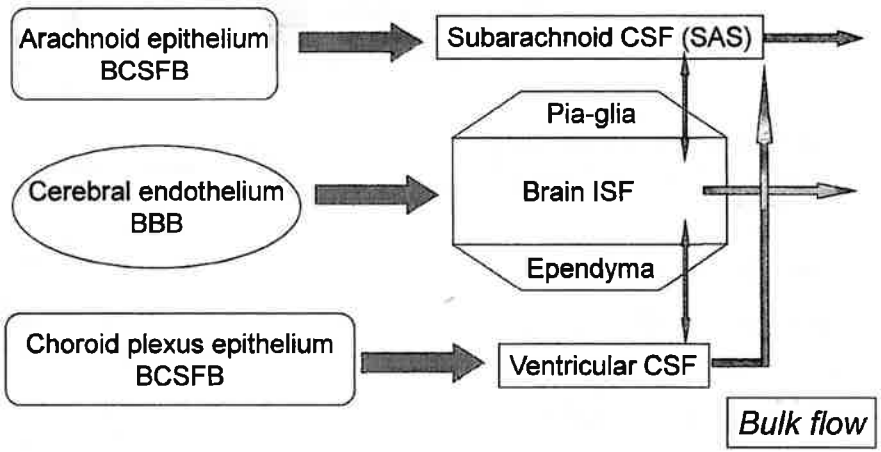
## Barriers and compartments

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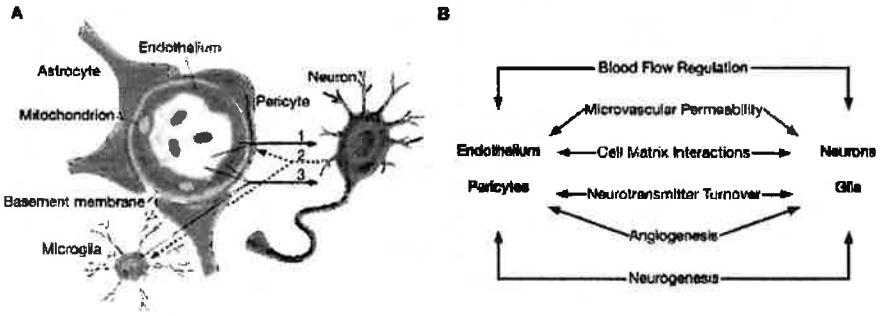
## Neurovascular Unit (NVU): Cell associations




**Key barrier features:** Tight junctions, transporters, enzymes
- Cooperation between cell types of NVU

*Zlokovic 2008 Neuron 57:178-201*

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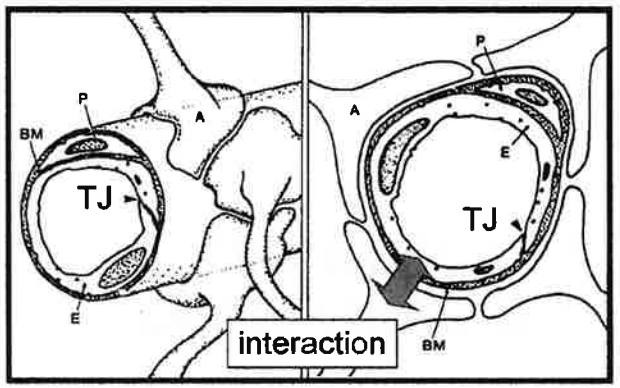
## Blood-Brain Barrier Structure




A = astrocyte E = endothelial cell
P = pericyte BM = basement membrane

*+ other cell types: microglia, macrophages, leukocytes*

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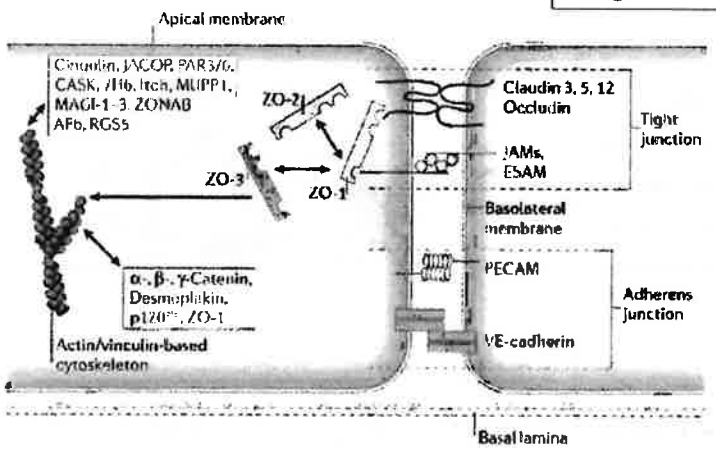
# Molecular structure of BBB tight junctions

*Regulatory sites*




Tight junction

Adherens junction

*Abbott et al 2006 Nat Rev Neurosci 7:41*

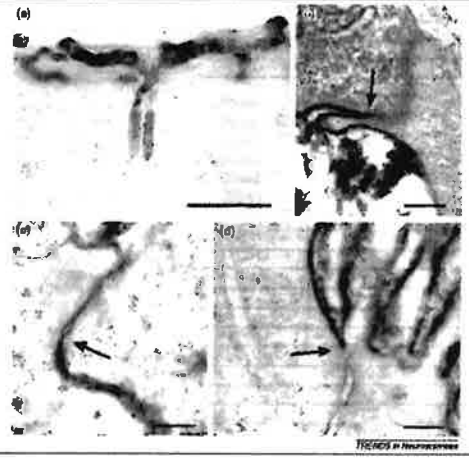


# DEVELOPMENT OF CNS BARRIERS



# Development of BBB – at the time of vessel ingrowth

Tight junctions (TJ)



Newborn opossum

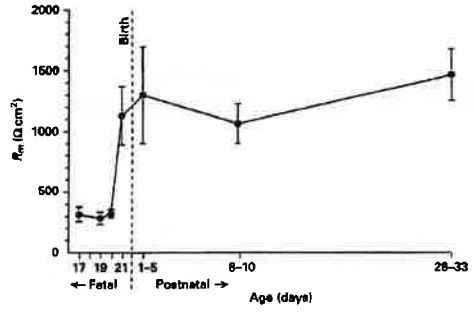
*Wnt* signalling : angiogenesis + barrier development

3 kDa biotin-dextran amine 30 min IP

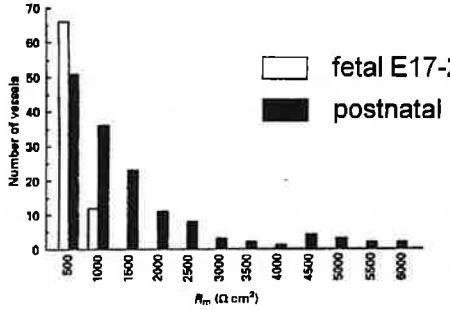
*Saunders et al. 2008. TINS 31:279*



# Tightening of BBB in rat pial vessels at birth: tight junctions




TEER: transendothelial electrical resistance $R_m$




fetal E17-20

postnatal E21 and older

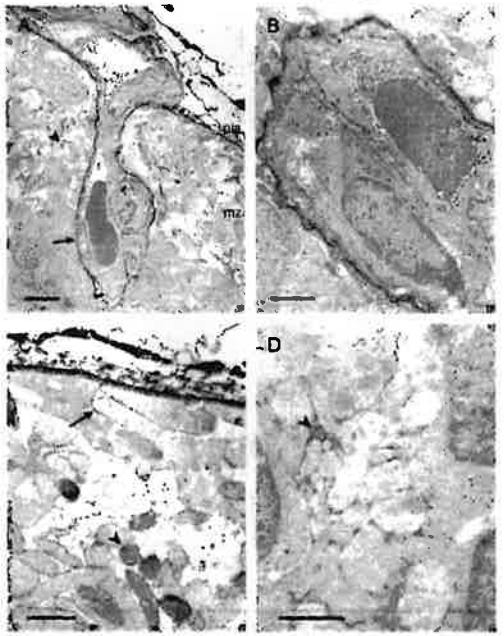
*Butt et al. 1990 J Physiol 429:47*

## Evidence for well-developed perinatal BBB

- BBB to proteins and macromolecules present in fetal and neonatal brain (1,2,3,4,5,6,7)
- TJs develop between E11 and E17 in mouse, are barrier to proteins (6,7)
- Efflux transporters mdr1a,mdr1b and mdr2 appear between E13 and E18 in mouse (8)
- High electrical resistance present at E21 in rat (9)
- Permeability to $^{86}Rb$ declines from 42.5 to 12.2 $uL.g^{-1}.min^{-1}$ from E21 to P2 (3)
- $K_{in}$ for mannitol is between 0.19 and 0.22ul.$uL.g^{-1}.min^{-1}$ at one week in rat (same as adult) (7)
- **Occludin and claudin-5 expressed in brain capillaries of 14 week human fetus with same cellular distribution as adult (10)**
- **Work with stillborns and perinatal deaths showed BBB to trypan blue present in human from 12 weeks gestation, comparable to adult (11)**

(1) Ballabh et al. (2001); (2) Olsson et al. 1968; (3) Tauc et al. (1984; (4) Saunders 1992; (5) Moos and Möllgård 1993; (6) Keep et al. 1995; (7) Preston et al. 1995; (8) Scheingold et al. 2001; (9) Butt et al. 1990; (10) Virgintino et al. 2004; (11) Gröntoft 1964






Perinatal CNS barriers – BBB effective, but some leak from choroid plexus or brain surface (pial) vessels

Biotin ethylenediamine tracer (BED, 286Da) 25 min after i.p. injection

Opossum P5 (~=E19 rat)

*Ek et al. 2006*
*J Comp Neurol 496:13*



## FUNCTIONS OF BBB & BSCB

* 1. Control molecular traffic, keep out toxins (precise connectivity: low cell death)

* 2. Ion homeostasis - optimal neural signalling

3. Low protein environment, limit proliferation

* 4. Separate CNS:PNS neurotransmitter pools; allow non-synaptic signalling

5. Immune surveillance with minimal inflammation, cell damage

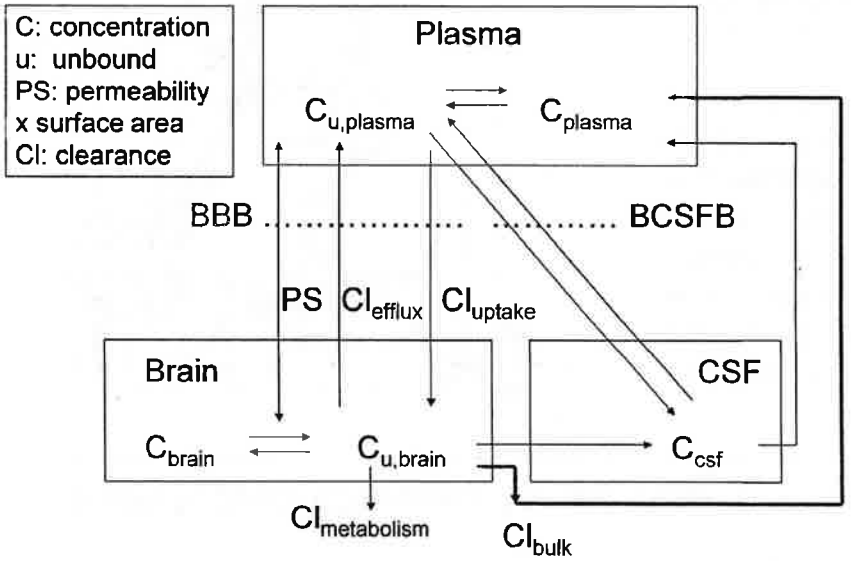
Selective advantage

* *Functions needing tightest endothelium*

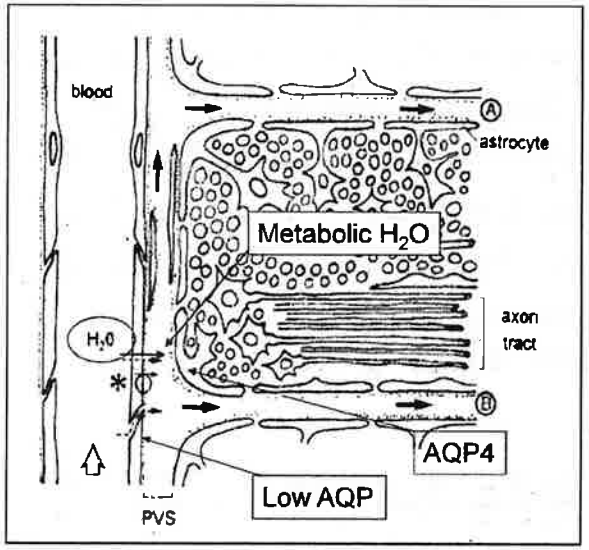


## Compartmental analysis: CNS Drug distribution

C: concentration
u: unbound
PS: permeability x surface area
Cl: clearance



# Perivascular space – flow of interstitial fluid (ISF)




*brain endothelial Na,K,ATPase

PVS, perivascuar space

Source of $H_2O$ :
- Plasma
- Metabolism

+ some recycled CSF

*Abbott 2004 Neurochem Int 45:545*

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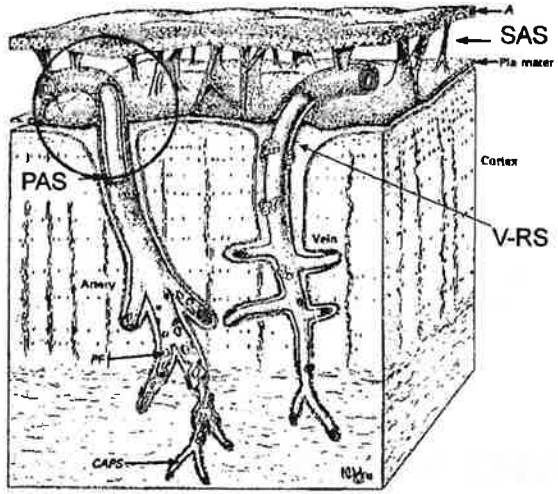
# Perivascular channels for ISF flow: human




Relation of subarachnoid space (SAS), Virchow-Robin space (V-RS), and periarterial space (PAS) in human brain

A, arachnoid
PF, perforations
CAPS, capillaries

*Zhang et al 1990 J Anat 170:111*

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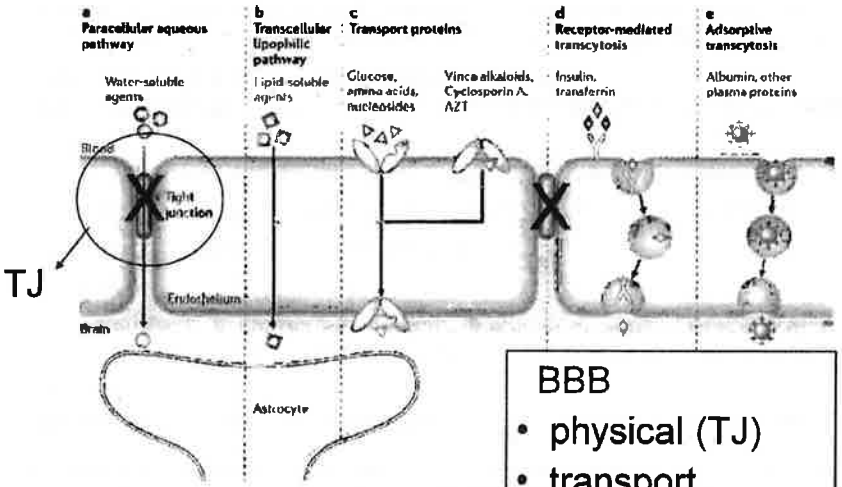
# Pathways across the blood-brain barrier




BBB
- physical (TJ)
- transport
- enzymatic

*Abbott et al [illegible] Nat Rev Neurosci 7:41*

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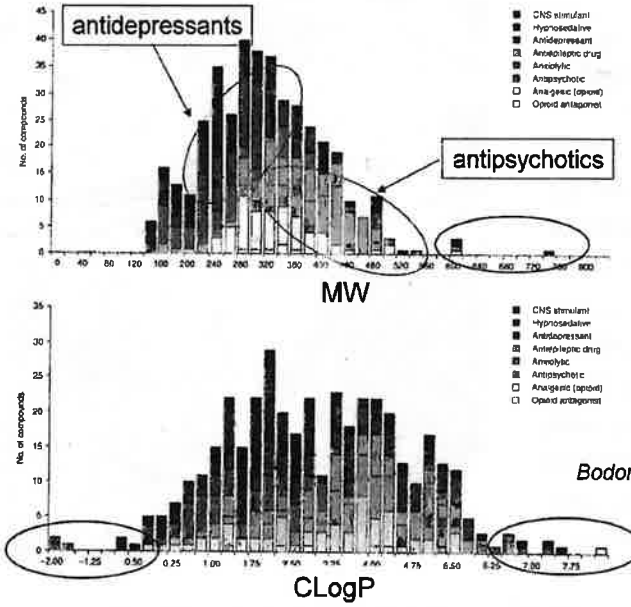
# CNS drugs : Molecular weight and lipophilicity




Learning from successful drugs : Darwinian approach

*Bodor: Am J Drug Del 1: 2003*

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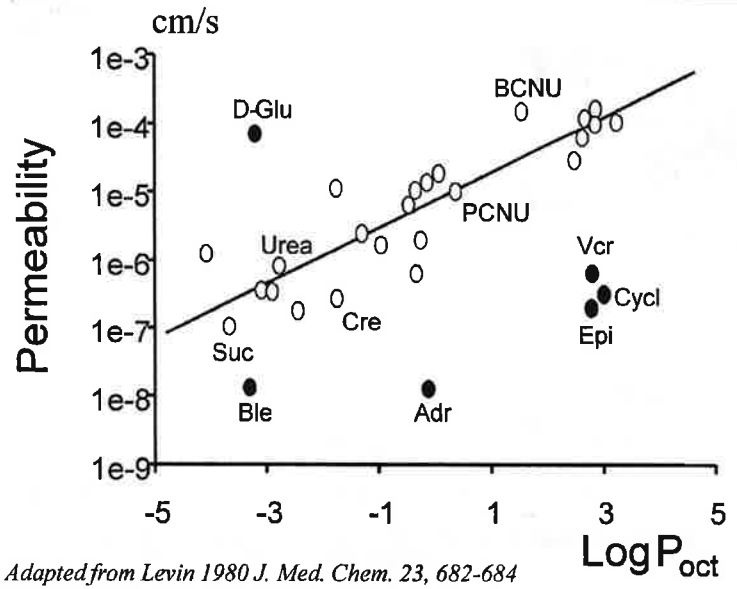
# BBB permeability and lipid solubility




*Adapted from Levin 1980 J. Med. Chem. 23, 682-684*



# PC properties benefiting CNS penetration

**Common properties of CNS drugs**

| | |
|---|---|
| • small | < 400-500 |
| • lipophilic | log $D_{7.4} \geq 2$ |
| • neutral | uncharged at pH7.4 |
| • slim (x-sectional area) | $A_D < 80A^2$ |

**Deteriorating properties**

| | |
|---|---|
| • Polarity | PSA > 60 $A^2$ |
| • Lewis base strength | |
| • Hydrogen-bonding | esp. HBA, $\beta^H_2$ |
| • Branching | |
| • # of rotatable bonds | |

**HBA > AR ~ HBD ~ κ > mw ~ logP > RotB**

HBA - hydrogen bond acceptors
AR - aromatic density
HBD - hydrogen bond donors
κ - branching index
mw - size
logP - lipophilicity
RotB - No of rotatable bonds (flexibility)

**Improving properties**

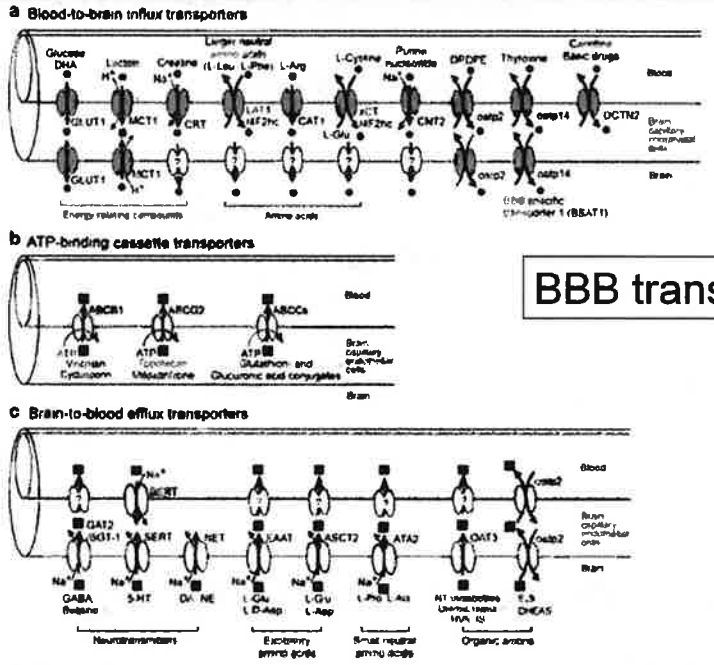
- optimimum lipophilicity *and* relatively high membrane diffusivity
- presence of polarizable surface electrons,
  e.g. conjugated or aromatic substructures or larger halogens
- aromatic density

Pardridge (1998)
Fischer et al. (1998)
Ajay et al. (1999)
Norinder et al. (1998)
Lombardo et al. (1996)
Clark (1999)



# BBB TRANSPORT Specific carriers

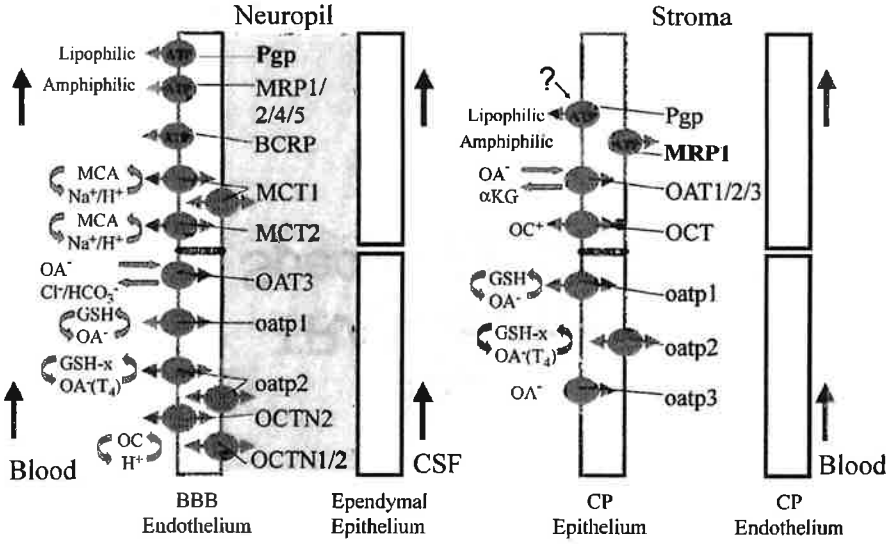





BBB transporters

*Ohtsuki 2007*
*Pharm Res*
*24:1745-58*

## Efflux transporters in the BBB and B-CSF-B




*Begley 2004 In Sharma & Westman volume (Spinal Cord)*

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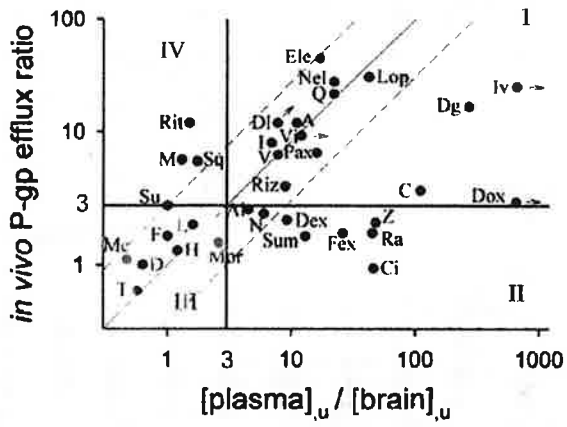
## Measurements to segregate drugs




Quadrants I-IV

Quadrant III
Enter brain, not Pgp substrate

As yet unidentified transporters?

*Dobson & Kell 2008: Nat Rev Drug Disc 7:205*

*Kalvass et al. 2007 Drug Metab Dispos 35:660*

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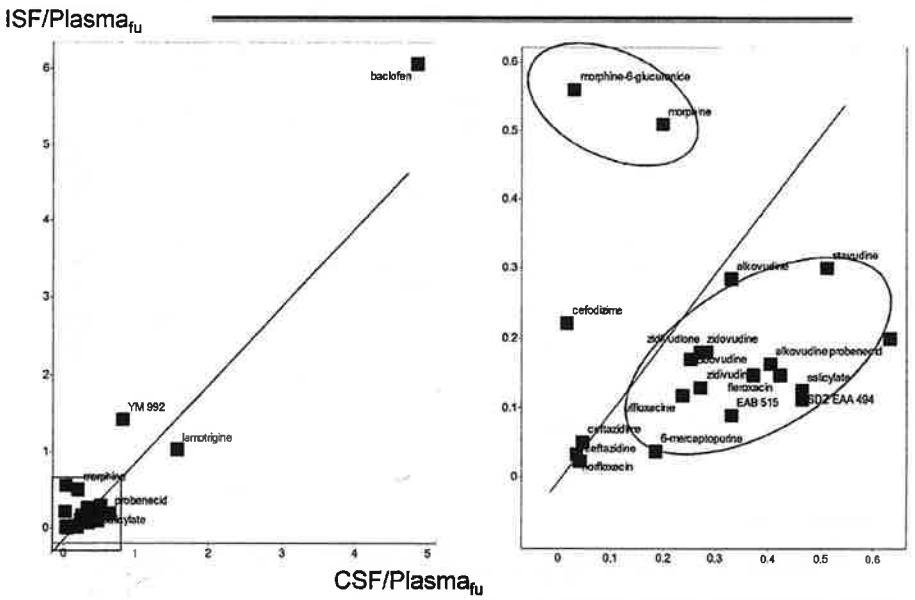
## Comparison of ISF(microdialysis) with CSF




*Data : Shen et al. 2004 Adv Drug Del Rev 56: 1825*

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# Drug metabolism in BBB and choroid plexus

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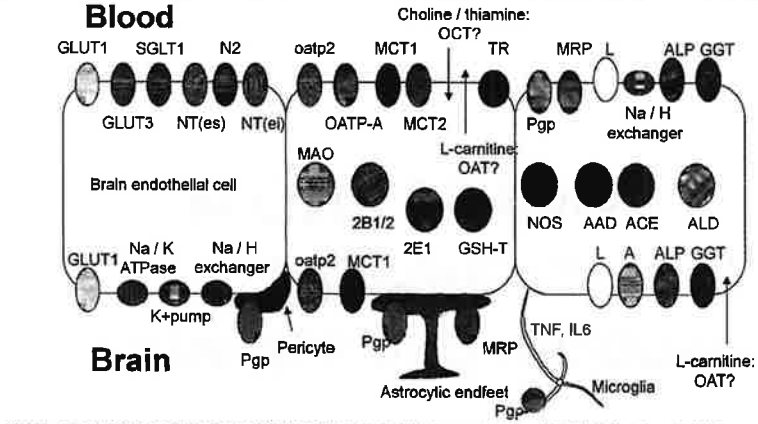
# Transporters and enzymes of the BBB




Transporters: GLUT1-glucose transporter; SGLT1-sodium-dependant glucose transporter; N2, NT(es), NT(ei)-nucleoside transporters; Oatp2-rat organic anion transporter polypeptide 2; OATP-A-human organic anion transporting polypeptide-A; MCT1, 2-monocarboxylic acid transporters; TR-receptors for transferrin, insulin, leptin, lectins IGF's; Pgp-P-glycoprotein (MDR1); MRP-multidrug resistance associated protein; L-amino acids L-system (LAAT); A-amino acids A-system; $Na^+/H^+$ exchanger -Sodium-proton exchanger; ALP-alkaline phosphatase; GGT-γ-glutamyl-transpeptidase; OCT-organic cation trasnporter; OAT-organic anion transporter; $Na^+/K^+$-ATPase-sodium-potassium ATPase; $K^+$-pump - potassium pump. Agents : TNFα, IL-6 - cytokines.
Enzymes : MAO-monoamine oxidase; 2B1/2-CYP enzymes: 2E1-CYP enzyme; GSH-T-GSH-S-transferase; NOS-nitric oxide synthase; AAD-aromatic acid decarboxylase; ACE-angiotensin converting enzyme; ALD-aldehyde dehydrogenase

*From: Mertsch & Maas (2002) Curr. Med. Chem. - Central Nervous System Agents 2, 187*



# Metabolic conversion in the CNS

## Phase 1 (Functionalisation)

MAO [1] : flavone dependant mono-oxygenases [2] : cytochromes P450 [1, 2, 3]
NADPH-cytochrome P450 reductase [1, 3] : epoxide hydrolases [1, 2, 3]

## Phase 2 (Conjugation)

UDP-glucuronyltranferases [1,2,3] : glutathione S-transferases [1,2,3]
sulphotransferases [2,3] : catechol O-methyltransferases [1,2,3]

## Phase 3 (Export)

ATP-dependant transport of drugs and metabolites catalysed by Pgp [1,2] and MRP [1,2,3] and also by OAT, OCT and oatp [1,2]

1 BBB endothelium
2 Parenchyma (neurones and glia)
3 CP epithelium

*Adapted from Minn 2000*



# Drug metabolizing enzyme activity in brain, liver

Ratio of drug metabolism activity compared to brain cortex homogenate

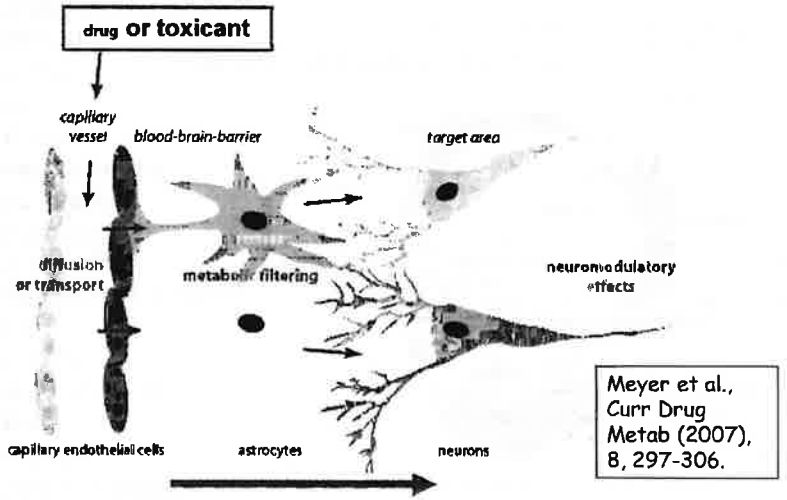
| Enzyme | Isoform | Brain Microvessels | Choroid plexus | Olfactory Bulb | Liver |
|---|---|---|---|---|---|
| Monoamine Oxidase | B | 8.6 (rat) 0.2 (human) | low | | 2.9 |
| Cytochrome P450 | 1A and 2B | 3.9 | 20 | 4.2 | >100 |
| NADPH-450 reductase | | 1.3 | 2.4 | | <0.1 |
| UGT | 1A6 | 1.5 | ~65 | | ~50 |
| Epoxide Hydrolases | Micro-somal | 5.4 | 35 | 0.9 | 34 |
| Glutathione-S-Transferase | TSO | 1.8 | 2.1 | 1.8 | 6.2 |

UGT – Uridine diphosphate glucuronosyltransferase

*Data from: Minn (2000, Pers.comm.)*



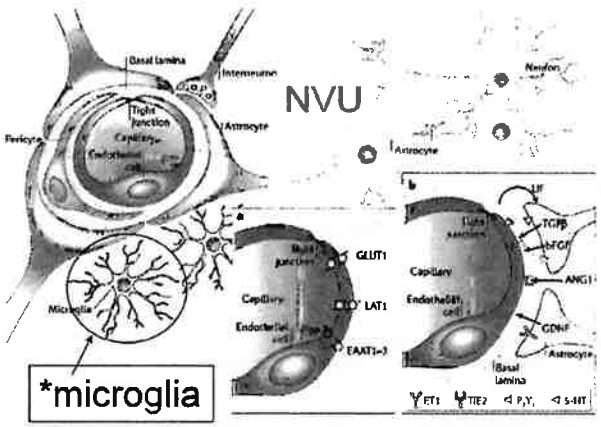
# Summary: BBB enzymes as metabolic filter




Meyer et al., Curr Drug Metab (2007), 8, 297-306.

Downstream signalling: (dis)integrated brain network function?

# Glial maintenance of BBB phenotype




*microglia

➢Tox context

*Abbott et al 2006 Nature Rev Neurosci. 7:41*

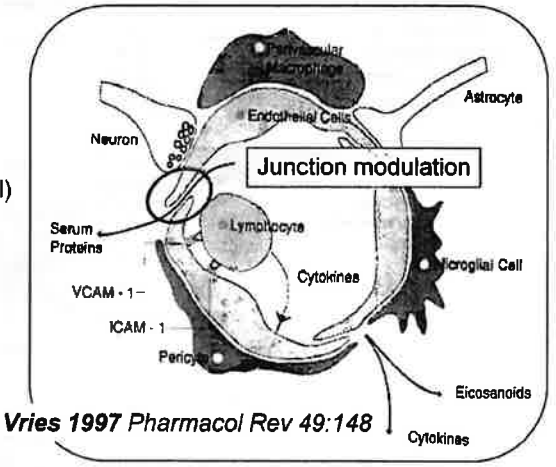
*Induction:*
Junctions
Transporters
Enzymes
Receptors

Receptors:
- Signalling within Neurovascular Unit (NVU)
- Regulating **permeability** and transport



# The BBB is involved in many CNS pathologies

- Traumatic brain injury
- Hypertension
- Diabetes
- Ischaemia, hypoxia
- Inflammation
- Migraine
- Pain (inflammatory, surgical)
- Multiple sclerosis
- HIV-encephalitis
- Cerebral malaria
- Bacterial meningitis
- Brain tumours
- Epilepsy
- Depression
- Schizophrenia
- Age-related dysfunctions
- Alzheimer's disease
- Parkinson's disease
- **Environmental toxins?**




***de Vries 1997** Pharmacol Rev 49:148*

Characteristics of brain endothelium

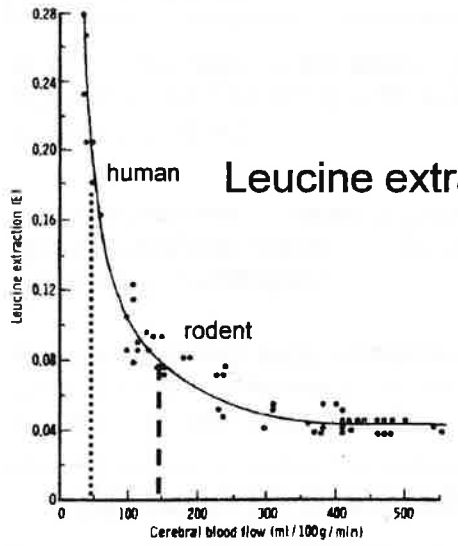
***Inflammation, ROS implicated in many***



# Species and regional differences in CNS barriers?



# Effect of species differences in blood flow




CBF
Human~0.5ml/g/min
Rat, mouse ~1.5ml/g/min

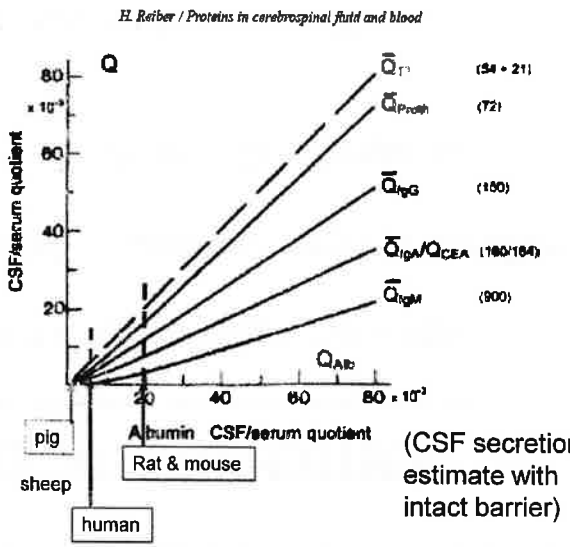
Leucine extraction

Slower flow, greater extraction during CNS transit

*Sage 1981 J Neurochem 36:1731*

# Species differences

Using CSF to estimate brain concentration




(CSF secretion estimate with intact barrier)



# Species differences

Cationic amino acid transporter, no CAT2 & 3 in human??

OAT1 OAT 3 (human CP)
Oat3 (rat BBB)

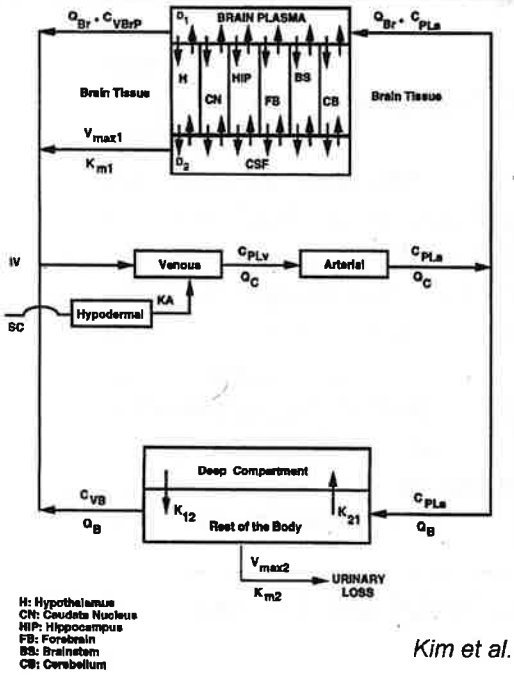
OCTN1 OCTN2 (human)
Octn1 octn2, octn3 (rodent)

MDR1 (human)
mdr1 mdr2 (rodent)

MRP1 4 5 6 (low3) (human)
mrp1 4 5 (low 2 & 3) (rodent)

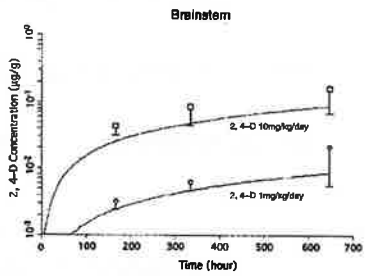
Löscher and Potschka. Blood-Brain Barrier Active Efflux Transporters: ATP-Binding Cassette Gene Family. NeuroRx. 2005 January; 2(1): 86–98
Kusuhara H, Sugiyama Y. Active efflux across the blood-brain barrier: role of the solute carrier family. NeuroRx. 2005 Jan;2(1):73-85.






# Physiologically-based pharmacokinetic (PBPK) modelling

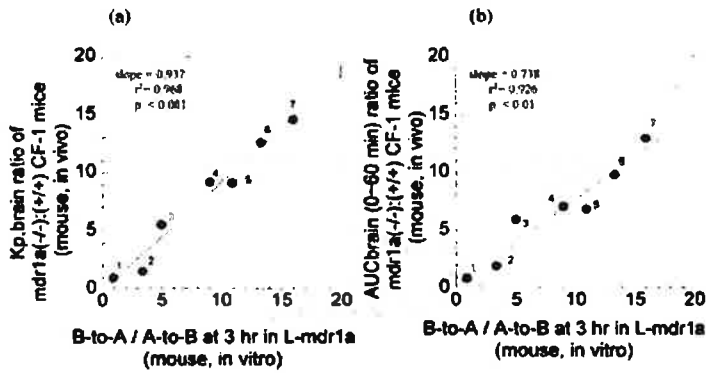
Low-dose, long-term exposures of organic acid toxicant in brain. 2,4-dichlorophenoxyacetic acid (2,4-D)




*Kim et al. 2001 Env Toxicol Pharmacol 9:153*



# Good correspondence mouse *in vivo* and *in vitro* Pgp function




➢Basis for cross-species PBPK modeling

*Yamazaki et al. 2001 JPET 296:723*

## Species differences: summary

1. Vascular: territories, CBF
2. CSF: secretion rate, leakiness of CP/subarachnoid vessels, scaling for size
3. Plasma binding of drugs – will affect 'free unbound' concentration, PK
4. BBB passive permeability – well conserved
5. Regional differences – may be some variation
6. 'Housekeeping' transport systems – similar if not identical (may affect rank order of uptake, efflux)
7. Metabolising enzymes – may differ in isoforms, activity
8. PBPK helpful for modelling species and regional differences



# PARAQUAT AND CNS BARRIERS

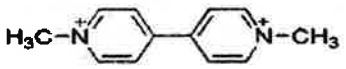


## PARAQUAT PROPERTIES

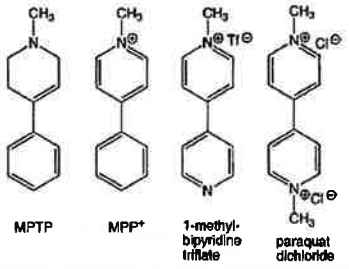
- 1,1-Dimethyl-4,4'-bipyridinium dichloride
- Para-substituted quaternary bipyridyl di-cation
- Non-selective herbicide. ICI - Syngenta (Gramoxone)
- One of most toxic herbicides (>40mg/kg)



## PARAQUAT (PQ) CHEMISTRY

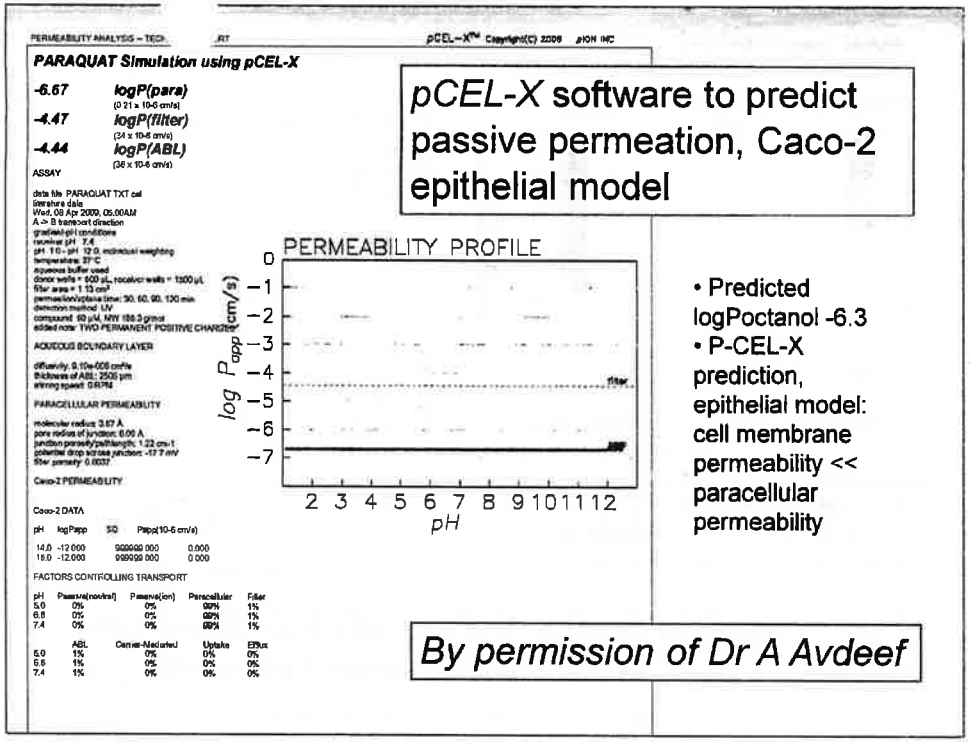



Similarities of PQ structure with $MPP^+$, neurotoxic metabolite of MPTP




Does paraquat cause a Parkinson-like syndrome?

*Bartlett et al. 2009 Brain [illegible] 1259: 74*




*pCEL-X* software to predict passive permeation, Caco-2 epithelial model

- Predicted logPoctanol -6.3
- P-CEL-X prediction, epithelial model: cell membrane permeability << paracellular permeability

*By permission of Dr A Avdeef*

SYNG-PQ-00471742

# PARAQUAT PROPERTIES

- Permanently dicharged highly soluble cation
- Predicted logPoctanol -6.3
- *P-CEL-X* prediction – epithelial/BBB model – cell membrane permeability << paracellular permeability.
- PQ oral bioavailability low -~10%.
- Not subject to metabolism in body, rapidly excreted in urine
- If enters brain appreciably, expect this to by via transporter(s) or leak into CSF
- What about efflux?

SYNG-PQ-00471743

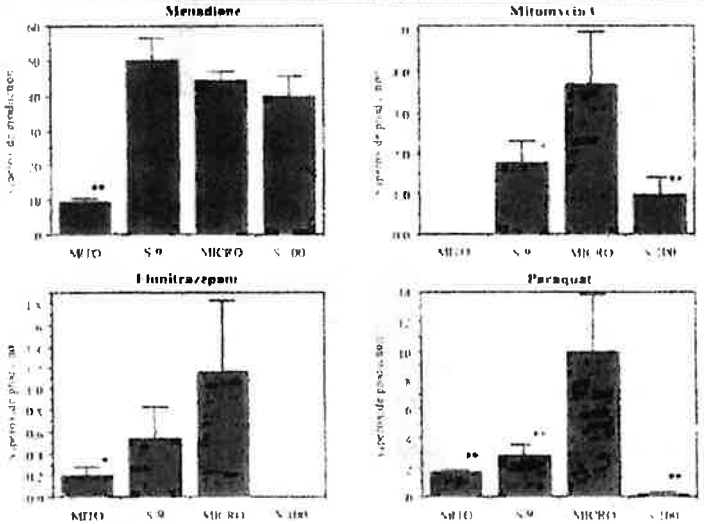
# Does Paraquat (PQ) get into the CNS ?

## *Early work*

- Smith et al. 1990 Lung toxicity PQ related to polyamine transporter (PAT), uptake into alveolar Type II cells
- Shin et al. 1985. Detectable polyamine transport rat BBB: BUI putrescine, spermidine, spermine 5.3-6.1% (baseline ~2%) 34% of this is carrier-mediated. But saturated at normal blood polyamine levels.
- Corasaniti et al. 1991. HPLC. PQ able to cross rat BBB, dose-related, entry higher in young (2 wks) and old (12,24 month) *cf.* 3 month.

SYNG-PQ-00471744

# Superoxide production by brain and microvessels

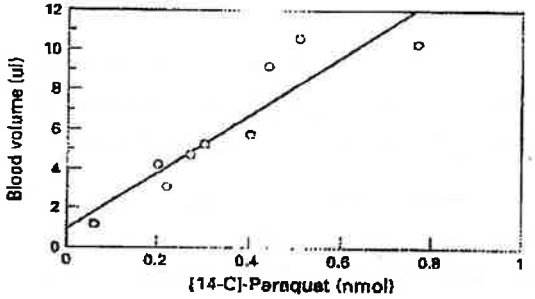



PQ on brain microvessels: $O_2^{\cdot-}$ production stimulated by NADH nucleotide cofactor

*Ghersi-Egea et al. 1991 Toxicol Appl Pharmacol 110:107*

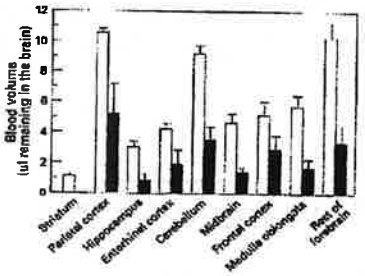
SYNG-PQ-00471745

A.




## Paraquat distribution in brain related to regional blood volume (inulin space, ul)

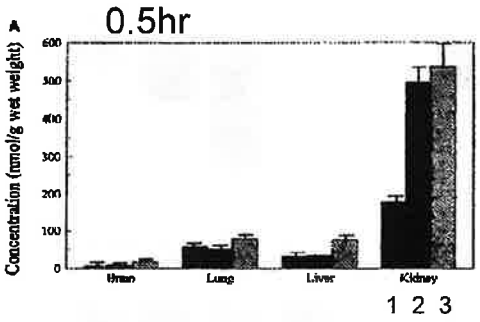
B. Inulin distribution (blood volume): black, perfused

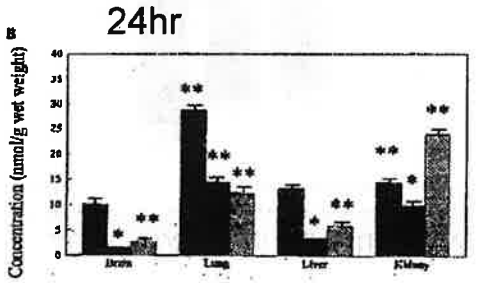



PQ remaining in brain at 24 hr mainly associated with vascular elements ?

Naylor et al. 1995 Hum Exp Toxicol 14:587

SYNG-PQ-00471748




## Age-dependent entry [$^{14}$C] paraquat to brain

20mg/kg s.c.(median lethal dose), rat

1: neonate (10 day)
2: adult (3 month)
3: elderly (24 month)

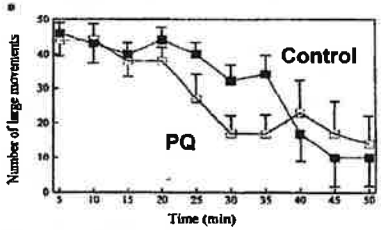
Initially in more permeable BBB regions, then in most of brain by 24 hr, especially in neonate

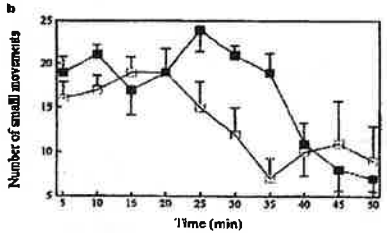
*Widdowson et al 1996a Hum Exp Toxicol 15:231*

SYNG-PQ-00471747

## PD? Multiple paraquat oral dosing in rat caused no detectable change in behaviour or neurochemistry

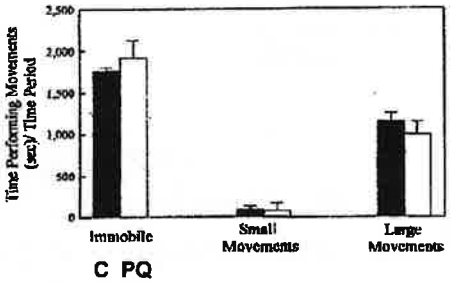
Locomotor activity




- 5mg/kg/day,14 days: conc in brain reached 10x higher than single dose.
- No change in behaviour, nigro-striatal neuronal cell death
- Neurochemistry, higher DA striatum

Time performing movements




Conclude: PQ not like MPP+

*Widdowson et al. 1996b Hum Exp Toxicol 15:583*

SYNG-PQ-00471748

## Does Paraquat (PQ) get into the CNS ?

- Ghersi-Egea et al. 1991. PQ + other xenobiotics cause generation of ROS in brain cells and endothelium, via NADPH-cytochrome P450 reductase. Potential toxicity on BBB, neurons, glia, microglia etc.
- Naylor et al. 1995. BBB impedes $^{14}$C PQ entry rat brain (c1/3 values HPLC method). Max entry (0.05% dose) within 1 hr, 13% residue at 24hr, associated with vascular elements? Especially pineal, lining of cerebral ventricles, olfactory bulb, hypothalamus, area postrema ('outside BBB or no BBB'). No evidence of neuronal damage.
- Widdowson et al, 1996a. $^{14}$C PQ entry rat brain, greater and more sustained in 10 day old cf. 3 and 18 month old. Most entry in regions with more permeable BBB (dorsal hypothal, area postrema, an olfactory bulb), though more diffusely by 24hr.
- Widdowson et al. 1996b. PQ causes no detectable change in motor behaviour, neuronal cell death; increase in striatal dopamine concentrations due to PQ-induced stress?
- * Poduslo et al. 1996, 1998. Polyamine modification of proteins including growth factors improves CNS access, via polyamine transporter BBB?

SYNG-PQ-00471749

## Does Paraquat (PQ) get into the CNS ?

- *Chen et al. 2007 Paraquat transported by renal hOCT2 (basolateral), hMATE1 (apical) (HEK-293 study). BBB expresses luminal OCT – would favour brain penetration. No MATE reported.
- Purisai et al. 2007. Microglial activation (LPS) priming – exacerbates PQ neurotoxicity against dopaminergic neurons.
- Gradinaru et al 2008. PQ upregulates choroid plexus UDP-glucuronosyltransferase UGT1A6.
- Bartlett et al. 2009. Rhesus macaque PET $^{11}$C-PQ bolus injection 0.1-0.4ug/kg iv. Some in CSF, little detectable in brain.
- Prasad et al. 2009 Toxicokinetics and toxicodynamics of PQ accumulation in brain. Plateau after multiple doses.



## PARAQUAT PROPERTIES

- 1,1-Dimethyl-4,4'-bipyridinium dichloride
- Para-substituted quaternary bipyridyl di-cation
- Non-selective herbicide. ICI - Syngenta (Gramoxone)
- One of most toxic herbicides (>40mg/kg)

- Permanently dicharged highly soluble cation
- Predicted logPoctanol -6.3
- P-CEL-X prediction – epithelial/BBB model – cell membrane permeability << paracellular permeability.
- PQ oral bioavailability low -~10%.
- Not subject to metabolism in body, rapidly excreted in urine

- If enters brain appreciably, expect this to by via transporter(s).
- Evidence for significant entry in rodents: rat, mouse. Primates?
- Candidates: L1, polyamine transporter, OCT? Problem – efflux transport? No metabolism in brain.



## CONCLUSIONS

- PQ enters rodent brain rapidly, cleared slowly
- BBB uptake transporters L1 (+OCT? PAT?) + leak into CSF implicated
- No metabolic breakdown in CNS
- No clear efflux transporter from CNS
- Very slow clearance suggests intracellular sequestering in CNS – potential for long-term toxicity
- Similarities between species in CNS barrier organisation and uptake transporters mean it is likely that similar toxicokinetics and dynamics will apply in humans. Low dose PQ bolus PET study in primates may not be sufficiently sensitive to show CNS distribution
- CNS barriers more leaky/vulnerable in neonate, possibly also in old age
- Entry PQ into brain can be increased by other toxicants, e.g. DTCs
- Neuronal damage by PQ exacerbated by LPS/infection – priming role of microglial activation
- Need more studies of mechanisms for CNS uptake (BBB, CP) and efflux/clearance of PQ, in combination with other potential compounding factors; PBPK

➢ *Combination of carrier-mediated uptake with very low clearance and known toxicity: problematic profile*



## Acknowledgements

**The BBB Group KCL**

*BBB physiology, development, toxicology, pathology*

- David Begley, Paul Fraser, Jane Preston, Mike Bradbury
- Arthur Butt, Nacho Romero, Andreas Reichel, Alex Easton, Rob Rist, Diana Dolman, Adjanie Patabendige
- Sarah Fredriksson, Siti Yusof

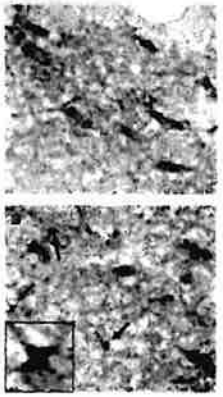
*Collaborators*

- KCL - Ken Smith, Catherine Rice-Evans, Marcus Rattray
- St Thomas' Hospital - Graham Hughes
- Budapest - Ester Domotor, Ildiko Sipos, Vera Adam-Vizi
- Cleveland - Damir Janigro
- Boston - Alex Avdeef (pION)

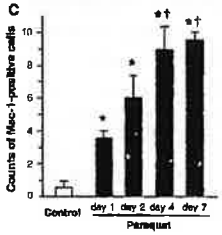
Supported by:

- The Wellcome Trust, BBB Consortium with Industry, UK MS Society and UCB

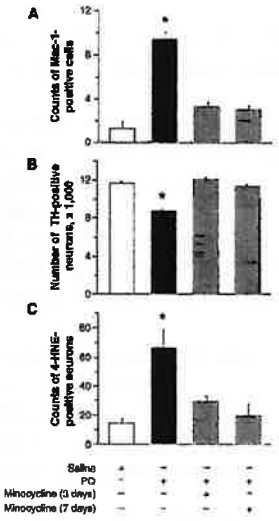
## Microglial activation: priming event leading to PQ-induced dopaminergic cell degeneration



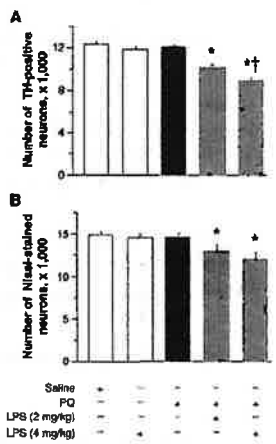





1. Activated microglia




2. Protection by minocycline




3. LPS + PQ: neurotox

*Purisai et al. 2007 Neurobiol Dis 25:392*



## Drug and xenobiotic-induced upregulation of choroid plexus metabolic enzymes

**Table 1** 1-Naphthol UGT1A6 and NADPH-cytochrome P450 reductase enzymatic activities in rat choroid plexus isolated after an in vivo treatment with either: 3-methylcholanthrene, phenobarbital, dexamethasone, cyclosporine or paraquat

| Treatment | 1-Naphthol UGT1A6 specific activity (nmol/h per mg protein) | NADPH-cytochrome P450 reductase specific activity (nmol cytochrome *c* reduced/min per mg protein) |
|---|---|---|
| Control | 115.32 ± 24.17 | 24.55 ± 8.27 |
| 3-Methylcholanthrene (50 mg/kg i.p. daily, for 2 days) | 354.25 ± 85.44* | 25.08 ± 7.17 |
| Phenobarbital (80 mg/kg i.p. daily, for 3 days) | 127.15 ± 40.11 | 26.12 ± 9.85 |
| Dexamethasone (50 mg/kg i.p. daily, for 4 days) | 110.45 ± 29.08 | 25.58 ± 10.05 |
| Cyclosporine (30 mg/kg i.g. daily, for 6 days) | 125.87 ± 34.49 | 23.66 ± 7.85 |
| Paraquat (25 mg/kg i.p. daily, for 2 days) | 257.51 ± 49.66* | 26.54 ± 9.92 |

Results are given as mean ± SD, $n = 10$; $n$ number of animals

*i.p.* Intraperitoneal administration, *i.g.* intragastric administration

* Significantly different from control rats ($P < 0.001$, Student $t$ test)

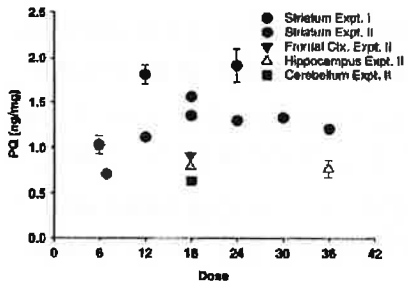
PQ: upregulation of choroid plexus UGT1A6 UDP-glucuronosyltransferase

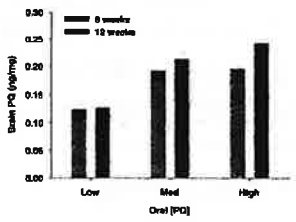
*Gradinaru et al. 2008 Arch Toxicol. Nov 21*



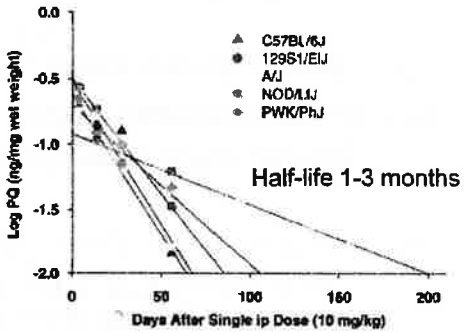
## Paraquat toxicokinetics and toxicodynamics in mouse brain

1. Concentration in brain



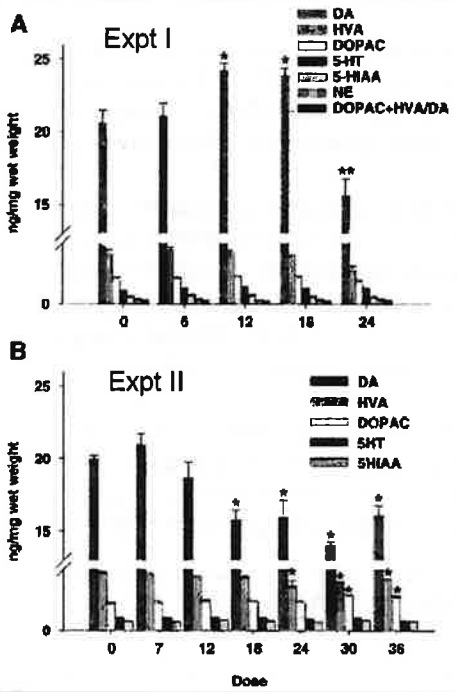




2. Strain differences in clearance




*Prasad et al. 2009 Exp Neurol 215:358*



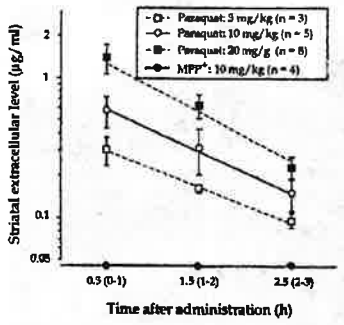



## Paraquat toxicokinetics and toxicodynamics in mouse brain

3. Effects on striatal neurochemistry, 7 days after dose 10 mg/kg ip C57BL/6J (B6J)

*Prasad et al. 2009 Exp Neurol 215:358*

# Paraquat crosses the rat BBB




Brain microdialysis, HPLC-UV detection

No detectable $MPP^+$ entry

| Dose | n | Serum concentration (nmol/ml) | Striatal extracellular level (nmol/ml) | Ratio |
|---|---|---|---|---|
| *Paraquat* | | | | |
| 5 mg/kg | 3 | 1.42±1.50 | 0.28±0.06 | 0.208±0.064 |
| 10 mg/kg | 5 | 3.88±0.79 | 0.36±0.09 | 0.126±0.031 |
| 20 mg/kg | 8 | 8.04±2.41 | 0.49±0.09 | 0.085±0.023** |
| *MPP⁺* | | | | |
| 10 mg/kg | 4 | 11.62±4.84* | Not detected | - |

*Shimizu et al. 2001*
*Brain Res 906: 135*



# Paraquat crosses BBB by L1 (LAT1) amino acid carrier

| | n | Striatal extracellular paraquat level 0–1 h | 1–2 h | 2–3 h | Serum paraquat 3 h | Ratio* |
|---|---|---|---|---|---|---|
| Paraquat (control) | 8 | 4.50±1.06 | 2.03±0.38 | 0.74±0.13 | 8.04±2.41 | 0.085±0.023 |
| Paraquat +L-valine | 8 | 1.32±0.29** | 0.71±0.13**† | 0.06±0.23 | 11.74±2.80 | 0.032±0.006†† |
| Paraquat +L-lysine | 8 | 3.47±0.90 | 1.90±0.26 | 0.84±0.26 | 7.59±1.74 | 0.100±0.028 |

Transport inhibited by L-valine, L-lysine.

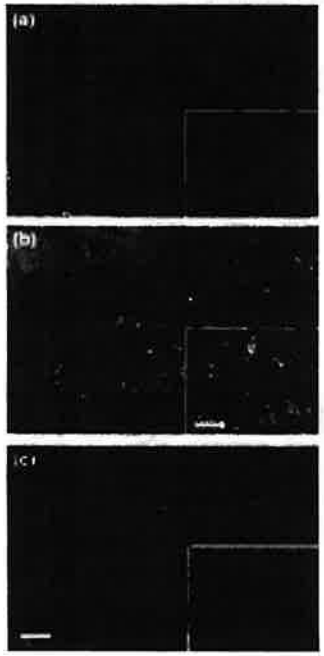
| | n | Ipsi lateral (pmol) | Contra lateral (pmol) |
|---|---|---|---|
| Paraquat (50 μM) | 5 | 172.5±53.9 | ND* |
| Paraquat (50 μM) with $Na^+$-free Ringer's solution | 5 | 76.3±6.8* | ND |
| Paraquat (50 μM) with putrescine (50 μM) | 3 | 169.1±60.5 | ND |
| $MPP^+$ (10 μM) | 5 | 23.1±8.2 | ND |

Locally injected PQ:
Striatal neurone
PQ uptake
$Na^+$-dependent, not blocked by putrescine
?not PAT

*Shimizu et al. 2001*
*Brain Res 906: 135*

➢ *PQ crosses 3 membranes*





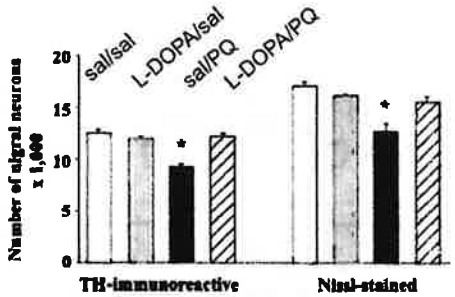

saline/ saline

saline/ PQ

L-val/ PQ

# Paraquat entry to brain reduced by competing L1 transporter substrates

30mg/kg PQ ip. Antibody to detect PQ.
L-DOPA, L-valine, L-phenylalanine pretreatment (30 min) reduced brain entry PQ

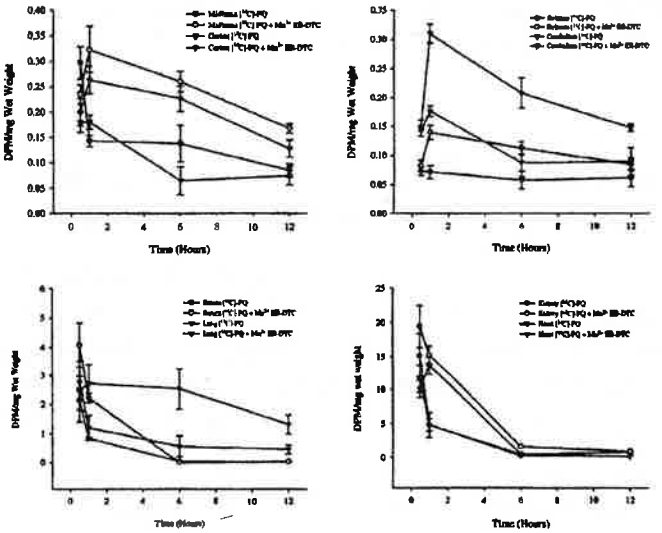



L-DOPA protects against PQ neurotoxicity

*McCormack J Neurochem 2003 85:82*

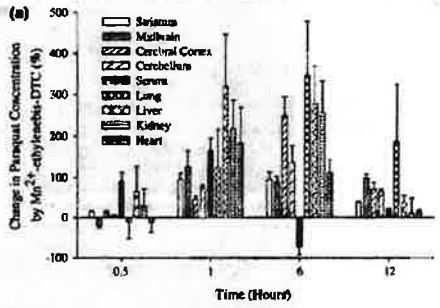


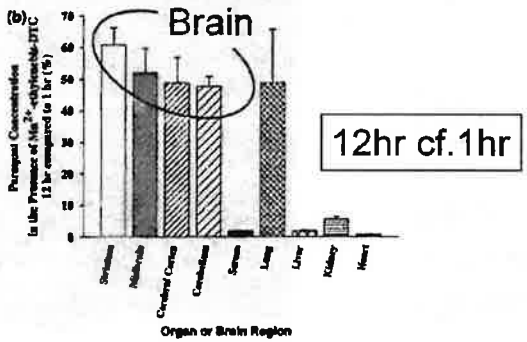
# $^{14}C$ PQ brain entry increased by $Mn^{2+}$-ethylenebis-DTC




DTC: Dithiocarbamate pesticide

*Barlow et al. 2003*
*J Neurochem 85:1075*




12hr cf.1hr

$^{14}$C PQ brain entry increased by $Mn^{2+}$-ethylenebis-DTC

Residual PQ in brain cleared slowly

➢ DTC inhibits efflux transporter?

*Barlow et al. 2003*
*J Neurochem 85:1075*

SYNG-PQ-00471754

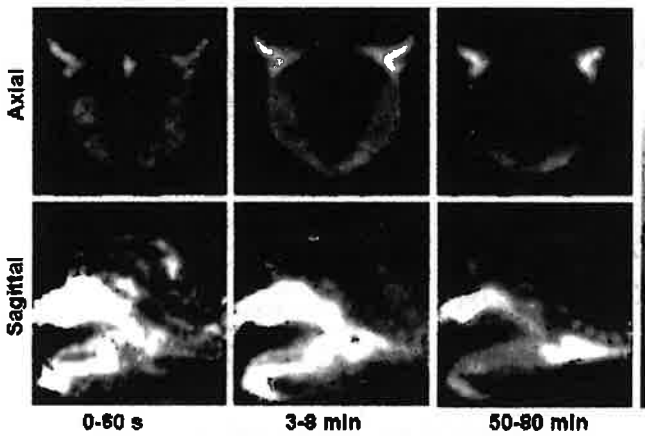
## Does Paraquat (PQ) get into the CNS ?

- Shimizu et al. 2001. Rat brain microdialysis/HPLC-UV. Detectable entry PQ but not MPP+; BBB intact after PQ treatment. Uptake PQ BBB inhibited by L1 substrates: L-valine, L-lysine. Uptake into striatal neurones $Na^+$-dependent, not PAT.
- McCormack & Di Monte 2003. 30mg/kg PQ. Antibody to detect PQ. L-DOPA, L-valine, L-phenylalanine pretreatment reduced brain entry PQ. PQ entry BBB via L1 amino acid carrier.
- Barlow et al. 2003 $^{14}$C PQ enters brain, peak levels ~1hr, certain dithiocarbamates increase brain uptake PQ - by inhibiting efflux transporter?

SYNG-PQ-00471755

## [$^{11}$C] Paraquat in primate: low CNS distribution

PET study Rhesus macaque



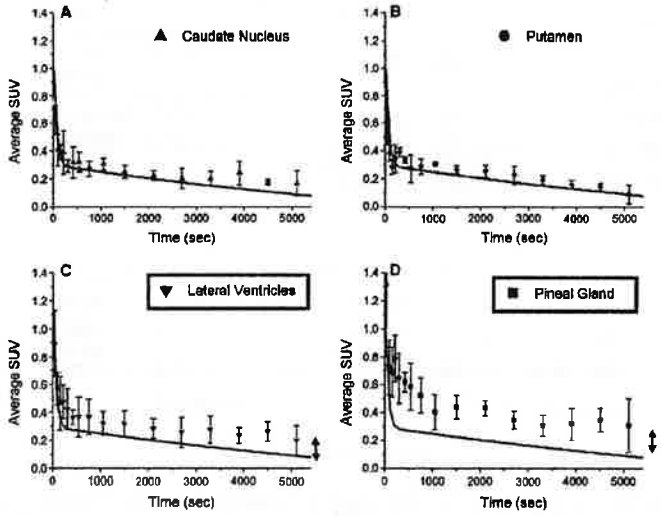

Single bolus injection PQ 0.1-0.4ug/kg iv

Rapid CNS entry, clearance.

*Bartlett et al. 2009 Brain Res 1259: 74*

SYNG-PQ-00471758

## [$^{11}$C] Paraquat in primate: low CNS distribution



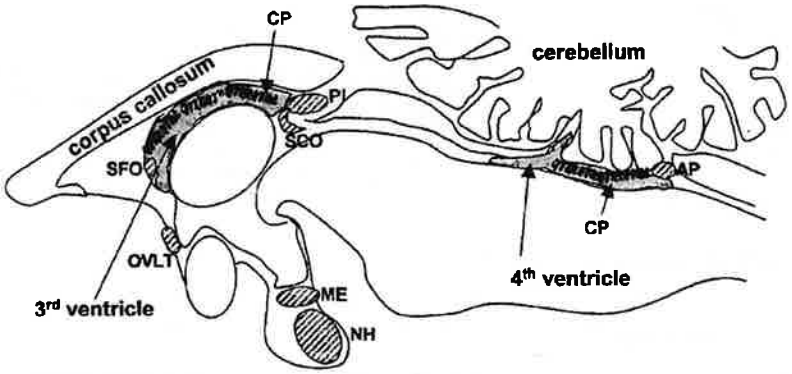

Standardized uptake values (SUV)
n=4 subjects

+ fitted plasma SUV curve (scaled to 5% CBV)

*Bartlett et al. 2009 Brain F 259: 74*

SYNG-PQ-00471757

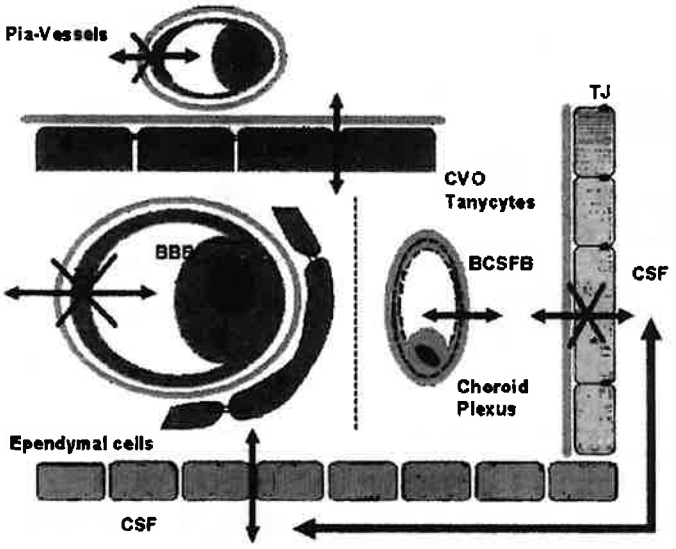
## CIRCUMVENTRICULAR ORGANS IN RAT




*Ueno 2007 Curr Med Chem 14: 1199*



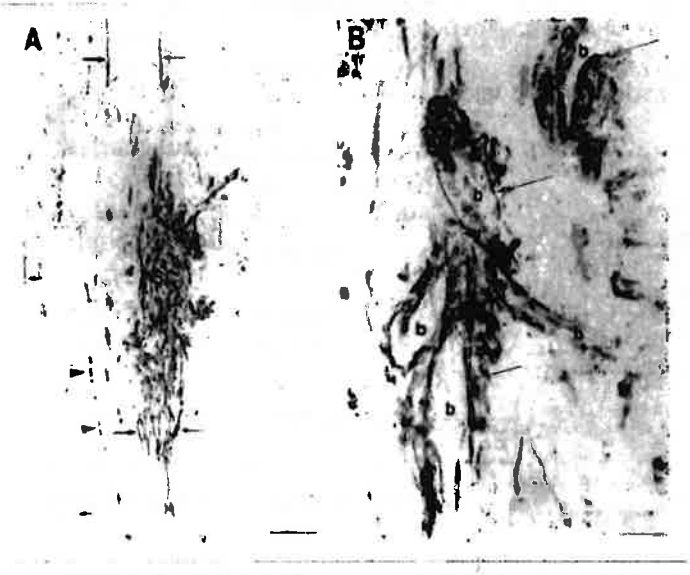
## CNS interfaces and barriers




*Wolburg et al. 2009 Cell Tissue Res 335:75*



## Migration of grafted Schwann cells along perivascular spaces

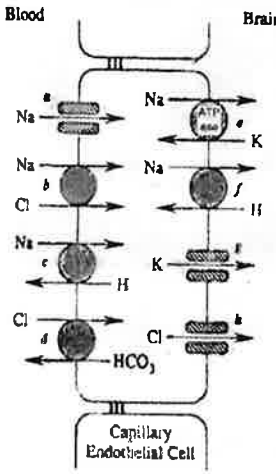


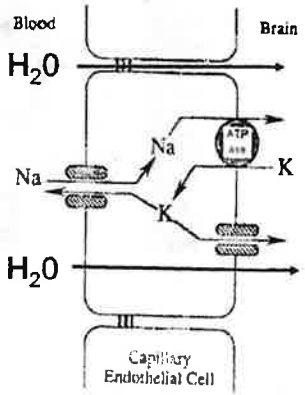

b,
blood vessel

PVS – also migration route for malignant cells

*Li & Raisman 1997 Exp Neurol 149:397*

# Source of ISF ? Model of ion transporters and channels in brain endothelium



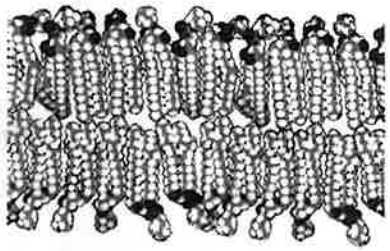




*Schielke & Betz 1992*

SYNG-PQ-00471770

# Properties of the brain endothelial cell membrane

*Anna Seelig 2007 J Mol Neurosci 33:32*



*Flexibility of membrane lipid chains*

- Lipid bilayer: liquid crystalline state, crystals perpendicular to surface
- Aniosotropic (*cf.* isotropic octanol, hexadecane).
- Average order fatty acyl chains: high close to head group, low in centre of membrane.
- ↑Order: high % lipids with small polar headgroups (PE, SM, cholesterol).
- ↓Order: high % unsaturated fatty acyl chains, increased concentrations amphiphilic guest molecules (e.g. drugs).
- Transmembrane proteins good match with lipid bilayer properties – hardly influence bilayer properties.

SYNG-PQ-00471771

# Properties of the brain endothelial cell membrane

Anna Seelig 2007 J Mol Neurosci 33:32

- Lateral packing density ($\Pi_M$) mN/m: intestinal barrier 28, BBB 35
- Energy required for membrane insertion $\Delta W = A_D.\Pi_M$ (cross-sectional area x packing density).
- Lipid:water partition coefficient ↓ with ↑$A_D$ and ($\Pi_M$).
- Molecules spanning half membrane (long chain FA, lipids) move by flip-flop.
- Charged molecules can insert, but only uncharged form can cross hydrophobic core.
- For CNS entry: limiting cross-sectional area ~AD70-80Å$^2$, ionization constant p$K_a$ = 4 (acids) – 10 (bases).

SYNG-PQ-00471772

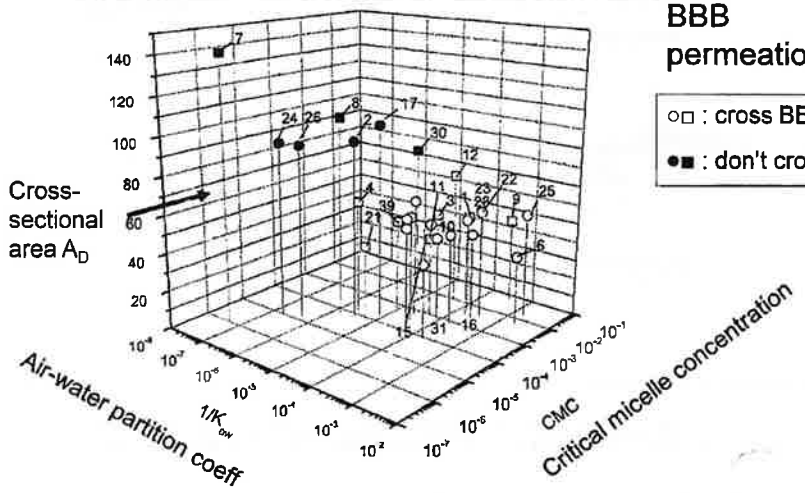
# Properties of the brain endothelial cell membrane

Anna Seelig 2007 J Mol Neurosci 33:32

3-D calibration diagram for BBB permeation

○□ : cross BBB

●■ : don't cross

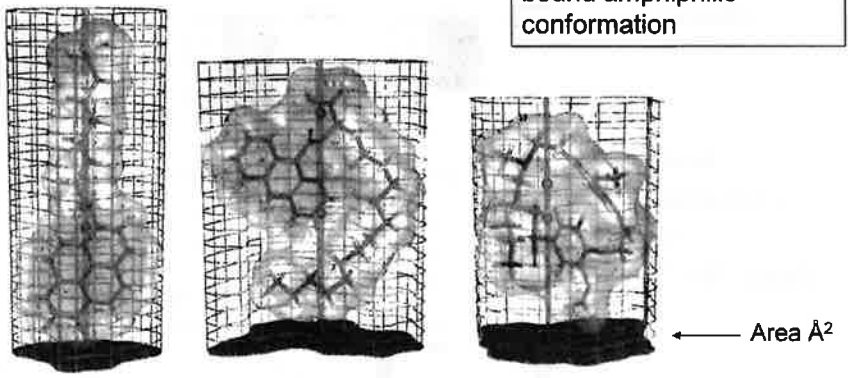



SYNG-PQ-00471773

# Shapes of Fatty Acids *vs.* Verapamil

Anna Seelig 2007 J Mol Neurosci 33:32

Molecules in membrane-bound amphiphilic conformation



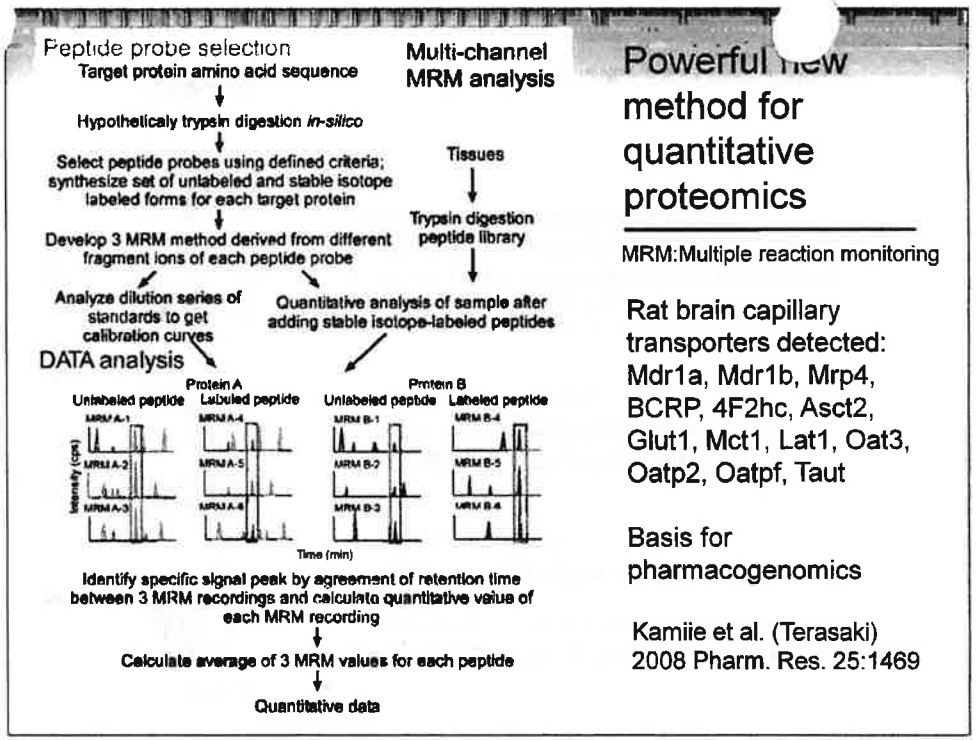
Area Å²

Pyrene nonanoic Acid (14) (high passive influx)

Antroyloxystearic acid (15) (low passive influx)

Verapamil (12) (Pgp efflux)






## Powerful new method for quantitative proteomics

MRM:Multiple reaction monitoring

Rat brain capillary transporters detected: Mdr1a, Mdr1b, Mrp4, BCRP, 4F2hc, Asct2, Glut1, Mct1, Lat1, Oat3, Oatp2, Oatpf, Taut

Basis for pharmacogenomics

Kamiie et al. (Terasaki) 2008 Pharm. Res. 25:1469

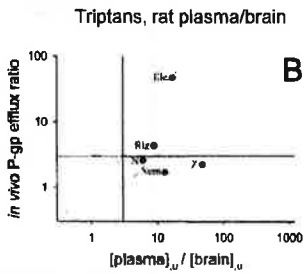


# Efficacy with low brain penetration

**Triptans**
- Zolmitriptan and sumatriptan
- $Brain_u/Plasma_u$ = 0.02, 0.08

(Kalvass et al 2007 Drug Metab Dis 35, 660-666)

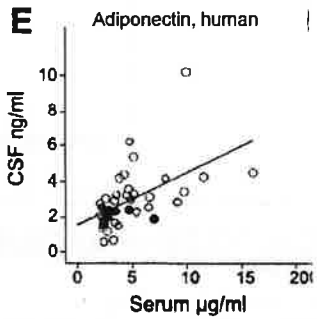
***Reasons?***
- Removal by CSF bulk flow
- Non-CNS target (vascular)?




**Adiponectin 150kDa**
- i.v. injection = weight loss in leptin-deficient obese mice
- Detected in CSF 3hrs after i.v.
- CSF/serum = 0.001

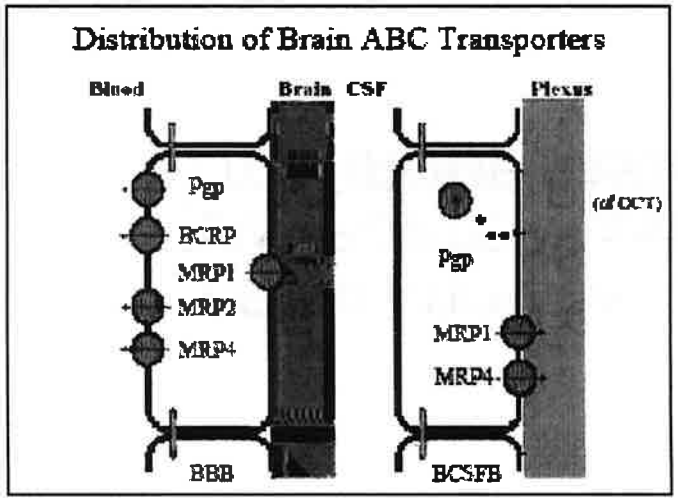
(Neumeier et al 2007 Am J Physiol Endo & Metab 293, E965-E969)

***Reasons?***
- Small blood-CSF barrier penetration
- Sites of action AdipoR in hypothalamus and paraventricular nucleus close to CSF
- Rapid degradation (therefore difficult to detect)?



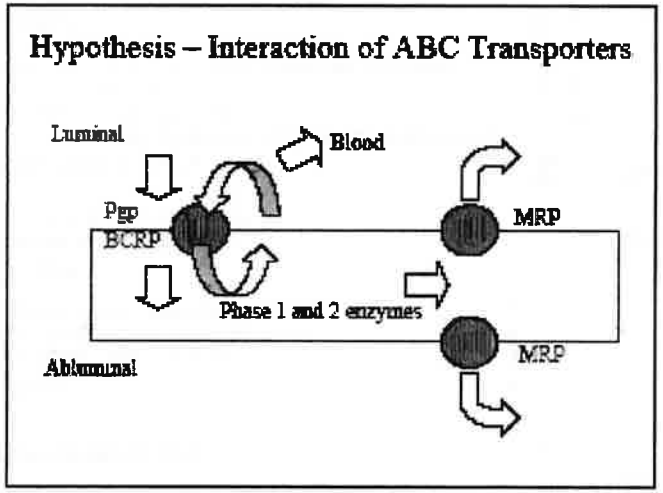



# Functional drug efflux transporters, rat




G Fricker

# ABC Transporters: efflux and metabolism

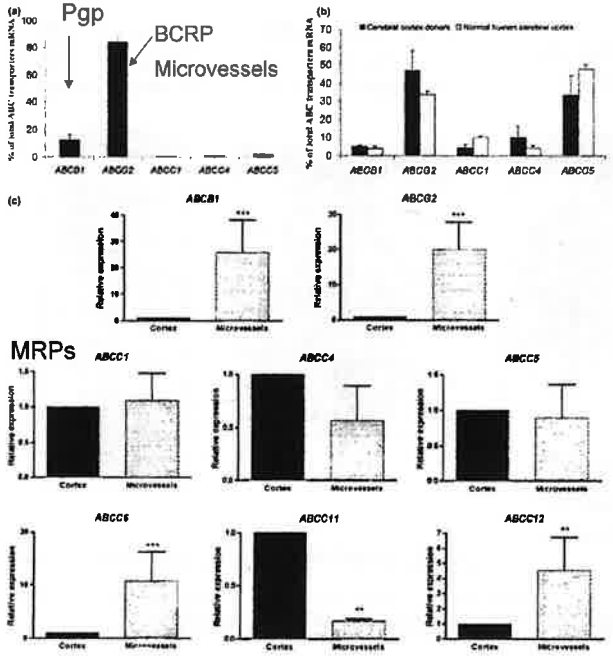



*G Fricker 2007*



# REGULATION OF TRANSPORTERS and CYTOCHROME P450s





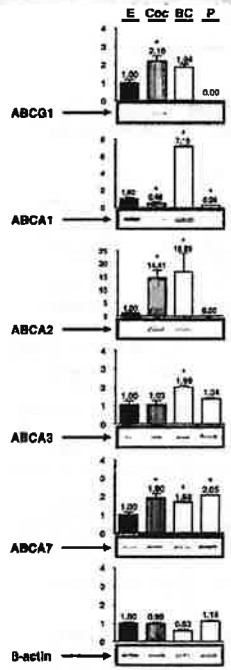

Cortex

Human brain: ABC transporter genes

Relative expression

*Dauchy et al. 2008 J Neurochem 107:1518*






## Role of BBB in cerebral sterol homeostasis

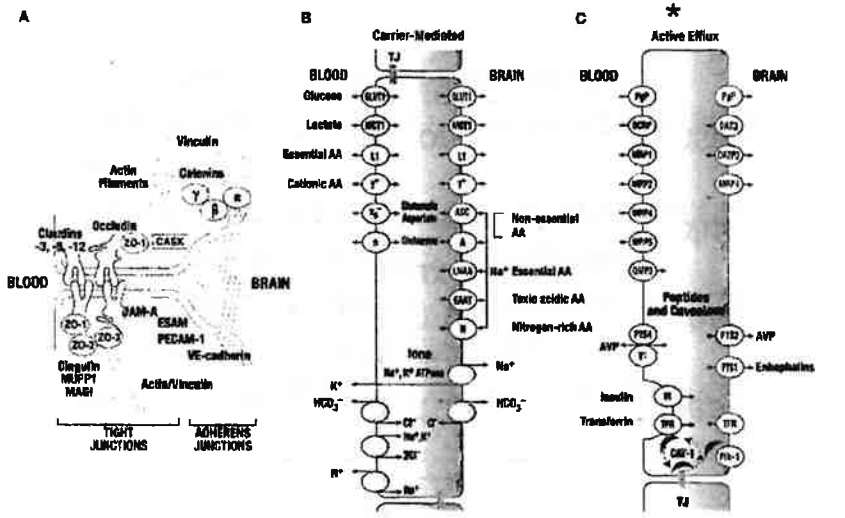
ABC Transporter expression bovine BCEC

- Coculture with astrocytes (Coc)
- with bovine capillary extracts BC)
- with pericytes (P)

ABCG1: cholesterol efflux
ABCA1: ApoE- dependent cholesterol release
ABCA7: ApoA1-dependent cholesterol release
ABCA2, 3,7 induced by cholesterol

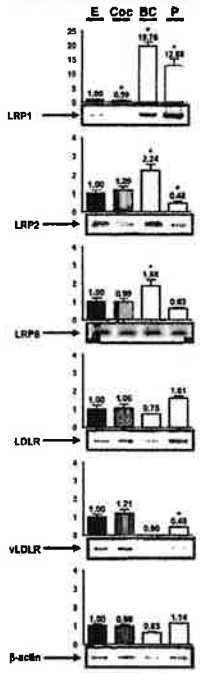
*Gosselet et al. 2009 Brain Res. 1249:34*

# Regulation of transporter expression




*Zlokovic 2008 Neuron 57:178-201*






# Receptors of the LDL-R family

Lipoprotein receptor expression bovine BCEC

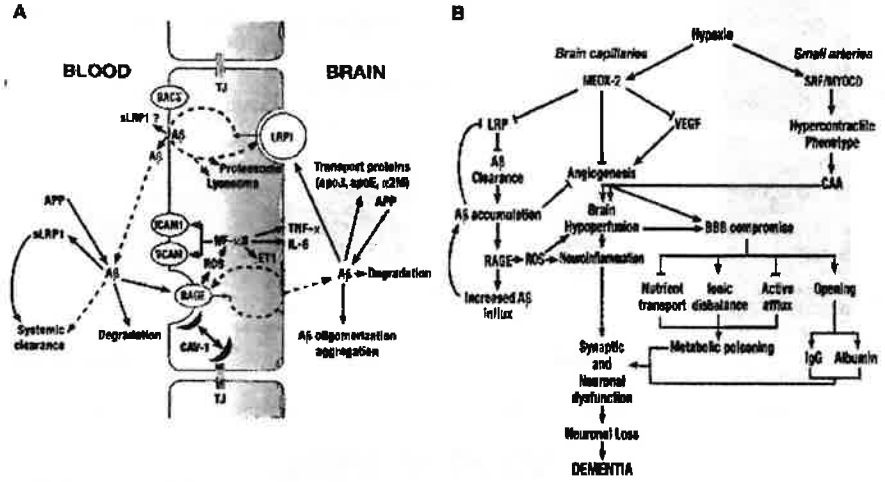
- Coculture with astrocytes (Coc)
- with bovine capillary extracts BC)
- with pericytes (P)

*Gosselet et al. 2009 Brain Res. 1249:34*



# Role of BBB in Alzheimer's Disease : Aβ transport

*Modulation in hypoxia*

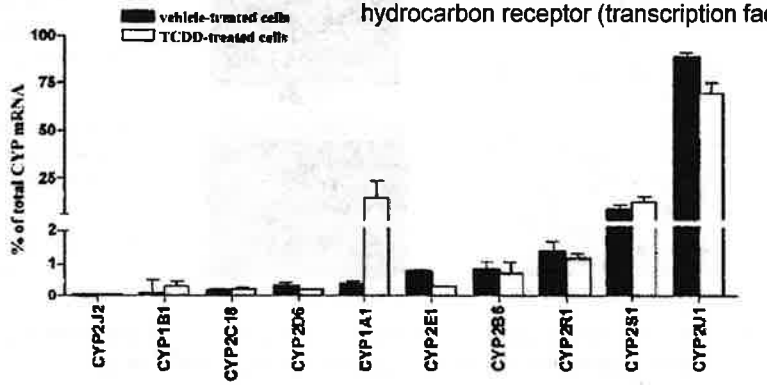



*Zlokovic 2008 Neuron 57:178-201*



# Expression profiles of CYP genes in hCMEC/D3 cells

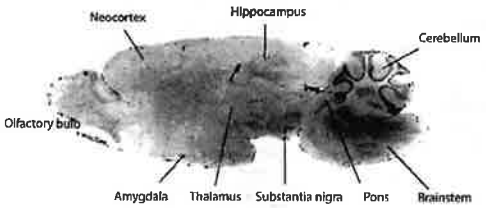
TCDD - agonist for AhR – aryl hydrocarbon receptor (transcription factor)




*Dauchy et al. 2009 Biochem Pharmacol 77:897*

# P450 in brain

Mouse brain

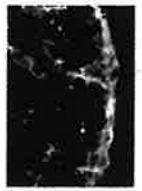



CYP2C29, phenytoin-treated mouse

BBB

CYP2B1 (Rat)

Pons – Pia Mater


Regions with strong P450 expression:

Barrier regions (Blood-brain barrier & CSF- brain barrier)
Steroidogenic & steroid-metabolising regions of the brain

*RP Meyer et al., Curr Drug Metab (2007), 8(4), 297-306.*

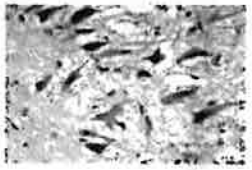
SYNG-PQ-00471788

# P450 & AR in human hippocampus

Androgen receptor- and CYP3A4-expression are enhanced in hippocampal pyramidal neurons of patients with temporal lobe epilepsy (TLE)

CA2 pyramidal neurons

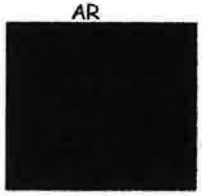
Patient with TLE, treated with carbamazepine

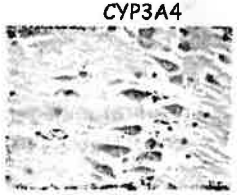


AR

CYP3A4

Control patient, (accident victim) untreated, no neurological symptoms





N. Killer et al. Epilepsia (2009) in press

SYNG-PQ-00471787

# SUMMARY

- Wnt signalling important in angiogenesis, development of BBB phenotype
- Significant regulation of expression (and activity) for active efflux transporters
- Some reported regulation of other solute transporters
- Regulation of receptor-mediated transcytosis
- Regulation of cytochrome P450s

➢ *Physical, transport and enzymatic barrier – all regulated*

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# SPECIES DIFFERENCES

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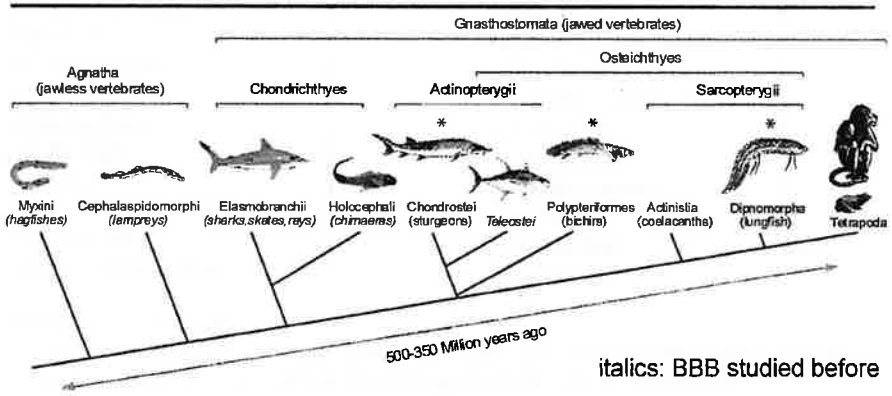
# Evolution of BBB



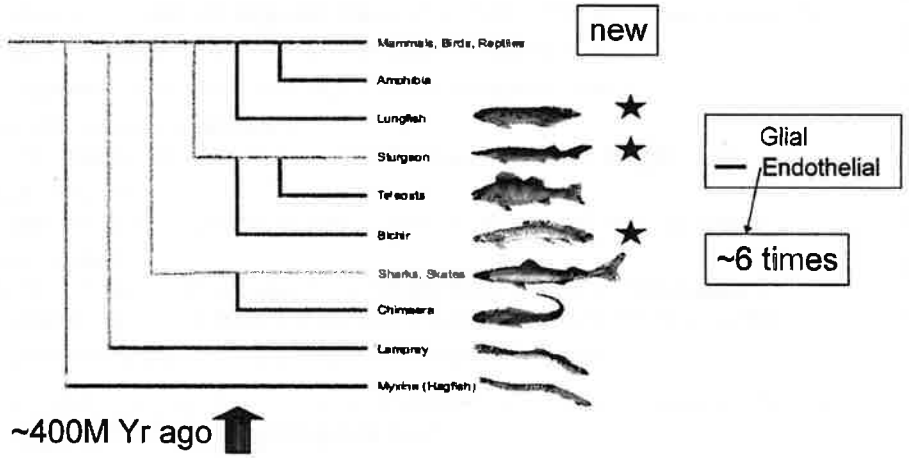

italics: BBB studied before

*New studies : Sturgeon, Bichir, Lungfish

*Bundgaard & Abbott (2008) Glia 56 (7):699-708*

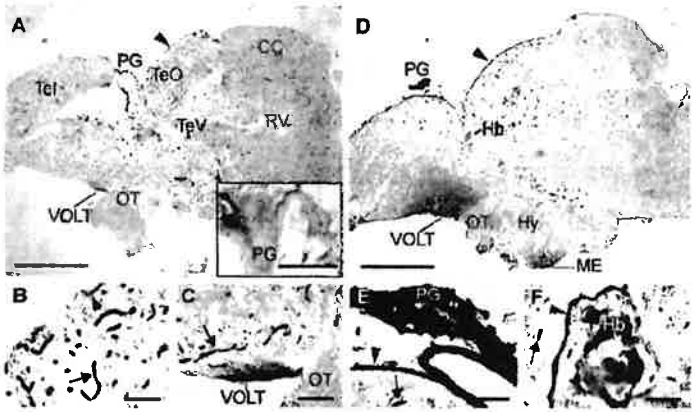


# Shift of barrier layers during evolution






# Adult Zebrafish BBB (Teleost, endothelial)




Tight junction proteins:
ZO-1
Claudin 5

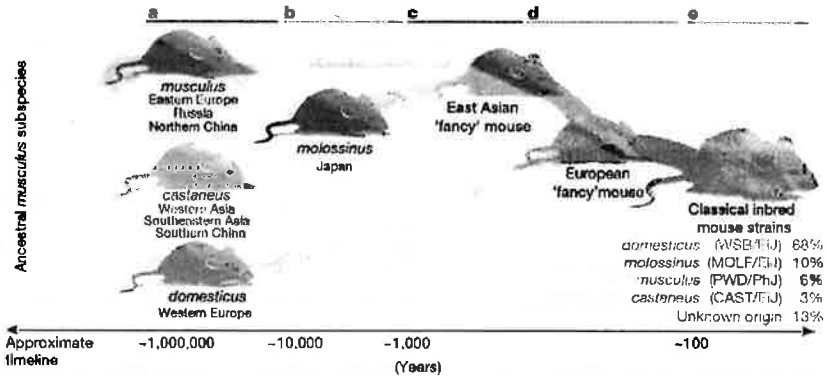
HRP

Sulfo-NHS-biotin

Jeong et al. 2008
Brain Res Bull 75:619



# Limited origin of inbred mice strains




Frazer et al. 2007 Nature 4448:1050

# Variables in uptake

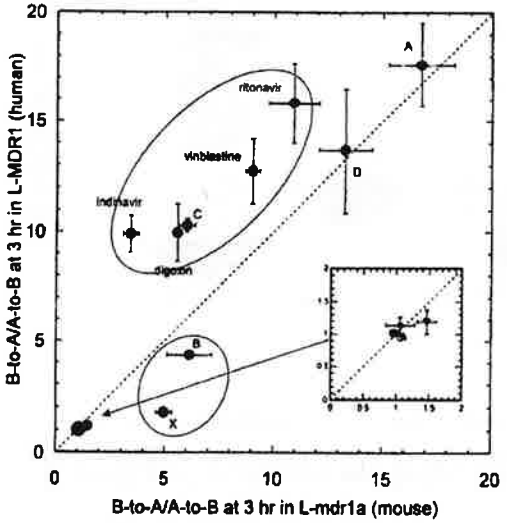
**Age**
↓Pgp (Toornvliet et al 2004)

**↑Body mass index** (↑venous pressure)
Reduced CSF drainage, elevated CSF drug concentration
(Seyfert et al 2002)

**Pathology**
'Sticky' lumen – binds protein, increases Vi
(Poduslo et al 2007)
BBB breach (AD etc)



# Differences human and mouse Pgp drug specificity




Basal to apical vs Apical to basal transport ratios

Transfected LLC-PK1 porcine renal epithelial cell line

*Yamazaki et al. 2001 JPET 296:723*



# Regional differences

**Leucine transport (rat)**
Vmax Highest in cortex (greater capillary surface area?)
No differences in Km or Kd (*Smith et al J Cereb Blood Flow Metab. 1985 5:300)*
**Blood flow (rat)**
Highest in grey matter, caudate nucleus & occipital lobe (0.030ml/g/s)
Lowest in cerebellum and white matter (0.018ml/g/s) but highest sucrose space (*Rapoport et al 1979)*

**Sucrose space (rat)**
Olfactory bulb 4.68%
medulla
cerebellum
frontal lobe
**Parietal grey**
pons
**occipital lobe**
colliculus
thalamus + hypothalamus
hippocampus
**caudate nucleus**
white matte 1.14%

**Blood flow**
Parietal grey 0.040 ml/g/s
Colliculus
caudate nucleus
occipital lobe
frontal lobe
thalamus + hypothalamus
hippocampus
pons
**medulla**
white matter
**Olfactory bulb**
**cerebellum** 0.017 ml/g/s



# Species differences

- Evolution of BBB – insights into origin mammalian barrier
- Need to document, understand species differences among mammals and human, to build in to modelling (including PBPK) and to avoid problems in extrapolation
- Take into account history of specific animal models, e.g. rodents, mouse strains, KO etc
- Size differences – will lead to scaling factors, especially for CBF, fluid compartments and dynamics
- Differences in anatomy and territory of cerebral vasculature
- PK (concentration *vs* time curve plasma); protein binding
- Passive permeability BBB well conserved. Justification for *in vitro* and animal models, PAMPA
- Uptake transporters – some differences, e.g. amino acid transporters, but functionally similar
- Efflux transporters – most variability especially MRPs (may be linked to metabolic differences)

** Note - some reported differences may relate to techniques used, se* *ity*

## Species differences

- Disease models – many, few mimic closely the clinical condition – e.g. stroke, multiple sclerosis, Alzheimer's. Differ among themselves, and between species
- How to choose model? Need to check against human disorder to decide on relevance. May get mechanistic information from animal models, but finally require validation and methods to monitor therapy in humans (e.g. imaging, biopsies, post-mortem, blood and CSF sampling, microdialysis in patients)
- Some treatments effective in rodent models, not in humans (e.g. stroke therapies, AD..) Check and understand better the timeframe, window for intervention, balance of elements in pathology
- New therapeutic targets in early stages of disease, including BBB. Couple with early diagnosis of BBB disorder.
- Extrapolation, toxicology etc. Warrants BBB Club Symposium on this topic. Examples of current practice from companies?



## BBB MODULATION



## Agents that increase permeability

| | BBB | Perineurium |
|---|---|---|
| Histamine ($H_2$), 5HT, bradykinin | + | - |
| Arachidonic acid | + | - |
| A23187 | + | - |
| Bile salts (DOC) | + | + |
| Non-ionic detergents | + | + |
| Reactive oxygen species (ROS) | + | + |
| *Nitric oxide (NO high) | + | + |
| *Glutamate | + | ? |
| *ATP, ADP, AMP | *+ | ? |
| *ET-1, Substance P | + | ? |
| *Cytokines, *MIPs | + | ? |
| *VEGF | + | ? |

*Secreted by astrocytes



## Agents that decrease BBB permeability

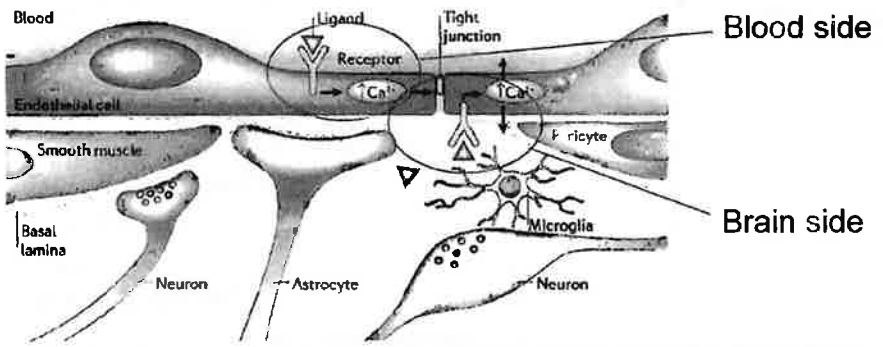
↑cAMP
- Histamine ($H_1$, high)
- Adrenomedullin
- Noradrenaline
- Dopamine
- Adenosine A2

Nitric oxide (NO, low)
Bradykinin? (low)

Corticosteroids
Fluvastatin

➢ BBB can be tightened by endogenous and exogenous agents

# Source of modulating influences at BBB




*Abbott et al 2006 Nature Rev Neurosci 7:41*

- Physiology, pathology
- Therapeutic BBB modulation for drug delivery

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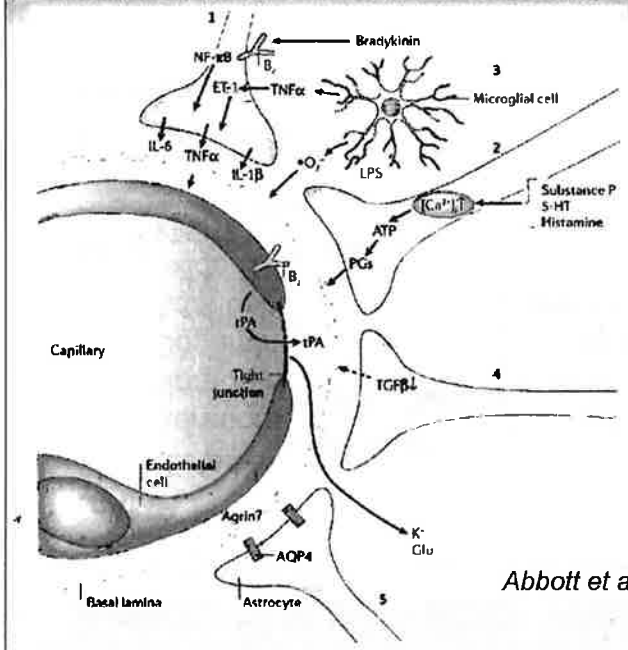


# Role of astrocytes and microglia in BBB modulation in pathology

*Abbott et al 2006 Nature NS Revs*

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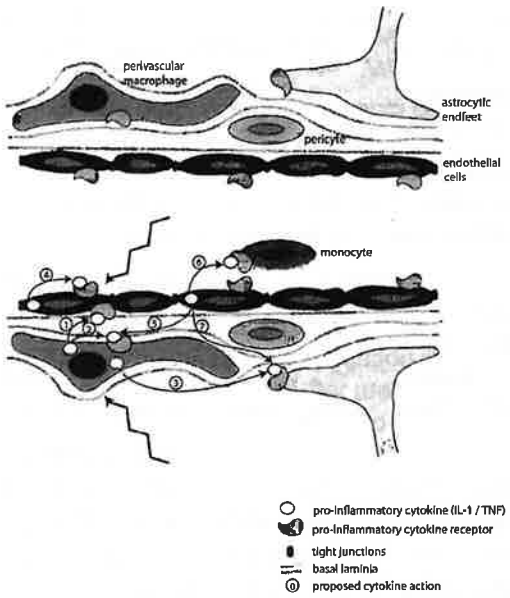



# Signalling within the *perivascular space: role in pathology

**especially post-capillary venules*

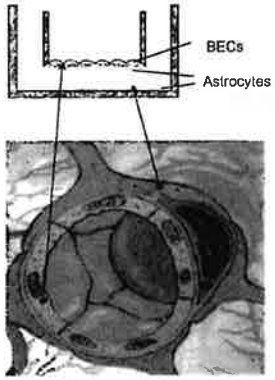
*Konsman et al. 2007 Clin. Sci. 112, 1-25*

SYNG-PQ-00471804

# *In vitro* Models of the Blood-Brain Barrier

- **ECV304: Endothelial-epithelial phenotype, co-cultured with C6 glioma cells.** Hurst and Fritz. (1996) *J. Cell Physiol.* **167:** 81-88; Easton & Abbott (2001) *Brain Res.* **953**:157
- **Primary rat brain EC (RBEC) cultured with rat astrocytes.** Abbott et al. (1992) *J. Cell. Sci.* **103:** 23-37. Perrière et al. (2005) *J Neurochem* **93**:279.

*S Fredriksson*

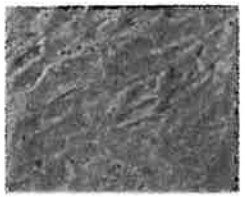



Cross Section Brain Microvessel

Glial cells

WPI EVOM STX-100
TEER 50-600 ohm.cm$^2$

Use at ~4 weeks +



Endothelial cells

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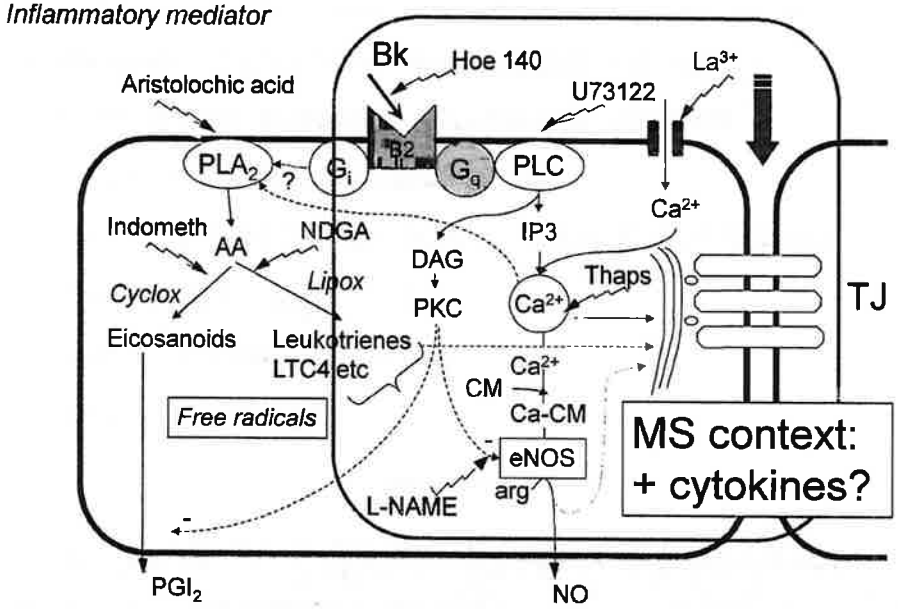
## Bradykinin modulation of rat brain endothelial cells

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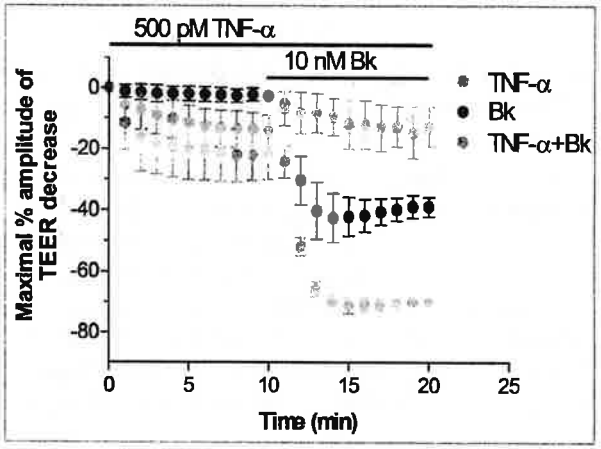
## Effect of TNFα on bradykinin response ?




Cytokine modulation

TNF-α augments Bk-induced TEER decrease

Can this be blocked ?

*S Fredriksson*

Mean ± SEM n=3

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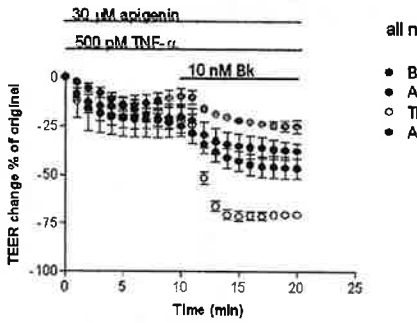
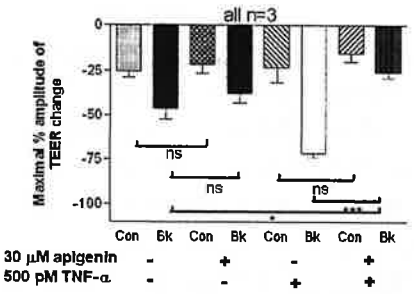



all n=3

- Bk
- Apigenin+Bk
- TNF-α+Bk
- Apigenin+TNF-α+Bk

## *In vitro* BBB

Flavonoid (flavone) Apigenin blocks augmenting effect of TNFα on Bk response

Mean ± SEM n=3

*S Fredriksson*

SYNG-PQ-00471808

## Functions of BBB & BSCB: Effects of barrier breakdown?

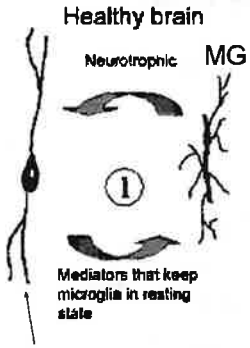
1. Control molecular traffic, keep out toxins (precise connectivity: low cell death)
2. Ion homeostasis - optimal neural signalling
3. Low protein environment, limit proliferation
4. Separate CNS:PNS neurotransmitter pools; allow non-synaptic signalling
5. Immune surveillance with minimal inflammation, cell damage

*Disturbed CNS barriers : increased neuronal damage*

SYNG-PQ-00471809

# MICROGLIA (MG)

- Resident immunocompetent cells of brain
- Derived from monocytes/macrophages
- Quiescent, resting morphology: dendritic
- Contribute to BBB induction in endothelium
- Activated in pathology - amoeboid




*Vilhardt 2005 Int J Biochem Cell Biol 37:17*





# MICROGLIA AND THE BBB

- Resident immunocompetent cells of brain
- Derived from monocytes/macrophages
- Contain $COX_2$
- Synthesise prostaglandins: $PGH_2$-synthetase
- Target for NSAIDS: Indomethacin and aspirin block $PGE_2$ release with LPS

Resting microglia
↓ ← cytokines/adenosine
Proliferation
↓ ← chemokines
Migration and differentiation
↓
Activated = Macrophages (phagocytic)



# FOCUS on ENDOTHELIAL ACTIVATION

Triggering stress:

- Viral or bacterial infection
- LPS, TNFα, IFNγ, IL-1β
- Anti-endothelial antibodies, immune complexes
- Injury, trauma, thrombin, toxins
- Heat shock, exercise, ischaemia, hypoxia
- Regional differences within CNS – leads to vulnerable sites? *(nerve roots, brain stem, subventricular zone)*



# Pattern of endothelial activation in MS

- ↑ Adhesion molecules including ICAM-1, E-selectin, leukocyte traffic, access encephalitogenic T cells, attack neuronal and glial antigens, can lead to demyelination and neuron/axon loss
- ↑ Protein traffic, mitochondrial activity, ROS
- ↑ Secretion IL-1β, IL-6, $PGE_2$, SubsP
- Activation macrophages, microglia
- Opening endothelial TJ, loss of homeostasis, entry of toxins
- Mechanism(s) – may involve TNFα, IL-1β, NFκB…

➢ *If transient, mild, get BBB resealing, protection against further stress (pre-conditioning, requires endothelial-astrocyte interaction)*

➢ *If severe or chronic, get positive feedback cascades, neuronal damage…*

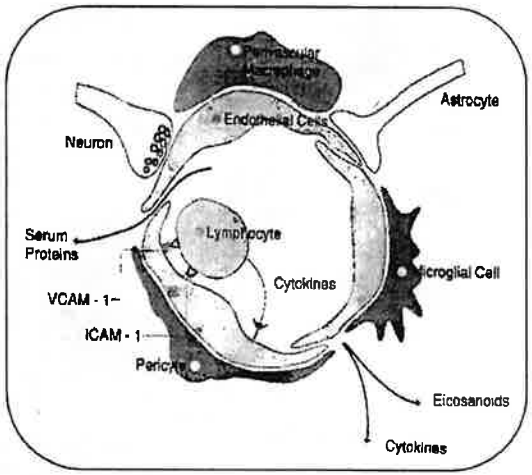
➢ *Hypothesis* : MS is a chronic BBB diseases

## Therapies for BBB in CNS pathologies

BBB as target

*Aim to maintain healthy BBB, and to repair BBB if damaged.*

- Block reactive-oxygen species (ROS)-mediated damage
- Block leukocyte attachment and migration
- Block cytokine production




Characteristics of the brain endothelium

Value in MS – other CNS disorders?



## The BBB in CNS pathologies

- Traumatic brain injury; BK ⇒ astrocyte release IL-6, BBB opening
- Hypertension; focal BBB opening
- Ischaemia, Stroke; disturbed BBB ion transport, two phase BBB opening, upregulation AQP4 astrocytes
- Inflammation, pain; changes in TJ, BBB permeability
- Multiple sclerosis; BBB opening, changes in laminin
- HIV-related dementia; BBB disruption
- Bacterial meningitis, encephalitis, sepsis; LPS ⇒ ROS, IL-6, IL-1β
- Brain tumours; Changes in TJs and glial endfeet
- Epilepsy; Changes in TJs and expression of MDR proteins
- Alzheimer's disease; changed expression Glut-1, AQP4, reduced P-gp ⇒ reduced Aβ efflux
- Parkinson's disease; reduced efficacy P-gp

➢ *Target and strategies for therapy*



## CONCLUSIONS

- Damage to CNS barriers may be triggering or exacerbating event in CNS pathologies (MS, lupus, epilepsy...)
- Chronic low-grade BBB dysfunction could explain many features of disease progression
- Need better and earlier diagnostic tests for barrier damage; treat at-risk individuals
- Target prophylaxis and therapies to CNS barriers

➢ ***Future perspectives***



## Acknowledgements

**The BBB Group KCL**

*BBB modulation, physiology and pathology*

- Paul Fraser
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- Sarah Fredriksson, Adjanie Patabendige, Siti Yusof

*Collaborators*

- KCL - Ken Smith, Catherine Rice-Evans, Marcus Rattray
- St Thomas' Hospital - Graham Hughes
- Budapest - Ester Domotor, Ildiko Sipos, Vera Adam-Vizi
- Cleveland - Damir Janigro

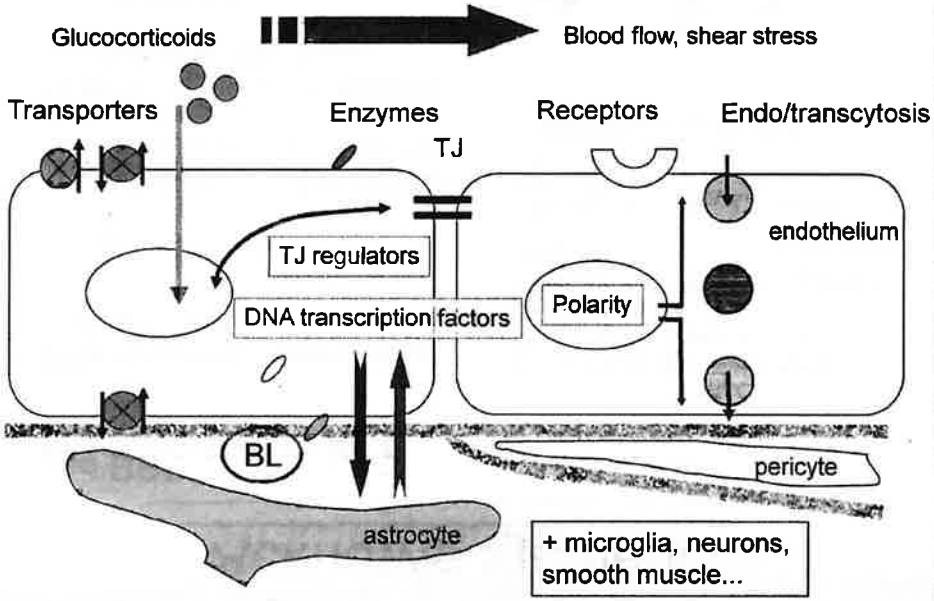
Supported by:

- The UK MS Society and UCB

# BBB FUNCTION and DEVELOPMENT



## BBB INDUCTION : SUMMARY





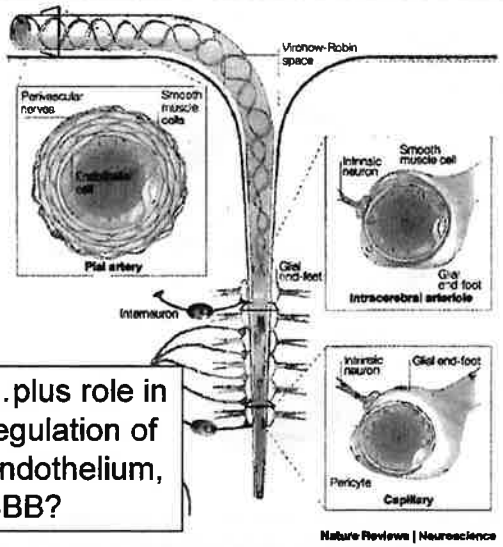

## The Neurovascular Unit

Artery, arteriole, capillary

Neurons
Glial cells
Endothelial cells
+ *associated cells*

Role in regulation of regional blood flow : *Neural activity increases flow*

…plus role in regulation of endothelium, BBB?

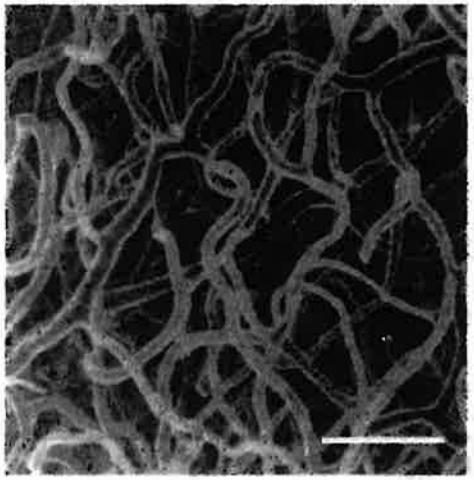



*Iadecola 2004 Nat Rev Neurosci 5:347*



## BRAIN MICROVESSELS

Cast of rat thalamus



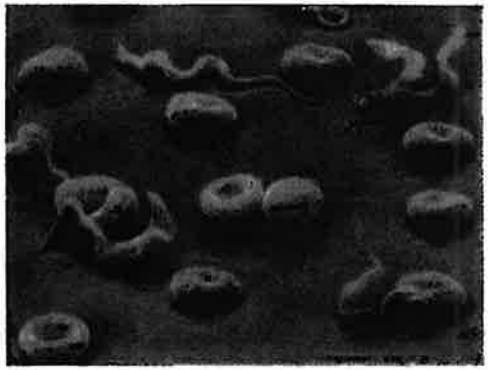
Human brain: ~ 1200 g
Capillary length: ~ 650 km
Endothelial volume: ~ 1 ml
Mean distance: ~ 40 µm
Lumen diameter: ~ 3 µm
Surface area: ~ 12 $m^2$

Rat Brain: ~ 2 g
Endothelial volume: ~ 1 µl

*Scanning EM*
*Bar = 50µm*

## THE CONCEPT OF A BLOOD-BRAIN BARRIER

Paul Ehrlich 1914
*d.20/08/1915*



*T. cruzei* From: "die blut" Frankfurt 2001

Trypan Blue (C60D)

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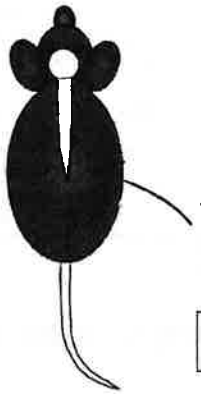
## GOLDMANN'S 1st and 2nd EXPERIMENTS

1st (Ehrlich)



Trypan Blue
intravenous
injection

**body stained**
CNS unstained

2nd

Trypan Blue
intrathecal
injection

body unstained
**CNS stained**

SYNG-PQ-00471823

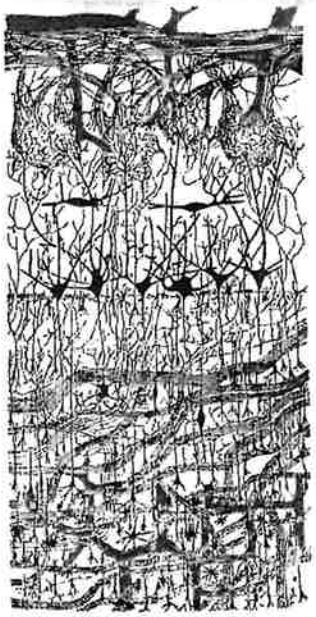
## Critical role of synapses in integrated CNS function

Golgi 1875: regional specialisations in synaptic zones :
*dog olfactory bulb*

- *High activity*
- *High vascularity*

⇒

*∴Need barrier*



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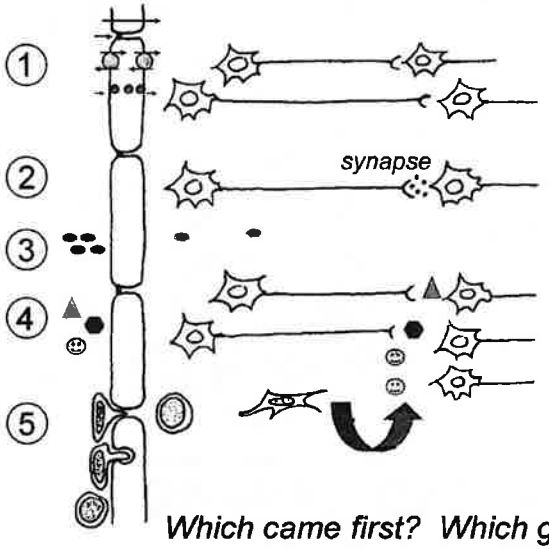
## FUNCTIONS OF BBB




Molecular traffic

Ion regulation

Low protein

Signalling

'Silent' immune surveillance

*Which came first? Which gave most advantage?*

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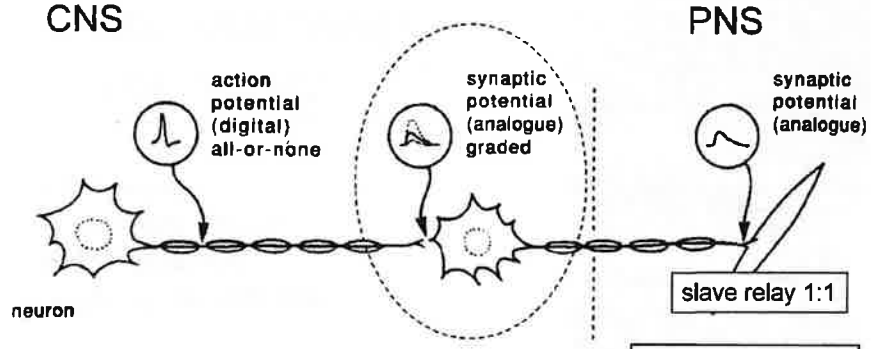
## SIGNALLING IN THE NERVOUS SYSTEM




- Greatest need for ionic homeostasis around CNS synapses

*Key selective advantage : evolution of BBB*

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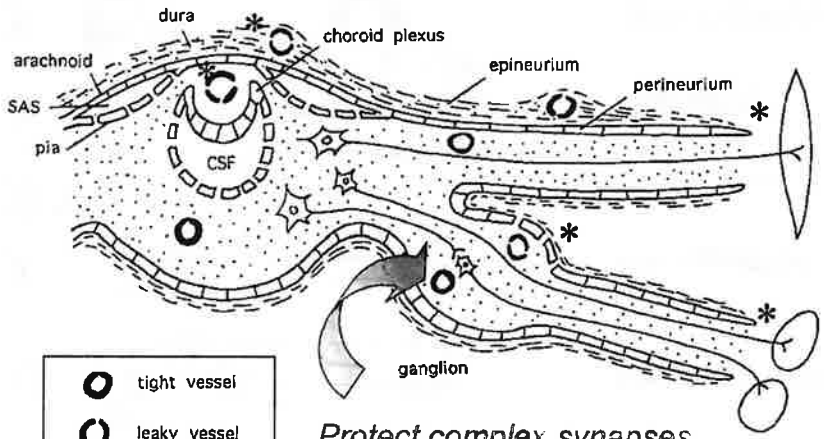
## Barriers in CNS and PNS




*Protect complex synapses, sense organs*

*Relevance to drug delivery, pathologies*

SYNG-PQ-00471827

KING'S College LONDON

Myth 1

The BBB in the neonate is immature and leaky compared with that of the adult

SYNG-PQ-00471828

## Development of BBB

1. Evidence for leaky perinatal BBB

| **Injected Tracer Horseradish Peroxidase (HRP)** | Species | Stage | Body wt g | Injection volume (% blood vol.) | Increase in plasma protein |
|---|---|---|---|---|---|
| Wakai and Hirokawa (1981) | Chick | E9 | 1.56 | 5-10% | 2x |
| Risau et al. (1986) | Mouse | E13 | 0.083 | 100-300% | 1% |
| Vorbrodt et al (1986) | Mouse | Newborn | 1.4 | 100% | 2-3x |
| Stewart and Hayakawa (1987) | Mouse | E15 | 0.26 | Not stated | 4x |

From: Saunders 1992

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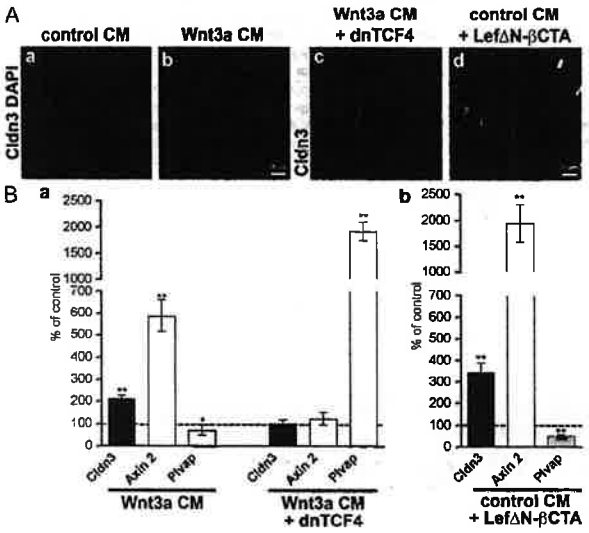
# Tight junctional Claudin-3 upregulation by β-catenin



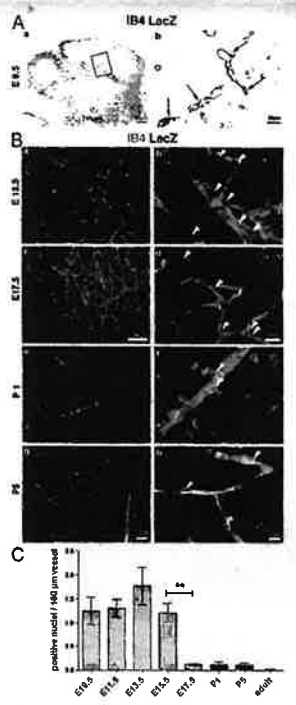

Claudin-3 upregulation requires transcriptionally active rather than junctional β-catenin in mouse brain endothelial cells in vitro

*Liebner et al. J. Cell Biol. 2008:183:409-417*



Cldn3 up-regulation requires transcriptionally active rather than junctional β-cat in MBEs in vitro. (A, a–c; and B, a) MBEs were either treated with Wnt3aCM or control CM and infected with dnTCF4 adenovirus or a control. (A, a–c) IF stainings for Cldn3. dnTCF4 abrogated the effect of Wnt3aCM on junctional Cldn3. Quantitative RT-PCR for Cldn3 (n = 3; **, P = 0.0095), Plvap (n = 3; *, P = 0.035), and Axin2 (n = 3; **, P = 0.004) with Wnt3aCM and for Plvap (n = 3; **, P = 0.002) with control CM and dnTCF4. (A, d; and B, b) MBEs infected with LefΔN-βCTA lentivirus or a control. Junctional localization of Cldn3 increased in IF stainings. Quantitative RT-PCR for Cldn3 (n = 3; **, P = 0.002), Plvap (n = 3; **, P = 0.008), and Axin2 (n = 3; **, P < 0.0001). Controls are set as 100% (dashed lines). Green nuclei show an anti–VS-TAG (c) and an anti–HA-TAG (d) staining of dnTCF4 and LefΔN-βCTA, respectively, confirming infection of the target cells. Error bars represent SEM.






# Wnt signalling in angiogenesis

Mouse brain
IB4 : endothelial marker
LacZ : Wnt

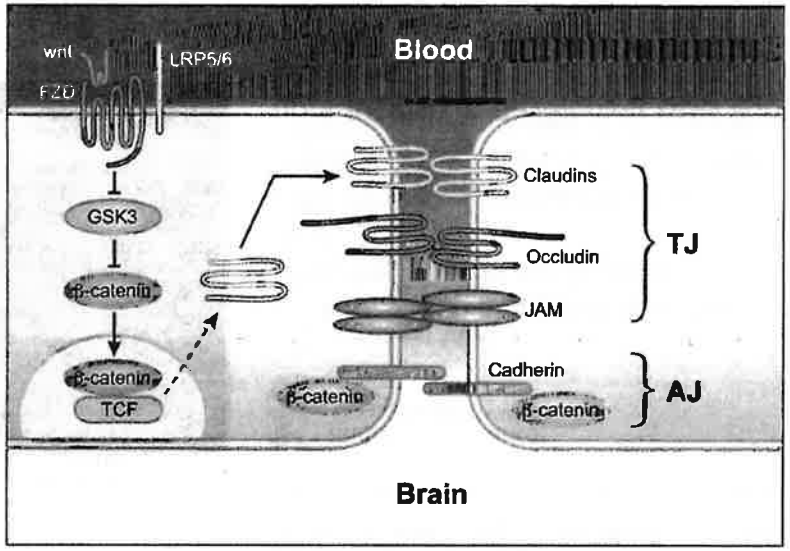
- Canonical Wnt signaling active in endothelial cells during brain angiogenesis
- Becomes progressively down-regulated during vessel maturation
- Corresponds to period of Claudin-3 upregulation in developing BBB tight junctions

*Liebner et al. 2008 J. Cell Biol. 183:409-417*



Canonical Wnt signaling is active in ECs during brain angiogenesis and becomes progressively down-regulated during vessel maturation. (A) LacZ whole-mount stained (blue) E9.5 BAT-Gal embryos sectioned and counterstained for Isolectin B4 (IB4; red). Panel b is a higher magnification of the boxed area in panel a. Arrows point to nuclear LacZ reporter gene staining. (B, a–d) Whole-mount hindbrain staining for LacZ (reflection; red) and IB4 (green) of BAT-Gal embryos (E13.5 and 17.5) analyzed by confocal microscopy. Arrowheads indicate LacZ-positive nuclei. (B, e–h) Staining of brain cryosections from postnatal BAT-Gal pups (P1 and P5) for LacZ (immunofluorescence [IF], red) and IB4 (green). Arrowheads indicate LacZ-positive nuclei. Positive nuclei outside the vascular system indicate active Wnt signaling in the brain parenchyma. (C) Quantification of LacZ-positive nuclei per 100-µm vessel length shows a significant decrease from E15.5 to 17.5 (five fields per hindbrain; three brains; **, P = 0.0003). Error bars represent SEM.

# Wnt signaling and the BBB




*Polakis J. Cell Biol. 2008:183:371-373*

SYNG-PQ-00471834

Wnt signaling and the BBB. Depiction of the primary constituents of the tight junction (TJ) and the adherens junction (AJ) at the interface between endothelial cell plasma membranes. Activation of Wnt receptors FZD and LRP5/6 inhibits GSK3 to stabilize β-catenin that in turn enters the nucleus to activate T cell factor (TCF)–dependent transcription. This drives Cldn3 gene activation either directly or indirectly (dashed line arrow), and the resulting Cldn protein reinforces the tight junction. JAM, junctional adhesion molecule.

SYNG-PQ-00471835

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KINGS College LONDON

# Enzyme-Replacement Therapy from Birth Delays the Development of Behavior and Learning Problems in Mucopolysaccharidosis Type IIIA Mice

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*Lysosomal Diseases Research Unit, Women's and Children's Hospital, North Adelaide, South Australia 5006, Australia*

## ABSTRACT

Mucopolysaccharidosis type IIIA (MPS IIIA; Sanfilippo syndrome) is a lysosomal storage disorder characterized by severe CNS degeneration, resulting in behavioral abnormalities and loss of learned abilities. Early treatment is vital to prevent long-term clinical pathology in lysosomal storage disorders. We have used naturally occurring MPS IIIA mice to assess the effects of long-term enzyme-replacement therapy initiated either at birth or at 6 wk of age. MPS IIIA and normal control mice received weekly i.v. injections of 1 mg/kg recombinant human sulfamidase until 20 wk of age. Sulfamidase is able to enter the brain until the blood-brain barrier completely closes at 10–14 d of age. MPS IIIA mice that were treated from birth demonstrated normal weight, behavioral characteristics, and ability to learn. MPS IIIA mice that wer[illegible]d from birth performed significantly better in the Morri[illegible]ze than MPS IIIA mice that were treated from 6 wk of age or left untreated. A reduction in storage vacuoles in cells of the CNS in MPS IIIA mice that were treated from birth is consistent with the improvements observed. These data suggest that enzyme that enters the brain in the first few weeks of life, before the blood-brain barrier matures, is able to delay the development of behavior and learning difficulties in MPS IIIA mice. (*Pediatr Res* 56: 65–72, 2004)

**Abbreviations**

**BBB**, blood-brain barrier
**ERT**, enzyme-replacement therapy
**LSD**, lysosomal storage disorder
**MPS**, mucopolysaccharidosis
**rhNS**, recombinant human sulfamidase

SYNG-PQ-00471836