

CNS barriers: critical interfaces for CNS entry of paraquat

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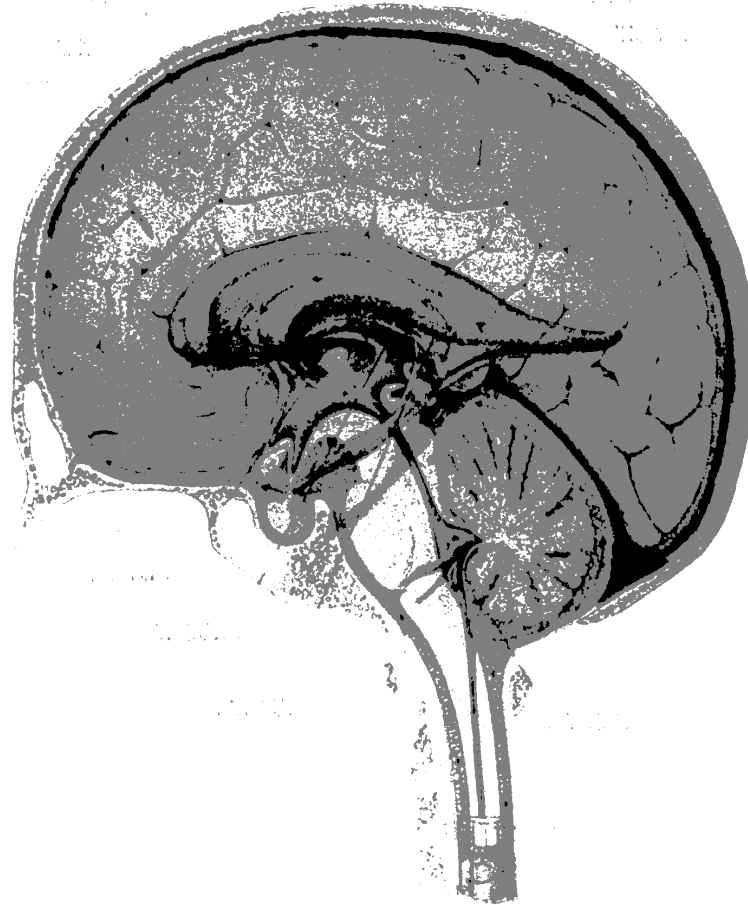
*Syngenta Discussion
Marlow
20 April 2009*

Case ID: 220300429

SYNG-PQ-01148991

BRAIN FLUID COMPARTMENTS

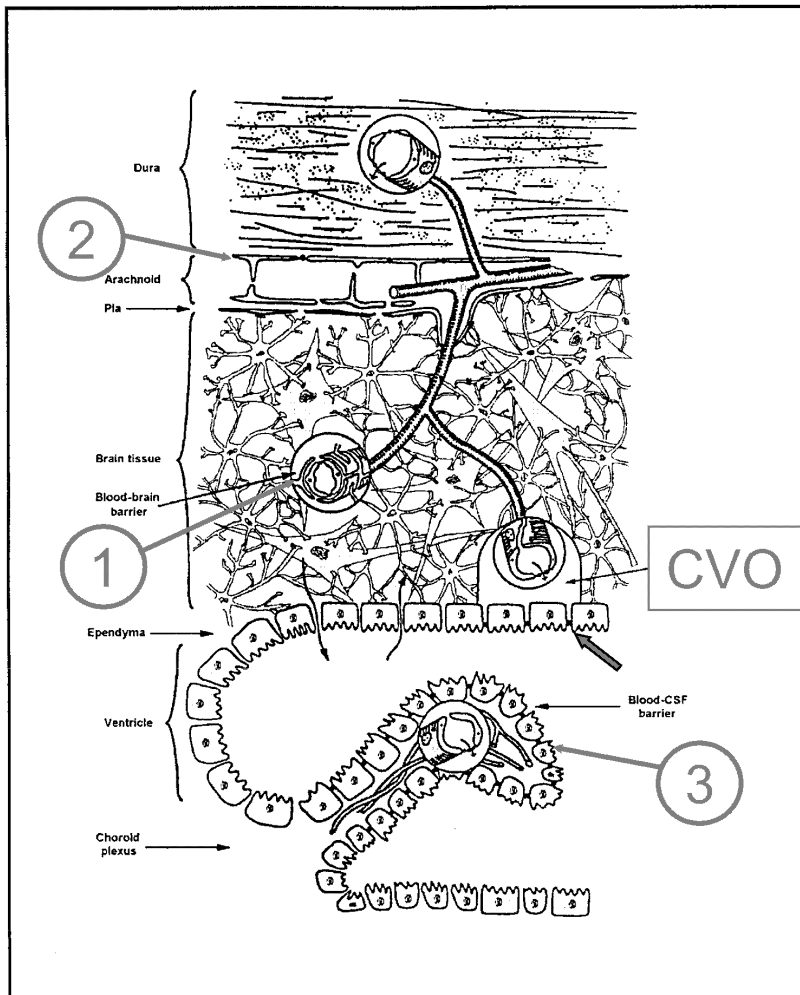
- Blood
- Cerebrospinal fluid (CSF)
- Interstitial fluid (ISF = ECF)



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



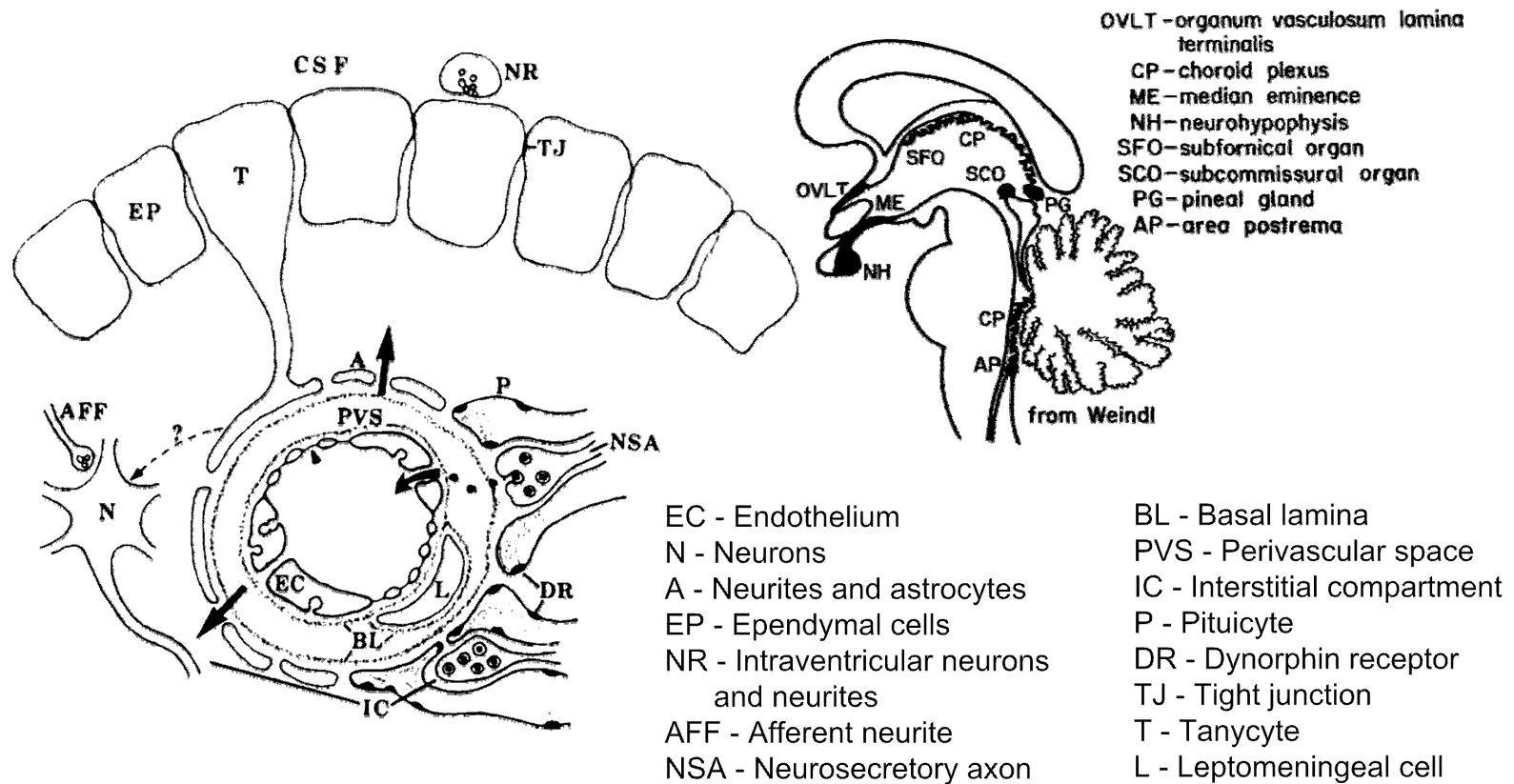
SITES OF CNS BARRIERS

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

CVO :
circumventricular organs

*Segal and Zlokovic 1990,
modified by A Reichel*

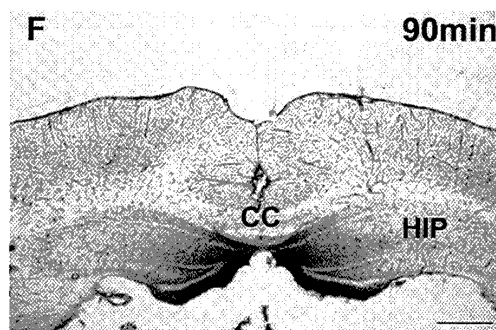
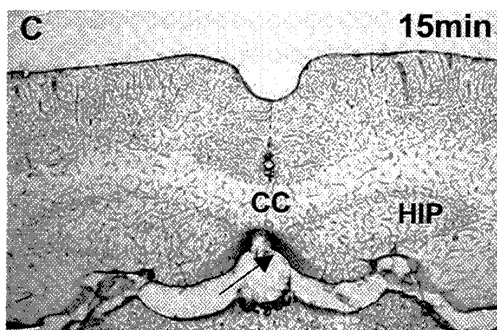
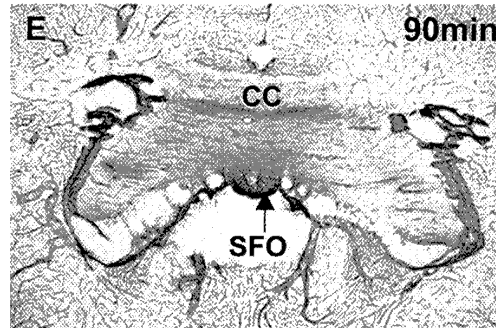
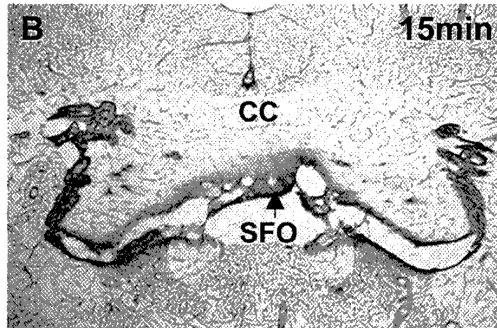
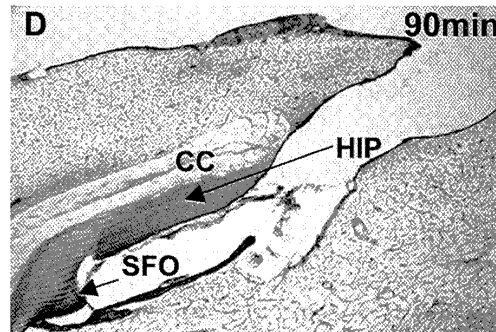
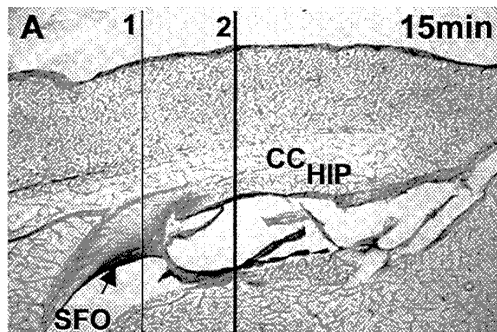
CIRCUMVENTRICULAR ORGANS (CVO)



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

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HRP tracer distribution in brain after iv injection

..often in regions adjacent to CVOs

Mouse
SFO: subfornical organ (CVO)

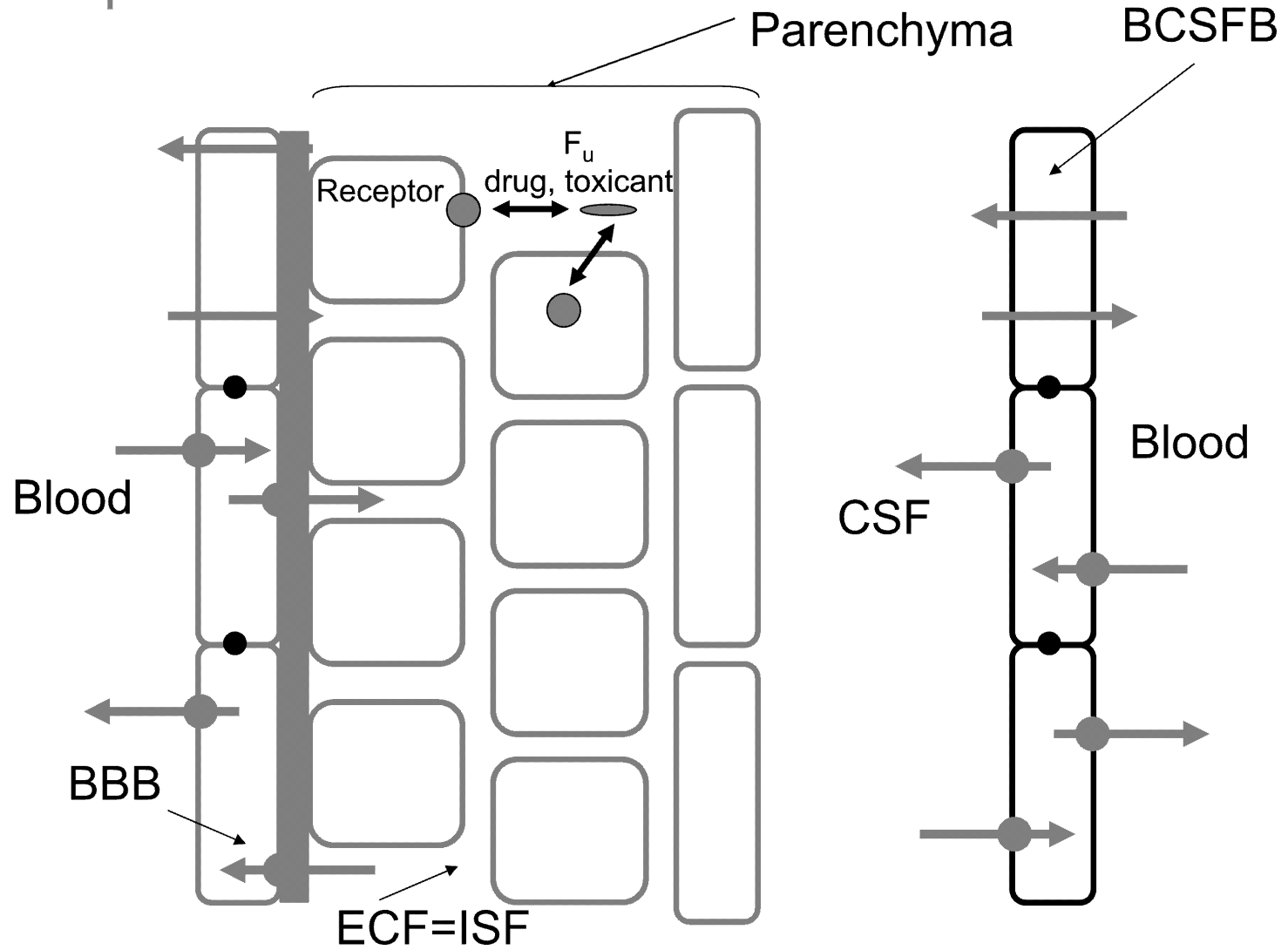
CC: corpus callosum
HIP: hippocampus

Ueno 2007 Curr Med Chem 14: 1199

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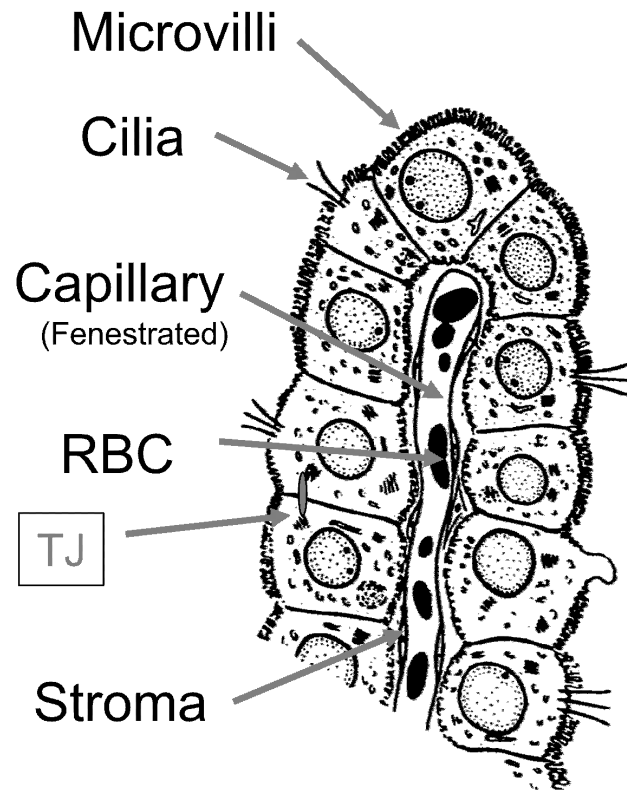
Compartments of the CNS



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CHOROID PLEXUS EPITHELIUM

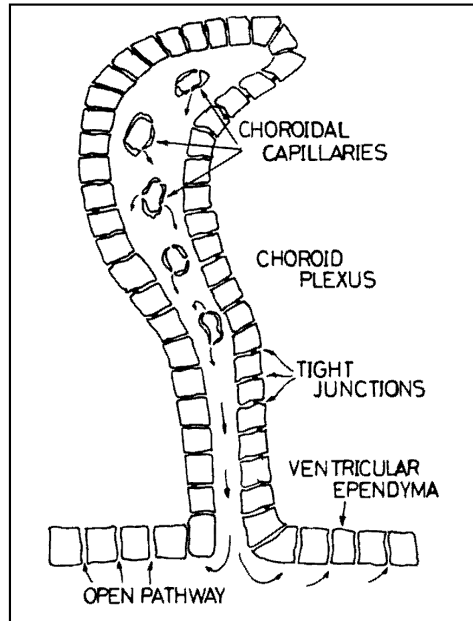


Anatomical barrier site:

- 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

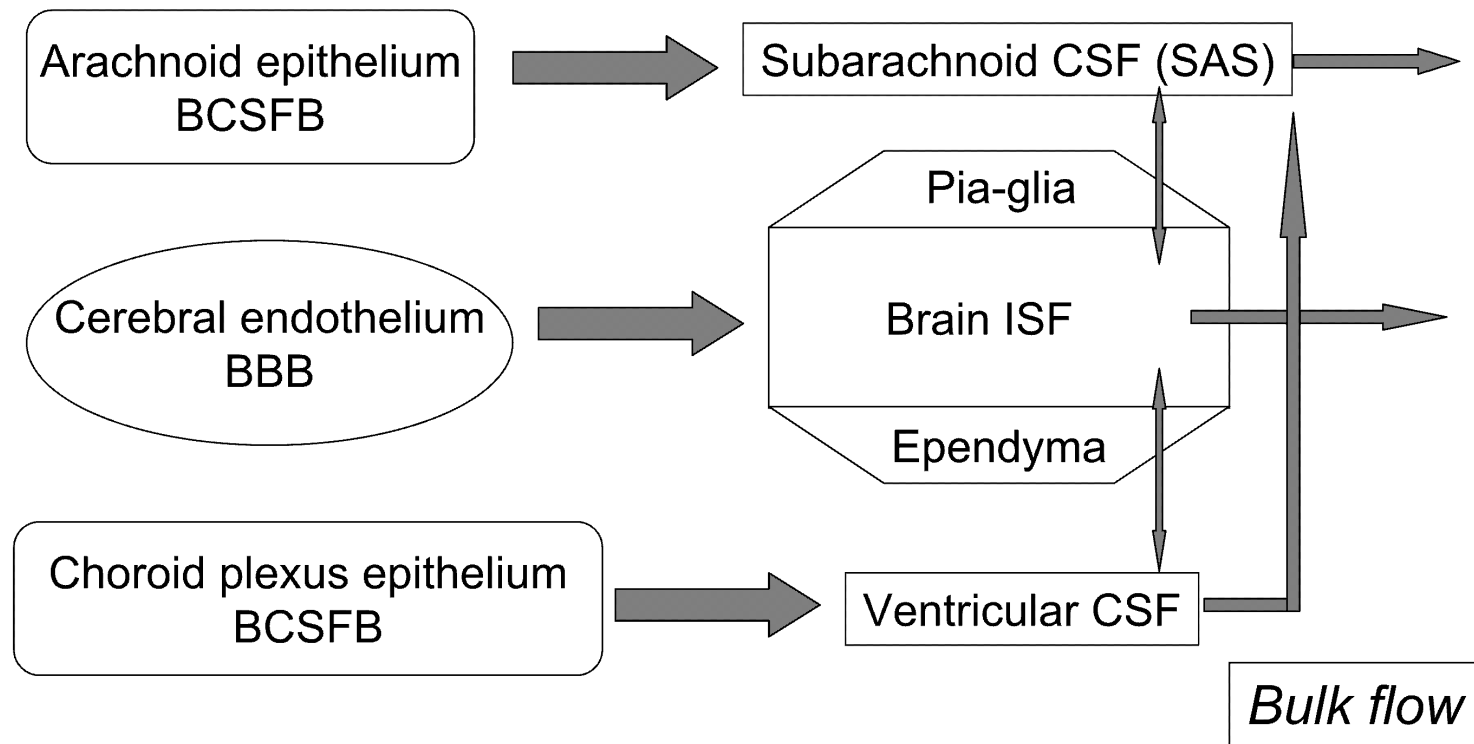
Solute	Units	Plasma	CSF	Ratio
Na	mM	140	131	~1
K	mM	4.6	2.9	0.63
Ca	mM	5.0	2.5	0.5
Mg	mM	1.2	2.4	1.4
Cl	mM	101	124	1.23
HCO ₃	mM	23	21	0.91
Osmolality	mOsmol	305.2	298.5	~1
pH		7.4	7.3	
Glucose	mM	5.0	3.0	0.6
Total amino acid	μM	2540	280	0.11
Leucine	μM	100	10.1-14.9	0.10-0.14
Arginine	μM	30	14.2-21.6	0.18-0.27
Glycine	μM	242	4.5-8.5	0.012-0.034
Alanine	μM	230	23.2-32.7	0.07-0.1
Serine	μM	140	23.5-37.8	0.16-0.25
Glutamic acid	μM	25	1.79-14.7	0.02-0.19
Tyrosine	μM	75	3.6-5	0.07-0.066
Total Protein	mg/ml	70	0.477	0.006
Albumin	mg/ml	42	0.192	0.004
Immunoglobulin G (IgG)	mg/ml	2.57	0.017	0.001
Transferrin	mg/ml	2.6	0.014	0.005
Plasminogen	mg/ml	0.7	0.000074	0.00001
Fibrinogen	mg/ml	2.5	0.00075	0.00003
α ₂ -macroglobulin	mg/ml	1	0.0046	0.0015
Cystatin C	mg/ml	0.001	0.001	1.0

Compiled from various sources, values mostly human.

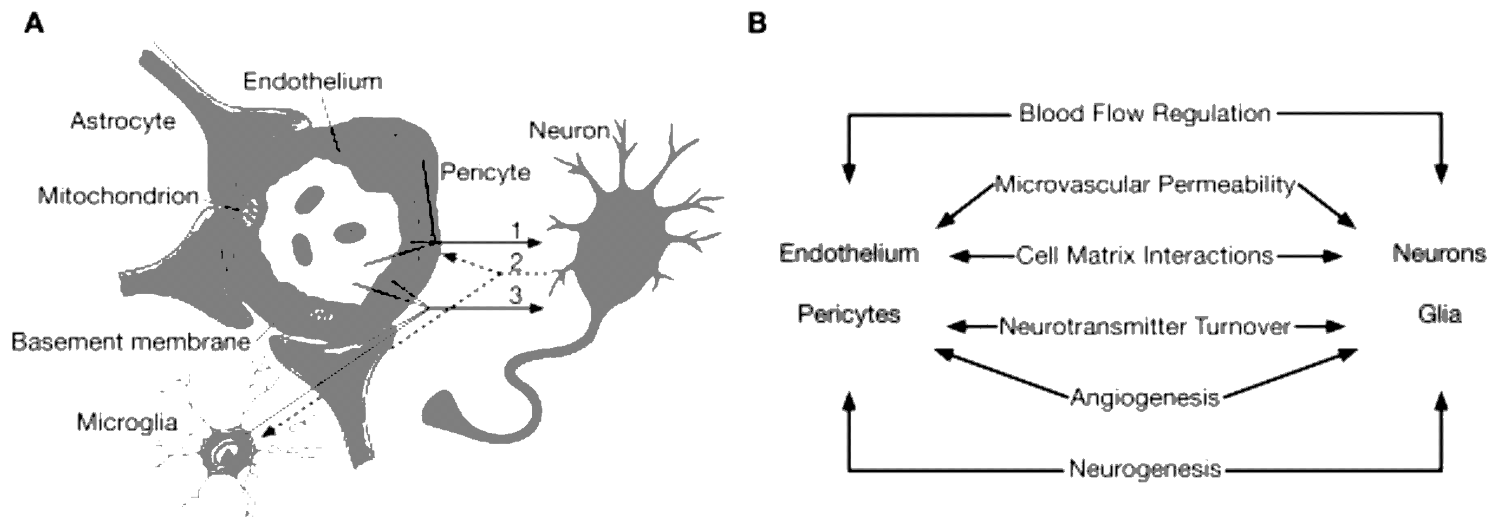
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Barriers and compartments



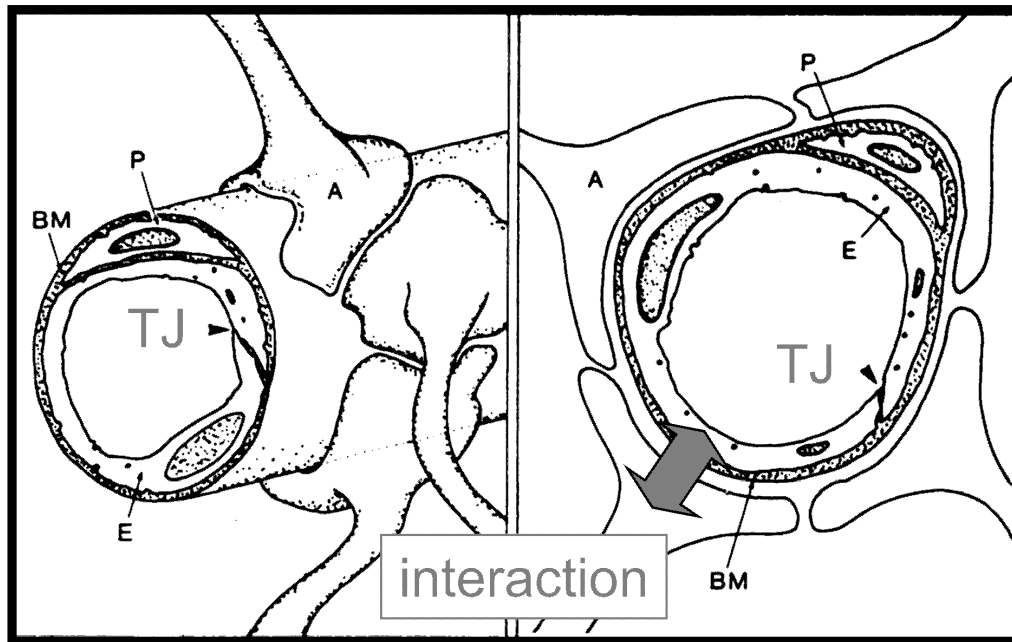
Neurovascular Unit (NVU): Cell associations



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

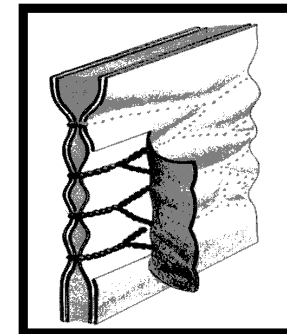
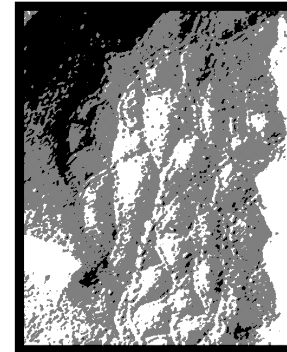
Zlokovic 2008 Neuron 57:178-201

Blood-Brain Barrier Structure



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

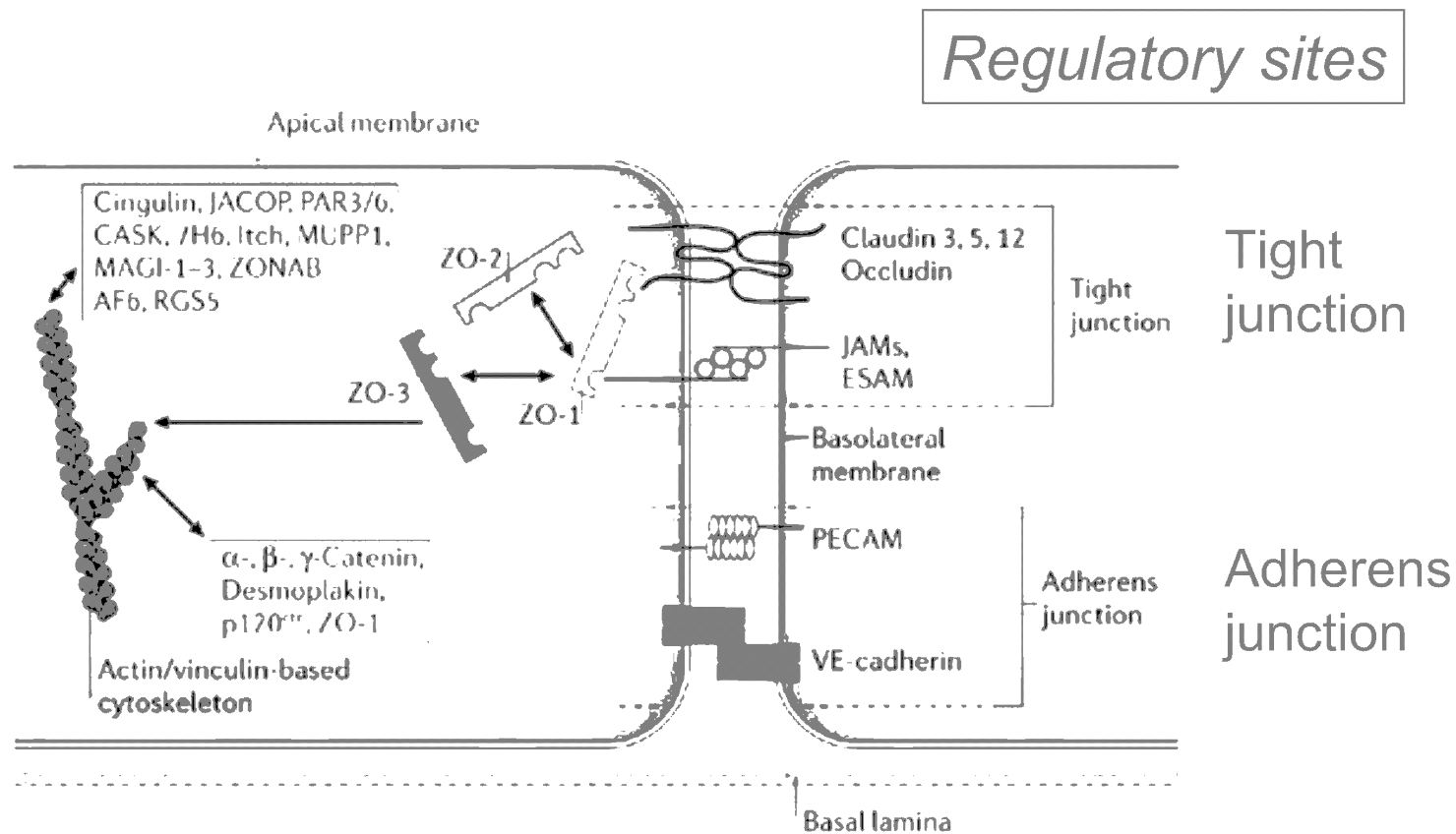
+ other cell types: microglia, macrophages, leukocytes



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Molecular structure of BBB tight junctions



Abbott et al 2006 Nat Rev Neurosci 7:41

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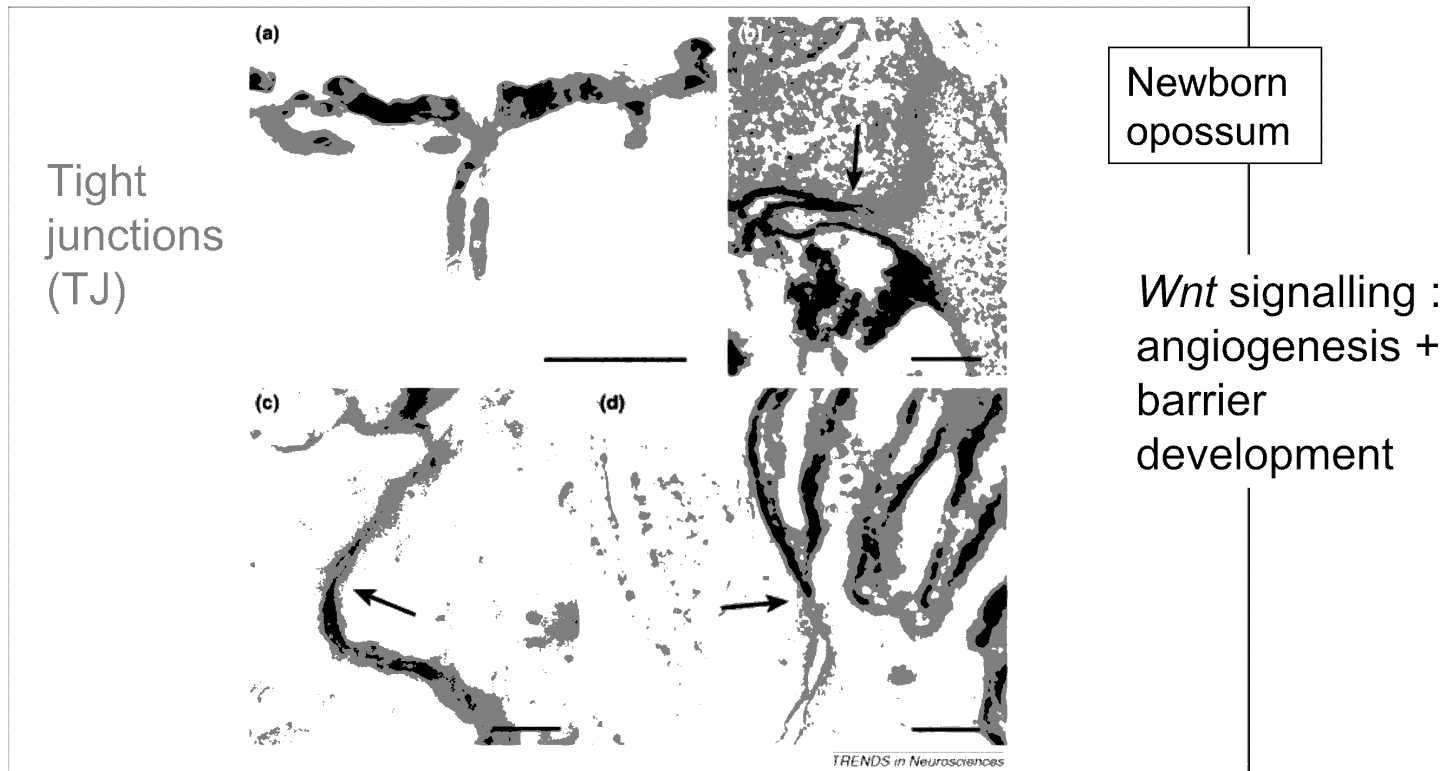
SYNG-PQ-01149003

DEVELOPMENT OF CNS BARRIERS

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Development of BBB – at the time of vessel ingrowth

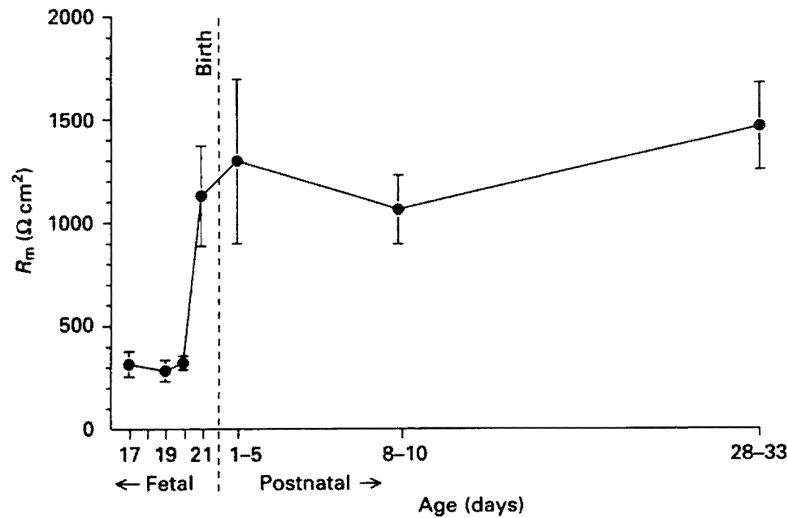


3 kDa biotin-dextran amine 30 min IP

Saunders et al. 2008. TINS 31:279

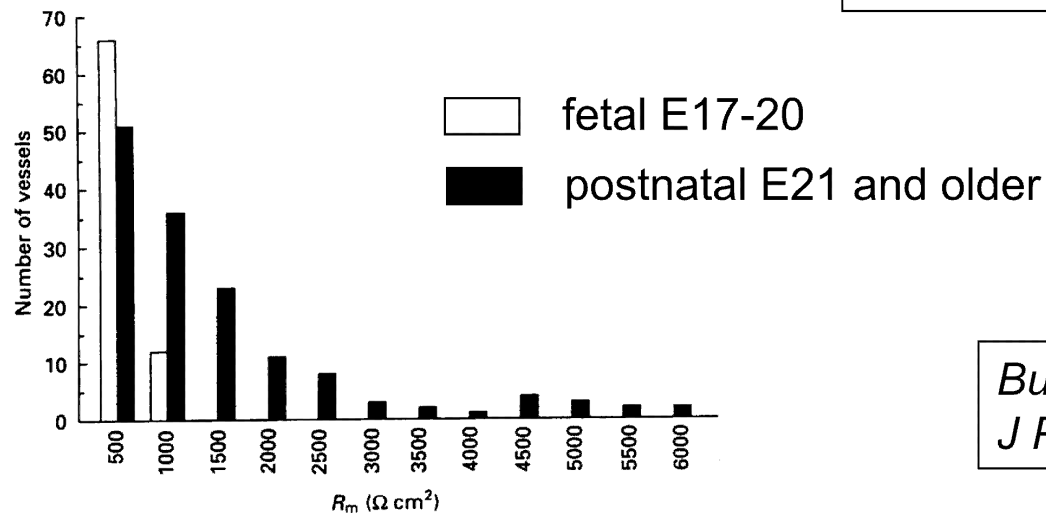
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Tightening of BBB
in rat pial vessels at
birth: tight junctions

TEER: transendothelial
electrical resistance R_m

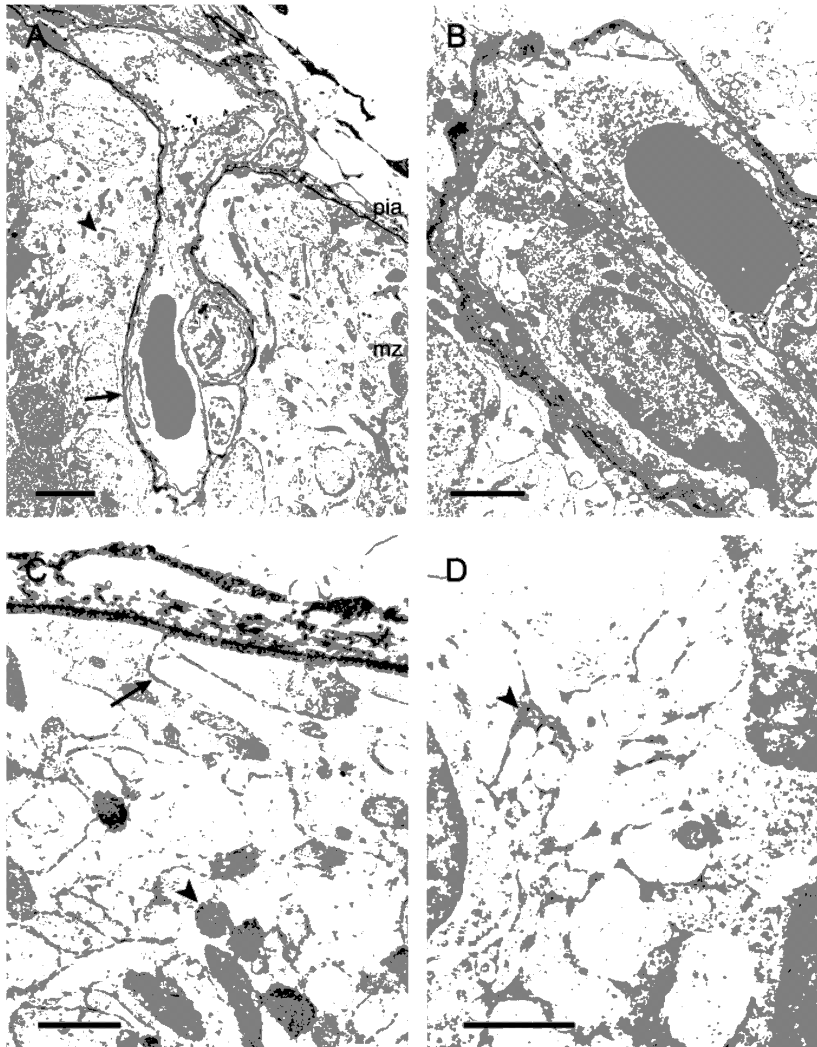


Butt et al. 1990
J Physiol 429:47

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 $\mu\text{L.g}^{-1}.\text{min}^{-1}$ from E21 to P2 (3)
- K_{in} for mannitol is between 0.19 and 0.22 $\mu\text{L}.\mu\text{L.g}^{-1}.\text{min}^{-1}$ 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) Grøntoft 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

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FUNCTIONS OF BBB & BSCB

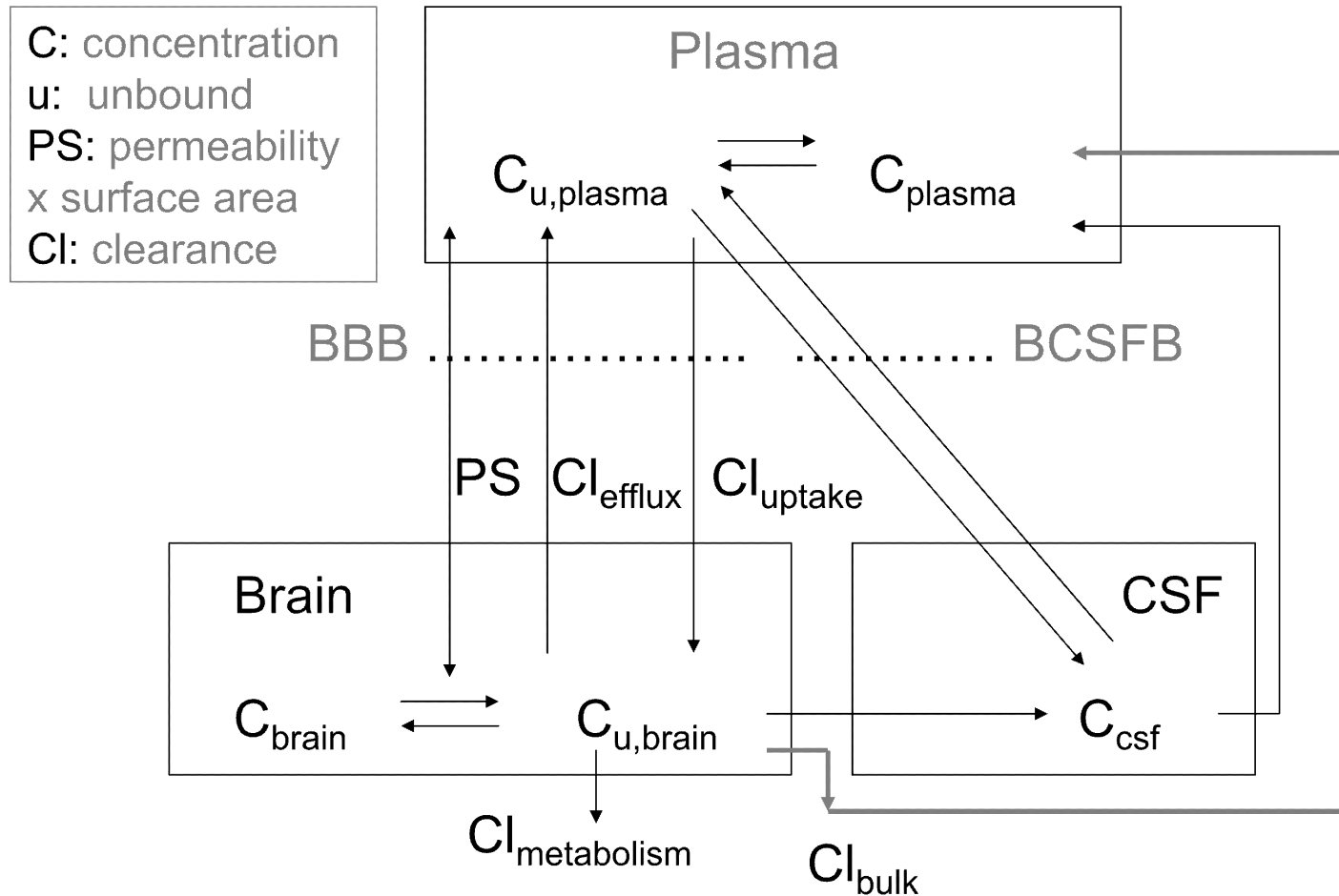
- * 1. Control molecular traffic, keep out toxins
(precise connectivity: low cell death)
- * 2. Ion homeostasis - optimal neural signalling
- 3. Low protein environment, limit proliferation
- * 4. Separate CNS:PNS neurotransmitter pools;
allow non-synaptic signalling
- 5. Immune surveillance with minimal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



Selective
advantage

** Functions needing tightest endothelium*

Compartmental analysis: CNS Drug distribution

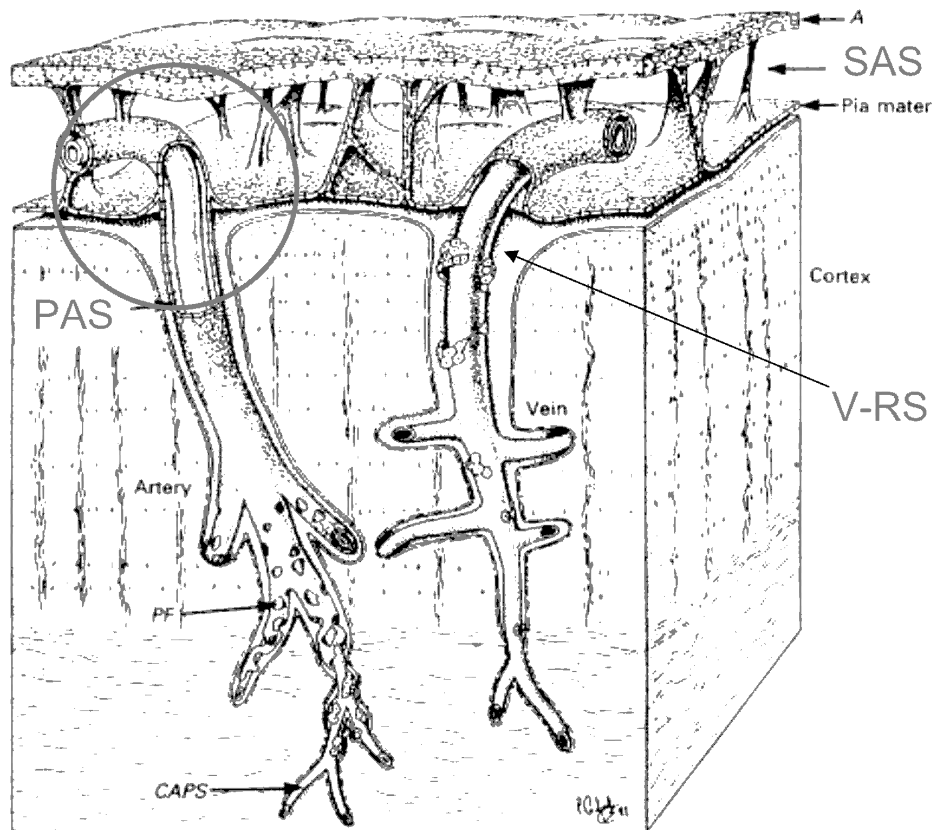


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Abbott 2004
Neurochem Int 45:545

Perivascular channels for ISF flow: human



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

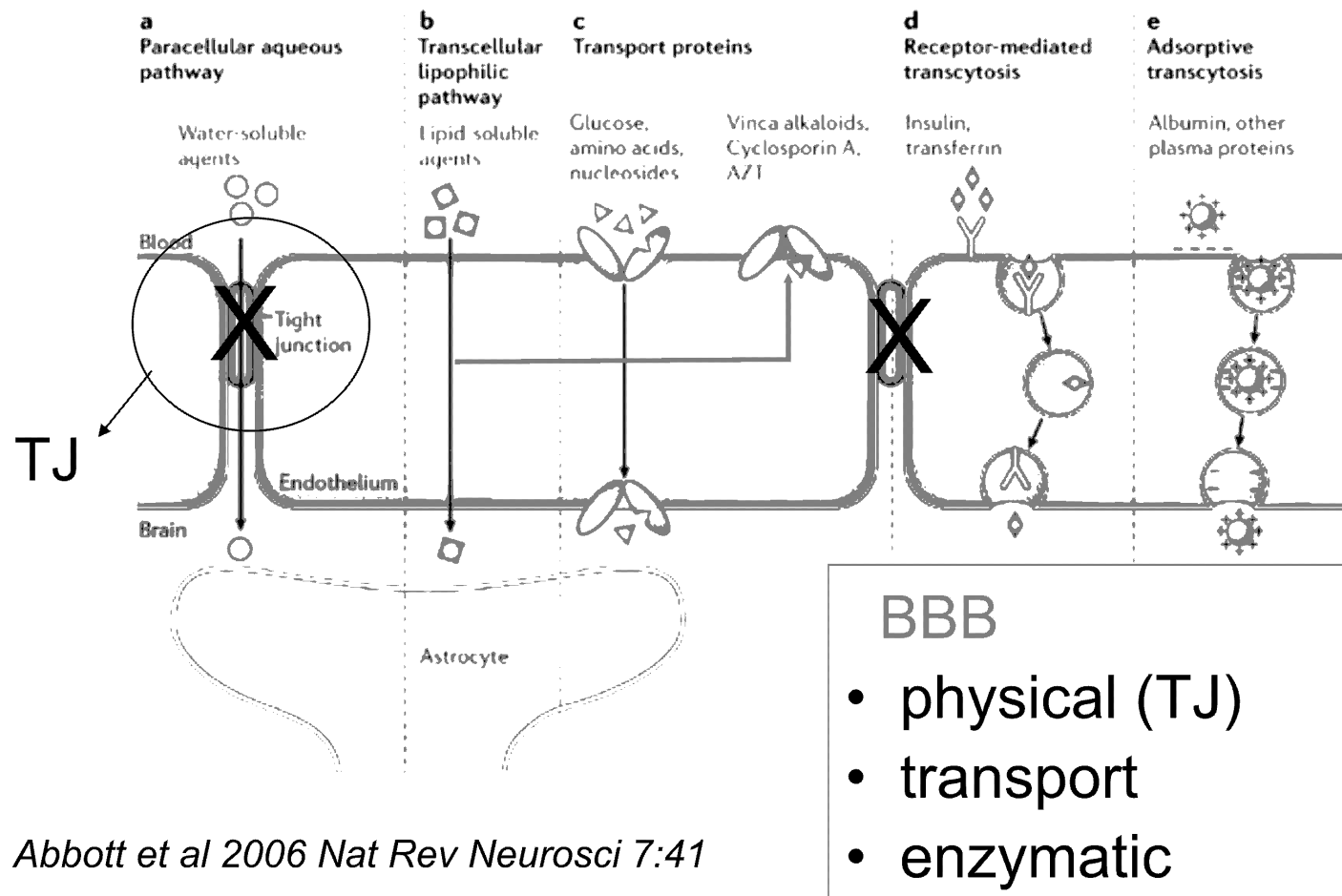
A, arachnoid
PF, perforations
CAPS, capillaries

Zhang et al 1990 J Anat 170:111

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Pathways across the blood-brain barrier

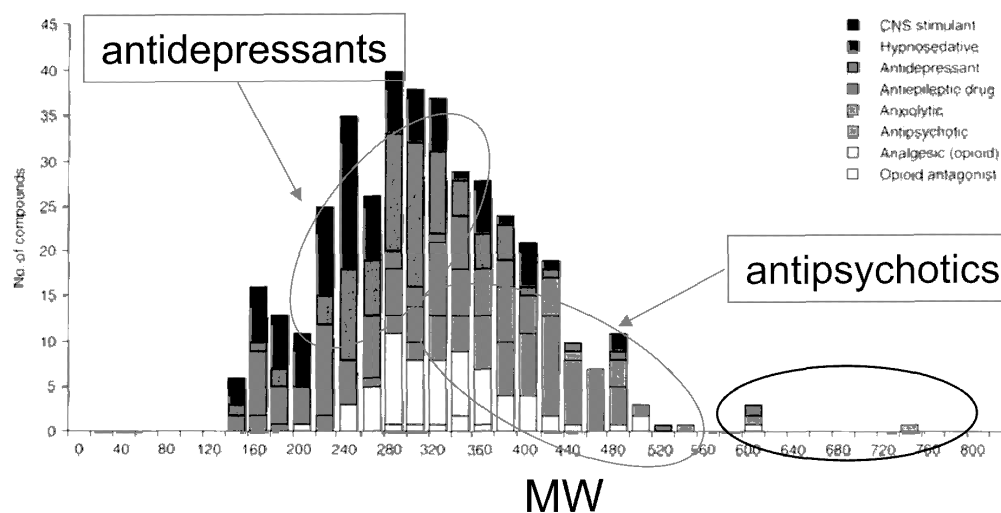


Abbott et al 2006 Nat Rev Neurosci 7:41

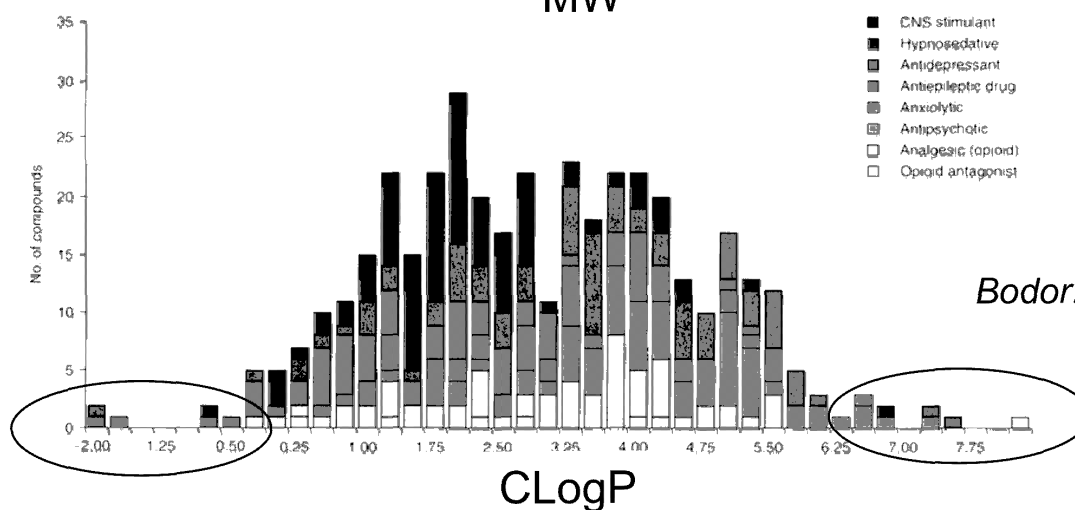
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CNS drugs : Molecular weight and lipophilicity



Learning from
successful
drugs :
Darwinian
approach

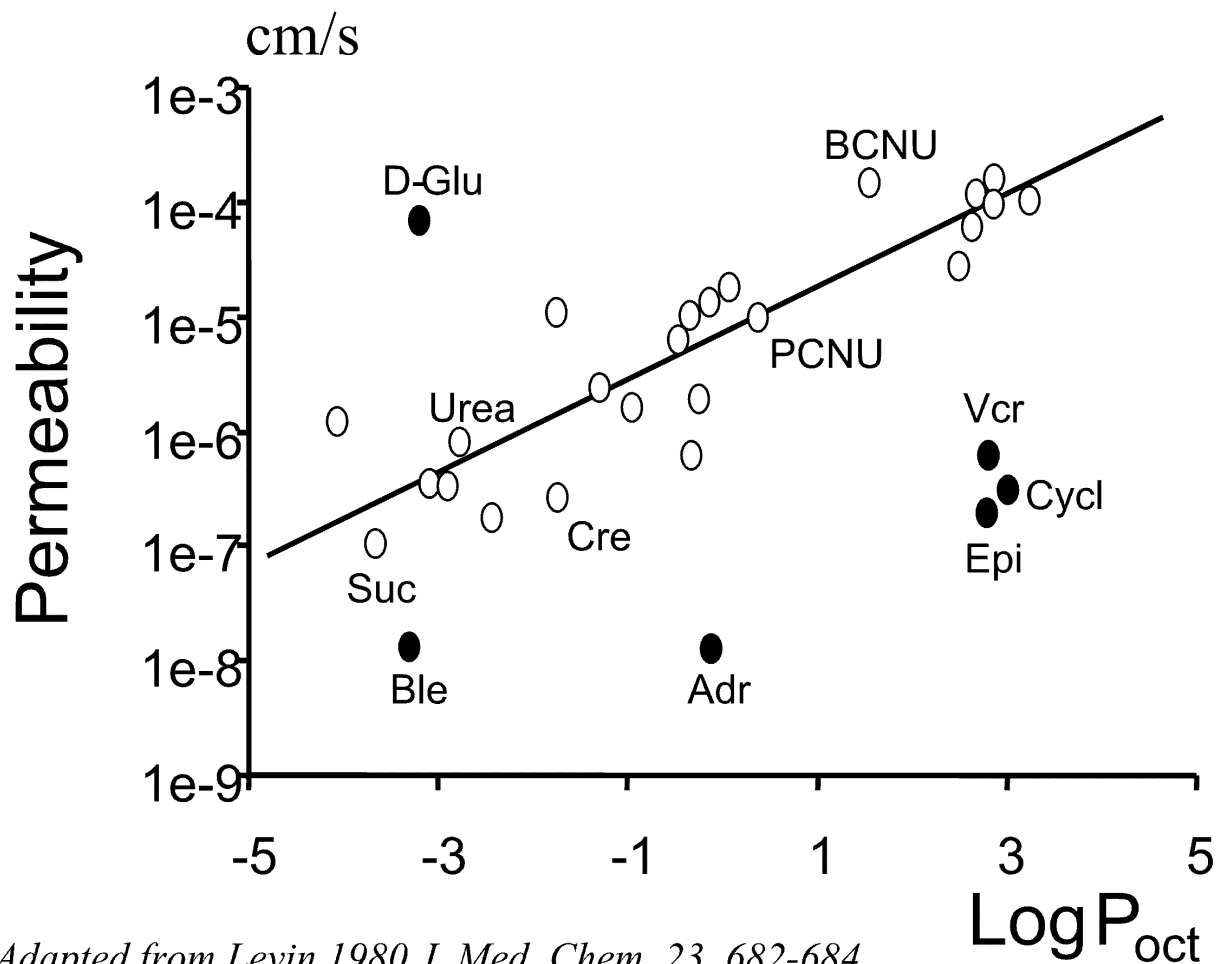


Bodor: Am J Drug Del 1: 2003

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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) $A_D < 80 \text{\AA}^2$

Deteriorating properties

- Polarity $\text{PSA} > 60 \text{\AA}^2$
- Lewis base strength
- Hydrogen-bonding esp. HBA, β^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)

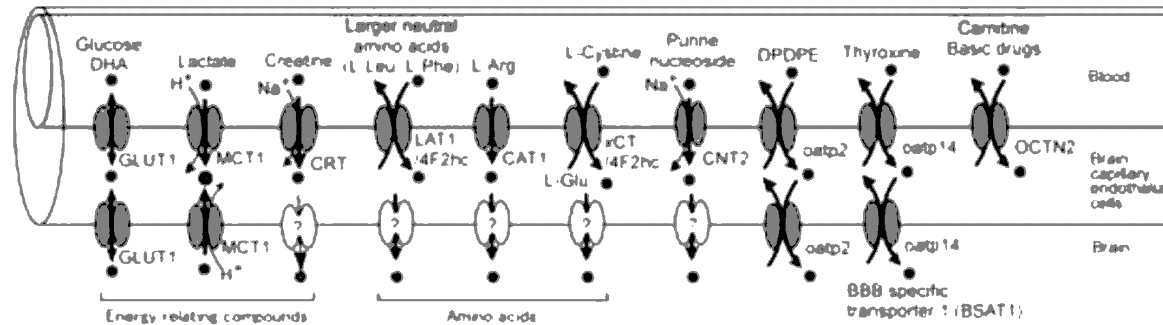
Improving properties

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

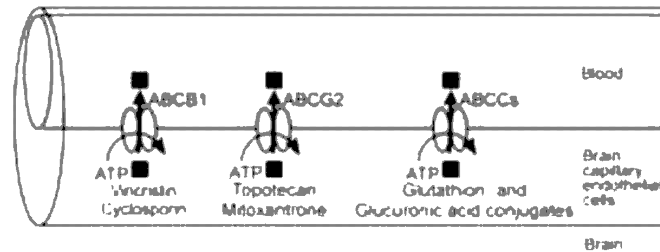
Pardridge (1998)
Fischer et al. (1998)
Ajay et al. (1999)
Norinder et al. (1998)
Lombardo et al. (1996)
Clark (1999)

BBB TRANSPORT Specific carriers

a Blood-to-brain influx transporters

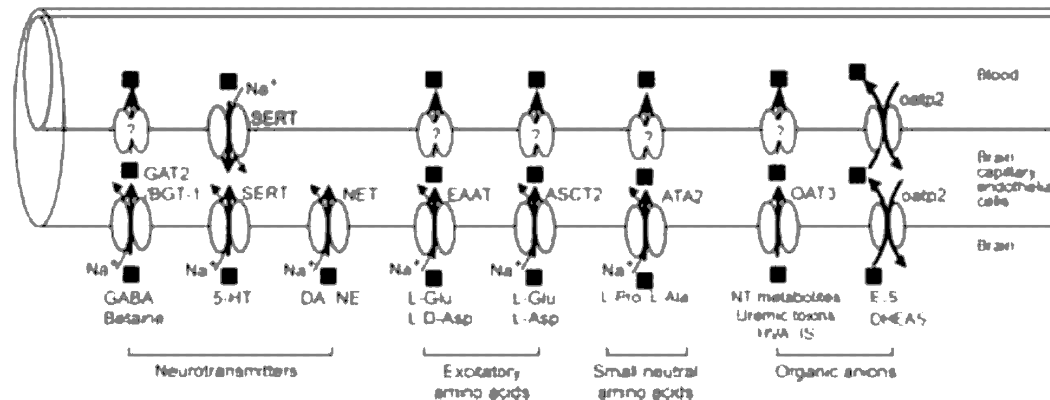


b ATP-binding cassette transporters



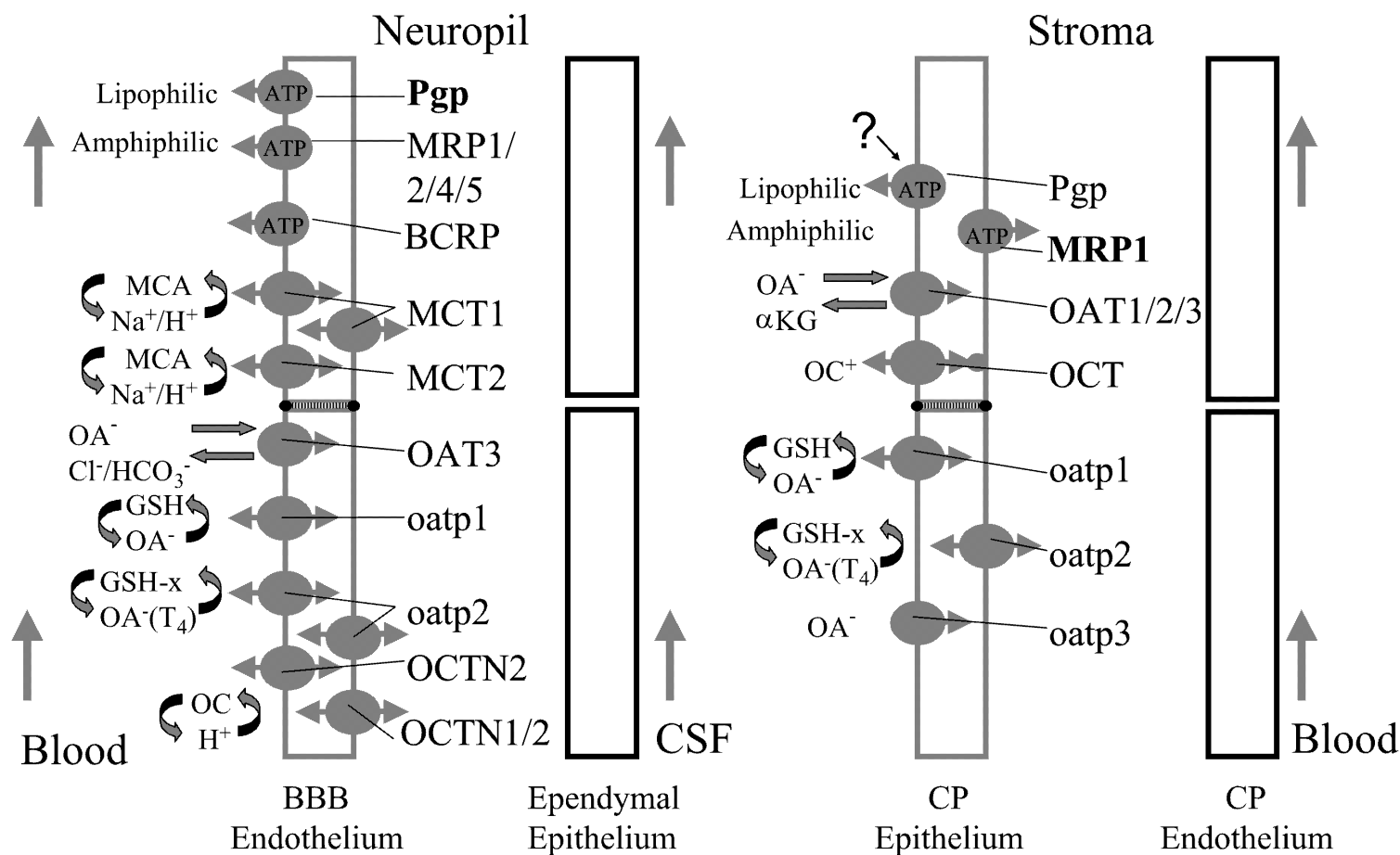
BBB transporters

c Brain-to-blood efflux transporters



Ohtsuki 2007
Pharm Res
24:1745-58

Efflux transporters in the BBB and B-CSF-B

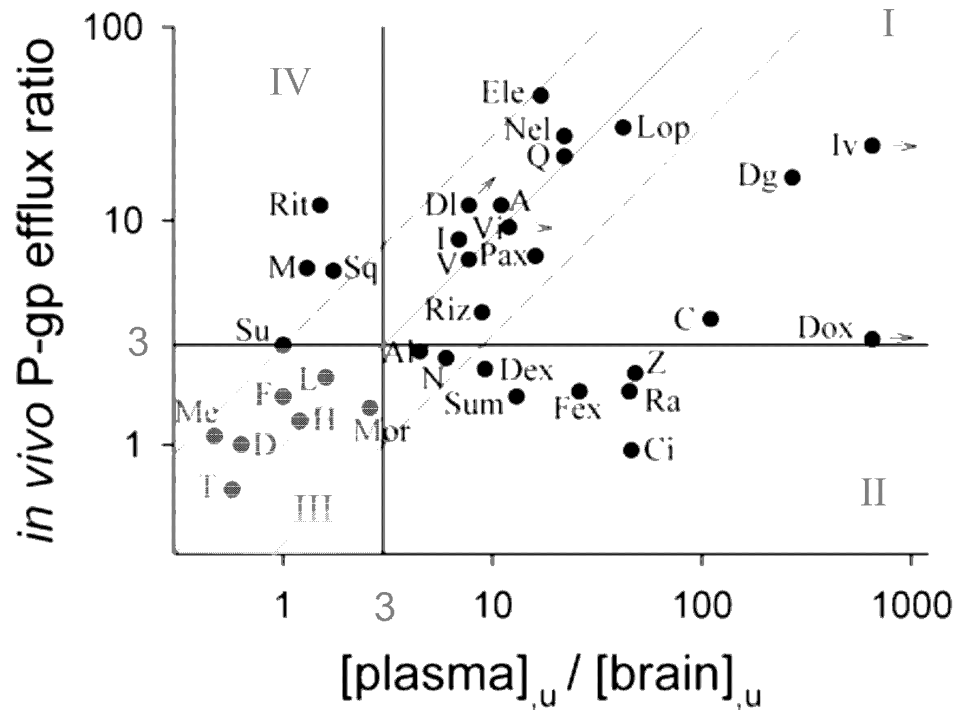


Begley 2004 In Sharma & Westman volume (Spinal Cord)

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Measurements to segregate drugs



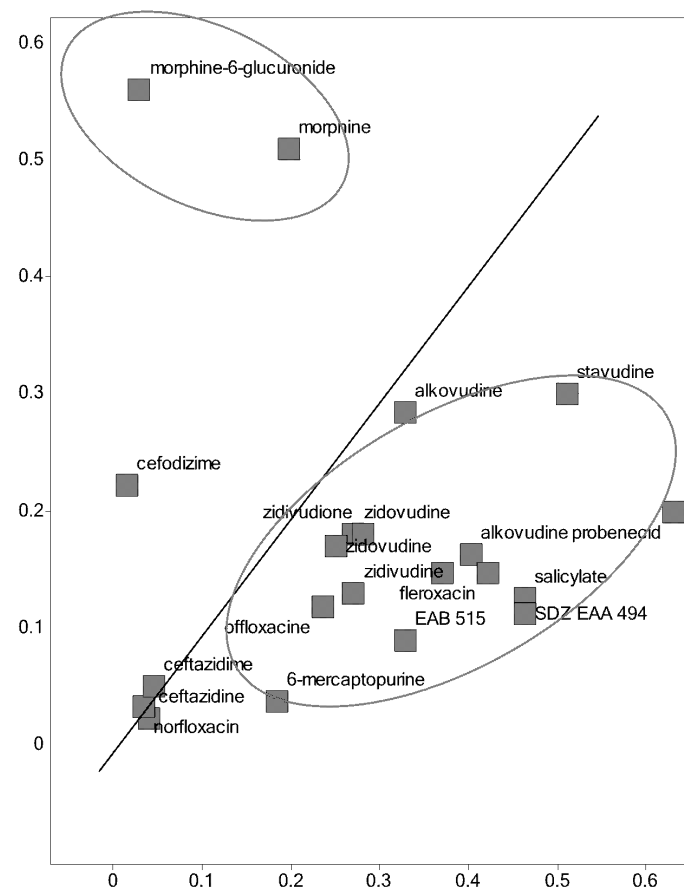
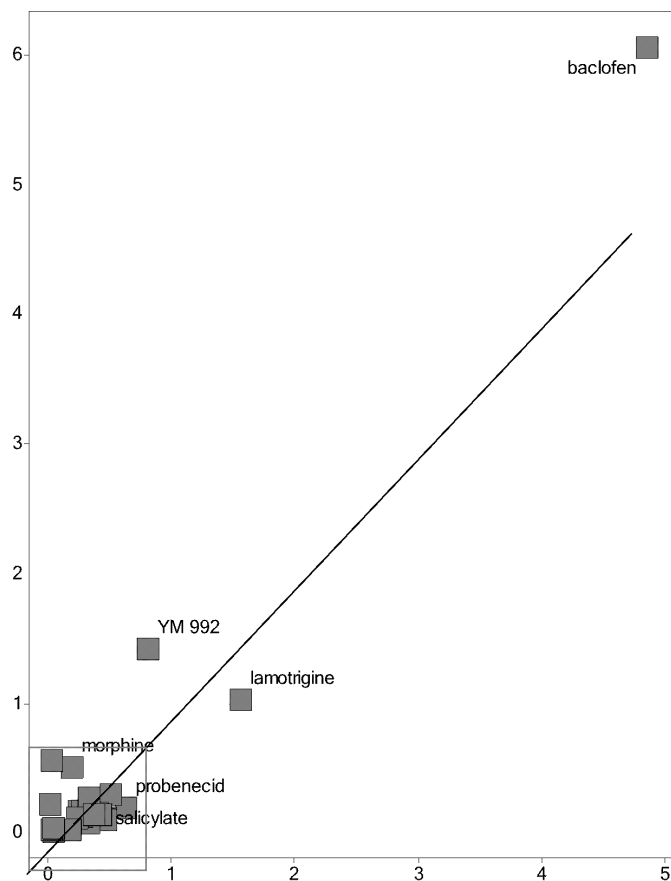
Kalvass et al. 2007 Drug Metab Dispos 35:660

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Comparison of ISF_(microdialysis) with CSF

ISF/Plasma_{fu}



CSF/Plasma_{fu}

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

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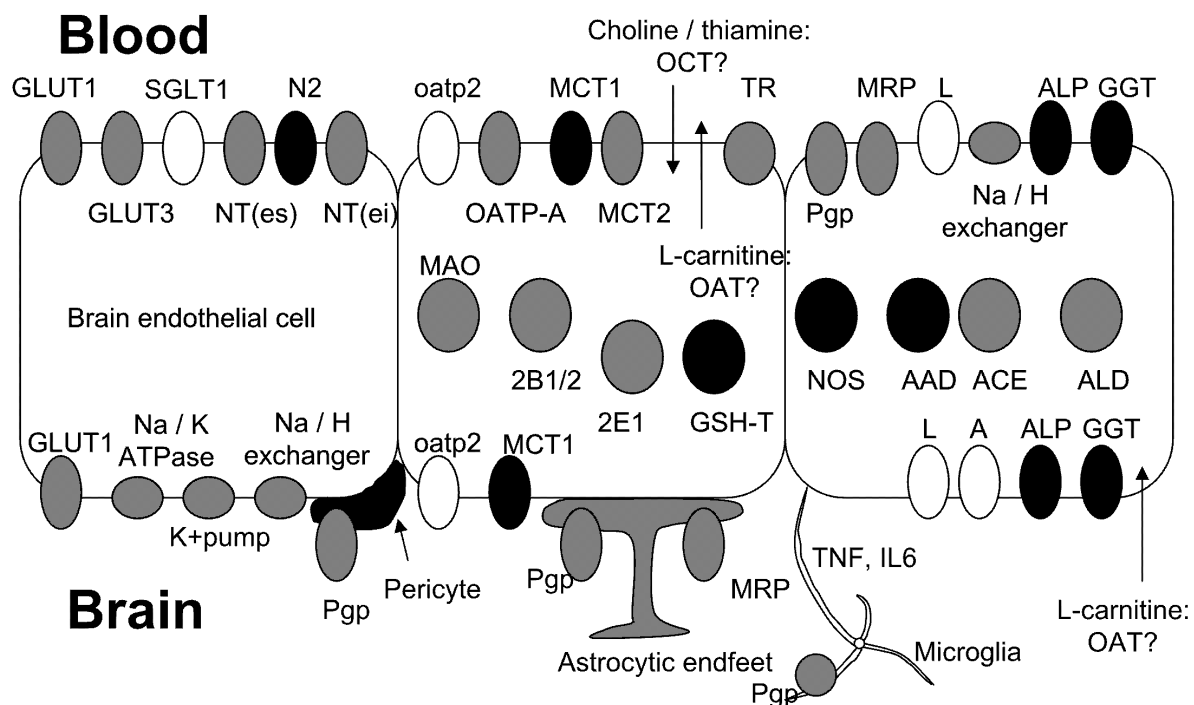
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Drug metabolism in BBB and choroid plexus

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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 transporter; OAT-organic anion transporter; Na⁺/K⁺-ATPase-sodium-potassium ATPase; K⁺-pump - potassium pump.

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

Agents : TNF α , IL-6 - cytokines.

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

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Metabolic conversion in the CNS

Phase 1 (Functionalisation)

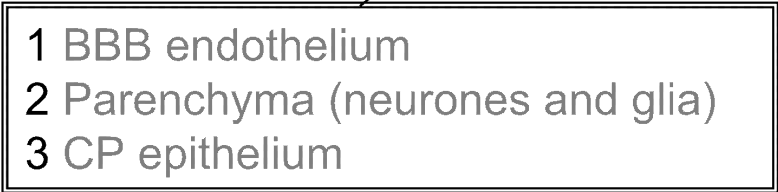
MAO ¹ : flavone dependant mono-oxygenases ² : cytochromes P450 ^{1, 2, 3}
NADPH-cytochrome P450 reductase ^{1, 3} : epoxide hydrolases ^{1, 2, 3}

Phase 2 (Conjugation)

UDP-glucuronyltransferases ^{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

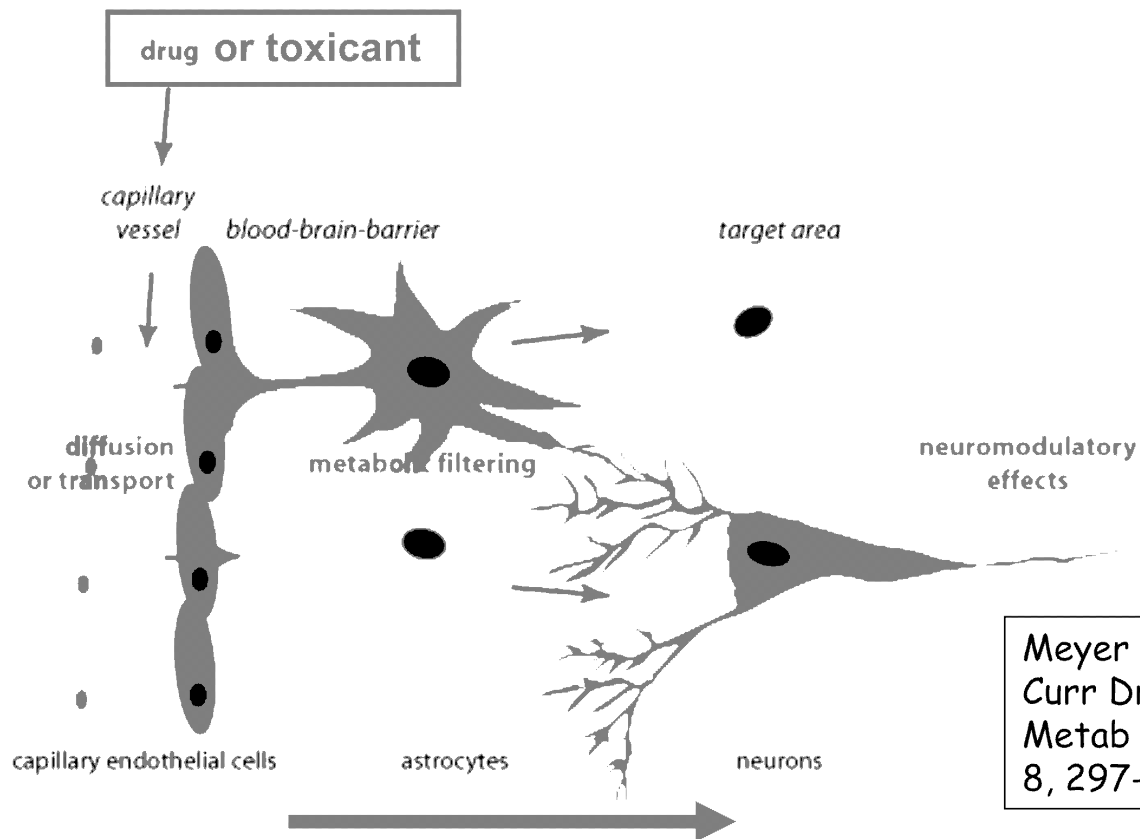
UGT – Uridine diphosphate glucuronosyltransferase

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

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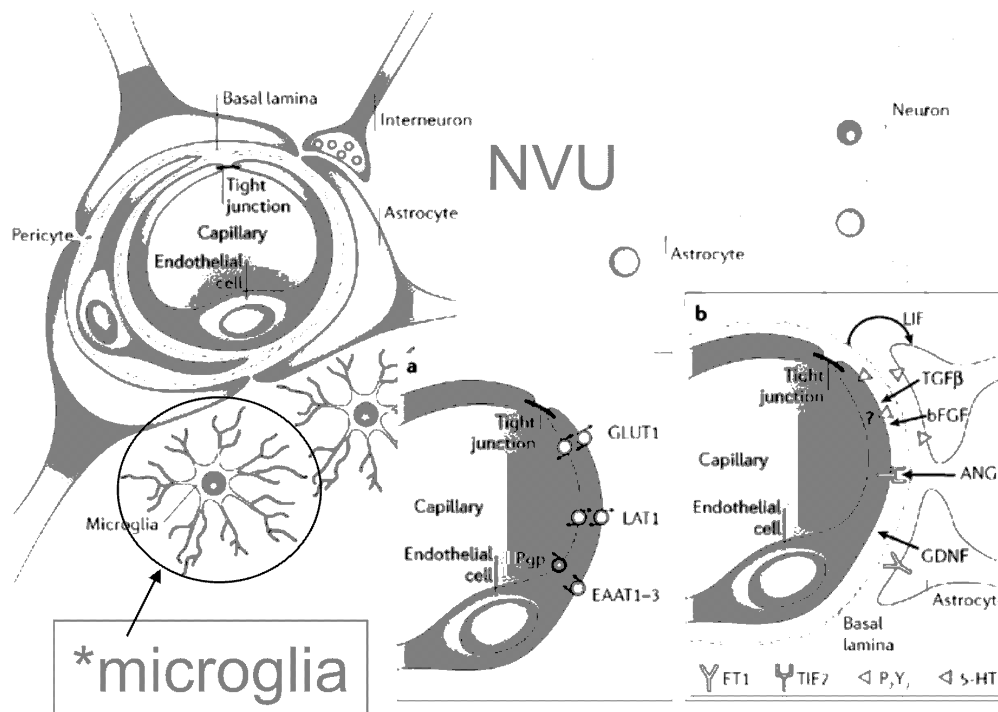
SYNG-PQ-01149025

Summary: BBB enzymes as metabolic filter



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

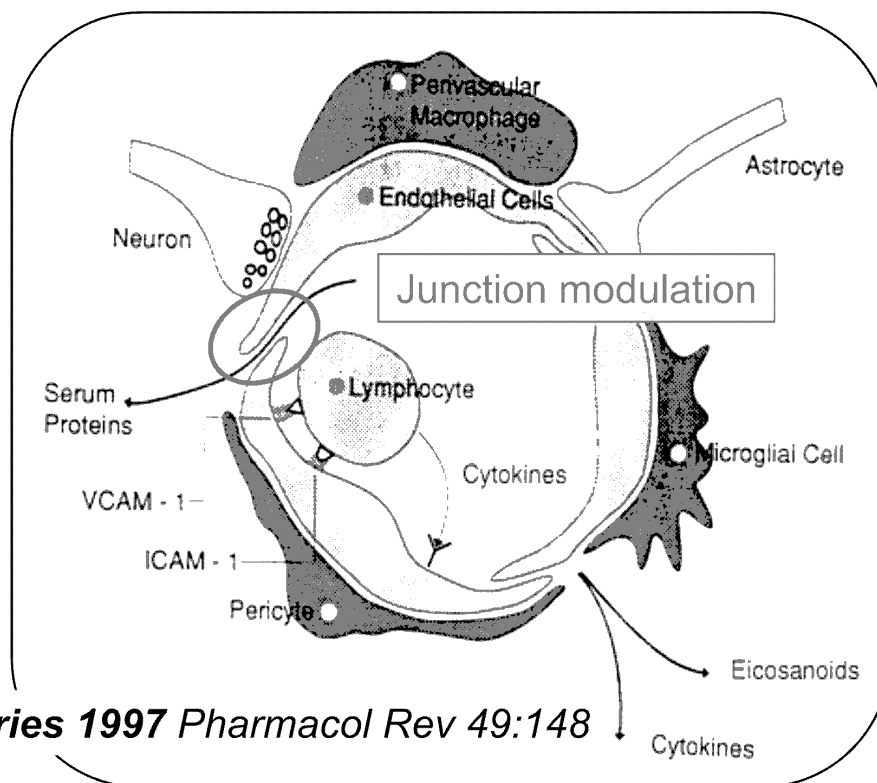
Receptors:
• Signalling
within
Neurovascular
Unit (NVU)
• Regulating
permeability
and transport

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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?**

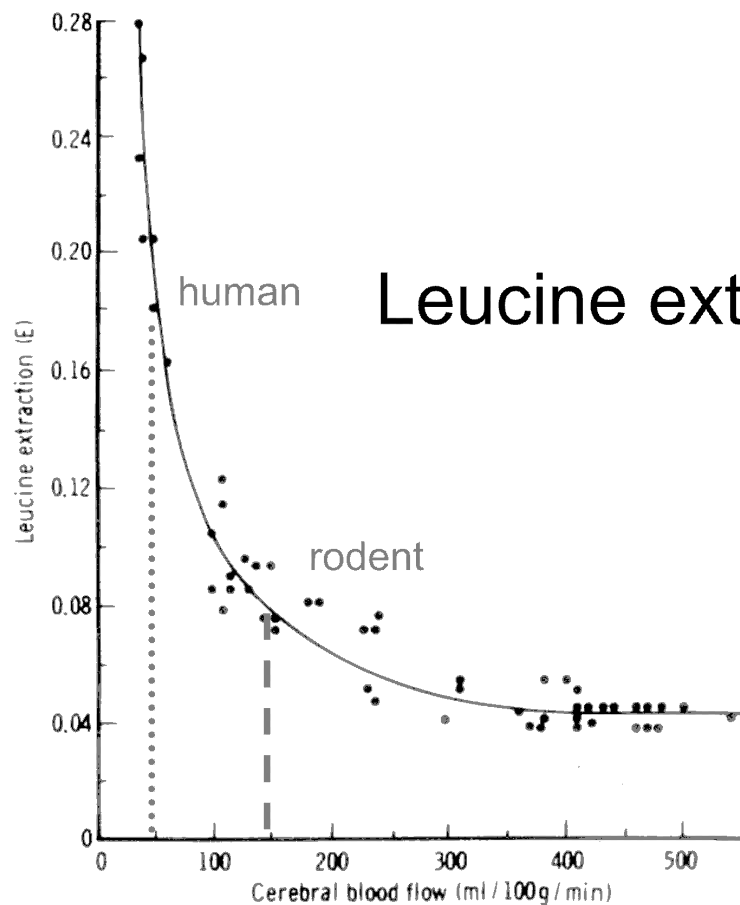


Characteristics of brain endothelium

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

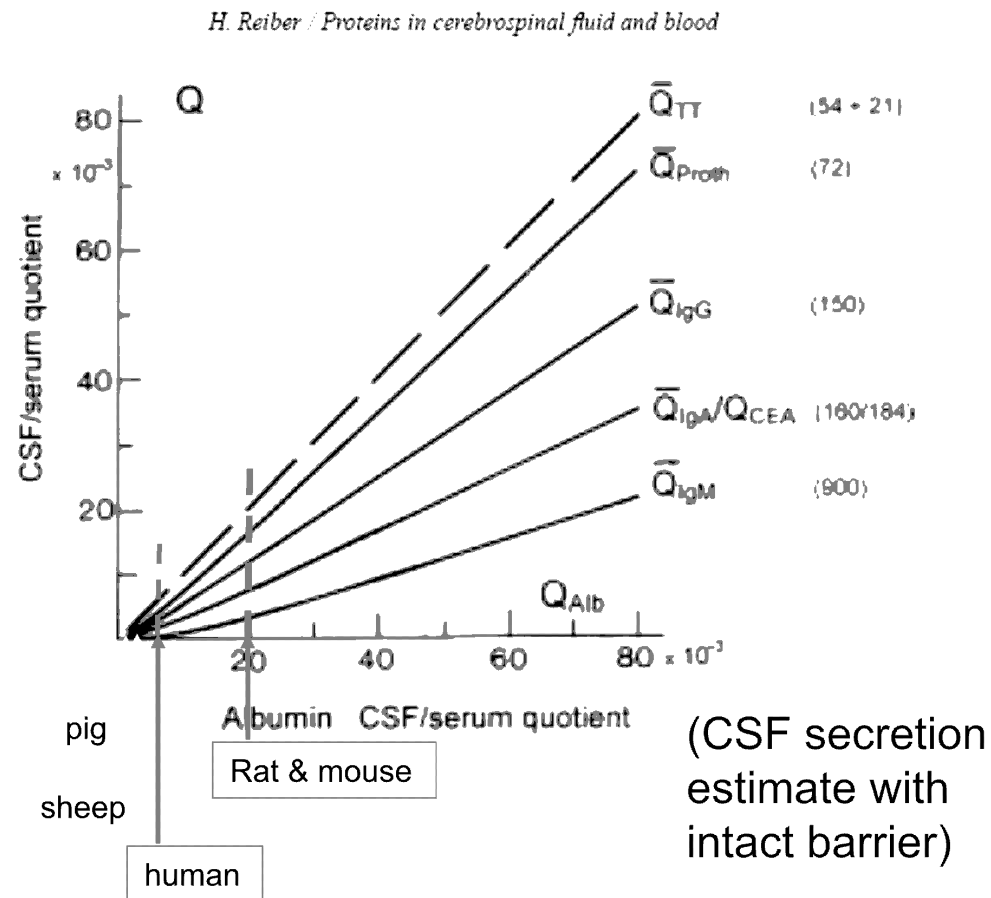
Sage 1981 J Neurochem 36:1731

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Species differences

Using CSF to estimate brain concentration



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SYNG-PQ-01149031

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)

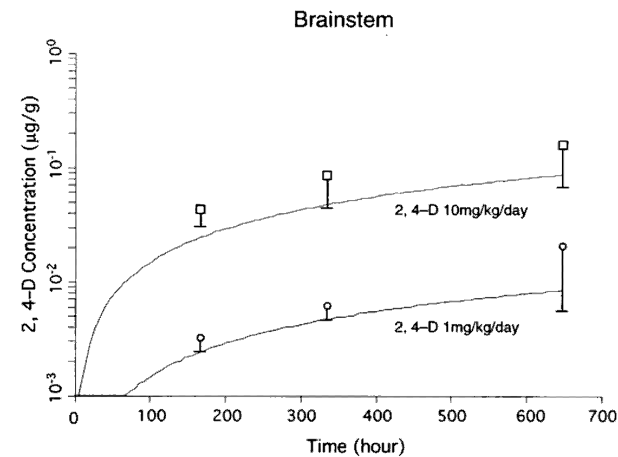
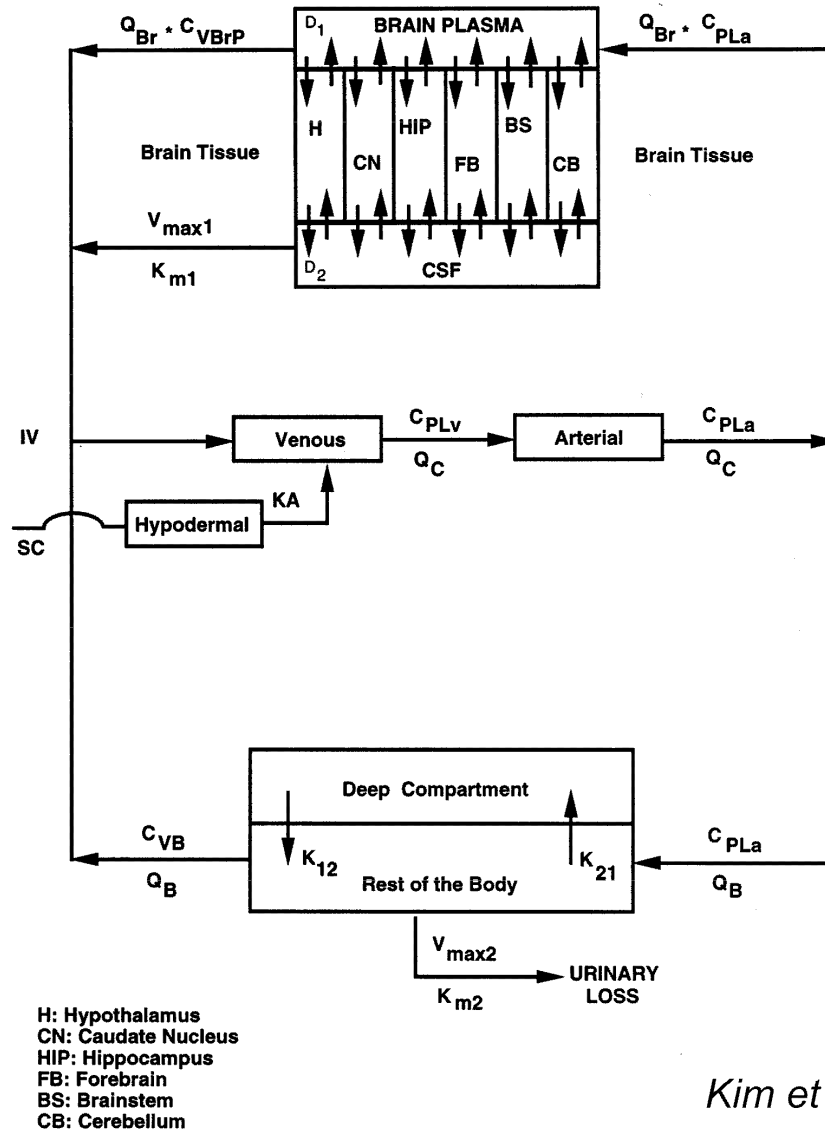
MDR1 (human)
mdr1 mdr2 (rodent)

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)

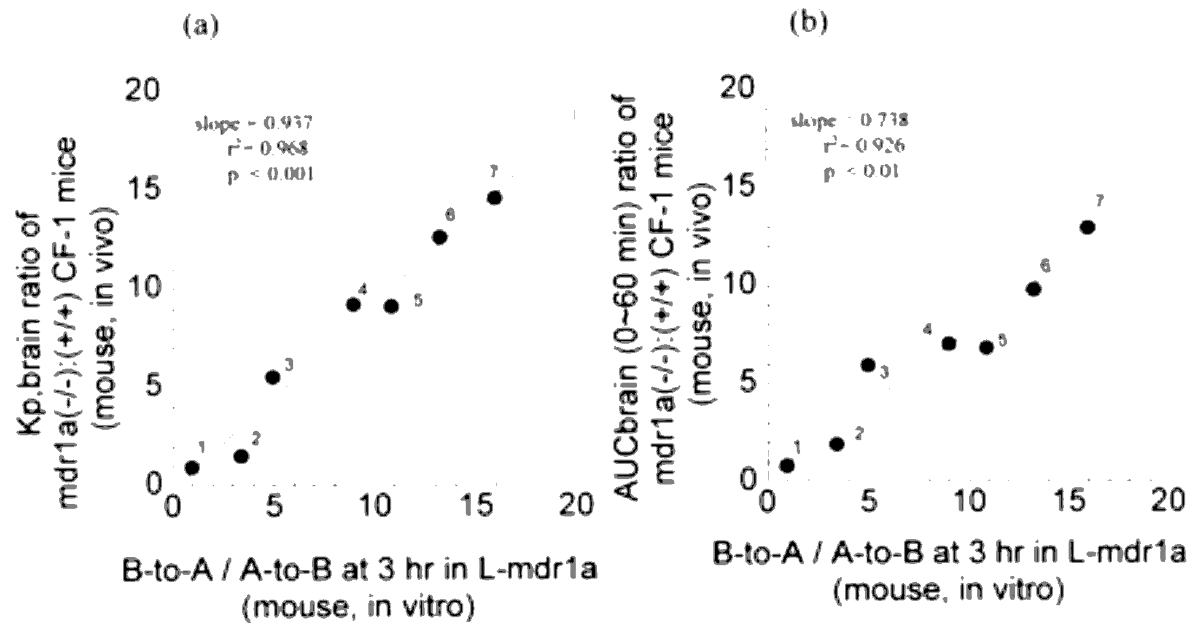


Kim et al. 2001 Env Toxicol Pharmacol 9:153

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SYNG-PQ-01149033

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

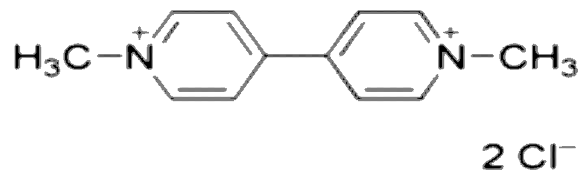
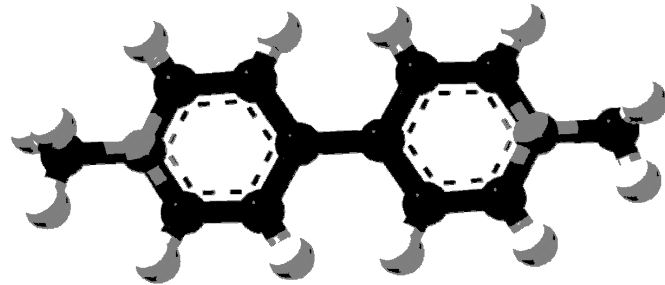
Case ID: 220300429

SYNG-PQ-01149036

PARAQUAT PROPERTIES

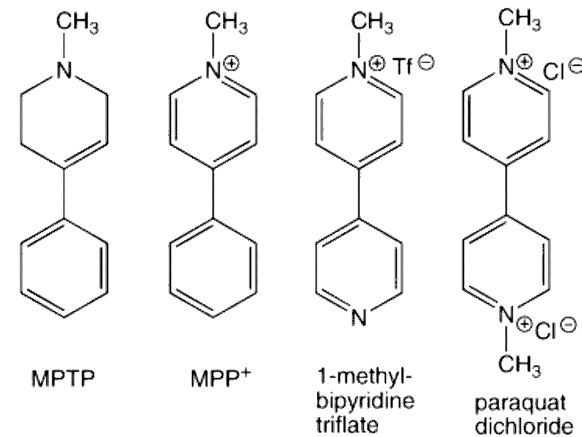
- 1,1-Dimethyl-4,4'-bipyridinium dichloride
- Para-substituted quaternary bipyridyl di-cation
- Non-selective herbicide. ICI - Syngenta (Gramoxone)
- One of most toxic herbicides (>40mg/kg)

PARAQUAT (PQ) CHEMISTRY



Does paraquat cause
a Parkinson-like syndrome?

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



Bartlett et al. 2009 Brain Res 1259: 74

Case ID: 220300429

SYNG-PQ-01149038

PARAQUAT Simulation using pCEL-X

-6.67 *logP(para)*
(0.21 x 10⁻⁶ cm/s)
-4.47 *logP(filter)*
(34 x 10⁻⁶ cm/s)
-4.44 *logP(ABL)*
(36 x 10⁻⁶ cm/s)

ASSAY

data file: PARAQUAT.TXT cel
literature data
Wed, 08 Apr 2009, 05:00AM
A -> B transport direction
gradient-pH conditions
receiver pH: 7.4
pH: 1.0 - pH: 12.0, individual weighting
temperature: 37°C
aqueous buffer used
donor wells = 500 µL, receiver wells = 1500 µL
filter area = 1.13 cm²
permeation/uptake time: 30, 60, 90, 120 min
detection method: UV
compound: 50 µM, MW 186.3 g/mol
added note: TWO PERMANENT POSITIVE CHARGES

AQUEOUS BOUNDARY LAYER

diffusivity: 9.10e-006 cm²/s
thickness of ABL: 2506 µm
stirring speed: 0 RPM

PARACELLULAR PERMEABILITY

molecular radius: 3.57 Å
pore radius of junction: 6.00 Å
junction porosity/pathlength: 1.22 cm⁻¹
potential drop across junction: -17.7 mV
filter porosity: 0.0037

Caco-2 PERMEABILITY

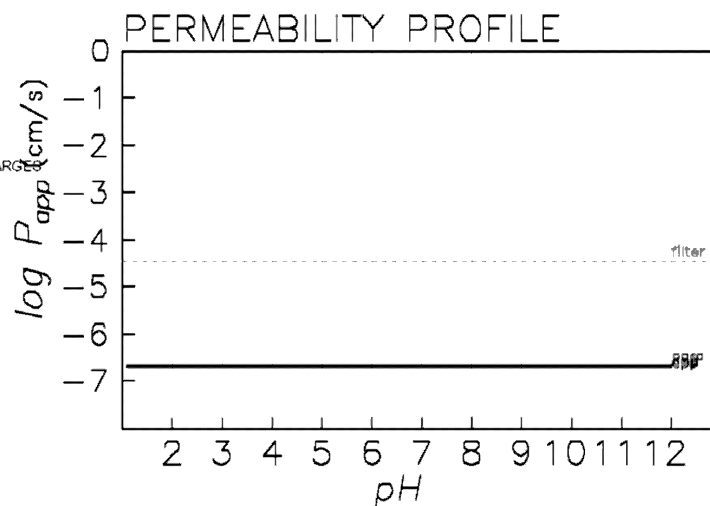
Caco-2 DATA

pH	logPapp	SD	Papp(10 ⁻⁶ cm/s)
14.0	-12.000	999999.000	0.000
15.0	-12.000	999999.000	0.000

FACTORS CONTROLLING TRANSPORT

pH	Passive(neutral)	Passive(ion)	Paracellular	Filter
5.0	0%	0%	99%	1%
6.5	0%	0%	99%	1%
7.4	0%	0%	99%	1%
	ABL	Carrier-Mediated	Uptake	Efflux
5.0	1%	0%	0%	0%
6.5	1%	0%	0%	0%
7.4	1%	0%	0%	0%

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



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

By permission of Dr A Avdeef

PARAQUAT PROPERTIES

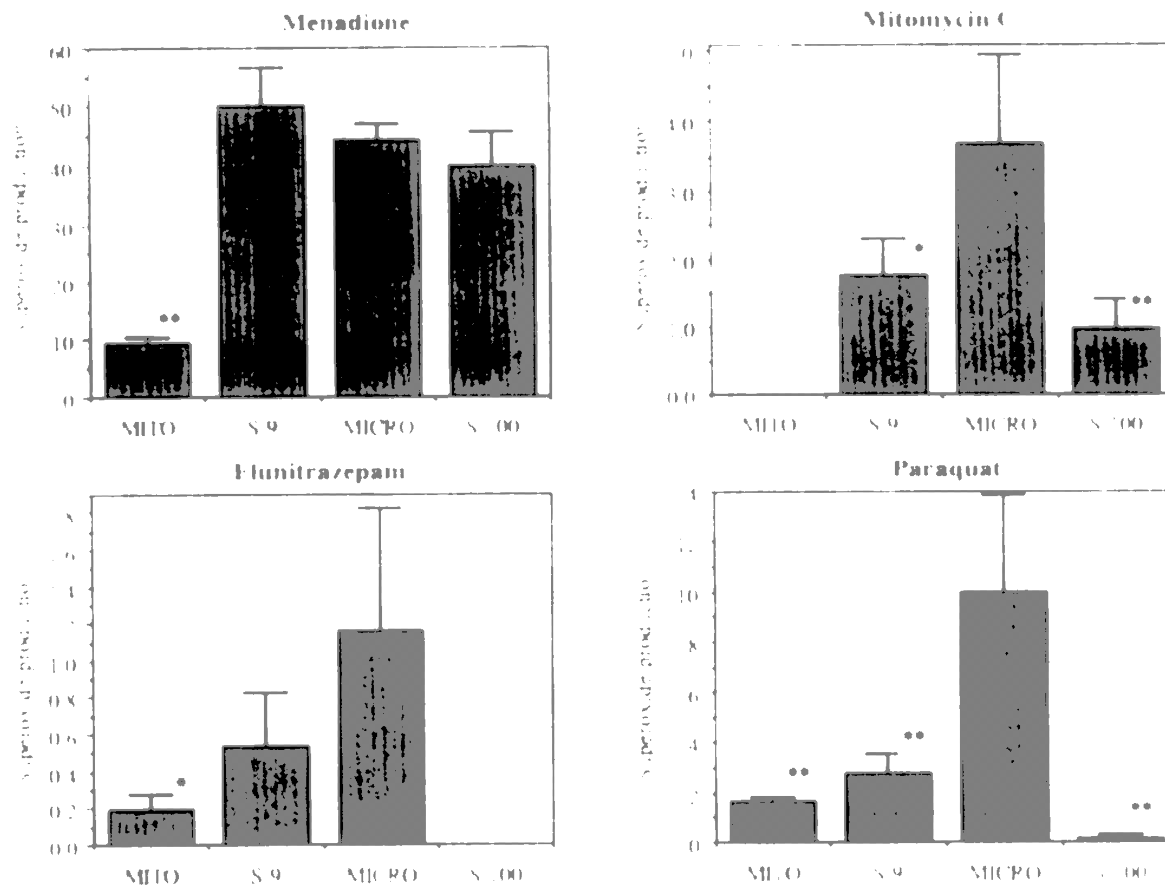
- Permanently discharged highly soluble cation
- Predicted logP_{octanol} -6.3
- *P-CEL-X* prediction – epithelial/BBB model – cell membrane permeability << paracellular permeability.
- PQ oral bioavailability low ~10%.
- Not subject to metabolism in body, rapidly excreted in urine
- If enters brain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

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.

Superoxide production by brain and microvessels

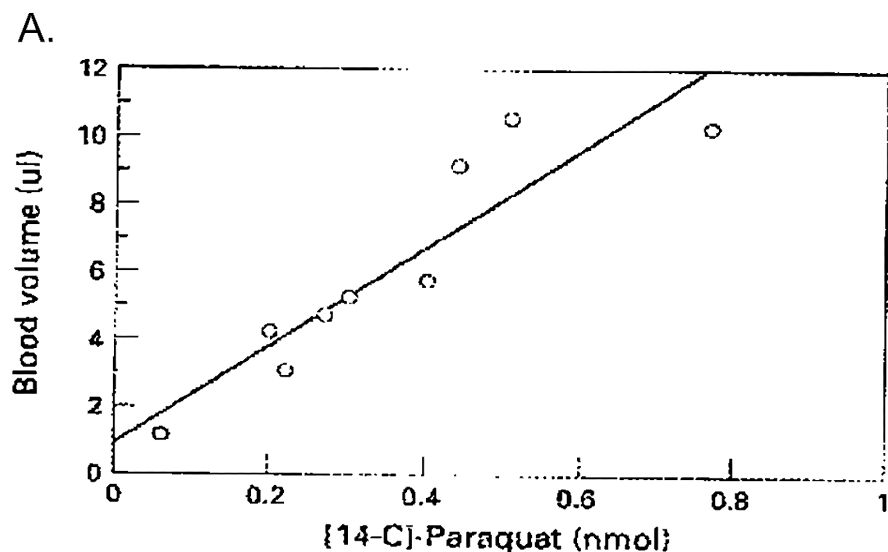


PQ on brain
microvessels:
O₂⁻ production
stimulated by
NADH nucleotide
cofactor

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

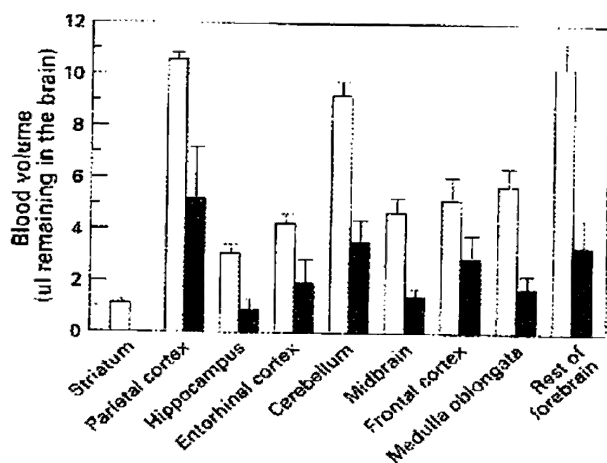
Case ID: 220300429

SYNG-PQ-01149042



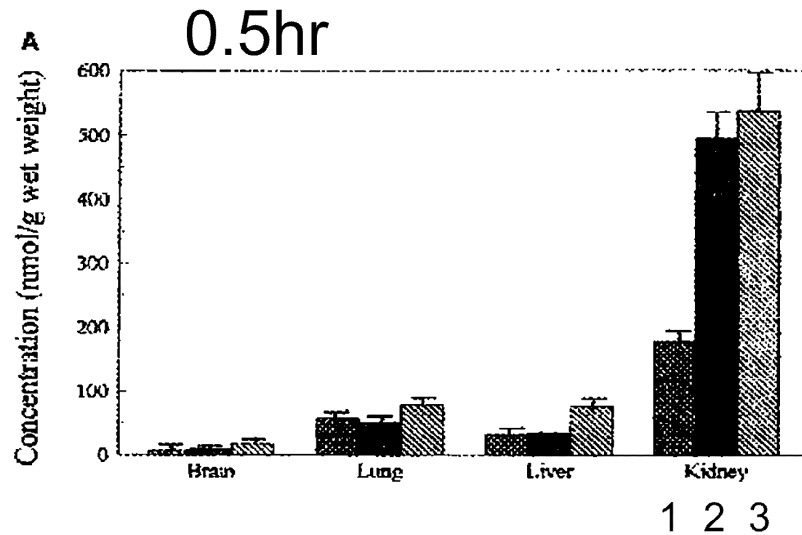
Paraquat
distribution in
brain related to
regional blood
volume (inulin
space, ul)

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



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

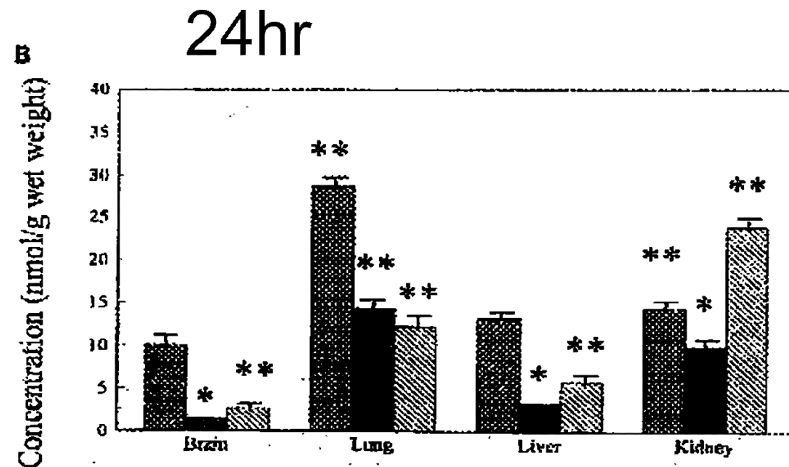
Naylor et al. 1995 Hum Exp
Toxicol 14:587



Age-dependent entry [¹⁴C] paraquat to brain

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

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

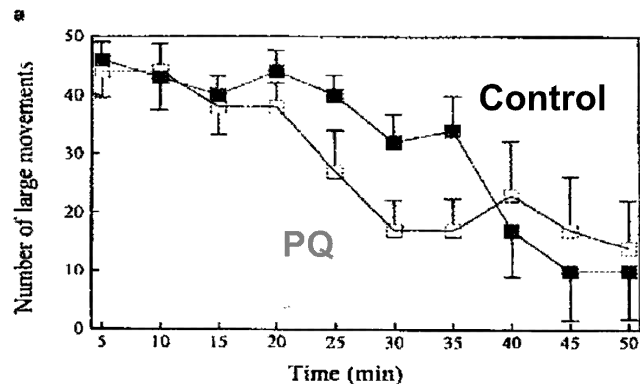


Initially in more permeable
BBB regions, then in most
of brain by 24 hr, especially
in neonate

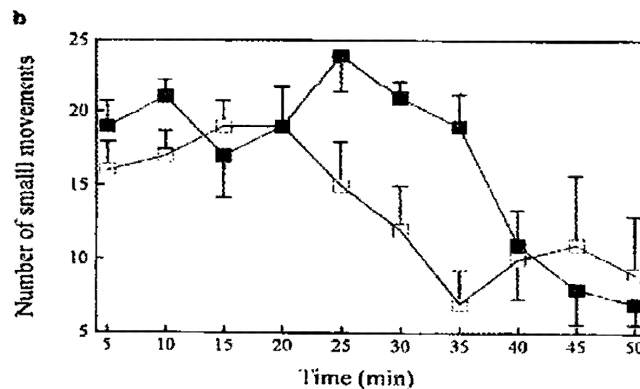
Widdowson et al 1996a
Hum Exp Toxicol 15:231

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

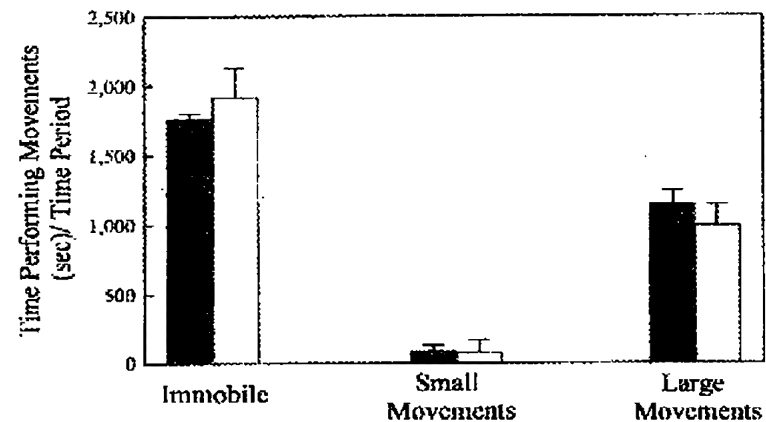
Locomotor activity



- 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



C PQ

Conclude: PQ not like MPP+

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

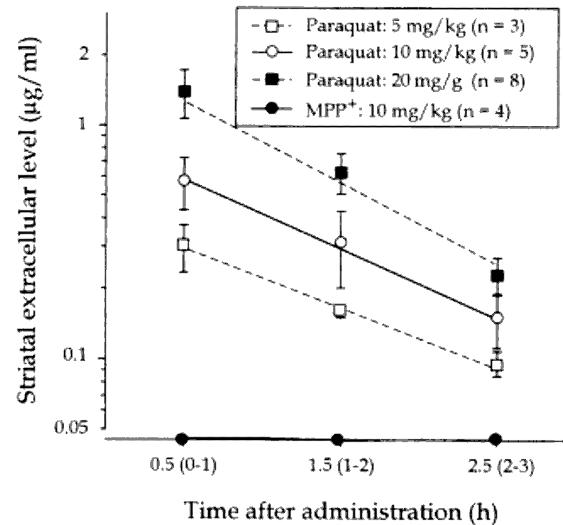
Case ID: 220300429

SYNG-PQ-01149045

Does Paraquat (PQ) get into the CNS ?

- Gherzi-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?

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
<i>Paraquat</i>				
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	8.01 ± 2.41	0.49 ± 0.09	0.085 ± 0.023**
<i>MPP⁺</i>				
10 mg/kg	4	11.62 ± 4.54*	Not detected	

Shimizu et al. 2001
Brain Res 906: 135

Paraquat crosses BBB by L1 (LAT1) amino acid carrier

	n	Striatal extracellular paraquat level			Serum paraquat 3 h	Ratio ^{a)}
		0-1 h	1-2 h	2-3 h		
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	0.052±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

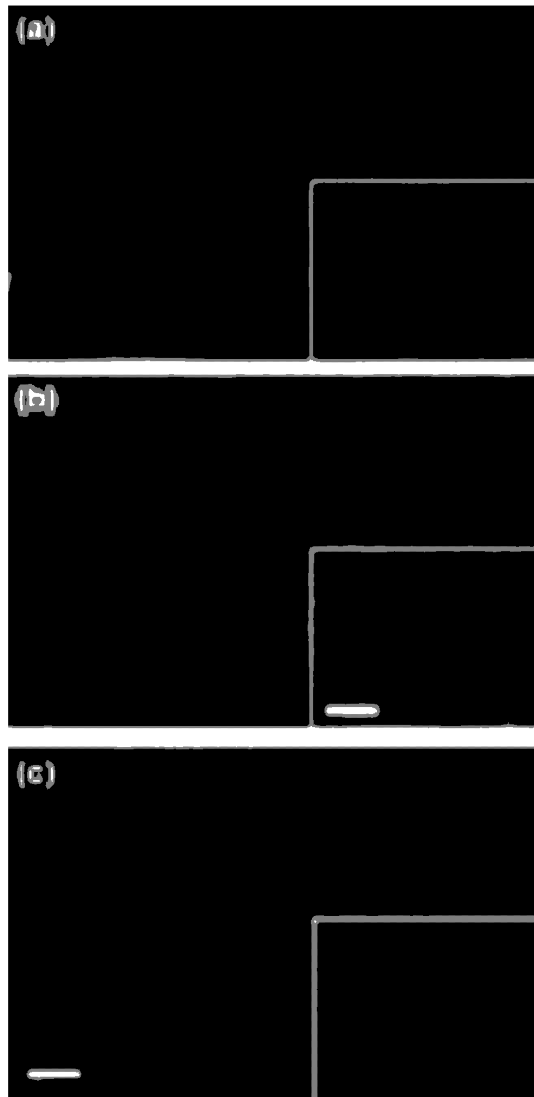
Locally injected PQ:
Striatal neurone
PQ uptake
Na⁺-dependent, not blocked by putrescine
?not PAT

Shimizu et al. 2001
Brain Res 906: 135

➤ PQ crosses 3 membranes

Case ID: 220300429

SYNG-PQ-01149048



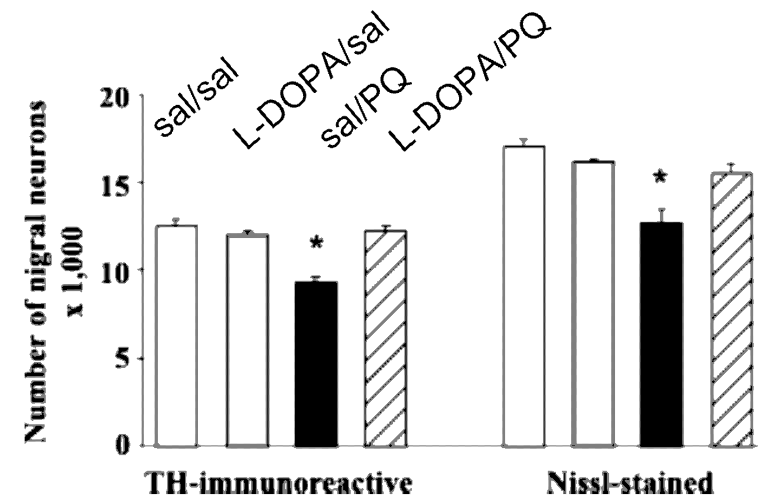
saline/
saline

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



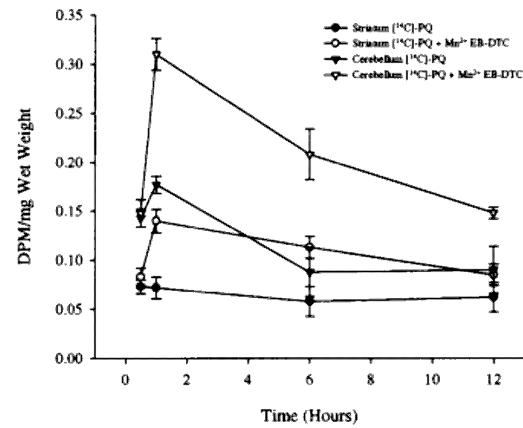
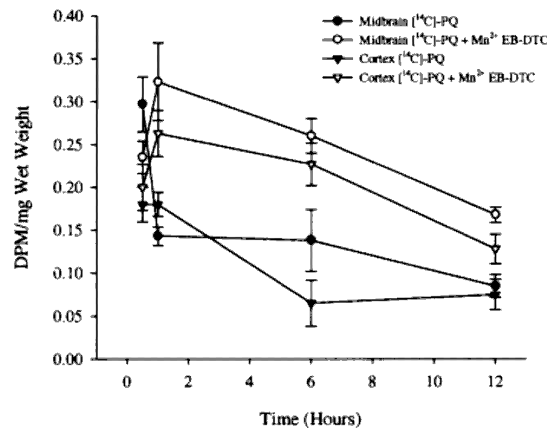
L-DOPA protects against PQ neurotoxicity

McCormack J Neurochem 2003 85:82

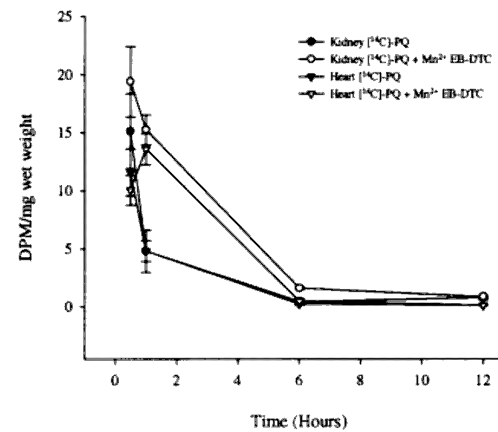
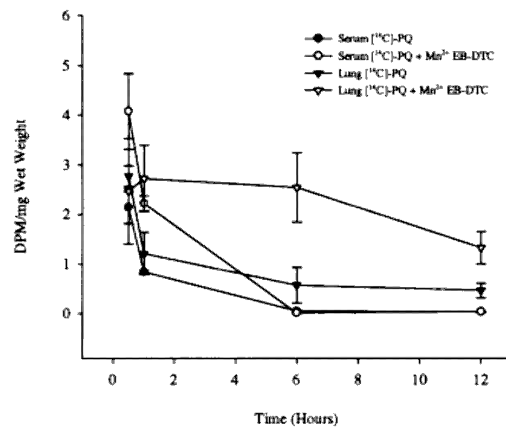
Case ID: 220300429

SYNG-PQ-01149049

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



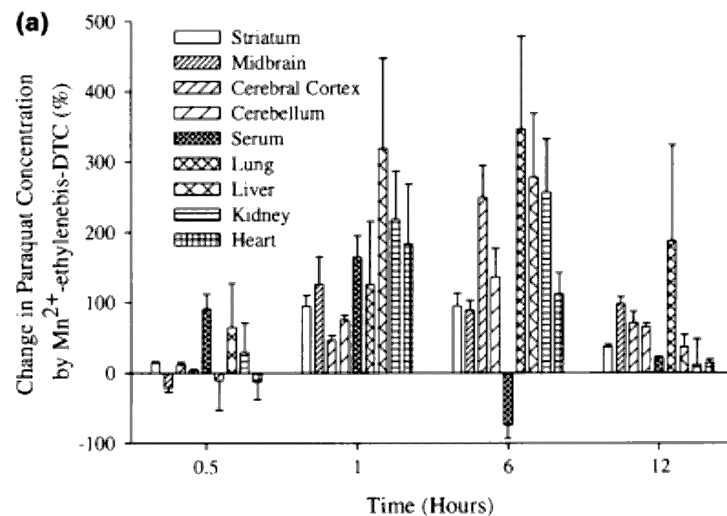
DTC:
Dithiocarbamate
pesticide



Barlow et al. 2003
J Neurochem
85:1075

Case ID: 220300429

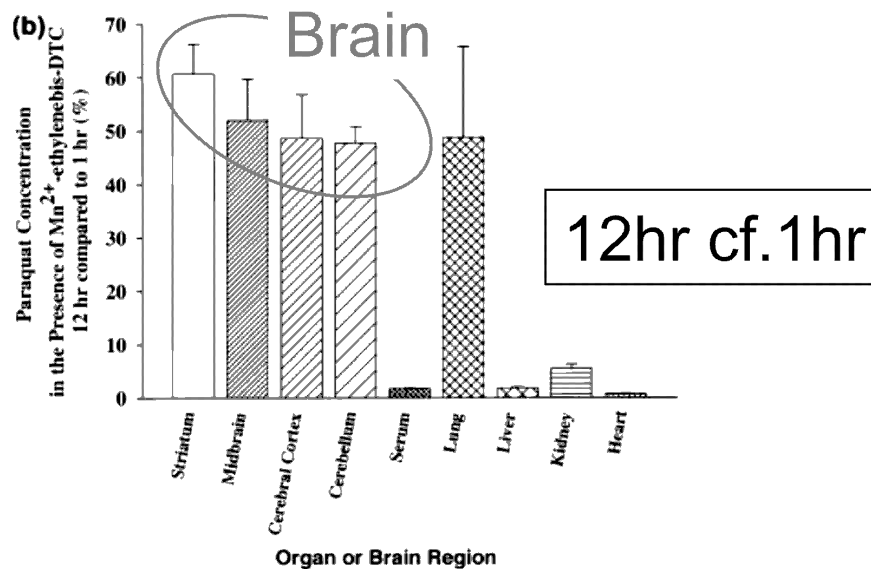
SYNG-PQ-01149050



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

Residual PQ in brain cleared slowly

➤ DTC inhibits efflux transporter?



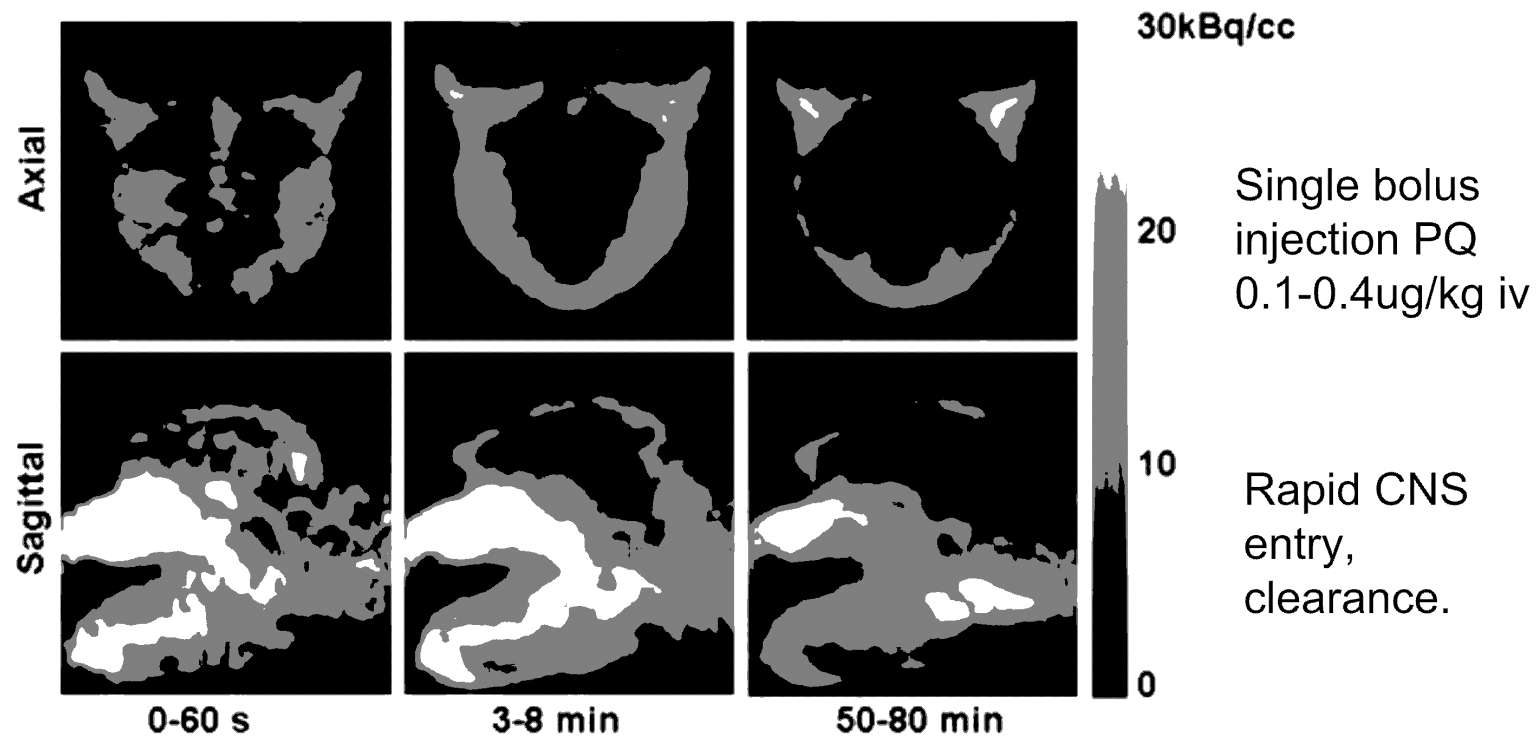
Barlow et al. 2003
J Neurochem 85:1075

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 ¹⁴C PQ enters brain, peak levels ~1hr, certain dithiocarbamates increase brain uptake PQ - by inhibiting efflux transporter?

[¹¹C] Paraquat in primate: low CNS distribution

PET study Rhesus macaque

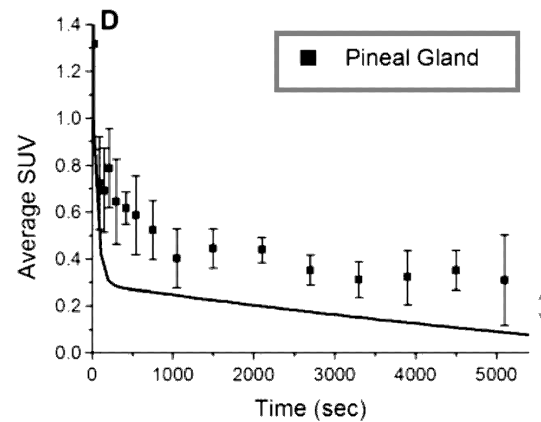
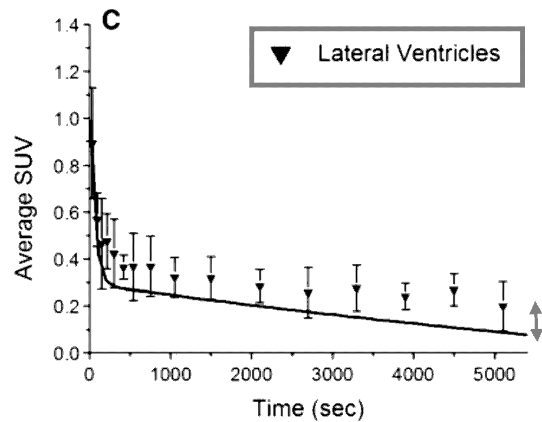
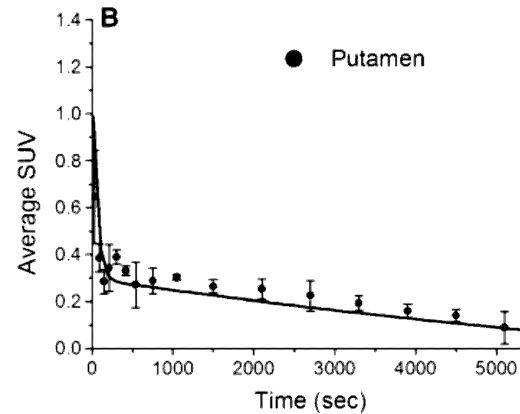
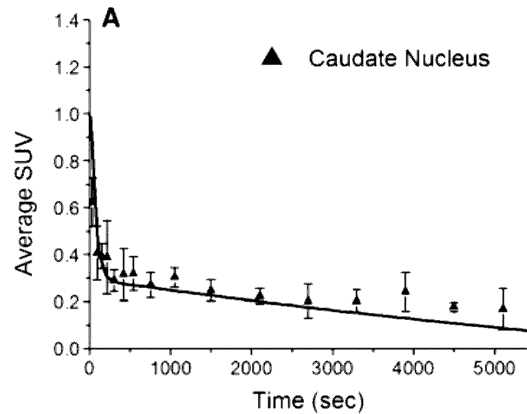


Bartlett et al. 2009 Brain Res 1259: 74

Case ID: 220300429

SYNG-PQ-01149053

[¹¹C] Paraquat in primate: low CNS distribution



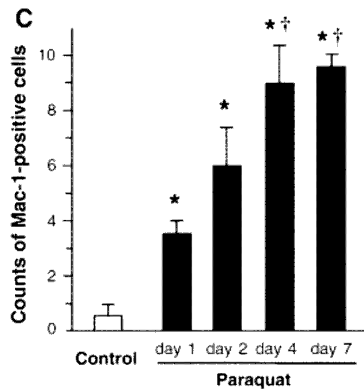
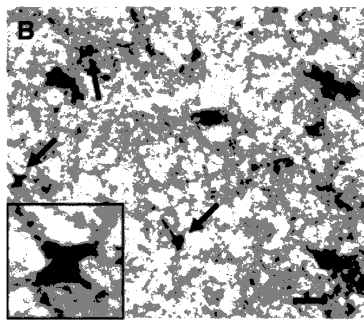
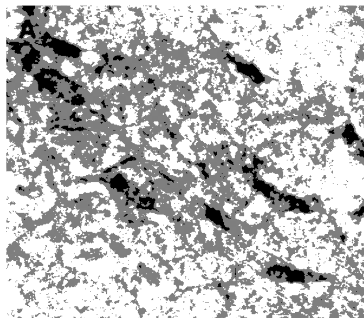
Standardized
uptake values
(SUV)
n=4 subjects

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

Bartlett et al. 2009 Brain Res 1259: 74

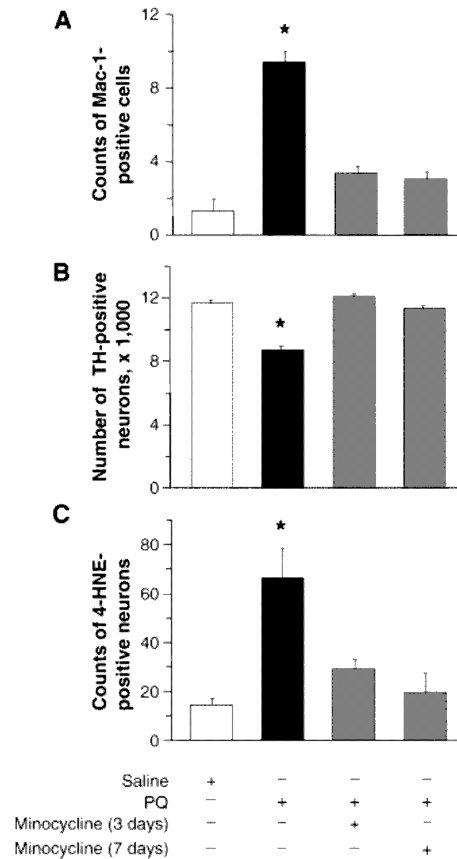
Case ID: 220300429

SYNG-PQ-01149054



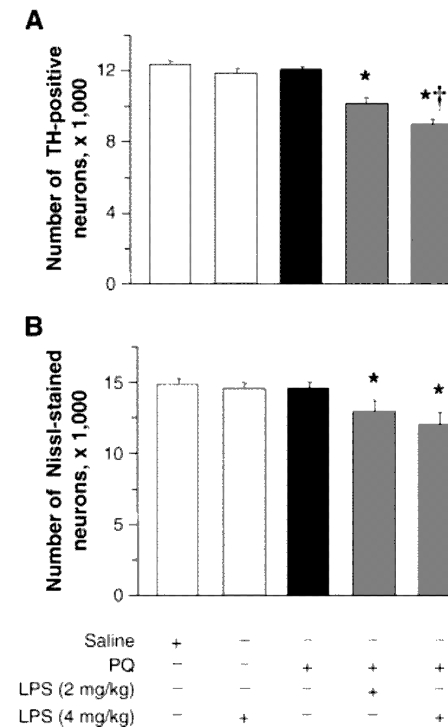
1. Activated microglia

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



Saline	+	-	-	-
PQ	-	+	+	+
Minocycline (3 days)	-	-	+	-
Minocycline (7 days)	-	-	-	+

2. Protection by minocycline



Saline	+	-	-	-	-
PQ	-	+	+	+	+
LPS (2 mg/kg)	-	-	+	+	-
LPS (4 mg/kg)	-	+	-	-	+

3. LPS + PQ: neurotox

Purisai et al. 2007
Neurobiol Dis 25:392

Case ID: 220300429

SYNG-PQ-01149055

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

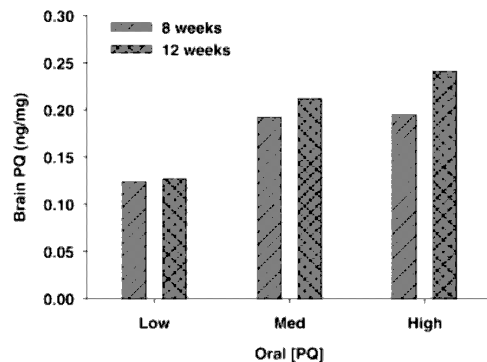
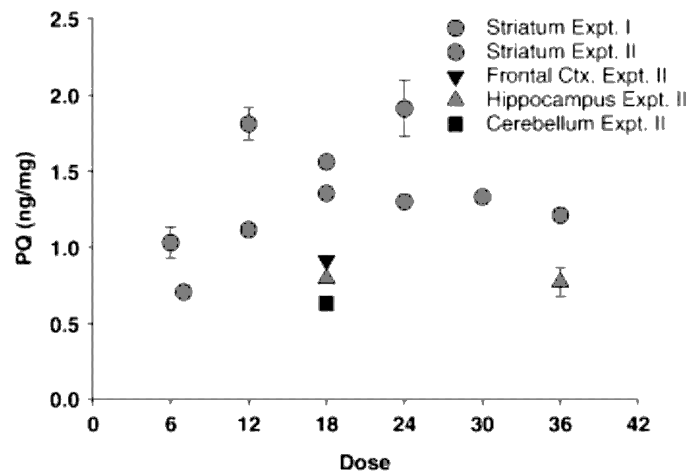
Gradinaru et al. 2008 Arch Toxicol. Nov 21

Case ID: 220300429

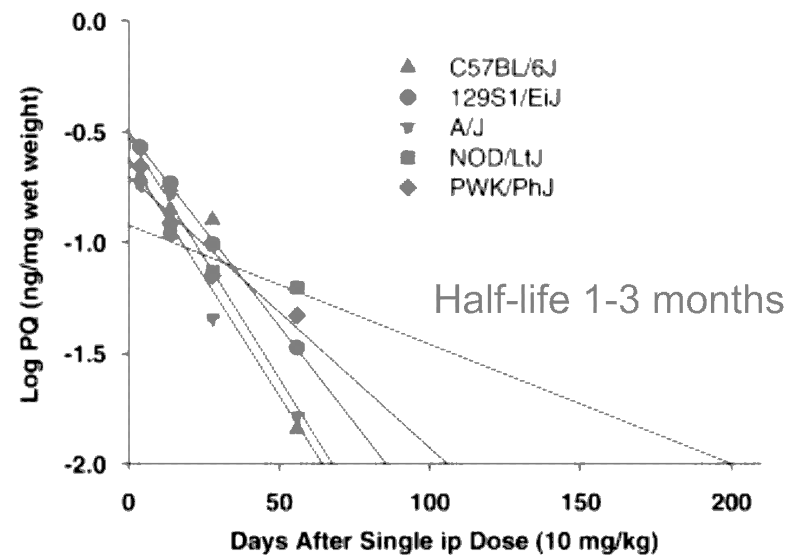
SYNG-PQ-01149056

Paraquat toxicokinetics and toxicodynamics in mouse brain

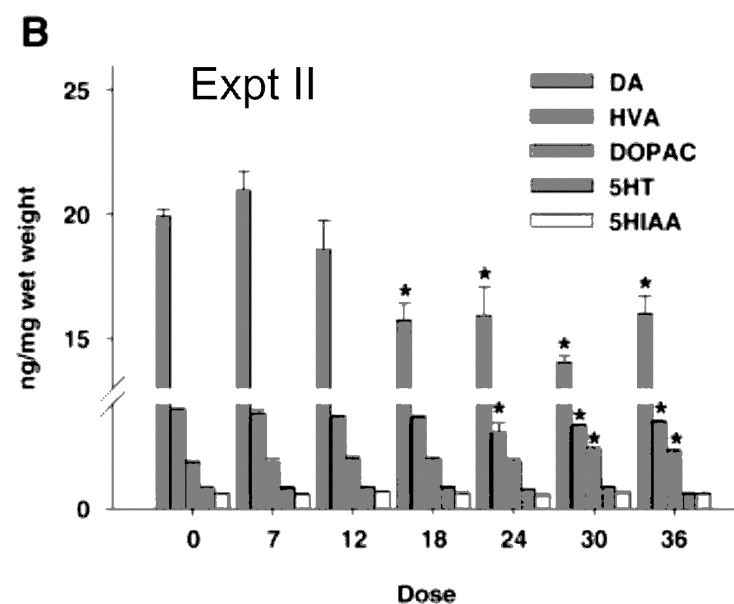
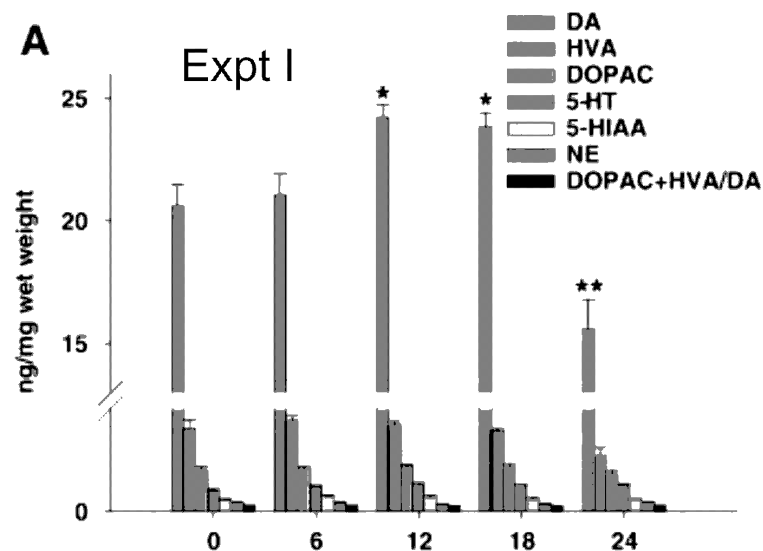
1. Concentration in brain



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*

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 discharged highly soluble cation
- Predicted logP octanol -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 be 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

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BBB physiology, development, toxicology, pathology

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- Sarah Fredriksson, Siti Yusof

Supported by:

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- St Thomas' Hospital - Graham Hughes
- Budapest - Ester Domotor, Ildiko Sipos, Vera Adam-Vizi
- Cleveland - Damir Janigro
- Boston - Alex Avdeef (pION)



Case ID: 220300429

SYNG-PQ-01149062