Assessment of unbound target site-concentration in brain and lungs: methodological considerations and practical implications

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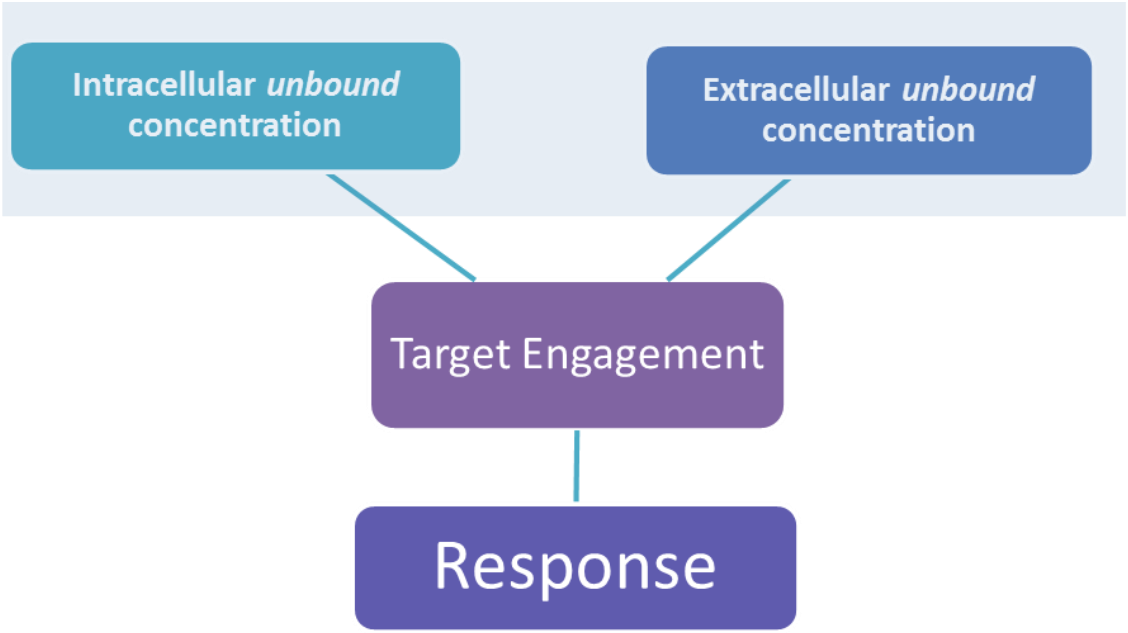
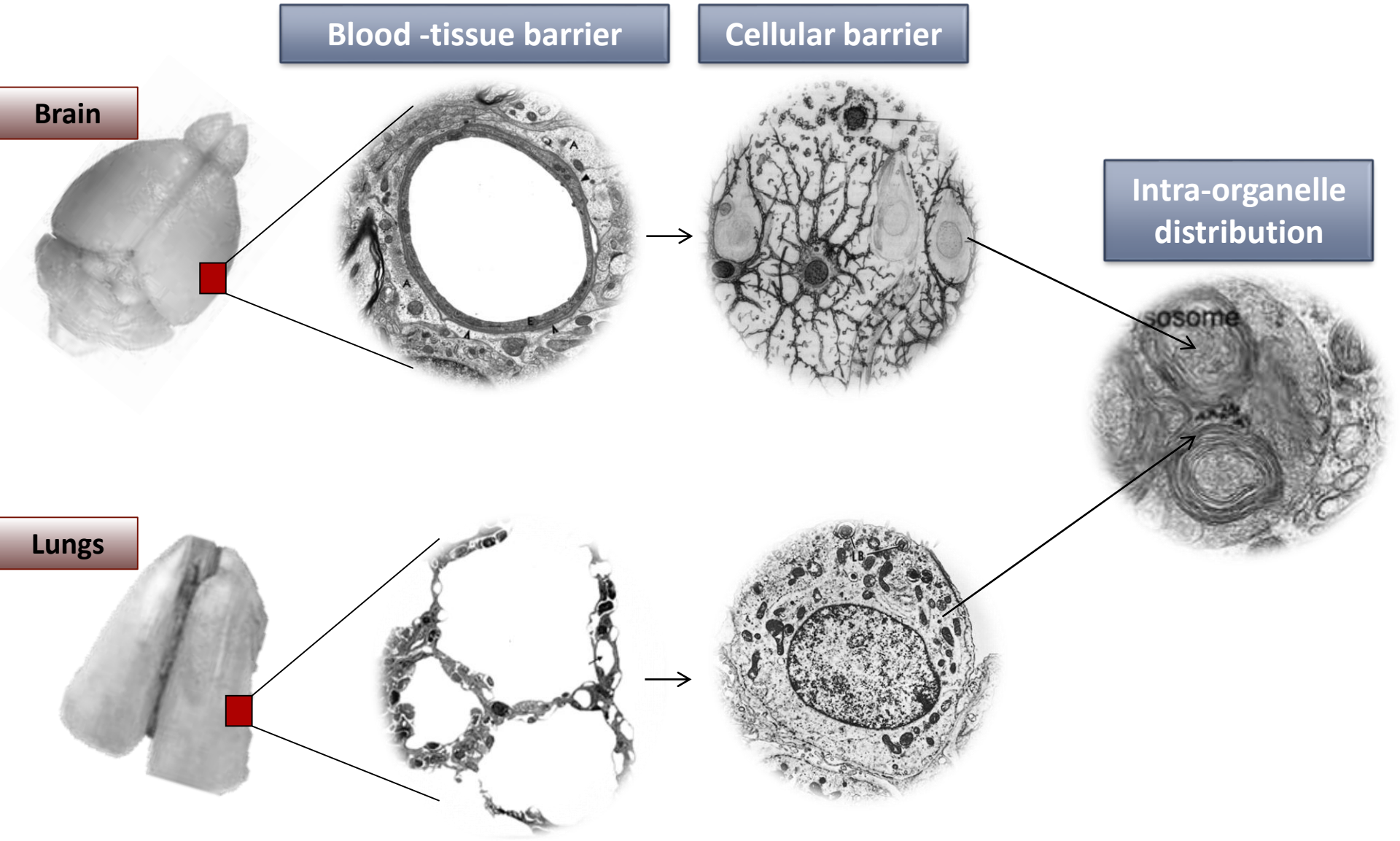
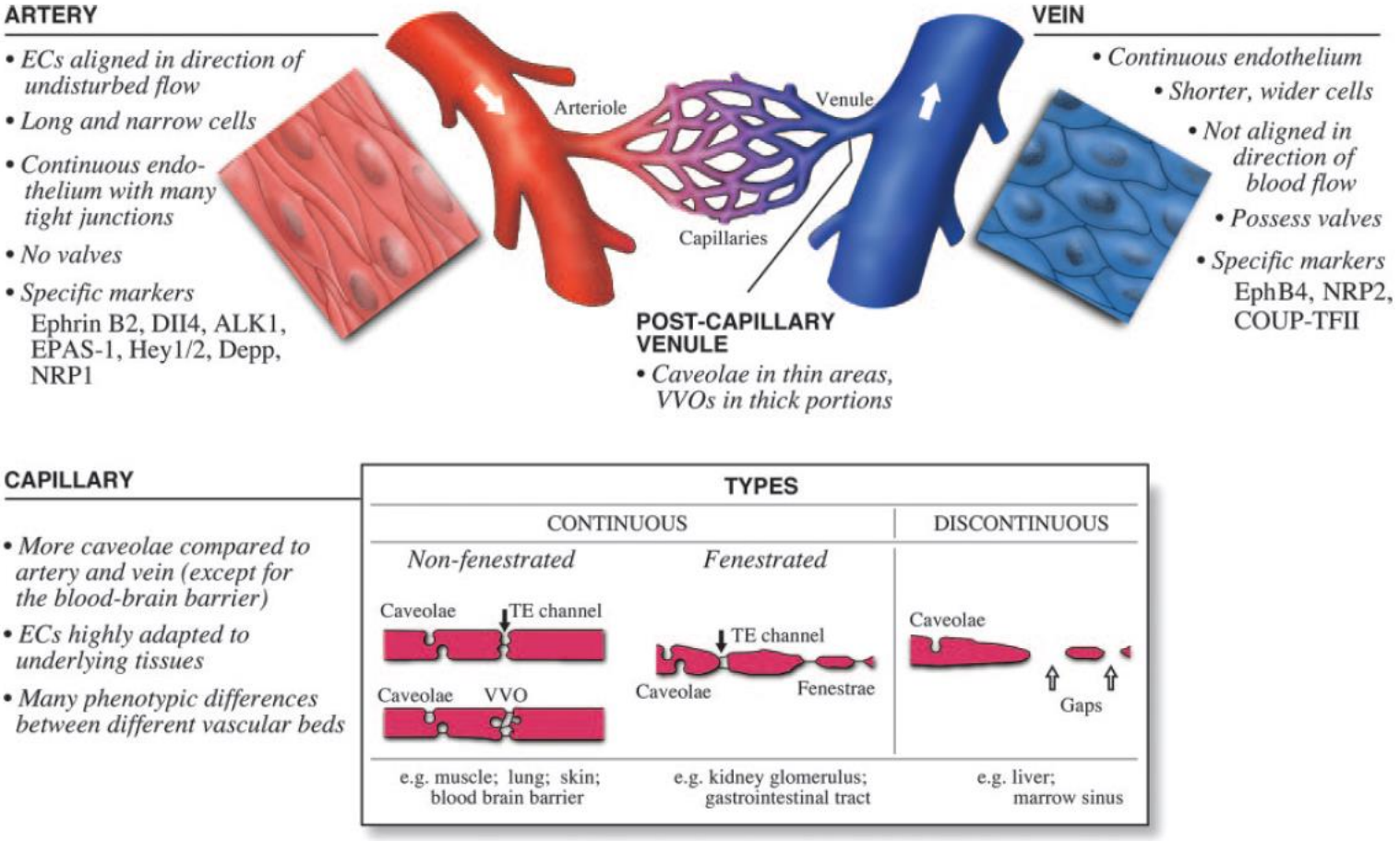
Drug target-site exposure as a driver of response



Assessment of target-site exposure

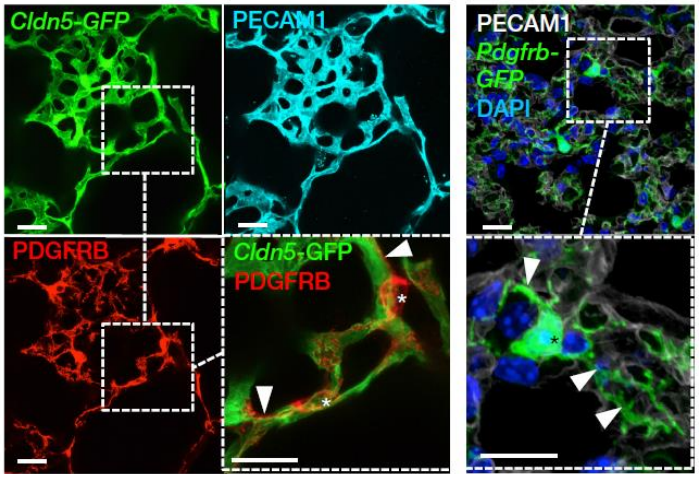


Heterogeneity of endothelium

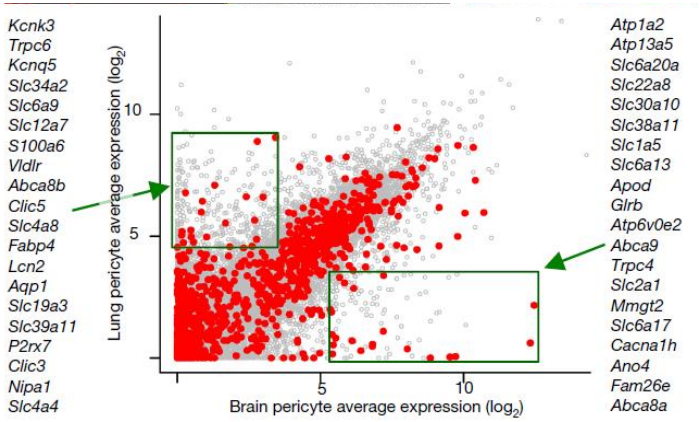


W. Aird, Circulation Res, 2007

Organotypicity of cells



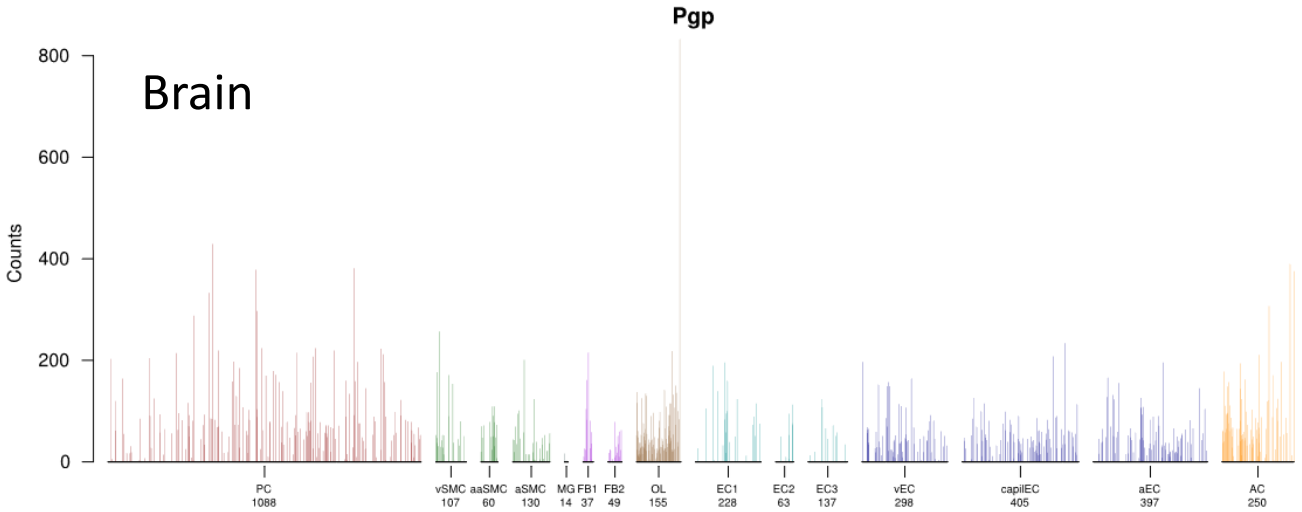
Cldn5-GFP and PECAM1 immunofluorescence mark endothelial cells, and *Pdgfrb*-GFP and PDGFRB immunofluorescence mark pericytes in mouse lung. Insets show pericytes at high magnification.

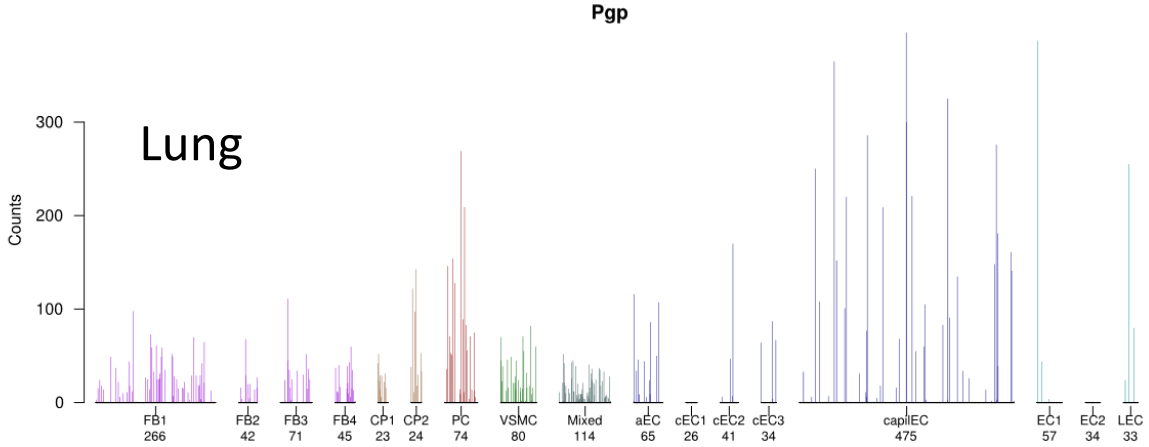


Transporters (red) in brain and lung pericytes. The 20 most differentially expressed transporters are indicated for each cell type.

Vanlandewijck et al, Nature, 2018

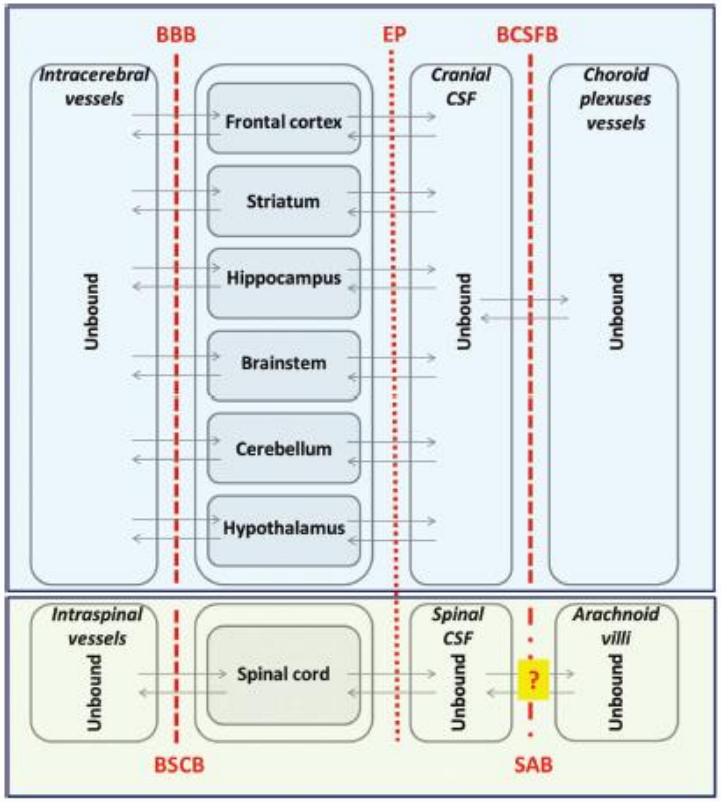
P-gp detailed expression in each cell in brain and lungs



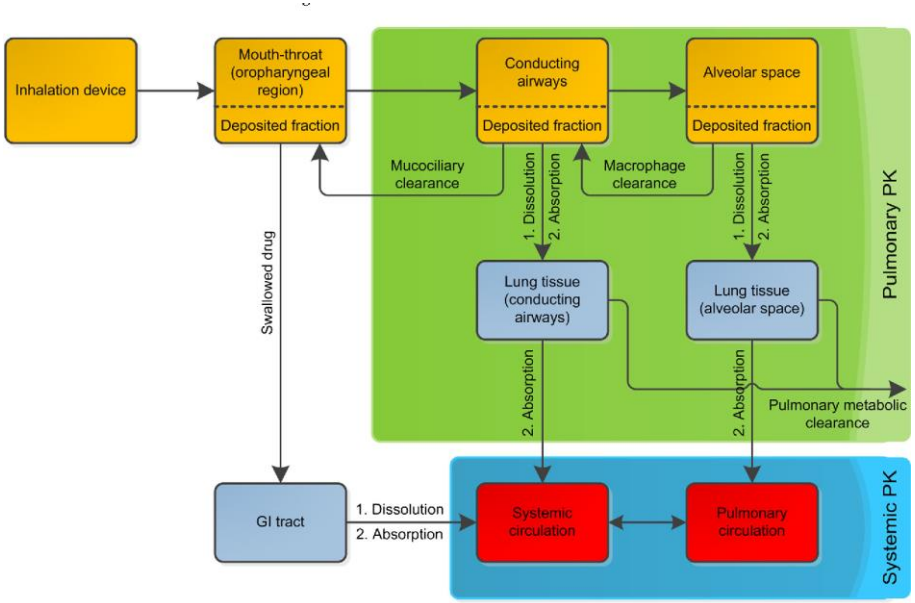


http://betsholtzlab.org/VascularSingleCells/database.html

Where, when and **how**
to measure drug concentration in CNS and lungs?

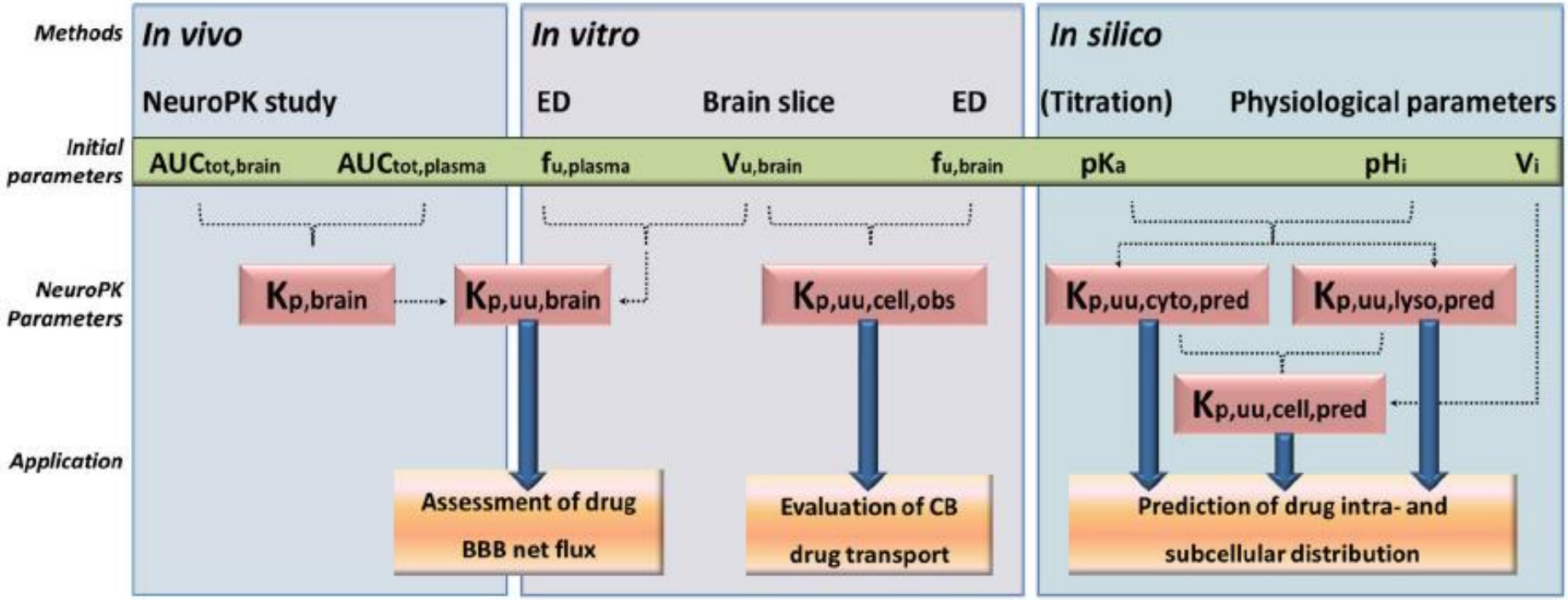


Loryan et al, MolPsychiatry, 2016



Borghardt et al, the AAPS journal, 2015

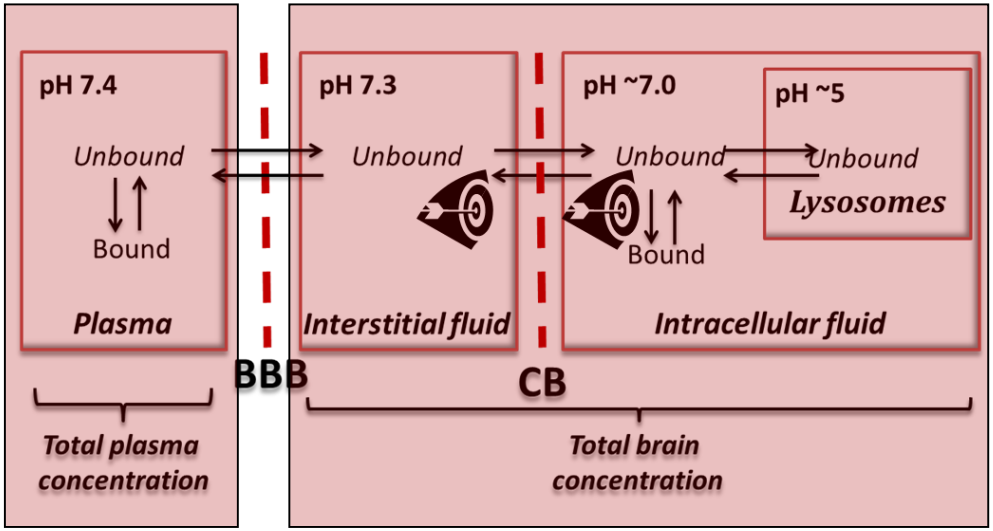
The combinatory mapping approach (CMA)



Loryan et al, PharmRes, 2014

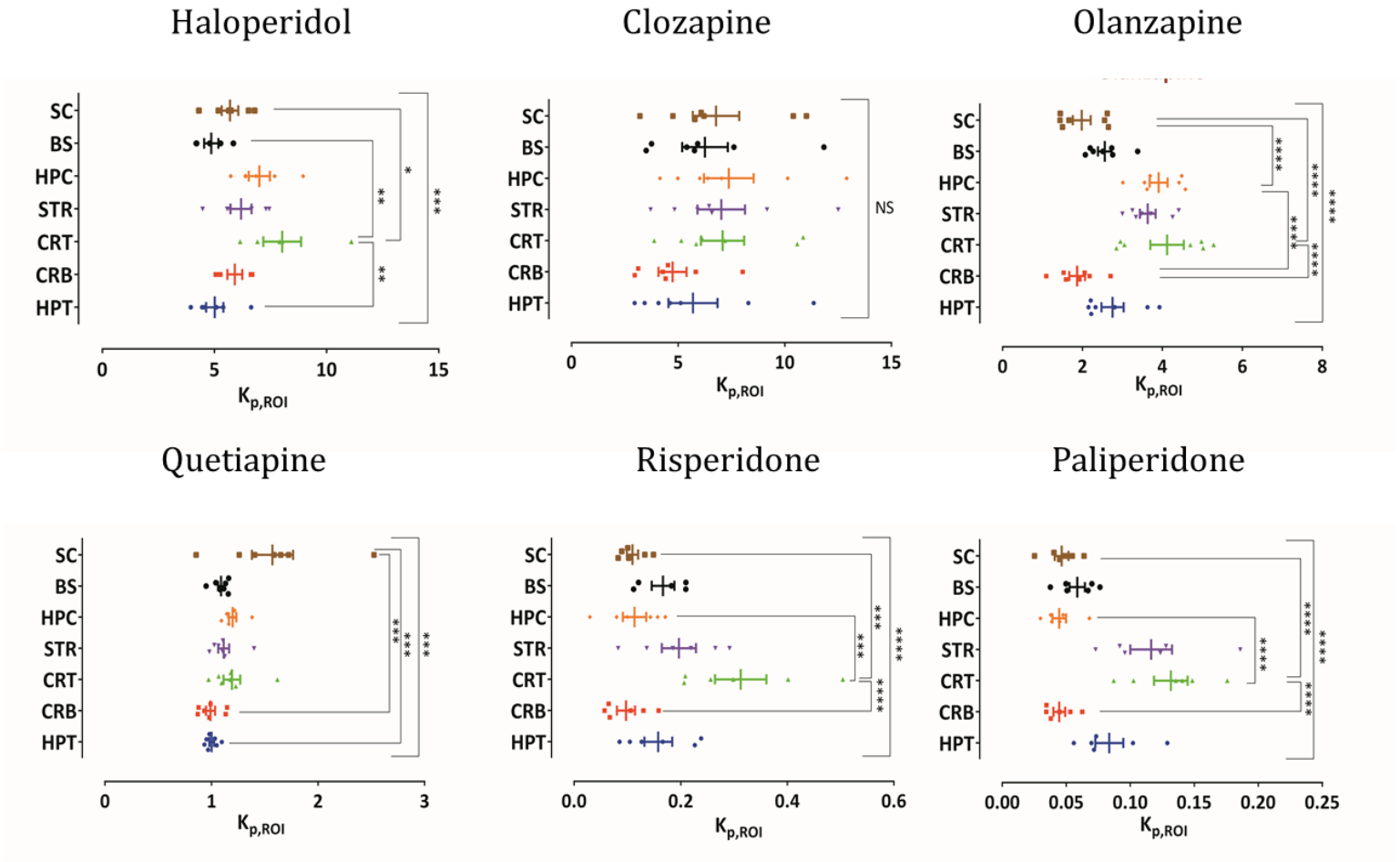
Assessment of equilibrium distribution ratio in the brain regions of interest (ROI), $K_{p,ROI}$

$$K_{p,ROI} = \frac{C_{tot,ROI,ss}}{C_{tot,plasma,ss}} = \frac{AUC_{tot,ROI}}{AUC_{tot,plasma}}$$



$K_{p,ROI}$ describes the extent of total drug transport across the BBB and is confounded by nonspecific binding

Equilibrium distribution ratio in brain regions and spinal cord, $K_{p,ROI}$



SC – spinal cord, BS – brainstem, HPC – hippocampus, STR – striatum, CRT – cortex, CRB – cerebellum, HPT – hypothalamus

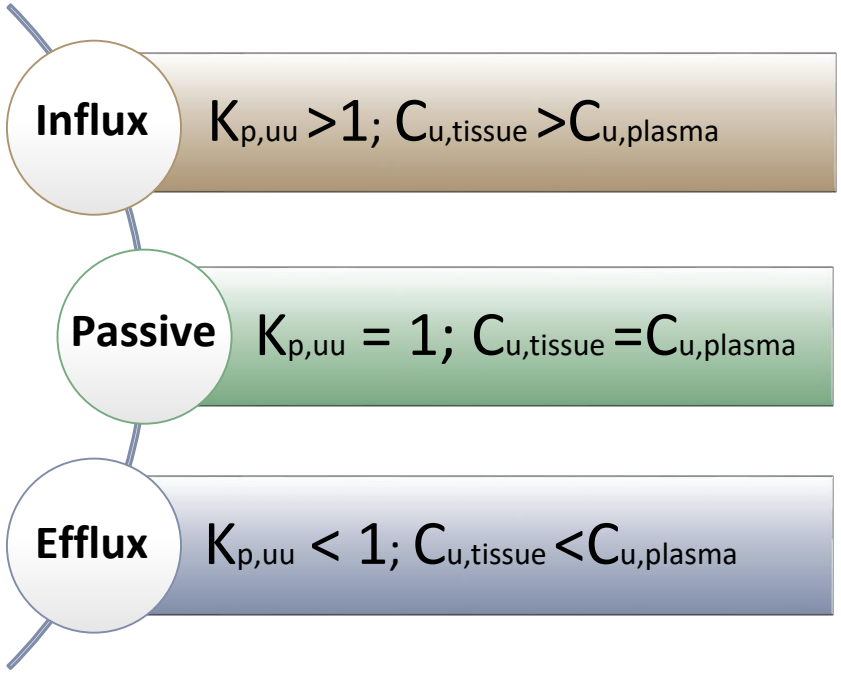
Loryan et al, MolPsychiatry, 2016

Tissue-specific transport across the barrier, **$K_{p,uu}$**

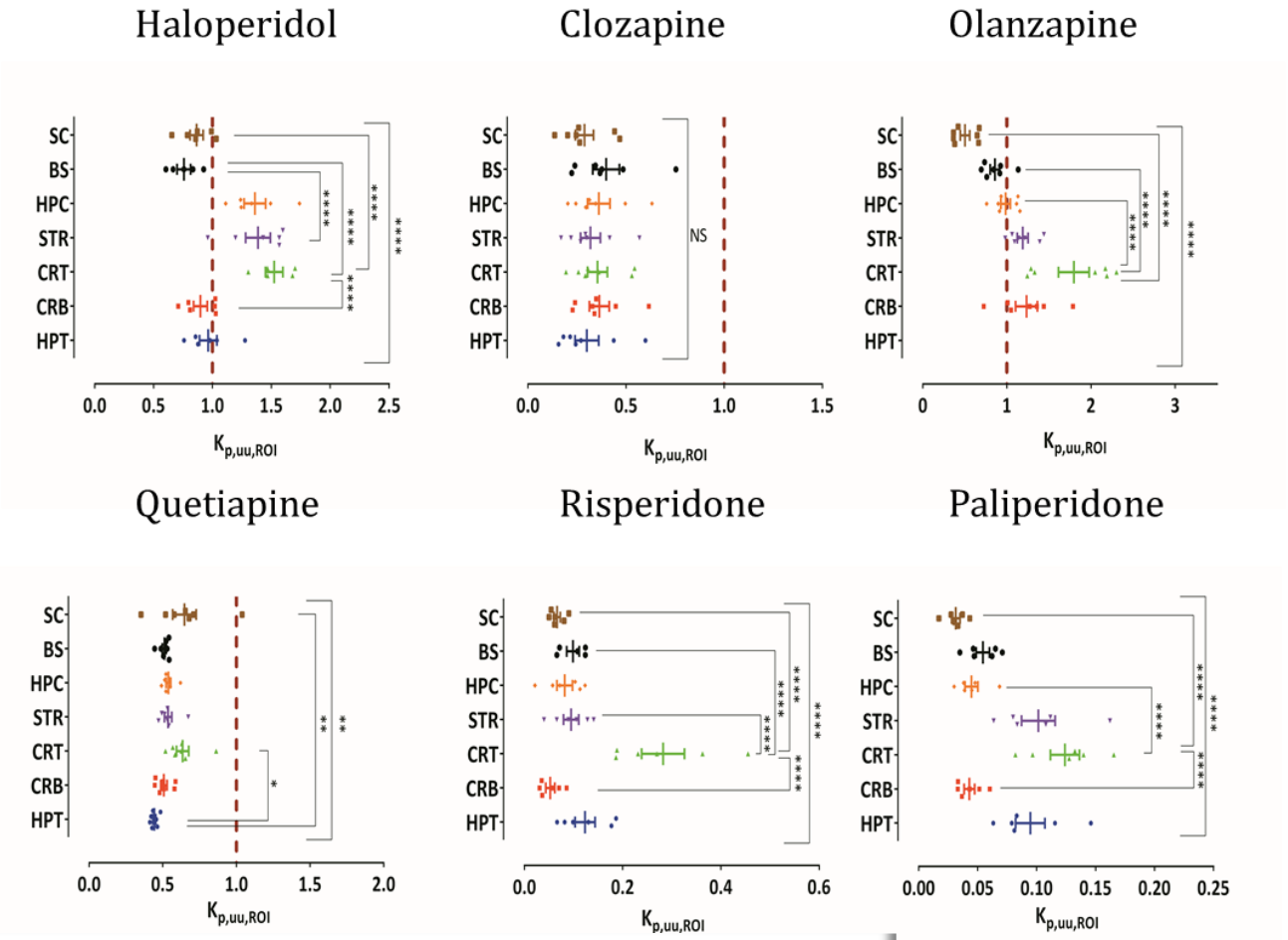
$K_{p,uu}$ quantifies the net flux of drug across the BBB, including the quantitative role of transporters, without being confounded by nonspecific binding in blood and brain tissues.

Gupta et al, DMD, 2006; Hammarlund-Udenaes et al, PharmRes, 2008

$$K_{p,uu,brain} = \frac{K_{p,brain}}{V_{u,brain} \cdot f_{u,plasma}}$$



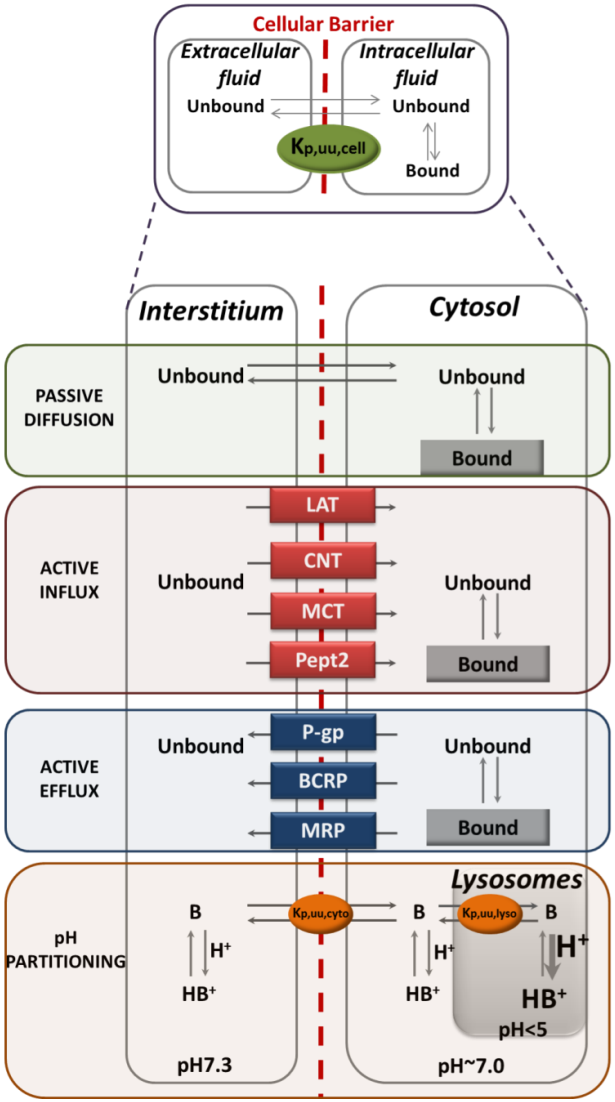
Comparative characteristics of the extent of BBB/BSCB transport ($K_{p,uu,ROI}$) of antipsychotics in CNS



SC – spinal cord, BS – brainstem, HPC – hippocampus, STR – striatum, CRT – cortex, CRB – cerebellum, HPT – hypothalamus

Loryan et al, MolPsychiatry, 2016

Post BBB intra-brain drug distribution

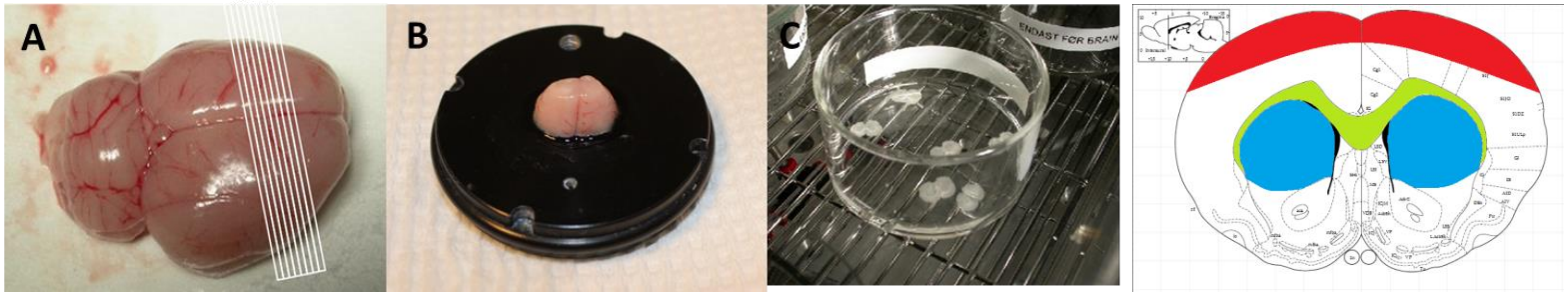


Processes governing intra-brain distribution:

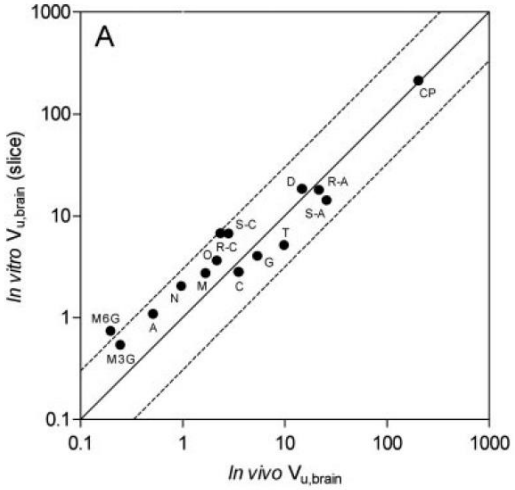
- nonspecific binding
- specific binding
- active transport at cellular barrier
- pH partitioning etc

Loryan et al, MolPsychiatry, 2016

Assessment of overall drug uptake into the brain



Preparation of fresh brain slices → **Equilibration** → **Measurement of C_{buffer} and $C_{brain\ slice}$**

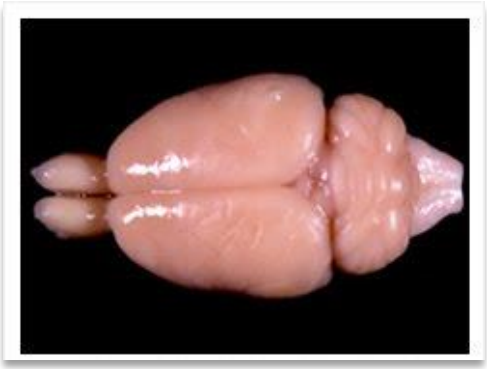


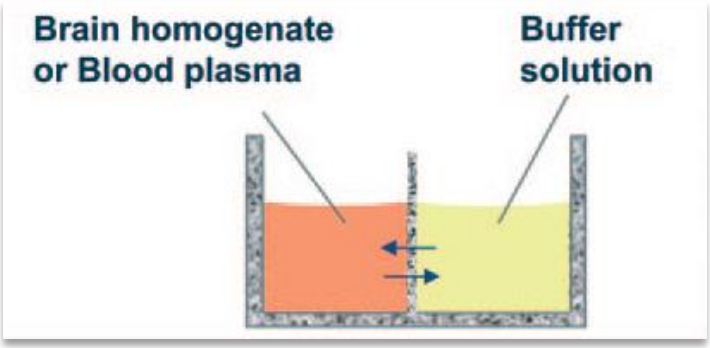
At equilibrium $C_{buffer} = C_{u,brainISF}$

$$V_{u,brain} = \frac{A_{brain}}{C_{buffer}}$$

Kakee et al, JPET, 1997; Fridén et al, DMD, 2007, 2009, 2011; Loryan et al, FBCNS, 2013; Loryan et al, MolPsychiatry, 2016

Assessment of regional brain tissue binding (nonspecific)





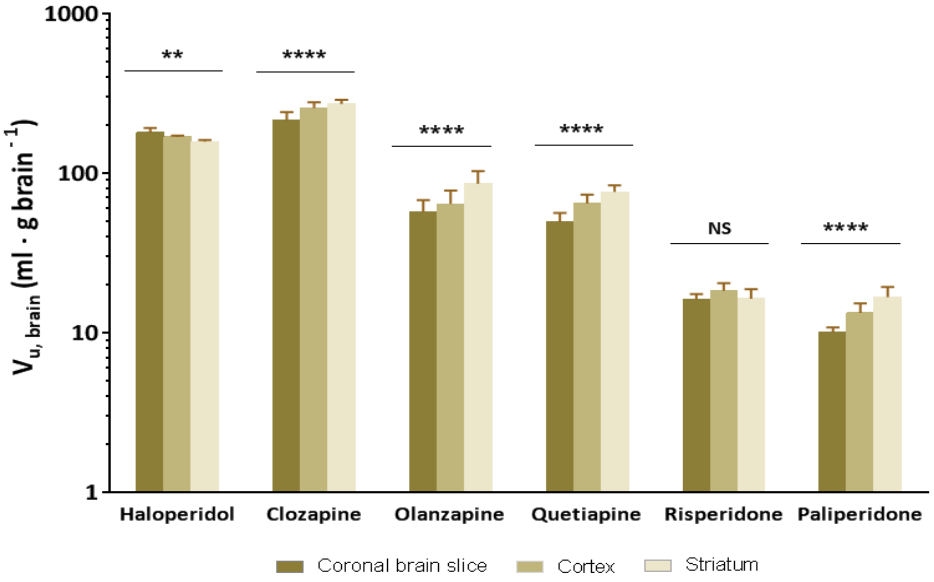
Preparation of brain homogenate → **Equilibrium dialysis** → **Measurement of C_{buffer} and $C_{homogenate}$**

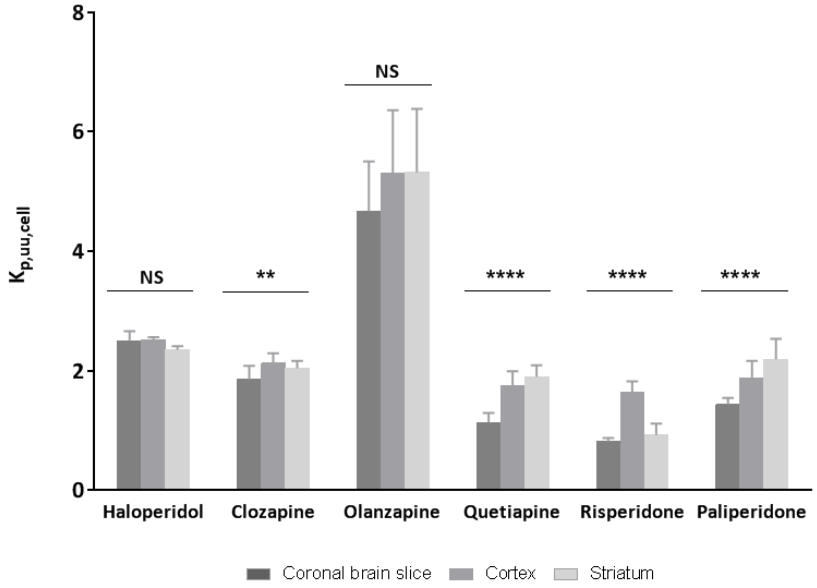
Fraction of unbound drug in brain homogenate, $f_{u,brain}$

$$f_{u,hD} = \frac{C_{buffer}}{C_{homogenate}} \Rightarrow f_{u,brain} = \frac{\frac{1}{D}}{\left(\left(\frac{1}{f_{u,hD}}\right) - 1\right) + \frac{1}{D}}$$

D – dilution factor

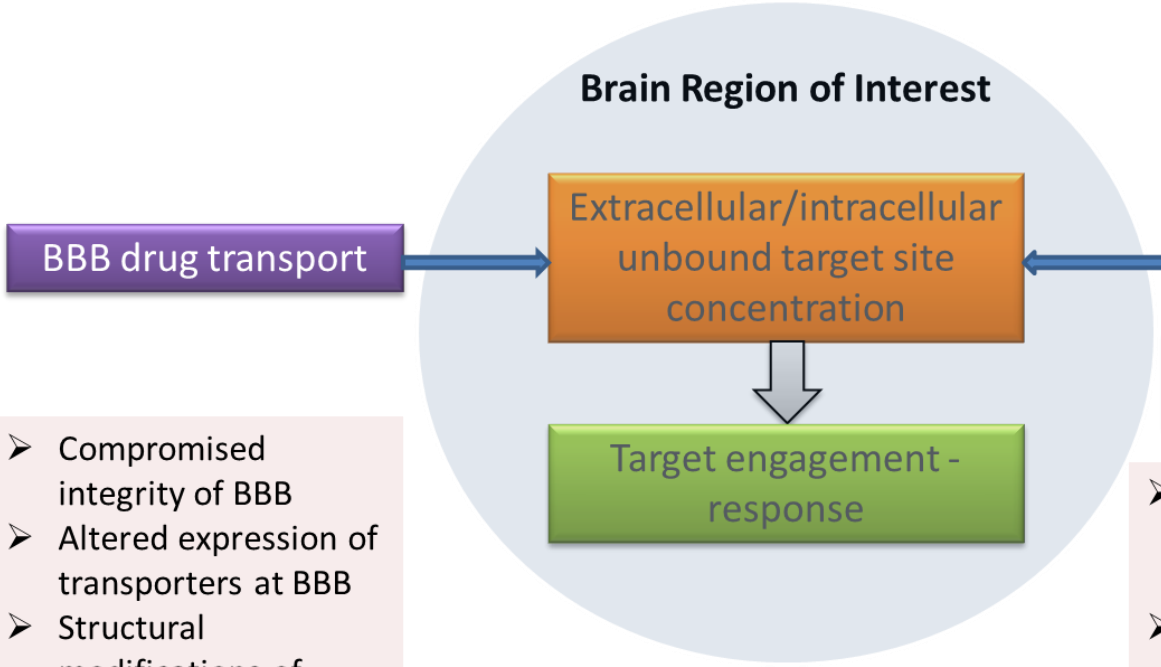
Intra-brain distribution of neuroleptics in cortex and striatum





Loryan et al, MolPsychiatry, 2016

Factors affecting the brain regional drug disposition



Loryan and Hammarlund-Udenaes (in manuscript)

Summary I

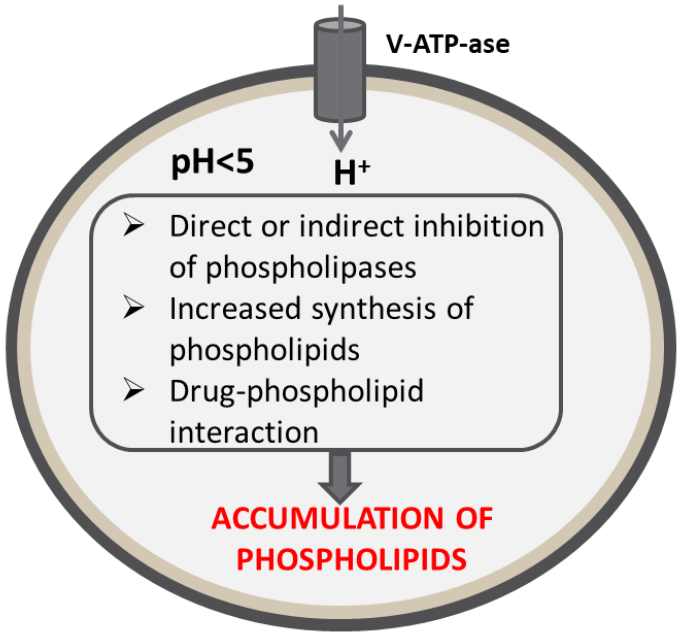
- ✓ CMA-ROI was used for the assessment of target-site concentration in brain regions of the interest.
- ✓ Significant spatial differences were observed in the extent of transport of antipsychotics across the BBB and BSCB.
- ✓ The dissimilarities were more pronounced for the P-gp substrates risperidone (5.4-fold) and paliperidone (4-fold).

Drug induced phospholipidosis (PLD): Interplay between drug-specific and tissue-specific features

PLD induction potential

- Lysosomal trapping
- Physicochemical properties:
  cLogP
  pK_a
  net charge at pH4

Potential mechanisms of Drug Induced Phospholipidosis (PLD)



Drug disposition in tissues

- Volume of distribution (V_d)
- Tissue barriers (BBB, BAB *etc.*)
- Tissue delivery of free drug
- Non-specific binding
- Lysosomal content in tissue

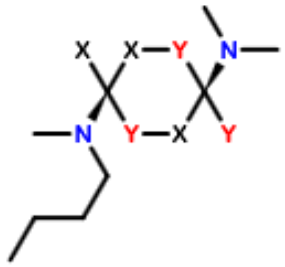
PLD

- Tissue lesions
- Electron dense depositions and myelin-like inclusions
- Foamy macrophages and flocculent material

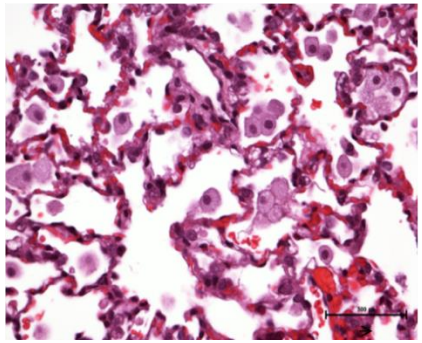
Loryan&Hoppe et al, MolPharm, 2017

Retrospective analysis on PLD in preclinical development

A

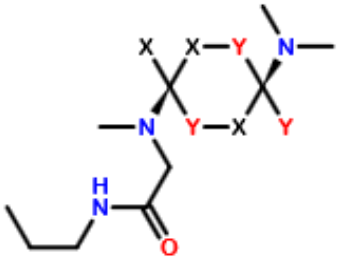


Log P= 5.6
pKa=7.06/9.73



Representative micrograph from histopathological investigation of lung tissue after 14-day repeated oral administration of 100 mg/kg of A to Wistar rats.

B

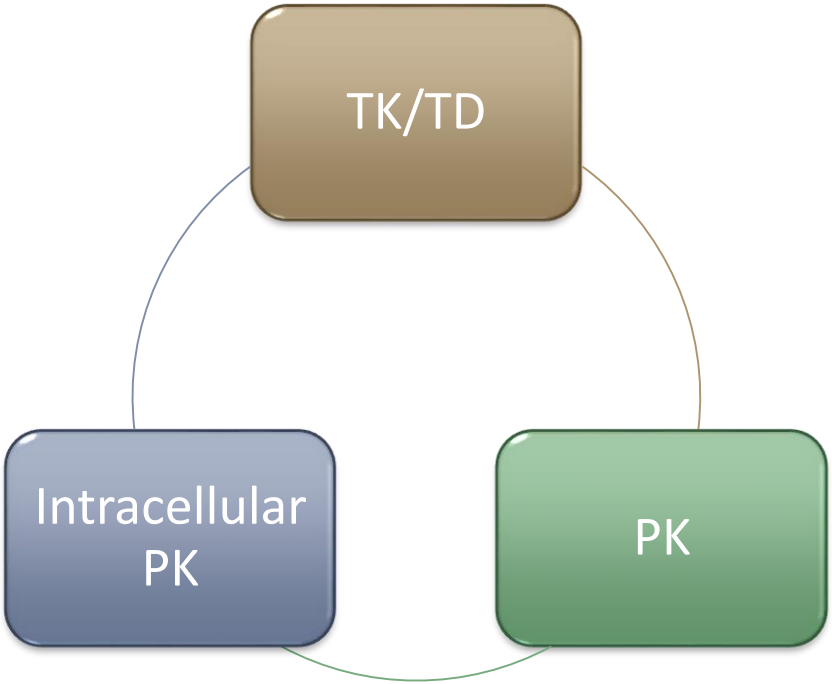


Log P= 4.7
pKa=3.75/9.33

Loryan&Hoppe et al, MolPharm, 2017

Aim & Specific tasks

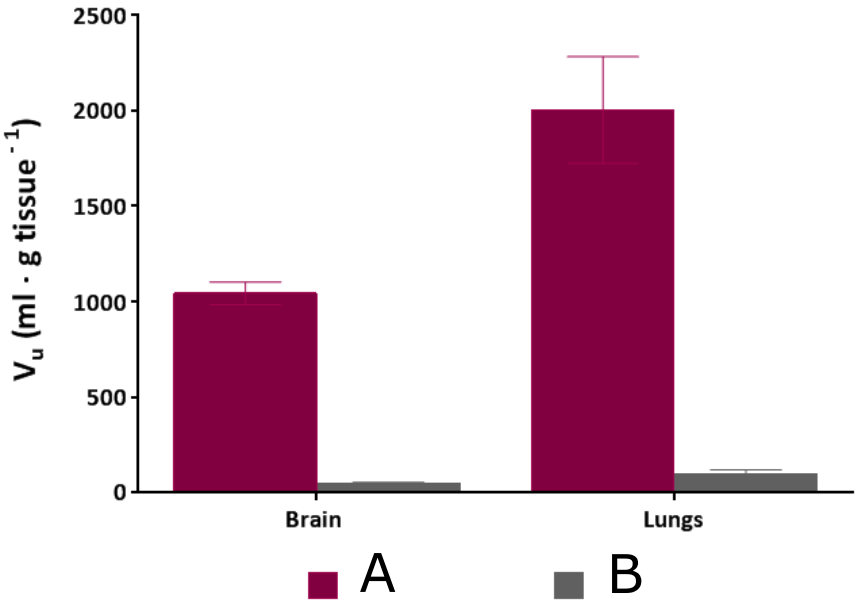
To provide pharmacokinetic basis for mechanistic understanding of tissue-specific PLD development using CMA approach.



Intra-cerebral and intra-pulmonary distribution, V_u

Preparation of fresh brain / lung slices → **Equilibration** → **Measurement of C_{buffer} and C_{slice}**

$$V_u = \frac{Amount_{slice}}{Concentration_{buffer}}$$



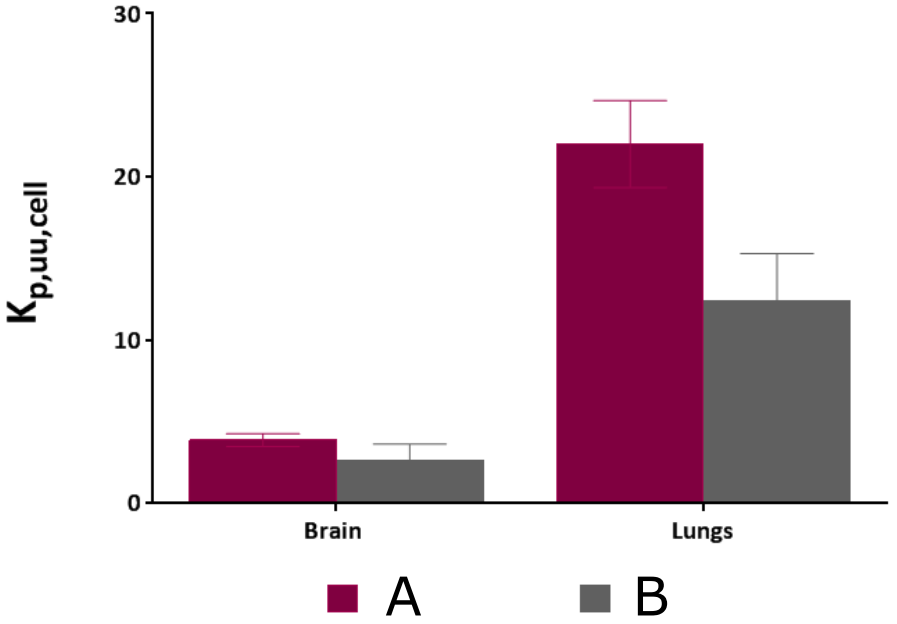
Kakee et al, JPET, 1997; Fridén et al, DMD, 2007, 2009, 2011; Loryan et al, FBCNS, 2013; Bäckström et al, JPharmSci, 2016

Tissue-specific cellular barrier transport, $K_{p,uu,cell}$

$K_{p,uu,cell}$ is intracellular-to-extracellular unbound drug concentrations ratio and it describes the average concentration ratio for all cell types within the brain/lung.

Friden et al, DMD, 2007; Bäckström et al, JPharmSci, 2016

$$K_{p,uu,cell} = V_u \cdot f_u$$



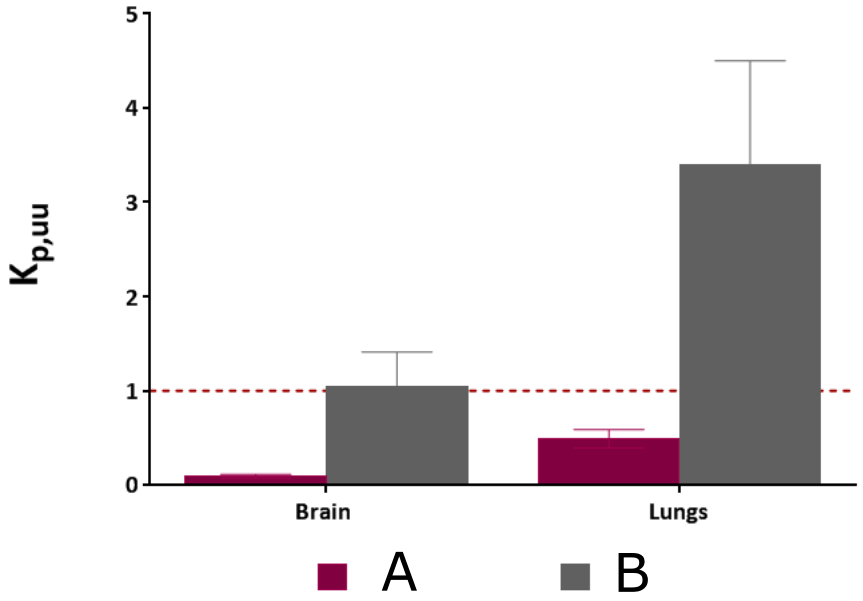
Tissue-specific transport across the barrier, **Kp,uu**

$$K_{p,uu,tissue} = \frac{K_{p,tissue}}{V_{u,tissue} \cdot f_{u,plasma}}$$

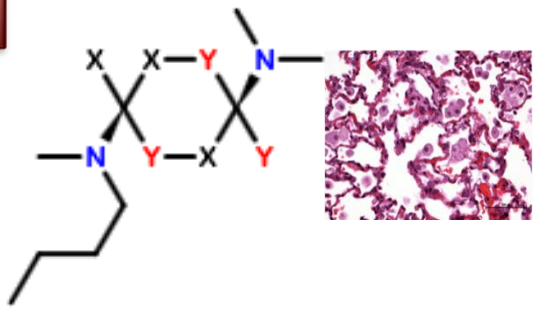
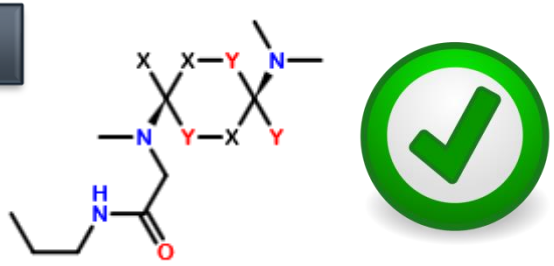
Influx $K_{p,uu} > 1$; $C_{u,tissue} > C_{u,plasma}$

Passive $K_{p,uu} = 1$; $C_{u,tissue} = C_{u,plasma}$

Efflux $K_{p,uu} < 1$; $C_{u,tissue} < C_{u,plasma}$



Summary

	Brain	Lungs
A 	**↓↓↓ BBB transport** ↑ cellular barrier uptake ↑↑ lysosomal trapping	↓ BAB transport **↑↑↑ cellular barrier uptake** **↑↑↑ lysosomal trapping**
B 	= BBB transport ↑ cellular barrier uptake **↑ lysosomal trapping**	↑ BAB transport ↑↑ cellular barrier uptake **↑↑ lysosomal trapping**

Summary II

- ✓ CMA was applied for assessment of tissue-specific target-site concentration.
- ✓ The compound and tissue-specific differences in the blood-tissue barrier and cellular barrier transport were observed.
- ✓ Tissue-specific target-site exposure assessment was beneficial for understanding of potential pharmacokinetic mechanisms of PLD.

Acknowledgments

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