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Introduction

- Brain distribution of drugs is hampered by **active efflux transporters** like P-glycoprotein (**P-gp**) at the blood-brain-barrier (BBB) [1][2]
- The LeiCNS-PK3.0 **PBPK model** allows **predictions of brain pharmacokinetics** (PK) in multiple central nervous system (CNS) compartments [3][4]
- LeiCNS-PK3.0 does not **explicitly** account for active transport processes, and to do so is reliant on the steady-state unbound brain to plasma ratio (**Kp_{uu,brain}**)
- In vitro* **transport assays** contain **mechanistic** information on P-gp functionality and might be promising for **use within LeiCNS** for **prediction** of BBB transport

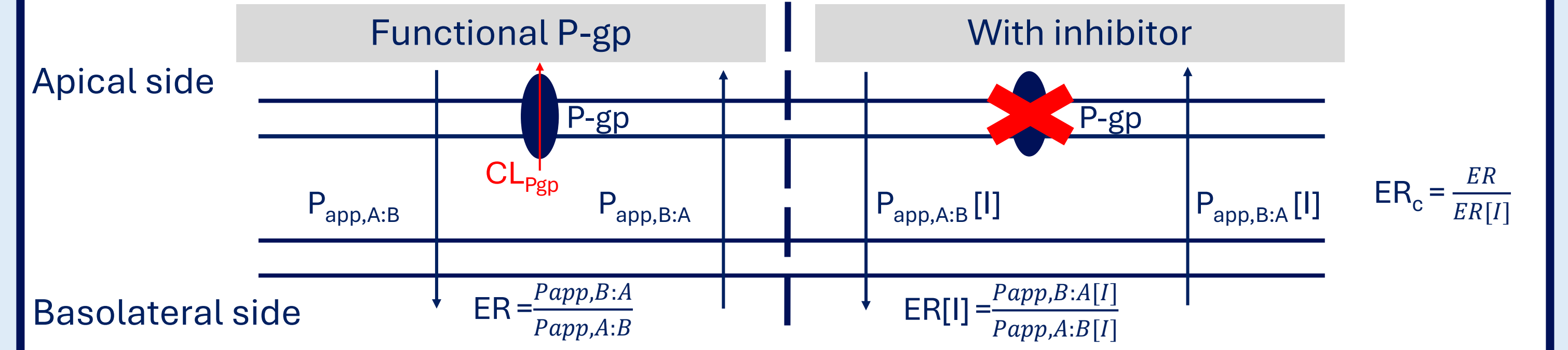
Aims

- Explicitly **describe P-gp mediated clearance (CL_{Pgp})** using *in vitro* transport data extracted from literature
- Evaluate the **suitability** of literature derived data to **predict CNS-PK** of P-gp substrates in **rats**

Methods

Description of drug clearances across the BBB

- P-gp mediated clearance (**CL_{Pgp}**) was calculated using **apparent permeability** (P_{app}) and **corrected efflux ratio** (ER_c) (figure 1) derived from literature
- In vitro* CL scaled to *in vivo* through **BBB surface area** (SA_{BBB}) and P-gp **relative expression factor**: REF = expression in vivo / expression in vitro
- Passive diffusion across BBB: **CL_{passive}** = P_{app,A:B}[I] * SA_{BBB}
- P-gp efflux at BBB **CL_{Pgp}** = (ER_c-1) * 2P_{app,A:B}[I] * SA_{BBB,trans} * REF [5]



Model validation

- Predictions were validated using rat **brain extracellular fluid (ECF)** data after short or continuous infusion of five P-gp substrates
- Median prediction errors (PE) **within 2-fold** were considered accurate

Results

- 32 drug transport values were extracted from literature, measured in **Caco-2**, **LLC-PK1**, and **MDCKII** cell lines
- The extracted transport values were **variable** between studies (figure 2)
- P-gp expression *in vivo* was **constant** between sources (mean = 19.4 fmol/μg protein), but **varied in vitro** (table 1)
- The model accurately predicted brainECF PK of 3 **passively diffusing** drugs (not shown) and showed **variable success** for P-gp substrates (figure 3)

Table 1: *In vitro* P-gp expression in different cell lines. Due to variability in the reported values, the expression values are categorized into the highest, average and lowest reported expression levels per cell line.

Cell line	Highest expression value (fmol/μg protein)	Average expression value (fmol/μg protein)	Lowest expression value (fmol/μg protein)
Caco-2	7.4	4.7	2
LLC-PK1-MDR1	-	-	13.1
LLC-PK1-mdr1a	61	38.1	15.2
MDCKII-MDR1	10.3	6.3	2.2

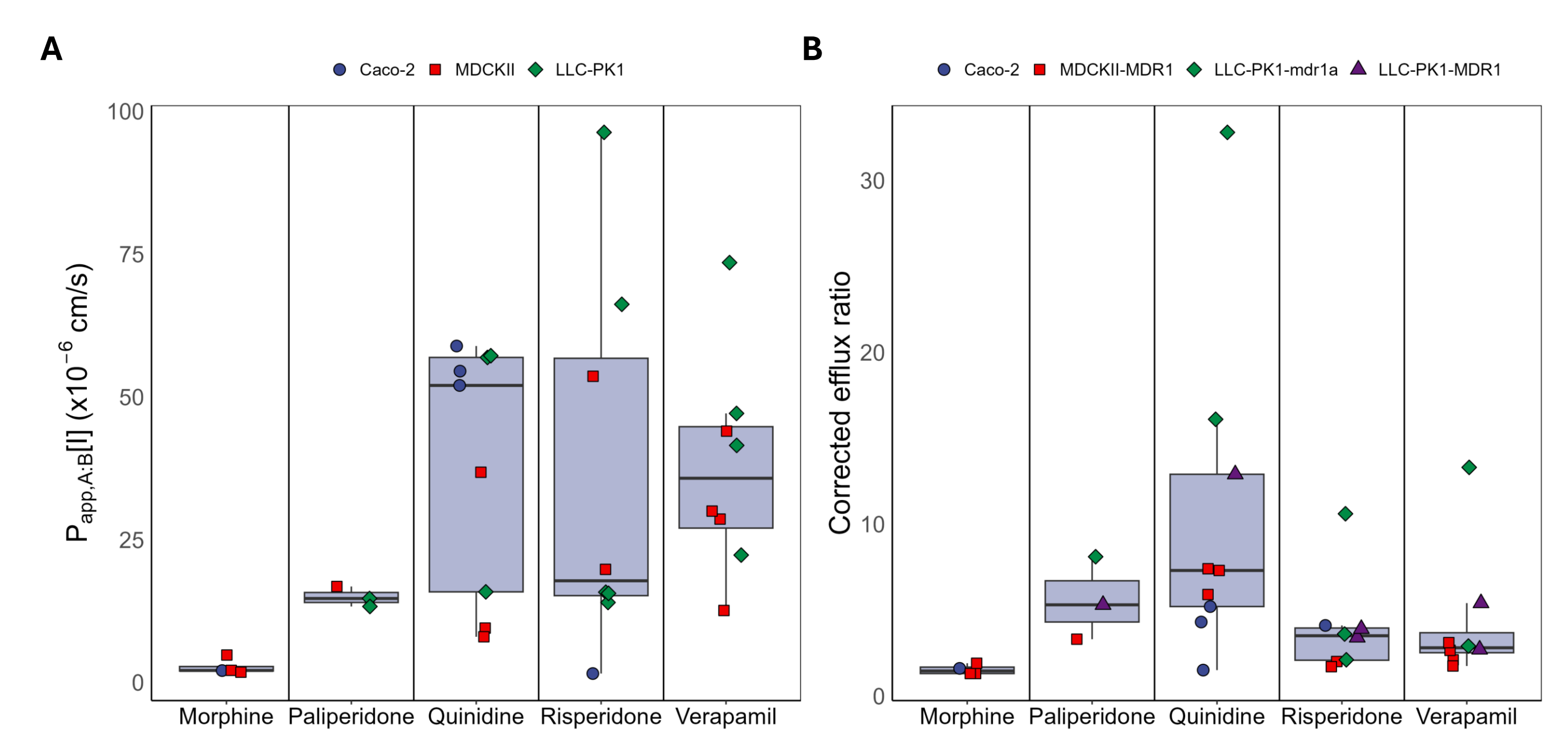


Figure 2: Overview of literature reported (A) $P_{app,A:B}[I]$ and (B) corrected efflux ratio (ER_c) per drug. Different cell lines are indicated by shapes and colours of the observed datapoints.

- Morphine, risperidone and verapamil brainECF PK were all **predicted well**
- Paliperidone and quinidine data were **underpredicted** after short infusion, but not after continuous infusion
- Matching transport values and P-gp expression reported **in conjunction** gave **poor predictions** (table 2)

Table 2: Median prediction errors (PE) of risperidone, quinidine and verapamil predictions made using LLC-PK1-mdr1a transport data from Uchida et al. [6] and Nicolai et al. [7], using the *in vitro* P-gp expression reported by Uchida et al. or Nicolai et al. Median PE values shaded in green, yellow and red fall in <2-fold error, <3-fold error and >3-fold error, respectively. PE was calculated as $\frac{Y_{pred,j} - Y_{obs,i,j}}{(Y_{pred,j} + Y_{obs,i,j})/2}$

	Risperidone (Subcutaneous)		Risperidone (Constant infusion)		Quinidine (Constant infusion)		Verapamil (Constant infusion)	
	Uchida transport data	Nicolai transport data	Uchida transport data	Nicolai transport data	Uchida transport data	Nicolai transport data	Uchida transport data	Nicolai transport data
Uchida expression	-1.14	0.56	-1.08	0.65	-1.56	-1.18	-1.2	0.28
Nicolai expression	-0.04	1.23	0.06	1.25	-0.71	-0.05	-0.1	1.09

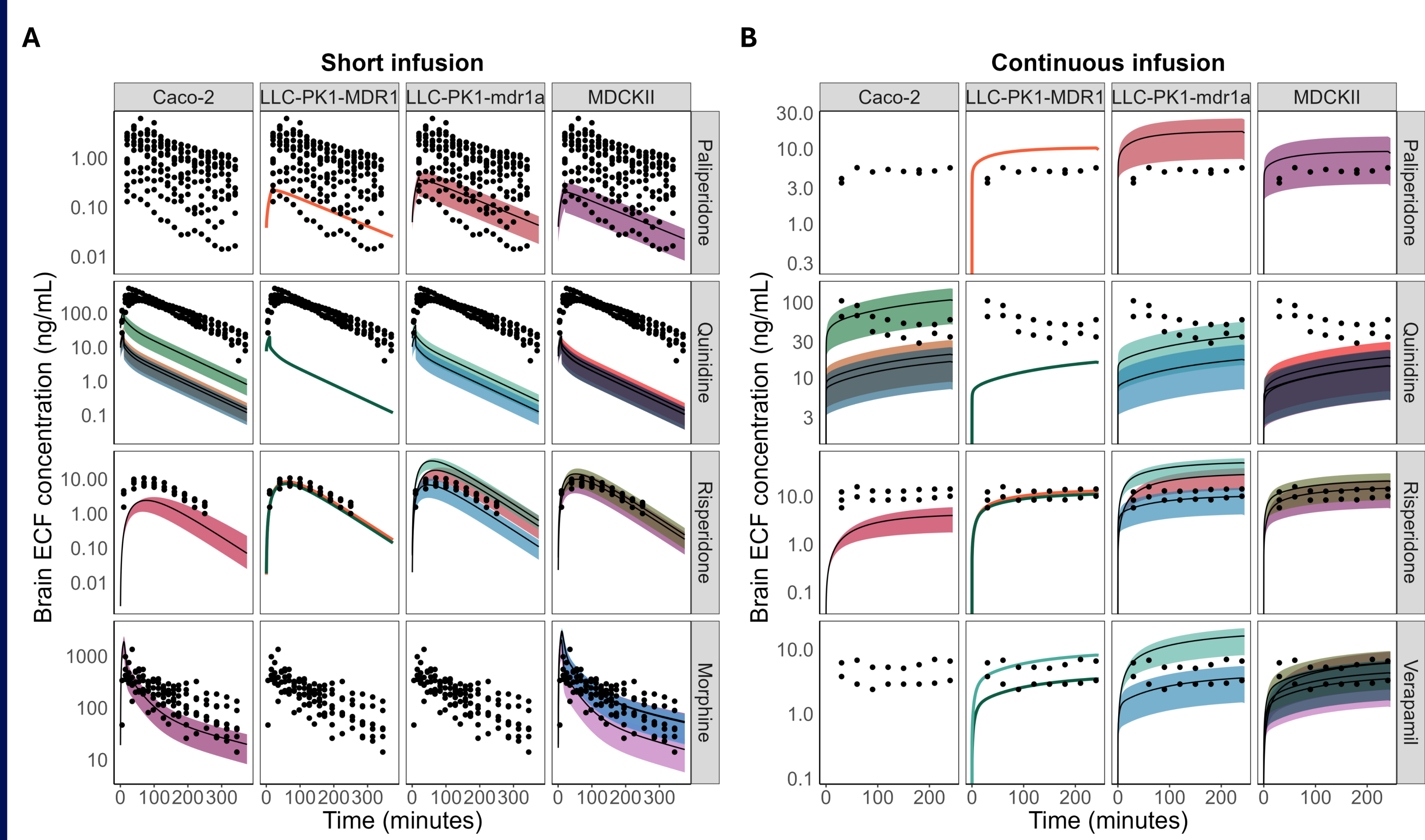


Figure 3: Rat brainECF PK predictions of (A) short infusion and (B) continuous infusion of the P-gp substrates paliperidone, quinidine, risperidone, morphine and verapamil. Each column shows which cell line was used to determine $P_{app}[I]$ and ER_c values that were used as input for the prediction. The colour of the prediction interval corresponds to the reference from which transport data were retrieved. The upper line of the prediction bands indicates the predictions made using the highest in vitro P-gp expression value for the given cell line, while the lowest line of the band corresponds to the lowest expression value. Black lines indicate the predictions made using the average in vitro P-gp expression. Each row indicates a different drug. Plots without predictions indicate lack of transport data in a cell line for a given drug. Observed unbound brainECF concentrations are shown as black points.

Discussion & Conclusions

- The model **accurately predicted** brainECF PK for risperidone and morphine (short infusion) and paliperidone, risperidone and verapamil (continuous infusion)
- Despite variability in transport data, many predictions **were in agreement** with each other
- A **bandwidth prediction** based on reported *in vitro* P-gp expression **gives an indication** of what happens *in vivo*
- The best scaling factor is not related to **actual in vitro P-gp expression**, highlighting missing information for **robust translation between in vitro and in vivo**
- Quinidine data shows large **underprediction after short infusion**, but not after continuous infusion. The reason for this is still unknown

References

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Acknowledgements and funding

- Attending PAGE was made possible through financial support by the PAGE conference.
- This project has received funding from the European Union's Horizon 2020 research and innovation programme QSPainRelief under grant agreement No 848068