Predicting Drug Concentration-Time Profiles in Multiple CNS Compartments Using a Comprehensive Physiologically-Based Pharmacokinetic Model

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Drug development targeting the central nervous system (CNS) is challenging due to poor predictability of drug concentrations in various CNS compartments. We developed a generic physiologically based pharmacokinetic (PBPK) model for prediction of drug concentrations in physiologically relevant CNS compartments. System-specific and drug-specific model parameters were derived from literature and in silico predictions. The model was validated using detailed concentration-time profiles from 10 drugs in rat plasma, brain extracellular fluid, 2 cerebrospinal fluid sites, and total brain tissue. These drugs, all small molecules, were selected to cover a wide range of physicochemical properties. The concentration-time profiles for these drugs were adequately predicted across the CNS compartments (symmetric mean absolute percentage error for the model prediction was <91%). In conclusion, the developed PBPK model can be used to predict temporal concentration profiles of drugs in multiple relevant CNS compartments, which we consider valuable information for efficient CNS drug development.

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Lack of knowledge of the target-site concentrations in the CNS is a major hurdle in the development of new CNS drugs.

WHAT QUESTION DID THIS STUDY ADDRESS?
☐ A generic PBPK model in the rat CNS was proposed.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE
☑ The developed PBPK model was able to predict time-dependent concentration profiles of many drugs with distinctively different physicochemical properties in multiple physiologically relevant compartments in the CNS.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?
☑ The developed model structure can be used to predict concentration-time profiles in rats and offers a scientific basis for the development of CNS drugs, in principle, without the need of using animals.

The development of drugs targeting diseases of the central nervous system (CNS) represents one of the most significant challenges in the research of new medicines. Characterization of exposure-response relationships at the drug target site may be of critical importance to reduce attrition. However, unlike for many other drugs, prediction of target-site concentrations for CNS drugs is complex, among other factors, due to the presence of the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB). Moreover, direct measurement of human brain concentrations is highly restricted for ethical reasons. Therefore, new approaches that can robustly predict human brain concentrations of novel drug candidates based on in vitro and in silico studies are of great importance.

Several pharmacokinetic (PK) models to predict CNS exposure have been published with different levels of complexity. The majority of these models depend on animal data. Furthermore, these models have typically not been validated against human CNS drug concentrations. We previously published a general multicompartmental CNS PK model structure, which was developed using PK data obtained from rats. Quantitative structure-property relationship (QSPR) models can be used to predict drug BBB permeability and $K_{pu,brain_{ECF}}$ (unbound brain extracellular fluid-to-plasma concentration ratio) without performing novel experiments, but these QSPR models have not taken into account the time course of CNS distribution. Therefore, there exists an unmet need for approaches to predict drug

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target-site concentration-time profiles without the need of in vivo animal experiments.

Physiologically based pharmacokinetic (PBPK) modeling represents a promising approach for the prediction of CNS drug concentrations. Previously, such models have been widely used to predict tissue concentrations. The PBPK models typically distinguish between drug-specific and system-specific parameters, therefore, enabling predictions across drugs and species. However, PBPK models for the CNS have been of limited utility due to a lack of relevant physiological details for mechanism of transport across the BBB and BCSFB, and for drug distribution within the CNS. 

Capturing the physiological compartments, flows, and transport processes in a CNS PBPK model is critically important to predict PK profiles in the CNS. The CNS comprises of multiple key physiological compartments, including brain extracellular fluid (brainECF), brain intracellular fluid (brainICF), and multiple cerebrospinal fluid (CSF) compartments. The brainECF and brainICF compartments are considered highly relevant target sites for CNS drugs, whereas CSF compartments are often used to measure CNS-associated drug concentrations, if brainECF and brainICF information cannot be obtained. Furthermore, cerebral blood flow (CBF) and physiological flows within the CNS, such as the brainECF flow and CSF flows, influence drug distribution across CNS compartments. Next to binding to protein and lipids, pH-dependent distribution in subcellular compartments, such as trapping of basic compounds in lysosomes, needs to be considered. With regard to the transfer processes across the BBB and BCSFB, passive diffusion via the paracellular and transcellular pathways and active transport by influx and/ or efflux transporters need to be addressed.

At both BBB and BCSFB barriers, the cells are interconnected by tight junctions, which limit drug exchange via the paracellular pathway. Paracellular and transcellular diffusion depend on the aqueous diffusivity coefficient and membrane permeability of the compound, which can be related to the physicochemical properties. The combination of these transport routes may differ between individual drugs, which complicate the prediction of plasma-brain transport.

System-specific information on physiological parameters can be used in scaling between species. Many of these system-specific parameters can or have been obtained from in vitro and in vivo experiments. Drug-specific parameters can be derived by in vitro and QSAR approaches, and can be used for the scaling between drugs. A comprehensive CNS PBPK model can integrate system-specific and drug-specific parameters to potentially enable the prediction of the brain distribution of drugs without the need to conduct in vivo animal studies.

The purpose of the current work is to develop a comprehensive PBPK model to predict drug concentration-time profiles in the multiple physiologically relevant compartments in the CNS, based on system-specific and drug-specific parameters without the need to generate in vivo data. We specifically consider the prediction of PK profiles in the CNS during pathological conditions, which may have distinct effects on paracellular diffusion, transcellular diffusion, and active transport. Therefore, we include a range of such transport mechanisms in our CNS PBPK model. This model is evaluated using previously published detailed multilevel brain and CSF concentration-time data for 10 drugs with highly diverse physicochemical properties.

### MATERIALS AND METHODS

We first empirically modeled plasma PK using available plasma PK data, which was used as the basis for the CNS PBPK model. This CNS model was based entirely on parameters derived from literature and in silico predictions. Model development was performed using NONMEM version 7.3.

### Empirical plasma PK model

Plasma PK models were systematically developed using in vivo data with a mixed-effects modeling approach. One, two, and three-compartment models were evaluated. Interindividual variability and interstudy variability were incorporated on each PK parameter using exponential models. Proportional and combined additive-proportional residual error models were considered. Model selection was guided by the likelihood ratio test ($P<0.05$), precision of the parameter estimates, and standard goodness of fit plots.

### CNS PBPK model development

A generic PBPK model structure was developed based on the previously published generic multicompartmental CNS distribution model (Figure 1), which consists of plasma, brainECF, brainICF, CSF in the lateral ventricle (CSF_LV), CSF in the third and fourth ventricle (CSF_TFV), CSF in the cisterna magna (CSF_CM), and CSF in the subarachnoid space (CSF_SAS) compartments. We added new components: (1) an acidic subcellular compartment representing lysosomes to account for pH-dependent drug distribution; (2) a brain microvascular compartment (brainMV) to account for CBF vs. permeability rate-limited kinetics; and (3) separation of passive diffusion at the BBB and BCSFB into its transcellular and paracellular components.

#### System-specific parameters

Physiological parameters for the distribution volumes of all the CNS compartments, flows, surface area (SA) of the BBB (SA_BB), SA of the BCSFB (SA_BCSFB), SA of the total brain cell membrane (BCM; SA_BCM), and the width of BBB (Width_BB) were collected from literature. The SA_BCSFB was divided into SA_BCSFB1, which is a surface area around CSF_yaw and SA_BCSFB2, which is a surface area around CSF_yaw. The lysosomal volume was calculated based on the volume ratio of lysosomes to brain intracellular fluid of brain parenchyma cells (1:80), and the SA of the lysosome (SA_LYS) is calculated by obtaining the lysosome number per cell using the lysosomal volume and the diameter of each lysosome. Transcellular and paracellular diffusion were separately incorporated into the models, therefore, the ratio of SA_BB and SA_BCSFB for transcellular diffusion and paracellular diffusion were required for the calculation. Based on electron microscopic cross-section pictures of brain capillary, the length of a single brain microvascular endothelial cell was estimated to be around 17 μm and the length of the...
The presence of tight junctions in the intercellular space of the BBB and BCSFB significantly reduces paracellular transport. Therefore, correcting for the effective pore size for paracellular diffusion is important. The transendothelial electrical resistance (TEER) is reported to be around 1,800 Ω cm² at the rat BBB, whereas the TEER is around 20–30 Ω cm² at the rat BCSFB. According to a study on the relationship between TEER and the pore size, the pore size at the BBB and BCSFB can be assumed to be around 0.0011 μm and 0.0028 μm, respectively. Thus, it was expected that 99.8% of total SABBB and 99.8% of total SABCSFB is used for the transcellular diffusion (SABBBt and SABCSFBt, respectively), whereas 0.006% of total SABBB and 0.016% of total SABCSFB are used for paracellular diffusion (SA BBBp and SABCSFBp, respectively). Note that, due to the presence of tight junction proteins, not all intercellular space can be used for paracellular diffusion.

**Drug-specific parameters**

**Aqueous diffusivity coefficient.** The aqueous diffusivity coefficient was calculated using the molecular weight of each compound with the following equation:

\[
\log D_{aq} = -4.113 - 0.4609 \times \log MW
\]  

where \( D_{aq} \) is the aqueous diffusivity coefficient (in cm²/s) and \( MW \) is the molecular weight (in g/mol).

**Permeability.** Transmembrane permeability was calculated using the log \( P \) of each compound with the following equation:

\[
\log P_{\text{transmembrane}}^0 = 0.939 \times \log P - 6.210
\]  

where \( P_{\text{transmembrane}}^0 \) is the transmembrane permeability (in cm/s), \( P \) is the n-octanol lipophilicity value.

**Active transport.** The impact of the net effect of active transporters on the drug exchange at the BBB and BCSFB was incorporated into the model using asymmetry factors (AFin1–3 and AFout1–3). The AFs were calculated from the Kp,uu,brainECF, Kp,uu,CSF LV, and Kp,uu,CSF CM, such that they produced the same Kp,uu values within the PBPK model at the steady-state. Therefore, the AFs were dependent on both the Kp,uu values and the structure and parameters of the PBPK model. If the Kp,uu values were larger than 1 (i.e., net active influx), then AFin1, AFin2, and AFin3 were derived from Kp,uu,brainECF, Kp,uu,CSF LV, and Kp,uu,CSF CM, respectively, whereas AFout1–3 were fixed to 1. If the Kp,uu values were smaller than 1 (i.e., net active efflux), then AFout1, AFout2, and AFout3 were derived from Kp,uu,brainECF, Kp,uu,CSF LV, and Kp,uu,CSF CM, respectively, whereas AFin1–3 were fixed to 1. In the

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**Figure 1** The developed model structure. The model consists of a plasma pharmacokinetic (PK) model and a central nervous system (CNS) physiologically based pharmacokinetic (PBPK) model with estimated plasma PK parameters, and system-specific and drug-specific parameters (colors) for CNS. Peripheral compartments 1 and 2 were used in cases where the plasma PK model required them to describe the plasma data adequately. AFin1–3, asymmetry factor into the CNS compartments 1–3; AFout1–3, asymmetry factor out from the CNS compartments 1–3; BBB, blood-brain barrier; BCSFB, blood-cerebrospinal fluid barrier; BF, binding factor; brainECF, brain extracellular fluid; brainICF, brain intracellular fluid; CSF CM, cerebrospinal fluid in the cisterna magna; CSF LV, cerebrospinal fluid in the lateral ventricle; CSF SAS, cerebrospinal fluid in the subarachnoid space; CSF TFV, cerebrospinal fluid in the third and fourth ventricle; PHF1–7, pH-dependent factor 1–7; QBCMB, passive diffusion clearance at the brain cell membrane; QCSFB, cerebrospinal blood flow; QCSFR, cerebrospinal fluid flow; QECF, brainECF flow; QPBBB, paracellular diffusion clearance at the BBB; QBCSFB1, paracellular diffusion clearance at the BCSFB1; QBCSFB2, paracellular diffusion clearance at the BCSFB2; QBBB, transcellular diffusion clearance at the BBB; QBCSFB1, transcellular diffusion clearance at the BCSFB1; QBCSFB2, transcellular diffusion clearance at the BCSFB2.
analysis, $K_{p,uu,brain_{ECF}}, K_{p,uu,CSF_{LV}},$ and $K_{p,uu,CSF_{CM}}$ were derived from previous in vivo animal experiments. The steady-state differential equations in the PBPK model were solved using the Maxima Computer Algebra System (http://maxima.sourceforge.net) to obtain algebraic solutions for calculating AFs from the $K_{p,uu}$ values. The detailed algebraic solutions for each AF are provided in Supplementary Material S1.

**Combined system-specific and drug-specific parameters**

*Passive diffusion across the brain barriers.* Passive diffusion clearance at the BBB and BCSFB ($Q_{BBB}$ and $Q_{BCSFB}$, respectively) was obtained from a combination of paracellular and transcellular diffusion, $Q_p$ and $Q_t$, respectively (Eq. 3).

$$Q_{BBB/BCSFB}(\text{mL/min}) = Q_{BBB} + Q_{t\text{BBB}/BCSFB}$$

where $Q_{BBB/BCSFB}$ represents the passive diffusion clearance at the BBB/BCSFB, $Q_{BBB}$ represents the paracellular diffusion clearance at the BBB/BCSFB, and $Q_{t\text{BBB}/BCSFB}$ represents the transcellular diffusion clearance at the BBB/BCSFB.

The paracellular diffusion clearance was calculated with the aqueous diffusivity coefficient ($D_{aq}$), $Width_{BBB/BCSFB}$, and $SA_{BBBp}$ or $SA_{BCSFBp}$ using Eq. 4.

$$Q_{BBB/BCSFB}(\text{mL/min}) = \frac{D_{aq}}{Width_{BBB/BCSFB}} \times SA_{BBBp/BCSFBp}$$

The transcellular diffusion clearance was calculated with the transmembrane permeability and $SA_{BBBh}$ or $SA_{BCSFBh}$ using Eq. 5.

$$Q_{t\text{BBB}/BCSFB}(\text{mL/min}) = \frac{1}{2} + P_{t\text{transacellular}} \times SA_{BBBh/BCSFBh}$$

where the factor $1/2$ is the correction factor for passage over two membranes instead of one membrane in the transcellular passage.

*Active transport across the brain barriers.* To take into account the net effect of the active transporters at the BBB and BCSFB, AFs were added on $Q_t\text{BBB}/BCSFB$ (Eqs. 6 and 7).

$$Q_{BBB/BCSFB}(\text{mL/min}) = Q_{t\text{BBB}/BCSFB} + Q_{t\text{BBB}/BCSFB} \times AFin$$

$$Q_{BBB/BCSFB}(\text{mL/min}) = Q_{t\text{BBB}/BCSFB} + Q_{t\text{BBB}/BCSFB} \times AFout$$

where $Q_{BBB/BCSFB, in}$ represents the drug transport clearance from brain_{MV} to brain_{ECF}/CSFs, and $Q_{BBB/BCSFB, out \text{withoutPHF}}$ represents the drug transport clearance from brain_{ECF}/CSFs to brain_{MV} without taking into account the pH-dependent kinetics (to be taken into account separately; see below).

*Cellular and subcellular distribution.* Passive diffusion at the BCM ($Q_{BCM}$) and at the lysosomal membrane ($Q_{LYSO}$) was described with the transmembrane permeability together with $SA_{BCM}$ or $SA_{LYSO}$, respectively (Eqs. 8 and 9).

$$Q_{BCM}(\text{mL/min}) = P_0 \times SA_{BCM}$$

$$Q_{LYSO}(\text{mL/min}) = P_0 \times SA_{LYSO}$$

where $Q_{BCM}$ represents the passive diffusion clearance at the BCM, and $Q_{LYSO}$ represents the passive diffusion clearance at the lysosomal membrane.

*pH-dependent partitioning.* We considered the differences in pH in plasma (pH 7.4) and in relevant CNS compartments, namely brain_{ECF} (pH_{ECF} 7.3), CSF (pH_{CSF} 7.3), brain_{ICF} (pH_{ICF} 7.0), and lysosomes (pH_{lyso} 5.0). The impact of pH differences on the passive diffusion clearance from brain_{ECF} to brain_{MV} (PHF1), from CSF_{LV} to brain_{MV} (PHF2), from CSF_{TVF} to brain_{MV} (PHF3), from brain_{ECF} to brain_{ICF} (PHF4), from brain_{ICF} to brain_{ECF} (PHF5), from brain_{ICF} to lysosomes (PHF6), and from lysosomes to brain_{ICF} (PHF7) were described by pH-dependent factors, which were defined as the ratio of the unionized fraction of each compound at the pH in a particular compartment and the unionized fraction in plasma. The PHFs were calculated from the $pK_a$ of each compound and the pH of a particular compartment. The equations are developed using the classical Henderson-Hasselbalch equation, and are based on the assumption that there is no active transport.

$$PHF_{base1} = \frac{10^{pK_a - pH_{ECF} + 1}}{10^{pK_a - pH_{ECF} + 1}}$$

$$PHF_{base2} = \frac{10^{pK_a - pH_{ECF} + 1}}{10^{pK_a - pH_{ECF} + 1}}$$

$$PHF_{base5} = \frac{10^{pK_a - pH_{ECF} + 1}}{10^{pK_a - pH_{ECF} + 1}}$$

$$PHF_{base7} = \frac{10^{pK_a - pH_{ECF} + 1}}{10^{pK_a - pH_{ECF} + 1}}$$

where PHF_{base1-7} are PHF1-7 for basic compounds, PHF_{acid1-7} are PHF1-7 for acidic compounds, and 7.4 is the pH in the plasma compartment.

The impact of pH differences on the drug distribution among brain_{ECF}, CSF, brain_{ICF}, and lysosomes was added on $Q_{BCM}$ and $Q_{LYSO}$ using PHFs with the following Eqs. 18–24 based on the assumption that the transport clearance is proportional to the unionized fraction of each compound.
where $Q_{\text{BBB, out}}$ represents the drug transport clearance from brainECF to brainMV, $Q_{\text{BCSFB1, out}}$ represents the drug transport clearance from CSFLV to brainMV, $Q_{\text{BCSFB2, out}}$ represents the drug transport clearance from CSF TFV to brainMV, $Q_{\text{BCM, in}}$ represents the drug transport clearance from brainECF to brainICF, and $Q_{\text{BCM, out}}$ represents the drug transport clearance from brainICF to brainECF.

**Drug binding.** Drug binding to brain tissue components was taken into account in the model using a binding factor (BF) under the assumption that drug binding to the tissue happens instantly. The BF was calculated from $K_p$ (total brain-to-plasma concentration ratio) by solving the BF that results in the same $K_p$ value in the model, using the Maxima program, as described above (Supplementary Material S1). The $K_p$ for each compound was calculated using the compounds’ log P, the composition of brain tissue and plasma, free fraction in plasma (fu,p) and free fraction in brain (fu,b) with the following equation:

$$K_p = \frac{10^{\log P} \times (V_{\text{nlb}} + 0.3 \times V_{\text{phb}} + 0.7 \times V_{\text{phb}} + V_{\text{wb}} \times f_{u,b})}{10^{\log P} \times (V_{\text{nlb}} + 0.3 \times V_{\text{phb}} + 0.7 \times V_{\text{phb}} + V_{\text{wb}} \times f_{u,p})}$$

(25)

where $V_{\text{nlb}}$, $V_{\text{phb}}$, $V_{\text{wb}}$, $V_{\text{nlp}}$, $V_{\text{php}}$, and $V_{\text{wp}}$ represent the rat volume fractions of brain neutral lipids (0.0392), brain phospholipids (0.0533), brain water (0.788), plasma neutral lipids (0.00147), plasma phospholipids (0.00083), and plasma water (0.96), respectively.

**In vivo data collection for model evaluation**

In vivo data obtained from multiple brain locations were used to evaluate the developed model. An overview of experimental design and data for 10 compounds with substantially different physicochemical characteristics is provided in Table 1. All data were previously published, except the remoxipride total brain tissue data. General animal surgery procedures, experimental protocol, and bioanalytical methods for remoxipride total brain tissue data are described in Supplementary Material S2, and experimental protocol details for each drug are summarized in Supplementary Table S1.

**Evaluation of the PBPK model**

The PBPK model performance was evaluated by the comparison of model predictions with the concentration-time profiles in brainECF, CSFLV, CSFCM, and total brain tissue of...
10 compounds. We performed 200 simulations for each compound, including random effect estimates for the plasma PK model. Based on these, we calculated the prediction error (PE) and symmetric mean absolute percentage error (SMAPE), see Eqs. 26 and 27.

\[
PE = \frac{Y_{OBS,ij} - Y_{PRED,ij}}{(Y_{OBS,ij} + |Y_{PRED,ij}|)/2}
\]

\[
SMAPE = \frac{1}{N} \sum_{i=1}^{N} |PE| \times 100
\]

where \(Y_{OBS,ij}\) is the \(i\)th observation of the \(i\)th subject, \(Y_{PRED,ij}\) is the \(i\)th mean prediction of the \(i\)th subject, and \(N\) is the number of observations.

RESULTS
Plasma PK model
The estimated parameters for the descriptive plasma PK models were obtained with good precision and summarized in Table 2. The models describe plasma concentration-time profiles very well for all compounds except risperidone (Supplementary Figure S1). For remoxipride, a small underprediction was observed at later time points.

CNS PBPK model
The NONMEM model codes for the 10 compounds are provided in Supplementary Material S3–S13. The values of the system-specific and drug-specific parameters are summarized in Tables 3–4 and 5, respectively. The combined system-specific and drug-specific parameters are summarized in Table 5. Overall, the developed generic PBPK model could adequately data in brainECF, CSF Lv, CSFCM, and total brain tissue. Figure 2 shows the PE for each compound and each CNS compartment. The PE for risperidone brainECF and CSFCM showed modest underprediction. For the other drugs, the PEs were distributed within two standard deviations and no specific trends were observed across time, compounds, and CNS locations. The SMAPEs for the model prediction in brainECF, CSF Lv, CSFCM, and total brain tissue were 72%, 71%, 69%, and 91%, respectively, indicating that the model could predict concentration-time profiles in these compartments with less than twofold prediction error. The concentration-time plots of individual predictions vs. observations across drugs and dose levels are provided (Supplementary Figure S1).

Impact of cerebral blood flow
Cerebral blood flow (QCBF) is 1.2 mL/min. Therefore, for strong lipophilic compounds, for instance, quinidine, the drug transport clearance from plasma to the brainECF (BBB permeability) is limited by QCBF because QBBB_in and QBBB_out of quinidine were 9.1 and 5.1 mL/min, respectively (Tables 3 and 5).

Impact of distinct paracellular and transcellular pathways on total diffusion at the BBB, and BCSFB (QBBB, QBCSFB1, and QBCSFB2)
The QBBB, QBCSFB1, and QBCSFB2 were determined by the combination of paracellular and transcellular diffusion in the model. Even though the SAABBp is very small compared to the SAABBB (0.006: 99.8), paracellular diffusion had an impact on the values of QBBB: QBCSFB1, and QBCSFB2 especially for hydrophilic compounds. For instance, the values of transcellular diffusion (QtBBB) and paracellular diffusion (QpBBB) for methotrexate, which is the most hydrophilic compound in this study, were 0.000080 and 0.087 mL/min, respectively (Table 5). Thus, the QBBB of methotrexate was determined mainly by paracellular diffusion. For quinidine, which is the most lipophilic compound in the study, the QBBB was mainly determined by CBF limited transcellular diffusion (QtBBB and QpBBB were 7.6 and 0.10 mL/min, respectively).

Rate limiting drug transport clearance for intra-extracellular exchange (Q BCM, in and Q BCM, out)
The QBCM, in and QBCM, out were higher than QBBB, in and QBBB, out for acetaminophen, paliperidone, phenytoin, quinidine, raclopride, remoxipride, and risperidone. The QBCM, in and QBCM, out are lower than QBBB, in and QBBB, out for methotrexate (Table 5). This suggests that the transport clearance from brainMV, via brainECF, to brainCF, is limited by QBBB, in and QBBB, out for acetaminophen, paliperidone, phenytoin, quinidine, raclopride, remoxipride, and risperidone, whereas it is limited by QBCM, in and QBCM, out for methotrexate.

Surface area of BCSFB to determine the paracellular and transcellular diffusion clearance around CSF Lv and CSFTFV
In our model, we assumed that the SA of the BCSFB around CSF Lv (SABCSFBLv) and CSFTFV (SABCSFBSFV) are equal in size (50% of the total SA:BBCSFB for each). The SA is one of the key factors that determine the paracellular and transcellular diffusion clearance across the BCSFB1 and BCSFB2. However, the early-time predictions for CSF Lv for acetaminophen, quinidine, and remoxipride indicate an overprediction of the paracellular and transcellular diffusion clearance (Figure 2 and Supplementary Figure S1), suggesting that the SA of BCSFB1 is <50% of the total SA:BCSF.

Impact of active transporters to determine the extent of drug exposure in the CNS compartments
Active transporters govern the extent of drug exposure in the brain and CSFs. For most of the compounds, the impact of active transporters among Kp,uu,brainECF, Kp,uu,CSF Lv, and Kp,uu,CSF CM was assumed to be identical, except for methotrexate. Different Kp,uu,CSF Lv (0.0066) and Kp,uu,CSF CM (0.0024) were observed for methotrexate, which were taken into account in the PBPK model by asymmetry factors AFout2 and AFout3. The extent of drug entry into the brain and CSF was predicted well for all compounds, except for morphine at the 4 mg/kg dose (Supplementary Figure S1).

DISCUSSION
The developed CNS PBPK model resulted in adequate predictions of concentration-time courses for 10 diverse drugs
| Parameter Estimates (RSE, %) | Acetaminophen | Atenolol | Methotrexate | Morphine | Paliperidone | Phenytoin | Quinidine | Raclopride | Remoxipride | Risperidone |
|-----------------------------|---------------|----------|--------------|----------|--------------|-----------|-----------|------------|-------------|-------------|
| \( CL_{PL} \) mL/min       | 15.8 (9.10)   | 7.13 (20.6) | 8.04 (15.9) | 22.6 (7.70) | 196 (13.0) | 36.0 (8.90) | 162 (4.10) | 46.4 (4.30) | 42.2 (4.90) | 886 (33.2)   |
| \( Q_{PL,\text{PER1}} \) mL/min | 33.8 (33.7) | NA        | 28.5 (30.7) | 30.8 (10.0) | 61.5 (86.2) | 265 (12.7) | 829 (6.80) | 13.4 (27.5) | 33.8 (20.7) | NA          |
| \( Q_{PL,\text{PER2}} \) mL/min | NA           | 3.33 (34.8) | 7.21 (10.2) | NA        | NA          | NA        | 692 (7.50) | 14.0 (10.1) | NA          | NA          |
| \( V_{PL} \) mL            | 49.5 (59.0)   | 256 (27.0) | 28.0 (55.0) | 152 (11.1) | 26,400 (12.6) | 943 (21.5) | 670 (13.3) | 48.9 (16.3) | 83.7 (18.3) | 43,100 (28.1) |
| \( V_{\text{PER1}} \) mL   | 363 (33.1)    | NA        | 111 (14.6) | 530 (9.10) | 2,050 (7.50) | 11,300 (3.20) | 684 (19.2) | 253 (10.9) | NA          | NA          |
| \( V_{\text{PER2}} \) mL   | NA            | NA        | 83.5 (34.9) | 1,200 (10.8) | NA          | NA        | 493 (18.3) | 757 (4.00) | NA          | NA          |
| Fraction                    | 0.693 (19.6)  | NA        | NA          | NA        | NA          | NA        | NA        | NA         | NA          | NA          |

Interindividual variability

- \( \sigma_{CL_{PL}} \) %
- \( \sigma_{Q_{\text{PL,PER1}}} \)
- \( \sigma_{Q_{\text{PL,PER2}}} \)
- \( \sigma_{V_{\text{PER1}}} \)
- \( \sigma_{V_{\text{PER2}}} \)

Intercasual variability

- \( \sigma_{\text{study1}} \)
- \( \sigma_{\text{study2}} \)

Residual error

- \( \sigma_{\text{plasma proportional}} \) %
- \( \sigma_{\text{plasma additive}} \) ng/mL

\( CL_{PL} \), clearance from the central compartment; Fraction, percentage of the drug which is reabsorbed by enterohepatic circulation; NA, not applicable; \( Q_{\text{PL,PER1}} \), intercompartmental clearance between the central compartment and the peripheral compartment 1; \( Q_{\text{PL,PER2}} \), intercompartmental clearance between the central compartment and the peripheral compartment 2; RSE, relative standard error; \( V_{PL} \), distribution volume of the central compartment; \( V_{\text{PER1}} \), distribution volume of the peripheral compartment 1; \( V_{\text{PER2}} \), distribution volume of the peripheral compartment 2.

\(^a\) \( \eta_i \) represents the parameters of the \( i \)th subject and \( \eta_h \) represents the parameters of the \( h \)th study, \( \eta_i \) is the random effect of the \( i \)th subject under the assumption of a normal distribution with a mean value of 0 and variance of \( \sigma_i^2 \), and \( \eta_h \) is the random effect of the \( h \)th study under the assumption of a normal distribution with a mean value of 0 and variance of \( \sigma_h^2 \).

\(^b\) \( \sigma^2 \) is the random effect of the \( j \)th observed concentration of the \( i \)th subject under the assumption of a normal distribution with a mean value of 0 and variance of \( \sigma_j^2 \).
in the brainECF, CSF_LV, CSF_CM, and total brain tissue with less than twofold prediction error. In comparison, QSPR studies that predict Kp,uu,brainECF of drugs have similar prediction error magnitudes, even though only one parameter was predicted. Therefore, the twofold prediction error is considered to be a good result.

A small underprediction was observed in brainECF and CSF_CM for risperidone, and in brainECF for morphine at the 4 mg/kg dose. The underprediction of risperidone brainECF and CSF_CM concentrations (Figure 2) likely results from difficulties in the plasma PK modeling of risperidone, which leads to propagation of an error in the PBPK model. Risperidone plasma PK data appeared to follow a two-compartment PK model but data were insufficient to describe this two-compartment kinetics. The small underprediction for morphine brainECF profiles at a dosage of 4 mg/kg might be related to a large interstudy variability for morphine, because the predictions for morphine at the other dosage groups could adequately capture the observations (Supplementary Figure S1 and Table S1).

This is the first time that the transcellular and paracellular diffusion clearance at the BBB/BCSFB were addressed separately, by using the information of the intercellular space and the effective pore size. As the contribution of these pathways may depend on the condition of the barriers (i.e., in certain disease conditions the tight junctions may become less tight), therefore, assessment of these system-specific parameters is important. From the electron microscopic cross-section picture of brain capillary, the intercellular space was measured to be 0.03 μm, which is comparable to the 0.02 μm width reported. Based on the relationship of the pore size and TEER, which were obtained from in vitro studies, we assumed the effective pore size of the BBB and BCSFB to be 0.0011 μm and 0.0028 μm, respectively. The effective pore size derived for the rat BBB (0.0011 μm) is within the range reported in literature (0.0007–0.0018 μm). Therefore, it is reasonable to assume that our estimations for these system-specific parameter values are appropriate. In this study, no compound with sole paracellular transport (such as mannitol) has been used, as no such data were available in literature.

For the PBPK model, the drug-specific parameters were obtained from in silico predictions using the compounds’ physicochemical properties, except for AF values. The AF values were calculated using Kp,uu values, as obtained from the previously published in vivo animal experiments. It should be noted that Kp,uu values can also be obtained from several published QSPR models using the compound’s physicochemical properties.

Unlike previously developed PBPK models for the CNS, our PBPK model contains a number of key relevant physiological processes and compartments.

We discriminated between paracellular and transcellular diffusion processes. The relative impact of the paracellular diffusion on QBBB or QBCSFB for each compound varied from around 100% (methotrexate) to 1.3% (quinidine). For hydrophilic compounds, QBBB and QBCSFB were impacted most by paracellular diffusion, whereas transcellular diffusion largely determined the QBBB and QBCSFB of lipophilic compounds. The separation of the two processes is expected to be meaningful for the prediction of the CNS drug concentrations in disease conditions, because pathophysiological conditions may differently affect paracellular and transcellular diffusion.

### Table 3 System-specific parameters of the PBPK model

| Description          | Parameter       | Value       | Reference |
|----------------------|-----------------|-------------|-----------|
| Volumes              | BrainECF        | V脑ECF      | 1880 μl   | 30        |
|                     | BrainECF        | V大脑ECF    | 290 μl    | 31        |
|                     | BrainICF        | V脑ICF      | 1440 μl   | 32        |
|                      | Total lysosome  | V总       | 18 μl     | Calculated |
|                      | CSF_LV          | V脑CSF_LV   | 50 μl     | 33,34     |
|                      | CSF_TFV         | V脑CSF_TFV  | 50 μl     | 33,34     |
|                      | CSF_CM          | V脑CSF_CM   | 17 μl     | 35,36     |
|                      | CSF_SAS         | V脑CSF_SAS  | 180 μl    | 33,37     |
|                      | BrainNV         | V脑NV       | 60 μl     | 38        |
| Flows                | Cerebral blood flow | Q脑CF  | 1.2 mL/min | 44       |
|                      | BrainECF flow   | Q脑ECF      | 0.0002 mL/min | 39 |
|                      | CSF flow        | Q脑CSF      | 0.0022 mL/min | 31 |
| Surface areas        | BBB              | SA BBB      | 263 cm²   | 40        |
|                      | BCSFB            | SA BCSFB    | 25 cm²    | 41        |
|                      | Total BCM       | SA BCM      | 3000 cm²  | 42        |
|                      | Total lysosomal membrane | SA总   | 1440 cm²  | Calculated |
| Width                | BBB              | Width脑   | 0.3–0.5 μm | (0.5 was used in the model) | 43 |

BBB, blood-brain barrier; BCM, brain cell membrane; BCSFB, blood-cerebrospinal barrier; CBF, cerebral blood flow; CM, cisterna magna; CSF, cerebrospinal fluid; ECF, extracellular fluid; ICF, intracellular fluid; LV, lateral ventricle; LYSO, lysosome; MV, microvascular; SA, surface area; SAS, subarachnoid space; TFV, third and fourth ventricle; TOT; total; V, volume.
Table 4 Drug-specific parameters of the PBPK model

| Drug specific parameters                              | Acetaminophen | Atenolol  | Methotrexate | Morphine | Paliperidone | Phenyltoin | Quinidine | Radopride | Remoxipride | Risperidone |
|-------------------------------------------------------|---------------|-----------|--------------|----------|--------------|------------|-----------|------------|-------------|-------------|
| Transmembrane permeability (cm/min)                   | 1.1*10^-4     | 5.7*10^-5 | 6.1*10^-7    | 2.5*10^-4| 0.0018       | 0.0077     | 0.058     | 6.6*10^-4  | 0.0035      | 0.0082      |
| Aqueous diffusivity coefficient (paracellular diffusion) (cm²/min) | 4.6*10^-4     | 3.5*10^-4 | 2.8*10^-4    | 3.4*10^-4| 2.8*10^-4    | 3.6*10^-4  | 3.2*10^-4 | 3.1*10^-4  | 3.0*10^-4   | 2.9*10^-4   |
| AF                                                   | 0.37          | 0.018     | 0.38<sup>a</sup>, 0.23<sup>b</sup> | 0.50     | 0.26         | 1.5        | 1.1       | 0.80       | 0.97        |
| AF<sub>1</sub>                                        | 0.4           | 0.37      | 0.0066       | 0.38<sup>a</sup>, 0.23<sup>b</sup> | 0.50     | 0.26         | 1.5        | 1.1       | 0.80       | 0.97        |
| AF<sub>2</sub>                                        | 0.51          | 0.37      | 0.0024       | 0.38<sup>a</sup>, 0.23<sup>b</sup> | 0.50     | 0.26         | 1.5        | 1.1       | 0.80       | 0.97        |
| AF<sub>3</sub>                                        | 0.94          | NA        | 1.3          | NA       | 1.3          | 2.3        | 13        | 11         | 5.5         | 2.1         |
| Free fraction                                        | 0.81          | 0.91      | 0.45         | 0.83     | 0.080        | 0.090      | 0.14      | 0.070      | 0.74        | 0.070       |
| Physicochemical properties                           | 0.80          | 0.90      | NA           | 0.76     | 0.065<sup>d</sup> | 0.080     | 0.090     | 0.13      | 0.57<sup>c</sup> | 0.065       |
| Molecular weight                                     | 151           | 266       | 454          | 285      | 426          | 252        | 324       | 347        | 371         | 410         |
| log P                                                | 0.5           | 0.2       | -1.9         | 0.9      | 1.8          | 2.5        | 3.4       | 1.3        | 2.1         | 2.5         |
| pK<sub>a</sub> (acid)                                 | 9.5           | 14.1      | 3.4          | 10.3     | 13.7         | 9.5        | 13.9      | 5.9        | 13.1        | 13.1        |
| pK<sub>a</sub> (base)                                 | -4.4          | 9.7       | 2.8          | 9.1      | 8.8          | -9.0       | 9.1       | 9.0        | 8.4         | 8.8         |

AF, asymmetry factor; K<sub>p,u,brain<sub>ECF</sub></sub>, unbound brain extracellular fluid-to-plasma concentration ratio; K<sub>p,u,CSF<sub>LV</sub></sub>, unbound CSF<sub>LV</sub>-to-plasma concentration ratio; K<sub>p,u,CSF<sub>CM</sub></sub>, unbound CSF<sub>CM</sub>-to-plasma concentration ratio; K<sub>p</sub>, total brain-to-plasma concentration ratio; f<sub>u,p</sub>, free fraction in plasma; f<sub>u,b</sub>, free fraction in brain.

AF<sub>1</sub>-3 and AF<sub>4</sub> were calculated from K<sub>p,u,brain<sub>ECF</sub></sub>, K<sub>p,u,CSF<sub>LV</sub></sub>, and K<sub>p,u,CSF<sub>CM</sub></sub>, respectively.

<sup>a</sup>4 mg/kg.
<sup>b</sup>10, 40 mg/kg.
<sup>c</sup>Calculated from V<sub>u,brain</sub>, and K<sub>p,u,cell</sub>.
<sup>d</sup>Assumed to be the same as risperidone.
**Table 5 Combined parameters of system-specific and drug-specific parameters in the PBPK model**

| Parameter | Unit   | Acetaminophen | Atenolol | Methotrexate | Morphine | Paliperidone | Phenytoin | Quinidine | Raclopride | Remoxipride | Risperidone |
|-----------|--------|---------------|----------|--------------|----------|--------------|-----------|-----------|------------|-------------|-------------|
| \( Q_{BBB, in} \) | mL/min | 0.16          | 0.12     | 0.087        | 0.14     | 0.33         | 1.1       | 9.1       | 0.18       | 0.55        | 1.2         |
| \( Q_{BBB, out} \) | mL/min | 0.31          | 0.33     | 4.8          | 0.38²    | 0.65         | 4.4       | 5.1       | 0.18       | 0.69        | 1.2         |
| \( Q_{BBB} \) | mL/min | 0.014         | 0.0275   | 8.0·10⁻⁵    | 0.033    | 0.24         | 1.0       | 7.6       | 0.086      | 0.46        | 1.1         |
| \( Q_{B,max} \) | mL/min | 0.14          | 0.11     | 0.087       | 0.11     | 0.090        | 0.11      | 0.10      | 0.099      | 0.096       | 0.091       |
| \( Q_{PHF1} \) | mL/min | 1.0           | 0.80     | 1.3         | 0.80     | 0.80         | 1.0       | 0.80      | 0.80       | 0.80        | 1.2         |
| \( Q_{PHF2} \) | mL/min | 6.8·10⁻⁴      | 3.8·10⁻⁴ | 3.8·10⁻⁶    | 0.0016   | 0.011        | 0.048     | 0.36      | 0.0041     | 0.022       | 0.051       |
| \( Q_{PHF3} \) | mL/min | 0.018         | 0.014    | 0.011       | 0.014    | 0.011        | 0.014     | 0.013     | 0.012      | 0.012       | 0.012       |
| \( Q_{PHF4} \) | mL/min | 1.0           | 0.80     | 1.3         | 0.80     | 1.0          | 0.80      | 0.81      | 0.80       | 0.80        | 1.2         |
| \( Q_{PHF5} \) | mL/min | 0.33          | 0.14     | 0.0023      | 0.61     | 4.4          | 23        | 140       | 1.6        | 8.4         | 20          |
| \( Q_{PHF6} \) | mL/min | 0.33          | 0.068    | 0.0046      | 0.31     | 2.2          | 23        | 70        | 0.80       | 4.4         | 10          |
| \( Q_{PHF7} \) | mL/min | 0.16          | 0.033    | 0.0022      | 0.15     | 1.1          | 11        | 33        | 0.38       | 2.1         | 4.8         |
| \( Q_{BCM, in} \) | mL/min | 0.16          | 0.00033  | 0.21        | 0.015    | 0.011        | 0.11      | 0.34      | 0.0039     | 0.022       | 0.049       |
| \( Q_{BCM, out} \) | mL/min | 1.0           | 0.40     | 2.5         | 0.40     | 0.41         | 1.0       | 0.40      | 0.40       | 0.42        | 0.41        |
| BF | mg/kg | 1.1           | 0.92     | NA          | 1.8²⁵  | 0.91         | 8.2       | 7.2       | 8.5        | 5.3         | 0.49        |

**Note:**

- \( Q_{BBB, in} \) and \( Q_{BBB, out} \) are passive diffusion clearance at the BBB.
- \( Q_{B,max} \) is transcellular diffusion clearance at the BBB.
- \( Q_{PHF1}, Q_{PHF2}, Q_{PHF3}, Q_{PHF4}, Q_{PHF5}, Q_{PHF6}, Q_{PHF7} \) are calculated from pKa of each compound and pH of each compartment, respectively.
- BF was calculated from Kp of each compound.

- \( a \) mg/kg.
- \( b \) mg/kg.

**PBPK Model for Brain Target-Site Concentrations**

- \( \text{BF} = \frac{\text{K}_{ \text{p}}}{\text{K}_{ \text{d}}} \)
- \( \text{BF} \) was calculated from Kp of each compound.

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**References:**

- Yamamoto et al.
- CPT: Pharmacometrics & Systems Pharmacology
We also demonstrated the relevance of considering CBF-limited kinetics on the drug transfer at the BBB. For the lipophilic compounds, $Q_{\text{BBB, in}}$ and $Q_{\text{BBB, out}}$ are higher than $Q_{\text{CBF}}$, indicating that the drug transfer clearance on the BBB is largely determined by $Q_{\text{CBF}}$.

The importance of the separation between brain ECF and brain ICF compartments was shown. The $Q_{\text{BCM, in}}$ and $Q_{\text{BCM, out}}$ were either higher or lower than $Q_{\text{BBB, in}}$ and $Q_{\text{BBB, out}}$, depending on the molecular weight, the log P, and the pKa of the compound, which led to differences in drug distribution into brain ECF from brain ICF.

We identified differences in methotrexate drug concentration in CSF_LV and CSF_CM. Therefore, it is expected that the expression level (function) of some of the active transporters may be different between the BCSFB around CSF_LV and CSF_TPV. Methotrexate is known to be a substrate of various transporters, such as RFC1, MRP, BCRP, OATP, and OAT transporters, even though there is no detailed information about their exact location. Therefore, we incorporated this in our model by including $Q_{\text{BCSFB1}}$ and $Q_{\text{BCSFB2}}$ to describe transport for methotrexate.

Figure 2 Prediction accuracy of the physiologically based pharmacokinetic (PBPK) model. The plots were stratified by the central nervous system (CNS) compartments (panels). (a) Selected individual observed drug concentrations (dots) and 95% prediction interval (red lines). (b) Box-whisker plots for the prediction errors (PEs) across all 10 drugs evaluated. Blue dots are PEs for each observation.
All of the parameters for our CNS PBPK model can be derived from either literature or in silico predictions. Therefore, the model can be used to assess newly developed CNS drugs without in vivo data and contributes to the refinement, reduction, and replacement of animals in drug research. Although the reported values of the system-specific parameters for humans are sparse and variable, theoretically, the model can be scaled to humans by replacing the system-specific parameters to predict target-site concentrations in the human brain, representing an important tool for translational development of new CNS drugs.

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