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# **Microdialysis: the key to physiologically based model prediction of human CNS target site concentrations**

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## ABSTRACT

Despite the enormous research efforts that have been put into the development of central nervous system (CNS) drugs, the success rate in this area is still disappointing. To increase the successful rate in the clinical trials, first the problem of predicting human CNS drug distribution should be solved.

As it is the unbound drug that equilibrates over membranes and is able to interact with targets, especially knowledge on unbound extracellular drug concentration-time profiles in different CNS compartments is important. The only technique able to provide such information *in vivo* is microdialysis. Also, obtaining CNS drug distribution data from human subjects is highly limited and therefore we have to rely on preclinical approaches combined with physiologically based pharmacokinetic (PBPK) modeling, taking unbound drug CNS concentrations into account. The next step is then to link drug concentrations in local CNS to target interaction kinetics and CNS drug effects.

In this review, system properties and small molecule drug properties that together govern CNS drug distribution are summarized. Furthermore, the currently available approaches on prediction of CNS pharmacokinetics are discussed, including *in vitro*, *in vivo*, *ex vivo* and *in silico* approaches, with special focus on the powerful combination of *in vivo* microdialysis and PBPK modeling. Also, sources of variability on drug kinetics in the CNS are discussed. Finally, remaining gaps and challenges are highlighted and future directions are suggested.

## INTRODUCTION

There is a huge unmet medical need for central nervous system (CNS) disease therapies because of the growing of chronic and complex diseases associated with aging. However development of CNS drugs is one of the most challenging tasks for the pharmaceutical industry (1). Actually, drug development for CNS drugs has suffered a higher attrition rate compared to that of other therapeutic areas drugs; it has been reported that only around 8-9% of CNS drugs that entered phase 1 were approved to launch (2). And around 50% of the attrition of potential CNS drugs has resulted due to a lack of efficacy and safety issues in phase 2 (2,3). Knowledge of human CNS drug concentrations forms the basis for understanding exposure-response relationships therefore the lack of appropriate consideration of these target-site drug concentrations is one of the factors contributing to this high degree of attrition.

Obtaining the target-site concentrations of CNS drugs is not straightforward because plasma concentrations do not adequately reflect CNS exposure, primarily due to the presence of the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barriers (BCSFB), and additional specific physiological characteristics of the CNS. Furthermore, significant variation in the rate and extent of mechanisms that govern target-site pharmacokinetics (PK), target engagement and signal transduction is known to exist, due to differences in system conditions such as species, gender, genetic background, age, diet, disease and drug treatment (4). Moreover, with regard to CNS drug action there is a lack of sufficiently established clinical biomarkers and proof-of-concept (5). Thus, it is clear that there is a need for more predictive approaches. These predictive approaches have to be interconnected to the system conditions and must be performed using adequate (including bound and unbound drug) concentrations. Also processes should preferably not be studied in isolation and then combined, but instead studied in conjunction with each other as this will provide insight about the interdependencies of these processes (4). Since measurement on CNS target-site concentration in the clinical setting is highly restricted, we have to develop an approach based on integrated preclinical data that is translatable to human.

Even though drug properties have been investigated well, information of CNS system properties (CNS physiology and biochemistry) is sparse and has a large variability. Drug PK in the CNS is determined by their interaction. System properties depend on the condition of the system, which means that we have to use approaches to distinguish between system and drug properties, as this would allow us to translate the model to other species and also other disease conditions, by using physiologically based pharmacokinetic (PBPK) modeling.

Currently many more or less complex semi-PBPK models have been published for CNS drug distribution. At present, 3 preclinical translational models have been validated with human CNS concentration profiles (6–8). In these models, however, the parameters were estimated using *in vivo* data to describe CNS distribution of individual drug in animals. Ultimate goal of the PBPK modeling is to build a generic PBPK model in which the parameters are derived from *in vitro* and/or *in silico* data. To achieve this, *in vivo* data is needed to validate the generic PBPK model. Furthermore, an investigation is needed on the relationship between drug physicochemical properties and CNS distribution.

In this review, system properties and small molecule drug properties that together govern CNS drug distribution are summarized, followed by currently available approaches on prediction of drug PK in the CNS, including *in vitro*, *in vivo*, *ex vivo* and *in silico* approaches, with special focus on the powerful combination of *in vivo* microdialysis and PBPK modeling. Also, sources of variability on drug kinetics in the CNS are discussed. Finally, remaining gaps and challenges will be discussed and future directions will be provided.

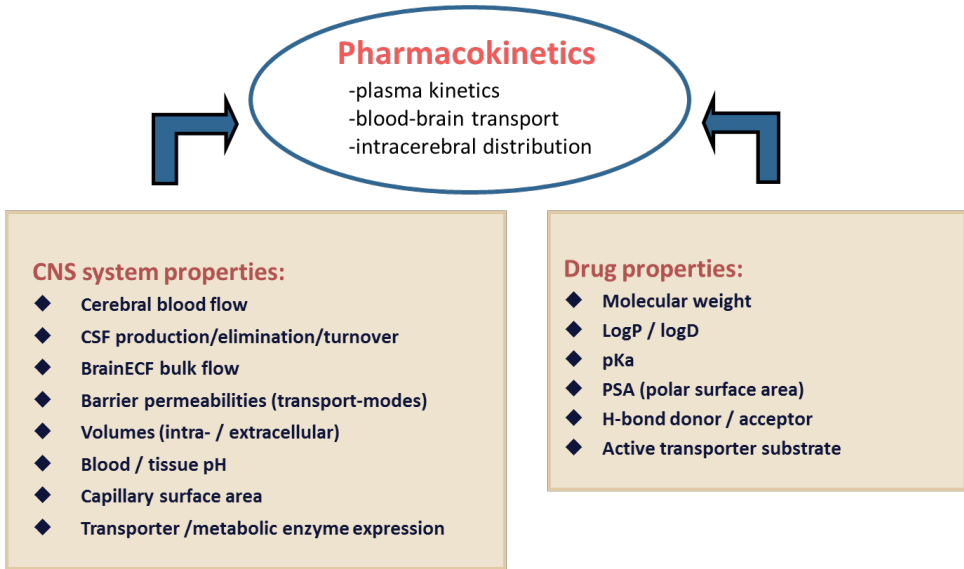
## INTERACTION BETWEEN CNS SYSTEM- AND DRUG PROPERTIES

Many CNS system properties and drug specific properties are known to influence drug kinetics in the brain, as shown in **Figure 1**. Here we focus on the relevant factors from each that contribute to the drug kinetics, and summarize their function.

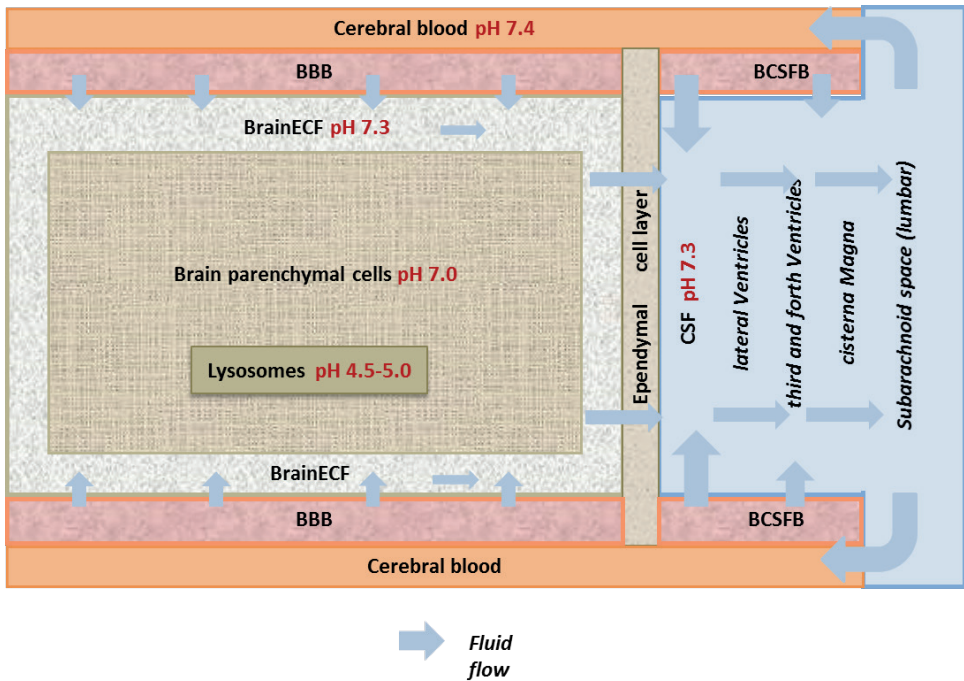
### CNS system properties

#### *Physiological compartments, flows and pH*

The CNS is a complex system composed of many physiological components and flows (**Figure 2**): Physiological compartments are the BBB, the BCSFB, brain extracellular fluid (brain<sub>ECF</sub>), cerebral blood, brain parenchymal cells, and the cerebrospinal fluid (CSF) in the ventricles, the cisterna magna, and the subarachnoid space (4). There are pH differences among the compartments (9–15). Then there are the CNS fluid flows that include the cerebral blood flow, brain<sub>ECF</sub> bulk flow, and CSF flow. All relevant physiological parameter values are summarized in **Table I**.



**Figure 1.** System and drug properties which govern drug kinetics in brain. The figure is modified from de Lange (4).



**Figure 2.** Brain physiological components and flow. The figure is modified from de Lange (4).



### ***Active transporters***

The localization of transporters, and their expression level are also important factors to determine drug distribution in the brain. Transporters are present at the BBB and at the BCSFB, also on the membrane of brain parenchyma. Active transporters on the BBB and BCSFB consist of facilitated transport and ATP-dependent transport. The solute carrier (SLC) family, such as organic anion-transporting polypeptide (OATP) and organic anion transporters (OATs) are categorized as a facilitated transport, while ABC transporters, such as P-glycoprotein (P-gp), multidrug resistance protein (MRPs) and breast cancer-resistant protein (BCRP) are categorized as an ATP-dependent transport (16). **Table II** summarizes an overview of transporters with their localization, and their endogenous and exogenous substrates.

### ***Metabolic enzymes***

Presence and localization of enzymes in the brain are also important factors to determine drug kinetics in the brain. In the brain the following enzymes are found: oxidoreductases such as cytochrome P450 (CYPs) and monoamine oxidase (MAO), membrane-bound and soluble catechol-O-methyltransferase (COMT), and transferases such as uridine 5-diphospho (UDP) -glucuronosyltransferases (UGTs) and phenol sulfotransferase (PST) (17). In **Table III**, an overview is provided of the different enzymes with their localization, and examples of their endogenous and exogenous substrates.

**Table I.** Values of CNS system properties for rat and human

	Parameter	Human	Refs	Rat	Refs
Volumes	BBB volume	8.25 mL (calculated using thickness endothelial cell of 550 nm)	(18)	5.02 $\mu$ L	(19)
	BCSFB volume	107.25 mL (calculated using thickness 14.3 $\mu$ m of endothelial cell)	(20)	37.5 $\mu$ L	(19)
	Brain volume	1400 g	(21)	1.8 g, 1880 $\mu$ L	(22,23)
	Brain <sub>ECF</sub> volume	240-280 mL	(24,25)	290 $\mu$ L	(26)
	Brain <sub>ICF</sub> volume	960 mL	(25)	1440 $\mu$ L	(25)
	CSF volume	130-150 mL	(27,28)	250 $\mu$ L	(22)
	CSF <sub>LV</sub> volume	20-25 mL	(27,29)	50 $\mu$ L	(30,31)
	CSF <sub>TFV</sub> volume	20-25 mL	(27,29)	50 $\mu$ L	(30,31)
	CSF <sub>CM</sub> volume	7.5 mL	(32,33)	17 $\mu$ L	(32,33)
	CSF <sub>SAS</sub> volume	90-125 mL	(27,29)	180 $\mu$ L	(34,35)
Flows	cerebral blood flow	610-860 mL/min	(36-38)	1.1-1.3 mL/min	(39,40)
	brain <sub>ECF</sub> flow	0.15-0.2 mL/min (50% of CSF production)	(28)	0.00018-0.00054 mL/min	(41)
	CSF flow	0.3-0.4 mL/min	(28)	0.0022 mL/min	(26,42)
Surfaces	BBB SA	12-18 m <sup>2</sup>	(18)	155-263 cm <sup>2</sup>	(43,44)
	BCSFB SA	6-9 m <sup>2</sup> (assumed 50% of BBB SA)	(18)	25-75 cm <sup>2</sup> (assumed 50% of BBB SA)	(43,45)
	brain ECF/ICF SA	228 m <sup>2</sup>	Calculated <sup>a)</sup>	3000 cm <sup>2</sup>	(19)
	brain ICF/lysosome SA	12 m <sup>2</sup>	Calculated <sup>a)</sup>	162 cm <sup>2</sup>	Calculated <sup>a)</sup>
pH	Plasma	7.4	(12)	7.4	(9)
	Brain <sub>ECF</sub>	NA		7.3	(10)
	Brain <sub>ICF</sub>	7.0	(13)	7.0	(10)
	lysosome	4.5-5.0	(14)	5.0	(10)
	CSF	7.3	(12)	7.3	(11)

<sup>a)</sup> Calculation was performed based on an assumption that the brain cells and lysosome are spherical.

brain<sub>ECF</sub>; a brain extracellular fluid compartment, brain<sub>ICF</sub>; a brain intracellular fluid compartment, CSF<sub>LV</sub>; a compartment of cerebrospinal fluid in lateral ventricle, CSF<sub>TFV</sub>; a compartment of cerebrospinal fluid in the third and fourth ventricle, CSF<sub>CM</sub>; a compartment of cerebrospinal fluid in the cisterna magna, CSF<sub>SAS</sub>; a compartment of cerebrospinal fluid in the subarachnoid space, SA; surface area

**Table II.** Transporters in the CNS

Transporter (Gene name in human) (Gene name in rat)	Tissue	Location			Substrates			Function		
		Human	Rat	Refs	Endogenous	Refs	Exogenous	Refs	Function	Refs
P-gp (ABCB1) (Abcb1a)	BBB	luminal membrane of the BCEC	luminal membrane of the BCEC	(46–48)			antineoplastic agents, anticancer drugs, corticoids, analgesics, hydrophobic neutral or cationic compounds		efflux	(51)
	BCSFB		apical side of the CPEC	(52)	cytokines	(49)			influx/ efflux	(52–54)
	BP	adjacent pericytes and astrocytes	astrocytes	(48,55)					efflux	(55)
MRPs (ABCC1) (Abcc1)	BBB	luminal and abluminal membranes of the BCEC	luminal and abluminal membranes of the BCEC	(52,56–58)	conjugated metabolites such as glutathione- and glucuronide- conjugates		anticancer drugs, organic anion compounds, 17 $\beta$ -estradiol-d-17 $\beta$ - glucuronide		efflux/ influx	(54,60)
	BCSFB	luminal and abluminal membranes of the CPEC	luminal and abluminal membranes of the CPEC	(61,62)		(59)			efflux	(63)
OTAPs (SLCO, formerly SLC21A) (Slco1a/b)	BP	astrocytes and microglial cells	astrocytes and microglial cells	(60)						
	BBB		luminal and abluminal membranes of the BCEC (Oatp1a4 and Oatp1a5 and OATP2)	(16,64,65)					efflux/ influx	(61)
	BCSFB		luminal membrane of the CPEC (Oatp1a4 and Oatp1a5, OATP2)	(16,64)	amphipathic organ anions	(16)	opioid peptides, E217bG	(66)		
OATs (SLC22A) (Slc22a)	BBB		brush border membrane of the CPEC (OATP1)	(16)					efflux/ influx	(16,61)
	BCSFB		abluminal membrane of the BCEC	(16)	organic anions	(67)		(67)		

BBB; blood–brain barrier; BCSFB; Blood–cerebrospinal fluid barrier; BP; brain parenchymal cells; BCEC; brain capillary endothelial cells; CPEC; choroid plexus epithelial cells

**Table III.** Metabolic enzymes in the CNS

Enzyme	Human		Rat		Endogenous Substrates	Refs	Exogenous substrates	Refs
	Location	Refs	Enzyme	Location				
<b>CYPs</b>								
CYP1A1		(68)	CYP1A1		Melatonin, estradiol, arachidonic acid, progesterone, all-trans-retinal acid	(69)		
CYP1A2		(68)	(CYP1A2)					
CYP1B1	cerebral microvessels at the BBB	(68,70)			Melatonin, estradiol	(69)		
			CYP2B		Arachidonic acid, testosterone, serotonin, anandamide, all-trans-retinoic acid,	(69)	Propofol	(71)
CYP2B6	pyramidal neurons of the frontal cortex and astrocytes surrounding cerebral blood vessels	(68,72)			17- $\beta$ estradiol, anandamide, arachidonic acid, estrone, serotonin, testosterone	(69)	Bupropion, diazepam, ketamine, methadone, meperidine, nicotine, pentobarbital, phencyclidine, propofol, sertraline selegiline, tramadol	(69)
CYP2C		(68)	CYP2C		Testosterone, progesterone, arachidonic acid, serotonin, harmaline, harmine, linoleic acid, melatonin, all-trans-retinoic acid	(69)		
			CYP2C13					
			CYP2D	neuron, glia cells, choroid plexus	5-methoxytryptamine, octopamine, synephrine, tyramine, progesterone, anandamide, harmaline, harmine	(69)		

Table III. (continued)

Enzyme	Human		Rat		Endogenous Substrates	Refs	Exogenous substrates	Refs
	Location	Refs	Enzyme	Location				
<b>CYPs</b>								
CYP2D6					5-methoxytryptamine, anandamide, progesterone, tyramine	(69)	Mirtazapine, brofaromine, clomipramine, codeine, citalopram, clozapine, desipramine, dextromethorphan, ethylmorphine, fluoxetine, fluvoxamine, haloperidol, hydrocodone, imipramine, mianserin, mirtazapine, nergoline, nortryptaline, oxycodone, paroxetine, perphenazine, risperidone, tramadol, tranylcypromine, venlafaxine, zuclopenthixol	(74–76)
						(68)		
						(68)		
CYP2E		(68)						
						(68)	Arachidonic acid, linoleic acid, oleic acid, 17- $\beta$ -estradiol, estrone, prostaglandin	(69)
CYP3A		(68)				(68)		
CYP3A51		(68)						
						(68)		
						(68)		
<b>COMT</b>								
membrane-bound	prefrontal cortex	(77)	membrane-bound	prefrontal cortex	Dopamine	(77)		(78)
soluble	prefrontal cortex	(77)	soluble	prefrontal cortex		(77)		

**Table III.** (continued)

Enzyme	Human		Rat		Endogenous Substrates	Refs	Exogenous substrates	Refs
	Location	Refs	Enzyme	Location				
<b>MAO</b>								
MAOA	Adrenergic neurons	(79)	MAOA		Noradrenaline, adrenaline, dopamine, $\beta$ -phenylethylamine and serotonin	(83)		
MAOB	Astrocytes and serotonergic neurons	(84)	MAOB					
<b>UGT</b>								
UGT2B7								
UGT1A6		(86)	UGT1A6			(87,88)	Morphine	(85)
<b>Miscellaneous</b>								
membrane-bound epoxide hydrolase		(86)						
benzoxresorufin-0-deethylase		(86)						
PST		(89–91)						

## Small molecule drug properties and interaction with the CNS system

A combination of CNS system properties and drug properties determines drug PK in the CNS, including the CNS target-site. Important physicochemical properties for determination of drug PK in the CNS are summarized in **Figure 1**.

Physicochemical properties of a drug, such as lipophilicity, size, charge, hydrogen bonding potential and polar surface area (PSA), are important determinants for drug distribution in the CNS. Many studies have investigated the influence of individual physicochemical properties on the BBB penetration in isolation. However, as physicochemical properties are highly inter-correlated, it is more appropriate to consider these properties in combination.

First of all it should be noted that it is the unbound and neutral form of drug molecules that is able to diffuse across barriers like the BBB and BCSFB, depending on the concentration gradient of the unbound and neutral form of the drug on either side of a membrane. Lipophilicity relates to the BBB permeability, as transcellular diffusion rate (92,93). Furthermore, as a rule of thumb, higher lipophilicity increases drug binding to brain tissue. Molecular size is an important factor for paracellular drug diffusion rate, and also has an impact on transcellular diffusion rate at the BBB (92,94,95). The degree of ionization depends on the pKa of the drug and actual pH in a body compartment. Thus, the BBB permeability rate is influenced by lipophilicity, size and pKa of a drug. (92,96). Using quantitative structure-activity relationship (QSAR) modeling, it has been shown that the descriptors for the prediction of BBB penetration, are different for different charge classes (97). As there are pH differences between plasma, brain<sub>ECF</sub> and CSF (**Figure 2**), charge is an important factor for CNS drug disposition (98).

The hydrogen bonding potential reflects the necessary energy for a molecule to move out of the aqueous phase into the lipid phase of a membrane. Recent studies have shown that the relationship between chemical structure and  $K_{p,uu,brain}$  (the ratio of the unbound concentration in the brain over that in plasma at equilibrium which measures the extent of CNS distribution) was dominated by hydrogen bonding (99).

PSA is generally defined as the sum of the van der Waals surface areas of oxygen and nitrogen atoms. Therefore, PSA of a compound can be related to its hydrogen bonding potential. Some studies have shown that PSA is highly correlated with the permeability coefficient of membranes (93,100,101). A recent study for  $K_{p,uu,brain}$  has been shown that PSA is one of the important factors to predict the  $K_{p,uu,brain}$  for each compound (102).

**BBB and BCSFB transport**

*Protein binding.* It is generally accepted that unbound drug in plasma is able to cross the BBB and BCSFB. Two major proteins in plasma are albumin and  $\alpha$ 1-acid glycoprotein (103). For passive diffusion, the free concentration gradient between plasma and brain determines the rate of transport. The extent of BBB and BCSFB transport are investigated using  $K_{p,uu,brain}$ : If there is only diffusion,  $K_{p,uu,brain}$  is 1. If there is active transport processes, then  $K_{p,uu,brain}$  is larger than 1 (active in) or  $K_{p,uu,brain}$  is smaller than 1 (active out).

*Ionization of the drug in plasma and in the brain.* There are similar pH differences among the CNS physiological compartments in human and in rat (**Table I**). Because of the pH differences, the ratio of neutral form of a compound among the compartments is different. It is generally accepted that neutral form can pass the barriers, therefore ionization that is determined by the pKa of a compounds and pH in the physiological compartments will have an impact on drug disposition in the brain.

*Cerebral blood flow- flow versus permeability limited transport rate.* Lipophilic compounds usually have a large permeability coefficient, therefore a permeability surface area product (PA), which is determined by the permeability coefficient and surface area of tissue, becomes large. If the PA is larger than the physiological cerebral blood flow, then the physiological cerebral blood flow determines the transport rate of the compound.

*Modes of BBB transport- different modes.* The combination of transport modes at the BBB, BSCFB and membrane of brain parenchyma determines the rate and extent of drug exchange at the BBB, BCSFB and membrane of brain parenchyma (104,105). Therefore, the operative transport mechanism(s) may differ for each drug. Each transport mode is summarized in **Table IV**.

*Active transporter function.* Active transporters mediate influx and efflux of drug transport. The magnitude of interaction of active transport is drug and species dependent (106). The functions of individual transporters are summarized in **Table II**.



**Table IV.** Blood-brain barrier main modes of transport and their characteristics

BBB/BCSFB transport mode	Characteristics	Concentration-dependent transport kinetics?	Drug concentration-gradient dependent?	Consumes energy?
Paracellular	Passive; Between tight junctions of the BCEC and the CPEC	No	Yes	No
Transcellular	Passive; Across the membranes of the BCEC and the CPEC	No	Yes	No
Facilitated	Passive;	Yes	Yes	No
Active influx	Active;	Yes	No	Yes
Active efflux	Active;	Yes	No	Yes
Transcytosis	Receptor (specific, low capacity) or absorptive mediated (non-specific, high capacity)	No	No	Yes

BCEC; brain capillary endothelial cells, CPEC; choroid plexus epithelial cells

### **Brain distribution and elimination**

*Extra-intracellular distribution.* Once having crossed the BBB, the drug is distributed by brain<sub>ECF</sub> bulk flow into the CSF compartments. At the same time, the drug in brain<sub>ECF</sub> is transported to brain parenchymal cell intracellular fluid (brain<sub>ICF</sub>). It should be noted that also on the brain parenchyma cell membranes active transport may occur (105).

*Tissue binding.* Tissue binding can occur as being specific at the target or non-specific to tissue components.

*Lysosomal trapping.* In the brain parenchyma cells, there is a physiological pH gradient between the intracellular compartment (cytoplasm) and the lysosome compartment (**Figure 2**). Especially basic compounds are known to be trapped in the lysosomes (10).

*Drug dispersion within CSF.* Some studies have shown that intrathecally administered drugs distribute faster than what can be accounted only by molecular diffusion (107,108). Thus, it is thought that molecular diffusion makes only a small contribution to the total drug dispersion within CSF. This leads to the need to take into account also the convection due to oscillatory CSF flow to adequately explain this dispersion (109). Recently the drug dispersion has been considered to be enhanced by the CSF pulsatility (heart rate and CSF stroke volume), and it leads to high inter- and intra-patient variability in drug distribution in the brain (109,110).

*Elimination from the brain.* Apart from transport across the BBB and BCSFB as discussed earlier, drug may leave the brain via the BBB, but also via CSF reflux into the blood stream at the level of the arachnoid villi.

*Metabolism.* In the brain, several metabolic enzymes are present. Enzyme interaction with drugs is important information not only on the drug PK profile but also on the drug pharmacological effect in the brain since it may create active metabolites. Presence and localization of several enzymes have been reported in the brain (**Table III**), although their activity is reported to be relatively small compared to the liver (17,86).

## **CURRENT APPROACHES TO INVESTIGATE CNS DRUG DISTRIBUTION**

Since obtaining a human drug target-site concentration in the brain is not feasible in most of the clinical studies, quantitative prediction of target-site concentration is important. To achieve this, we need information from *in vitro*, *ex vivo*, *in vivo*, and *in silico* approaches. Here we summarize the current approaches to obtain the necessary information to predict human drug target-site concentration.

### **In silico approaches**

For decades, QSAR studies have been performed using  $K_{p,brain}$  (total concentration ratio of the brain to plasma) or  $\log BB$ , either of which may not reflect the relevant drug exposure in the brain to assess the drug efficacy since drug efficacy is influenced by binding of compounds to plasma proteins and brain tissue. Eventually  $\log BB$  was replaced by the PA, as an estimate of the net BBB influx clearance (111). However, it has been argued that the PA cannot predict the unbound drug concentration in the CNS by itself. Recently the most relevant parameter  $K_{p,uu,brain}$  has been used, with QSAR being conducted to model this parameter (99,102,112,113). Other than  $K_{p,uu,brain}$ , physiological meaningful parameter,  $V_{u,brain}$  (the volume of distribution of the unbound drug in the brain) or  $K_{p,uu,cell}$  (unbound concentration ration between  $brain_{ECF}$  and  $brain_{ICF}$ ) are also reported using molecular descriptors (102).

### **In vitro approaches**

*In vitro* approaches to investigate the BBB permeability have been conducted using BBB models (114). BBB models can be classified into non-cell based surrogate models, such as parallel artificial membrane permeability assay (PAMPA), and cell-based models such as primary cultures cells, immortalized brain endothelial cells or human-derived stem cells (115). Although primary cultured cells from human tissue have been reported, acquiring human brain tissue is difficult as it can only be obtained postmortem and should be fresh enough (116). Therefore alternative models based on immortalized brain endothelial cells or human-derived stem cells are often used (117,118). Even though these models have been developed for measuring the BBB permeability, an ideal cell culture model of the BBB is yet to be developed. Furthermore, reliable *in vitro-in vivo* correlation data is needed to enable the use of *in vitro* results for the prediction of *in vivo* permeability. However, *in vitro* results have not been consistent in their ability to predict *in vivo* permeability, probably because of different *in vitro* models, and different sets of compounds used in the *in vitro* studies (119).

Currently, the biopharmaceutics classification system (BCS) and biopharmaceutics drug distribution classification system (BDDCS) are used for CNS drugs. The BDDCS is a modification of BCS that utilizes drug metabolism to predict drug disposition and potential drug-drug interactions in the brain (120). However, this classification approach needs to be further investigated because of inconsistencies. For example, it was proposed that 98% of BDDCS class 1 drugs would be able to get into the brain even though the drugs were P-gp substrates based on *in vitro* studies (121), while it has also been reported that the *in vitro* efflux ratio reflects the *in vivo* brain penetration regardless of the class in BDDCS (122).

### **Ex vivo approaches**

As mentioned before, it is the unbound drug molecules that are able to pass membranes and to interact with the target (22). Thus, measuring unbound drug concentrations is very important.  $V_{u,brain}$  or  $F_{u,brain}$  (the unbound fraction in the brain) are used to investigate unbound fraction of drugs in the brain.  $F_{u,brain}$  can be derived from brain homogenate (123), and  $V_{u,brain}$  can be obtained from the brain slice technique (124). The brain slice method is more physiologically relevant because the cell-cell interactions, pH gradients and active transport systems are all conserved (34).

## In vivo approaches

Microdialysis can be considered as a key technique to examine time-dependent information regarding unbound drug concentrations. With microdialysis both the rate and extent of drug transport and distribution processes can be determined (125,126). Thus, it can be used to obtain  $K_{p,uu,brain}$  in conjunction with the rate of transport processes. Moreover, this can be done at multiple locations and this feature has shown that even for a drug like acetaminophen that is not subjected to any active transport, substantial differences in pharmacokinetic profiles exist in different brain compartments (6). While there is some limit to use this water-based technique for the highly lipophilic drugs, lots of microdialysis experiments have contributed to a boost in the understanding on drug exchange across the BBB (125,127,128). Especially the use of microdialysis at multiple brain locations have provided insight into the relative contribution of CNS distribution and elimination processes to the local (differences in) PK of a compound (6,7,129).

Positron emission tomography (PET) is a valuable non-invasive *in vivo* monitoring technique that can be used to visualize drug CNS distribution in living animals and human. However, the PET technique cannot distinguish parent compounds from their metabolites, or bound and unbound drug. Furthermore it may also encounter difficulties in obtaining useful data when a very high non-specific binding (NSB) to non-target proteins and phospholipid membranes occurs (130). Recently a novel Lipid Membrane Binding Assay (LIMBA) was established as a fast and reliable tool for identifying compounds with unfavorably high NSB in the brain tissue (55).

## Combinatory mapping approach

Combinatory mapping is an approach that combines three compound-specific parameters obtained from *in vitro*, *ex vivo* and *in vivo* data:  $K_{p,brain}$ ,  $V_{u,brain}$  and  $F_{u,plasma}$ , for calculation of  $K_{p,uu,brain}$  (132). This approach can be used not only to obtain  $K_{p,uu,brain}$  but also to understand unbound drug disposition in the cell cytosol, and the lysosomes. Recently, this approach has been extended to predict drug exposure in different brain regions such as frontal cortex, striatum, hippocampus, brainstem, cerebellum and hypothalamus, in which also the impact of transporters and receptors in each region was taken into account (133). Although this approach is useful to support the selection of potential CNS drugs in drug discovery, it has two limitations. The first limitation is that it can only predict the parameters at steady state. The second limitation is that the approach cannot be translated to predict the parameters, for instance, inter-

species or inter-disease conditions because the processes to obtain the parameters in this approach are not connected with system properties which will be changed in these conditions.

## CONDITION DEPENDENCY AND PBPK MODELING

### Condition dependency

Drug distribution into and within the brain depends on the interaction between system and drug properties. Drug properties remain the same, whatever the species and conditions are in which the drug has been administered. This indicates that interspecies variability in drug distribution into and within the brain is the result of differences in physiological and biochemical parameters. Factors which cause variation in drug PK include: genetic background, species differences, gender, age, diet, disease states, drug treatment (4). Factors which cause variation in drug pharmacodynamics include: seasonal effect (134), age (135), gender (136), species (137). Effects of these conditions on CNS system properties are summarized in **Table V**.

### (Semi-) PBPK modeling

PBPK models need to be informed on system and on drug properties to model the interaction and predict the drug PK in different compartments. Especially as obtaining PK data from the human brain is highly restricted, working in the PBPK model framework is valuable as it can be translated to predict the target-site concentrations in inter-species and inter-disease situations (4). Some translational research has been reported by using an animal (semi-) PBPK model for CNS drugs but it is relatively sparse and ranges from simple to more advanced (**Table VI**).

For remoxipride, Stevens et al. have shown that drug concentration in brain<sub>ECF</sub> which was measured with microdialysis, represented the target-site concentrations, because these concentrations could be directly linked to the effect of remoxipride on plasma prolactin levels in an advanced mechanism-based model (138). After scaling to human, this indeed could also be concluded for human CNS remoxipride effects on human plasma prolactin levels. This underscores the importance of having information on PK at the CNS target region.

**Table V.** Sources of variability in CNS pharmacokinetics

Parameter	Location	Source of variability	Effect	Refs
Protein binding		aging	lower	(139)
		pathophysiological condition	higher with disease induced evaluation of plasma protein	(140,141)
Cerebral blood flow		aging	lower	(142)
		pathophysiological condition	lower in the multi-infarct group	(143)
		diurnal variation	change	(144)
BBB	membrane lipid	aging	change	(145)
		diet	change	(146)
	paracellular diffusion	pathophysiological condition	change in several disease conditions, such as Alzheimer's disease and schizophrenia	(147–149)
		stress	increase with hypoxic stress	(150)
	tight junction	pathophysiological condition	increase (due to loose of tight junctions)	see below
			disruption of the tight junctions by ischaemic brain stroke	(151)
			opening of the tight junctions in AD patients	(152)
	facilitated transport	pathophysiological condition	opening of tight junctions in multiple sclerosis patients	(153)
			diet	decreased in hypoglycemia condition
	vesicle based transport	pathophysiological condition	upregulation in the brain tumor	(155)
			active transporters	increase in experimental autoimmune encephalomyelitis
Brain <sub>ECF</sub>		pathophysiological condition	see below	see below
		pathophysiological condition	volume is enlarged in the patient with vasogenic type of brain	(157)
Brain Parenchyma		blockade of brain ECF flow in AD patient	(42)	
		aging	shrunk	(158)
BCSFB		aging	thinner	(159)
		pathophysiological condition	decrease in Alzheimer patients	(159)
CSF		aging	decrease in CSF production, increase in CSF outflow resistance	(160)
		pathophysiological condition	decrease in CSF production, CSF turnover and increase in CSF volume in AD patients	(161)
			increased resistance to CSF absorption and CSF pressure in the patients with normal-pressure hydrocephalus	(162)
Brain metabolic enzymes		aging	increase in the CYP2D6 enzyme level	(163)
		gender	higher MAO activity in women	(82)
		pathophysiological condition	higher MAOB activity in AD patients	(164)
			difference of COMT expression in schizophrenia patients	(77)
		gene	deficiency of CYP2D6 enzyme	(74)
			change of COMT function	(165,166)
smoking and alcoholism	change of CYP2B6 and CYP2E1 levels	(72,167)		

**Table V.** (continued)

Parameter	Location	Source of variability	Effect	Refs
Transporter		aging	decrease in P-gp activity	(168)
			decrease in glucose transporter activity	(169)
		pathophysiological condition	upregulation of P-gp and MRPs in epileptogenic brain	(170)
			upregulation of P-gp and MRP1 in the brain tumor	(171)
			Alteration of the levels of glutamate transporter in the various brain disorders, including cerebral ischemia, amyotrophic lateral sclerosis, AD, AIDS, traumatic brain injury, schizophrenia, and epilepsy (seizure)	(172,173)
		diurnal variation	change in P-gp activity	(174)





## REMAINING GAPS AND CHALLENGES ON PBPK MODELING, TOWARDS A GENERIC PBPK MODEL

The ultimate aim is to have a CNS PBPK model that can predict human brain compartment concentrations on the basis of the compounds physicochemical properties, which can be determined by *in vitro* measurements, or *in silico* prediction. Thus, in the overview in **Table VI** it can be seen that we still have a number of gaps in the currently available (semi-) PBPK models of CNS drugs. Most of the models require *in vivo* data on the compound(s), and most of the predictions have not been validated on human data. Thus, it can be seen that there is a need for further development of a generic, fully PBPK model for CNS drug distribution (185–187).

To have a PBPK model that would predict CNS drug distribution based the physicochemical properties of an individual drug, for different species and in different conditions, a number of challenges remain:

- Having a PBPK model structure with all relevant compartment/parameters, as physiological parameter values reported are sparse and variable (see **Table I**).
- Having drug physicochemical parameter values determined from *in vitro*, and/or *in silico*, or even some *in vivo* measurements, which may not necessarily be correct. For example, *in vitro* or *in vivo* data may depend on the experimental setting, while *in silico* information really depends on the data availability, used to obtain the equation.
- Obtaining human data sets for validating the model predictive performance is typically very difficult.
- Having information on pathophysiological changes in human CNS system properties in (the many) disease conditions. For example, BBB characteristics may change in Alzheimer's disease, multiple sclerosis, and pharmacoresistant epilepsies (188).

## DISCUSSION AND CONCLUSION

PK of drugs in the CNS is governed by a combination of CNS system physiology and drug properties. This means that variability in CNS system physiological parameters (condition dependency) may lead to variability of CNS drug PK. Therefore, it is important to explicitly distinguish between system physiology and drug properties, by either changing conditions and investigating the PK of one drug, or investigating the PK of different drugs in the same condition.

The currently available predictive approaches are based on total drug plasma and total tissue concentrations at equilibrium (SS), while more recent approaches include, at best, unbound plasma SS concentrations. However, as body processes are based on the interaction with the unbound drug and are time-dependent, it is crucial to measure the unbound drug in each compartment as a function of time (Mastermind Research Approach (MRA)) (4), for which microdialysis has been proven the key technique. Using the MRA, microdialysis will provide lots of valuable data that pave the way towards a generic CNS PBPK model.

One microdialysis experiment in a single freely-moving animal can provide a lot of data points, obtained under the same experimental condition of the animal, and thereby revealing the interrelationships of processes. With this microdialysis has already contributed to reduction and refinement in the use of animals. Furthermore, all this information can further be “condensed” into a generic PBPK model, and will thereby help in the reduction in the future use of animals (189).

In order to be able to predict CNS drug effects in human, next steps would be the development of a full PBPK CNS drug distribution model, and combining it with target binding kinetics, receptor occupancy and signal transduction (190,191), and including system changes by human disease condition.

## REFERENCES

1. Kola I, Landis J. Can the pharmaceutical industry reduce attrition rates? *Nat Rev Drug Discov.* 2004;3:1–5.
2. Hurko O, Ryan JL. Translational Research in Central Nervous System Drug Discovery. *J Am Soc Exp Neurother.* 2005;2(4):671–82.
3. Cook D, Brown D, Alexander R, March R, Morgan P, Satterthwaite G, et al. Lessons learned from the fate of AstraZeneca's drug pipeline: a five-dimensional framework. *Nat Rev Drug Discov.* 2014;13(6):419–31.
4. de Lange ECM. The mastermind approach to CNS drug therapy: translational prediction of human brain distribution, target site kinetics, and therapeutic effects. *Fluids Barriers CNS.* 2013;10(1):1–16.
5. Palmer AM, Stephenson FA. CNS Drug Discovery: Challenges and Solutions. *Drug News Perspect.* 2005;18(1):51–7.
6. Westerhout J, Ploeger B, Smeets J, Danhof M, de Lange ECM. Physiologically based pharmacokinetic modeling to investigate regional brain distribution kinetics in rats. *AAPS J.* 2012;14(3):543–53.
7. Westerhout J, van den Berg D-J, Hartman R, Danhof M, de Lange ECM. Prediction of methotrexate CNS distribution in different species - Influence of disease conditions. *Eur J Pharm Sci.* 2014;57:11–24.
8. Gaohua L, Neuhoff S, Johnson TN, Rostami-hodjegan A, Jamei M. Development of a permeability-limited model of the human brain and cerebrospinal fluid (CSF) to integrate known physiological and biological knowledge: Estimating time varying CSF drug concentrations and their variability using in vitro data. *Drug Metab Pharmacokinet.* 2016;31(3):224–33.
9. Mutch W, Hansen A. Extracellular pH changes during spreading depression and cerebral ischemia: mechanisms of brain pH regulation. *J Cereb blood flow Metab.* 1984;4(1):17–27.
10. Fridén M, Bergström F, Wan H, Rehngren M, Ahlin G, Hammarlund-Udenaes M, et al. Measurement of unbound drug exposure in brain: Modeling of pH partitioning explains diverging results between the brain slice and brain homogenate methods. *Drug Metab Dispos.* 2011;39(3):353–62.
11. Shu C, Shen H, Teuscher NS, Lorenzi PJ, Keep RF, Smith DE. Role of PEPT2 in peptide/mimetic trafficking at the blood-cerebrospinal fluid barrier: studies in rat choroid plexus epithelial cells in primary culture. *J Pharmacol Exp Ther.* 2002;301(3):820–9.
12. Shen DD, Artru AA, Adkison KK. Principles and applicability of CSF sampling for the assessment of CNS drug delivery and pharmacodynamics. *Adv Drug Deliv Rev.* 2004;56(12):1825–57.
13. Jwensen KE, Thomsen C, Henriksen O. In vivo measurement of intracellular pH in human brain during different tensions of carbon dioxide in arterial blood. A <sup>31</sup>P-NMR study. *Acta Physiol Scand.* 1988;134(2):295–8.

14. Mindell JA. Lysosomal acidification mechanisms. *Annu Rev Physiol.* 2012;74:69–86.
15. Touitou E, Barry BW. *Enhancement in Drug Delivery.* CRC Press; 2006.
16. Kusuhara H, Sugiyama Y. Active Efflux across the Blood-Brain Barrier: Role of the Solute Carrier Family. *J Am Soc Exp Neurother.* 2005;2:73–85.
17. Minn A, Ghersi-Egea J-F, Perrin R, Leininger B, Siest G. Drug metabolizing enzymes in the brain and cerebral microvessels. *Brain Res Rev.* 1991;16(1):65–82.
18. Wong AD, Ye M, Levy AF, Rothstein JD, Bergles DE, Searson PC. The blood-brain barrier: an engineering perspective. *Front Neuroeng.* 2013;6:1–22.
19. Trapa PE, Belova E, Liras JL, Scott DO, Steyn SJ. Insights from an Integrated Physiologically Based Pharmacokinetic Model for Brain Penetration. *J Pharm Sci.* 2016;105(2):965–71.
20. Serot JM, Béné MC, Foliguet B, Faure GC. Morphological alterations of the choroid plexus in late-onset Alzheimer's disease. *Acta Neuropathol.* 2000;99(2):105–8.
21. Dekaban A, Sadowsky D. Changes in brain weights during the span of human life: relation of brain weights to body heights and body weights. *Ann Neurol.* 1978;4(4):345–56.
22. Hammarlund-Udenaes M, Fridén M, Syvänen S, Gupta A. On The Rate and Extent of Drug Delivery to the Brain. *Pharm Res.* 2008;25(8):1737–50.
23. Kawakami J, Yamamoto K, Sawada Y, Iga T. Prediction of brain delivery of ofloxacin, a new quinolone, in the human from animal data. *J Pharmacokinet Biopharm.* 1994;22(3):207–27.
24. Begley DJ, Bradbury MW, Kreuter J. *The Blood-Brain Barrier and Drug Delivery to the CNS.* New York: Marcel Dekker, Inc. 2000.
25. Thorne RG, Hrabe S, Nicholson C, Robert G. Diffusion of Epidermal Growth Factor in Rat Brain Extracellular Space Measured by Integrative Optical Imaging. *J Neurophysiol.* 2004;92(6):3471–81.
26. Cserr H, Cooper D, Suri P, Patlak C. Efflux of radiolabeled polyethylene glycols and albumin from rat brain. *Am J Physiol.* 1981;240(4):319–28.
27. Sakka L, Coll G, Chazal J. Anatomy and physiology of cerebrospinal fluid. *Eur Ann Otorhinolaryngol Head Neck Dis.* 2011 Dec;128(6):309–16.
28. Kimelberg HK. Water homeostasis in the brain: basic concepts. *Neuroscience.* 2004 Jan;129(4):851–60.
29. Pardridge WM. Drug transport in brain via the cerebrospinal fluid. *Fluids Barriers CNS.* 2011;8(7):1–7.
30. Condon P, Wyper D, Grant R, Patterson J, Hadley D, Teasdale G, et al. Use of magnetic resonance imaging to measure intracranial cerebrospinal fluid volume. *Lancet.* 1986;327(8494):1355–7.
31. Kohn MI, Tanna NK, Herman GT, Resnick SM, Mozley PD, Gur RE, et al. Analysis of brain and cerebrospinal fluid volumes with MR imaging. Part I. Methods, reliability, and validation. *Radiology.* 1991;178(1):115–22.

32. Robertson EG. Developmental defects of the cisterna magna and dura mater. *J Neurol Neurosurg Psychiatry*. 1949;12(1):39–51.
33. Adam R, Greenberg JO. The mega cisterna magna. *J Neurosurg*. 1978;48(2):190–2.
34. Bass NH, Lundborg P. Postnatal development of bulk flow in the cerebrospinal fluid system of the albino rat: clearance of carboxyl-(14C)inulin after intrathecal infusion. *Brain Res*. 1973;52:323–32.
35. Levinger IM. The cerebral ventricles of the rat. *J Anat*. 1971;108(3):447–51.
36. Stange K, Greitz M, Ingvar T, Hindmarsh T, Sollevi A. Global cerebral blood flow during infusion of adenosine in humans: assessment by magnetic resonance imaging and positron emission tomography. *Acta Physiol Scand*. 1997;160(2):117–22.
37. Ito H, Inoue K, Goto R, Kinomura S, Taki Y, Okada K, et al. Database of normal human cerebral blood flow measured by SPECT: I. Comparison between I-123-IMP, Tc-99m-HMPAO, and Tc-99m-ECD as referred with O-15 labeled water PET and voxel-based morphometry. *Ann Nucl Med*. 2006;20(2):131–8.
38. Fagerholm U. The highly permeable blood-brain barrier: an evaluation of current opinions about brain uptake capacity. *Drug Discov Today*. 2007;12(23–24):1076–82.
39. Harashima H, Sawada Y, Sugiyama Y, Iga T, Hanano M. Analysis of nonlinear tissue distribution of quinidine in rats by physiologically based pharmacokinetics. *J Pharmacokinet Biopharm*. 1985;13(4):425–40.
40. Peng B, Andrews J, Nestorov I, Brennan B, Nicklin P, Rowland M. Tissue distribution and physiologically based pharmacokinetics of antisense phosphorothioate oligonucleotide ISIS 1082 in rat. *Antisense Nucleic Acid Drug Dev*. 2001;11(1):15–27.
41. Abbott NJ. Prediction of blood-brain barrier permeation in drug discovery from in vivo, in vitro and in silico models. *Drug Discov Today Technol*. 2004;1(4):407–16.
42. Abbott NJ. Evidence for bulk flow of brain interstitial fluid: significance for physiology and pathology. *Neurochem Int*. 2004;45(4):545–52.
43. Skipor J, Thiery JC. The choroid plexus-cerebrospinal fluid system: Undervaluated pathway of neuroendocrine signaling into the brain. *Acta Neurobiol Exp*. 2008;68(3):414–28.
44. Kumar G, Smith QR, Hokari M, Parepally J, Duncan MW. Brain uptake, pharmacokinetics, and tissue distribution in the rat of neurotoxic N-butylbenzenesulfonamide. *Toxicol Sci*. 2007;97(2):253–64.
45. Strazielle N, Ghersi-Egea JF. Choroid plexus in the central nervous system: biology and physiopathology. *J Neuropathol Exp Neurol*. 2000;59(7):561–74.
46. Schinkel AH. P-Glycoprotein, a gatekeeper in the blood–brain barrier. *Adv Drug Deliv Rev*. 1999;36(2–3):179–94.
47. Tsuji A, Tamai I. Blood-brain barrier function of P-glycoprotein. *Adv Drug Deliv Rev*. 1997;25(2–3):287–98.

48. Bendayan R. In Situ Localization of P-glycoprotein (ABCB1) in Human and Rat Brain. *J Histochem Cytochem.* 2006;54(10):1159–67.
49. Löscher W, Potschka H. Role of drug efflux transporters in the brain for drug disposition and treatment of brain diseases. *Prog Neurobiol.* 2005;76(1):22–76.
50. Schinkel AH, Jonker JW. Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: An overview. *Adv Drug Deliv Rev.* 2003;55:3–29.
51. Shapiro AB, Ling V. Stoichiometry of coupling of rhodamine 123 transport to ATP hydrolysis by P-glycoprotein. *Eur J Biochem.* 1998;254:189–93.
52. Rao VV, Dahlheime JL, Bardgett ME, Snyder AZ, Finch RA, Sartorelli AC, et al. Choroid plexus epithelial expression of MDR1 P glycoprotein and multidrug resistance-associated protein contribute to the blood-cerebrospinal-fluid drug-permeability barrier. *Med Sci.* 1999;96(7):3900–5.
53. Cordon-Cardo C, O'Brien JP, Casals D, Rittman-Grauer L, Biedler JL, Melamed MR, et al. Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. *Proc Natl Acad Sci U S A.* 1989;86(2):695–8.
54. Kassem NA, Deane R, Segal MB, Chen R, Preston JE. Thyroxine (T4) transfer from CSF to choroid plexus and ventricular brain regions in rabbit: contributory role of P-glycoprotein and organic anion transporting polypeptides. *Brain Res.* 2007;1181:44–50.
55. Ronaldson PT, Bendayan M, Gingras D, Piquette-Miller M, Bendayan R. Cellular localization and functional expression of P-glycoprotein in rat astrocyte cultures. *J Neurochem.* 2004;89(3):788–800.
56. Miller DS. Regulation of ABC transporters at the blood-brain barrier. *Clin Pharmacol Ther.* 2015;97(4):395–403.
57. Löscher W, Potschka H. Blood-brain barrier active efflux transporters: ATP-binding cassette gene family. *NeuroRx.* 2005;2(1):86–98.
58. Nies AT, Jedlitschky G, König J, Herold-Mende C, Steiner HH, Schmitt H-P, et al. Expression and immunolocalization of the multidrug resistance proteins, MRP1–MRP6 (ABCC1–ABCC6), in human brain. *Neuroscience.* 2004;129(2):349–60.
59. Hipfner DR, Deeley RG, Cole SPC. Structural, mechanistic and clinical aspects of MRP1. *Biochim Biophys Acta.* 1999;1461:359–76.
60. Hirrlinger J, König J, Dringen R. Expression of mRNAs of multidrug resistance proteins (Mrps) in cultured rat astrocytes, oligodendrocytes, microglial cells and neurones. *J Neurochem.* 2002;82:716–9.
61. Westerhout J, Danhof M, de Lange ECM. Preclinical Prediction of Human Brain Target Site Concentrations : Considerations in Extrapolating to the Clinical Setting. *J Pharm Sci.* 2011;100(9):3577–93.
62. Begley DJ. ABC transporters and the blood-brain barrier. *Curr Pharm Des.* 2004;10(12):1295–312.

63. Wijnholds J, de Lange ECM, Scheffer GL, van den Berg DD, Mol CAAM, van der Valk M, et al. Multidrug resistance protein 1 protects the choroid plexus epithelium and contributes to the blood-cerebrospinal fluid barrier. *J Clin Invest*. 2000;105(3):279–85.
64. Gao B, Stieger B, Noé B, Fritschy JM, Meier PJ. Localization of the organic anion transporting polypeptide 2 (Oatp2) in capillary endothelium and choroid plexus epithelium of rat brain. *J Histochem Cytochem*. 1999;47(10):1255–64.
65. Angeletti RH, Novikoff PM, Juvvadi SR, Fritschy J-M, Meier PJ, Wolkoff AW. The choroid plexus epithelium is the site of the organic anion transport protein in the brain. *Neurobiology*. 1997;94:283–6.
66. Gao B, Hagenbuch B, Kullak-Ublick GA, Benke D, Aguzzi A, Meier PJ. Organic Anion-Transporting Polypeptides Mediate Transport of Opioid Peptides across Blood-Brain Barrier. *J Pharmacol Exp Ther*. 2000;294(1):73–39.
67. Sekine T, Cha SH, Endou H. The multispecific organic anion transporter (OAT) family. *Pflugers Arch*. 2000;440(3):337–50.
68. Miksys SL, Tyndale RF. Drug-metabolizing cytochrome P450s in the brain. *J psychiatry Neurosci*. 2002;27(6):406–15.
69. Miksys S, Tyndale RF. Cytochrome P450-mediated drug metabolism in the brain. *J Psychiatry Neurosci*. 2013;38(3):152–63.
70. Declèves X, Jacob A, Yousif S, Shawahna R, Potin S, Scherrmann J. Interplay of drug metabolizing CYP450 enzymes and ABC transporters in the blood-brain barrier. *Curr Drug Metab*. 2011;12(8):732–41.
71. Favetta P, Degoute CS, Perdrix JP, Dufresne C, Bouliou R, Guitton J. Propofol metabolites in man following propofol induction and maintenance. *Br J Anaesth*. 2002;88(5):653–8.
72. Miksys S, Lerman C, Shields PG, Mash DC, Tyndale RF. Smoking, alcoholism and genetic polymorphisms alter CYP2B6 levels in human brain. *Neuropharmacology*. 2003;45(1):122–32.
73. Miksys S, Rao Y, Sellers EM, Kwan M, Mendis D, Tyndale RF. Regional and cellular distribution of CYP2D subfamily members in rat brain. *Xenobiotica*. 2000;30(6):547–64.
74. Zanger UM, Raimundo S, Eichelbaum M. Cytochrome P450 2D6: Overview and update on pharmacology, genetics, biochemistry. *Naunyn Schmiedeberg's Arch Pharmacol*. 2004;369(1):23–37.
75. D'empaire I, Guico-Pabia CJ, Preskorn SH. Antidepressant Treatment and Altered CYP2D6 Activity. *J Psychiatr Pract*. 2011;17(5):330–9.
76. Leysen J, Janssen P, Megens A, Schotte A. Risperidone: a novel antipsychotic with balanced serotonin-dopamine antagonism, receptor occupancy profile, and pharmacologic activity. *J Clin Psychiatry*. 1994;55:5–12.

77. Matsumoto M, Weickert CS, Beltaifa S, Kolachana B, Chen J, Hyde TM, et al. Catechol O-methyltransferase (COMT) mRNA expression in the dorsolateral prefrontal cortex of patients with schizophrenia. *Neuropsychopharmacology*. 2003;28(8):1521–30.
78. Huotari M, Gogos JA, Karayiorgou M, Koponen O, Forsberg M, Raasmaja A, et al. Brain catecholamine metabolism in catechol-O-methyltransferase (COMT)-deficient mice. *Eur J Neurosci*. 2002;15:246–56.
79. Pintar JE, Breakefield XO. Monoamine Oxidase (MAO) Activity as a Determinant in Human Neurophysiology. *Behav Genet*. 1982;12(1):53–68.
80. Bogdanski DF, Weissbach H, Udenfriend S. The distribution of serotonin, 5-hydroxytryptophan decarboxylase, and monoamine oxidase in brain. *J Neurochem*. 1957;1:272–8.
81. Luine VN, Khylichevskaya RI, Mcewen BS. Effect of gonadal steroids on activities of monoamine oxidase and choline acetylase in rat brain. *Brain Res*. 1975;86:293–306.
82. Robinson DS, Davis JM, Nies A, Ravaris CL, Sylwester D, Burlington V. Relation of Sex and Aging to Monoamine Oxidase Activity of Human Brain, Plasma, and Platelets. *Arch Gen Psychiat*. 1971;24:536–9.
83. Nagatsu T. Progress in Monoamine Oxidase (MAO) Research in Relation to Genetic Engineering. *Neurotoxicology*. 2004;25(1–2):11–20.
84. Levitt P, Pintar JE, Breakefield XO. Immunocytochemical demonstration of monoamine oxidase B in brain astrocytes and serotonergic neurons. *Neurobiology*. 1982;79:6385–9.
85. Ohno S, Kawana K, Nakajin S. Contribution of UDP-glucuronosyltransferase 1A1 and 1A8 to morphine-6-glucuronidation and its kinetic properties. *Drug Metab Dispos*. 2008;36(4):688–94.
86. Ghersi-Egea JF, Leninger-Muller B, Suleman G, Siest G, Minn A. Localization of Drug-Metabolizing Enzyme Activities to Blood-Brain Interfaces and Circumventricular Organs. *J Neurochem*. 1994;62:1089–96.
87. King CD, Rios GR, Assouline JA, Tephly TR. Expression of UDP-Glucuronosyltransferases (UGTs) 2B7 and 1A6 in the Human Brain and Identification of 5-Hydroxytryptamine as a Substrate. *Arch Biochem Biophys*. 1999;365(1):156–62.
88. Suleman FG, Abid A, Gradinaru D, Daval J-L, Magdalou J, Minn A. Identification of the Uridine Diphosphate Glucuronosyltransferase Isoform UGT1A6 in Rat Brain and in Primary Cultures of Neurons and Astrocytes. *Arch Biochem Biophys*. 1998;358(1):63–7.
89. Foldes A, Meek JL. Rat brain phenolsulfotransferase-Partial purification and some properties. *Biochim. Biophys. Acta* 1973. *Biochim Biophys Acta*. 1973;327:365–74.
90. Meek JL, Neff NH. Biogenic amines and their metabolites as substrates for phenol sulfotransferase (EC 2.8.2.1) of brain and liver. *J Neurochem*. 1973;21(9):1–9.
91. Renskers KJ, Feor KD, Roth JA. Sulfation of Dopamine and Other Biogenic Amines by Human Brain Phenol Sulfotransferase. *J Neurochem*. 1980;34(6):1362–8.



92. Levin VA. Relationship of octanol/water partition coefficient and molecular weight to rat brain capillary permeability. *J Med Chem.* 1980;23(6):682–4.
93. van de Waterbeemd er al H, van de Waterbeemd H, Camenisch G, Folkers G, Raevsky OA. Estimation of Caco-2 Cell Permeability using Calculated Molecular Descriptors. *Quant Struct-Act Relat.* 1996;15:480–90.
94. Lipinski C a., Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev.* 2012;64:4–17.
95. Chan OH, Stewart BH. Physicochemical and drug- delivery considerations for oral drug bioavailability. *Drug Discov Today.* 1996;1(11):461–73.
96. Upton RN. Regional pharmacokinetics I. physiological and physicochemical basis. *Biopharm Drug Dispos.* 1990;11(8):647–62.
97. Shayanfar A, Soltani S, Jouyban A. Prediction of Blood-Brain Distribution : Effect of Ionization. *Biol Pharm Bull.* 2011;34(2):266–71.
98. Macintyre AC, Cutler DJ. The potential role of lysosomes in tissue distribution of weak bases. *Biopharm Drug Dispos.* 1988;9:513–26.
99. Fridén M, Winiwarter S, Jerndal G, Bengtsson O, Wan H, Bredberg U, et al. Structure-brain exposure relationships in rat and human using a novel data set of unbound drug concentrations in brain interstitial and cerebrospinal fluids. *J Med Chem.* 2009;52(20):6233–43.
100. Palm K, Luthman K, Ungell A-L, Strandlund G, Beigi F, Lundahl P, et al. Evaluation of dynamic polar molecular surface area as predictor of drug absorption: comparison with other computational and experimental predictors. *J Med Chem.* 1998;41(27):5382–92.
101. Krarup LH, Christensen IT, Hovgaard L, Frokjaer S. Predicting drug absorption from molecular surface properties based on molecular dynamics simulations. *Pharmaceutical Res.* 1998;15(7):972–8.
102. Loryan I, Sinha V, Mackie C, van Peer A, Drinkenburg WH, Vermeulen A, et al. Molecular properties determining unbound intracellular and extracellular brain exposure of CNS drug candidates. *Mol Pharm.* 2015;12(2):520–32.
103. Peletier LA, Benson N, van der Graaf PH. Impact of plasma-protein binding on receptor occupancy: An analytical description. *J Theor Biol.* 2009;256(2):253–62.
104. Abbott NJ, Rönnbäck L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci.* 2006;7(41–53).
105. Lee G, Dallas S, Hong M, Bendayan R. Drug Transporters in the Central Nervous System : Brain Barriers and Brain Parenchyma Considerations. *Pharmacol Rev.* 2001;53(4):569–96.
106. Ohtsuki S, Terasaki T. Contribution of carrier-mediated transport systems to the blood-brain barrier as a supporting and protecting interface for the brain; importance for CNS drug discovery and development. *Pharm Res.* 2007;24(9):1745–58.

107. Plassat R, Verbe BP, Menei P, Menegalli D, Mathe J, Richard I. Treatment of spasticity with intrathecal Baclofen administration long-term follow-up, review of 40 patients. *Spinal Cord*. 2004;42:686–93.
108. Kroin JS. The Distribution of Medication along the Spinal Canal after Chronic Intrathecal Administration. *Neurosurgery*. 1993;33:226–30.
109. Hettiarachchi HDM, Hsu Y, Harris TJ, Penn R, Linninger AA. The effect of pulsatile flow on intrathecal drug delivery in the spinal canal. *Ann Biomed Eng*. 2011;39(10):2592–602.
110. Hsu Y, Hettiarachchi HDM, Zhu DC, Linninger AA. The Frequency and Magnitude of Cerebrospinal Fluid Pulsations Influence Intrathecal Drug Distribution. *Anesth Analg*. 2012;115(2):386–94.
111. Pardridge WM. Log(BB), PS products and in silico models of drug brain penetration. *Drug Discov Today*. 2004;9(9):392–3.
112. Chen H, Winiwarter S, Fridén M, Antonsson M, Engkvist O. In silico prediction of unbound brain-to-plasma concentration ratio using machine learning algorithms. *J Mol Graph Model*. 2011;29(8):985–95.
113. Loryan I, Fridén M, Hammarlund-Udenaes M. The brain slice method for studying drug distribution in the CNS. *Fluids Barriers CNS*. 2013;10(6):1–9.
114. Helms HC, Abbott NJ, Burek M, Cecchelli R, Couraud P-O, Deli MA, et al. In vitro models of the blood-brain barrier: An overview of commonly used brain endothelial cell culture models and guidelines for their use. *J Cereb Blood Flow Metab*. 2016;36(5):862–90.
115. Bicker J, Alves G, Fortuna A, Falcão A. Blood-brain barrier models and their relevance for a successful development of CNS drug delivery systems: A review. *Eur J Pharm Biopharm*. 2014;87(3):409–32.
116. Bernas MJ, Cardoso FL, Daley SK, Weinand ME, Campos AR, Ferreira AJG, et al. Establishment of primary cultures of human brain microvascular endothelial cells to provide an in vitro cellular model of the blood-brain barrier. *Nat Protoc*. 2010;5(7):1265–72.
117. Weksler BB, Subileau EA, Perrière N, Charneau P, Holloway K, Leveque M, et al. Blood-brain barrier-specific properties of a human adult brain endothelial cell line. *FASEB J*. 2005;19(13):1872–4.
118. Cecchelli R, Aday S, Sevin E, Almeida C, Culot M, Dehouck L, et al. A stable and reproducible human blood-brain barrier model derived from hematopoietic stem cells. *PLoS One*. 2014;9(6):1–11.
119. Stanimirovic DB, Bani-Yaghoub M, Perkins M, Haqqani AS. Blood-brain barrier models: in vitro to in vivo translation in preclinical development of CNS-targeting biotherapeutics. *Expert Opin Drug Discov*. 2015;10(2):141–55.
120. Larregieu CA, Benet LZ. Distinguishing between the permeability relationships with absorption and metabolism to improve BCS and BDDCS predictions in early drug discovery. *Mol Pharm*. 2014;11(4):1335–44.

121. Broccatelli F, Larregieu CA, Cruciani G, Oprea TI, Benet LZ. Improving the prediction of the brain disposition for orally administered drugs using BDDCS. *Adv Drug Deliv Rev.* 2012;64(1):95–109.
122. Kikuchi R, De Morais SM, Kalvass JC. In vitro P-glycoprotein efflux ratio can predict the in vivo brain penetration regardless of biopharmaceutics drug disposition classification system class. *Drug Metab Dispos.* 2013;41(12):2012–7.
123. Kalvass JC, Maurer TS. Influence of nonspecific brain and plasma binding on CNS exposure: Implications for rational drug discovery. *Biopharm Drug Dispos.* 2002;23(8):327–38.
124. Friden M, Gupta A, Antonsson M, Bredberg U, Hammarlund-Udenaes M. In Vitro Methods for Estimating Unbound Drug Concentrations in the Brain Interstitial and Intracellular Fluids. *Drug Metab Dispos.* 2007;35:1711–9.
125. Hammarlund-Udenaes M, Paalzow LK, de Lange ECM. Drug equilibration across the blood-brain barrier - Pharmacokinetic considerations based on the microdialysis method. *Pharm Res.* 1997;14(2):128–34.
126. Hammarlund-Udenaes M. The use of microdialysis in CNS drug delivery studies: Pharmacokinetic perspectives and results with analgesics and antiepileptics. *Adv Drug Deliv Rev.* 2000;45(2–3):283–94.
127. Elmquist W, Sawchuk R. Application of microdialysis in pharmacokinetic studies. *Pharm Res.* 1997;14(3):267–88.
128. de Lange ECM, Danhof M, de Boer AG, Breimer DD. Methodological considerations of intracerebral microdialysis in pharmacokinetic studies on drug transport across the blood-brain barrier. *Brain Res Brain Res Rev.* 1997;25(1):27–49.
129. Westerhout J, Smeets J, Danhof M, de Lange ECM. The impact of P-gp functionality on non-steady state relationships between CSF and brain extracellular fluid. *J Pharmacokinet Pharmacodyn.* 2013;40(3):327–42.
130. Neuwelt E, Abbott NJ, Abrey L, Banks WA, Blakley B, Davis T, et al. Strategies to advance translational research into brain barriers. *Lancet Neurol.* 2008;7(1):84–96.
131. Assmus F, Seelig A, Gobbi L, Borroni E, Glaentzlin P, Fischer H. Label-free assay for the assessment of nonspecific binding of positron emission tomography tracer candidates. *Eur J Pharm Sci.* 2015;79:22–35.
132. Loryan I, Sinha V, Mackie C, van Peer A, Drinkenburg W, Vermeulen A, et al. Mechanistic Understanding of Brain Drug Disposition to Optimize the Selection of Potential Neurotherapeutics in Drug Discovery. *Pharm Res.* 2014;32(8):2203–19.
133. Loryan I, Melander E, Svensson M, Payan M, König F, Jansson B, et al. In-depth neuropharmacokinetic analysis of antipsychotics based on a novel approach to estimate unbound target-site concentration in CNS regions: link to spatial receptor occupancy. *Mol Psychiatry.* 2016;1–10.
134. Murbach Freire C, Marques M, Costa M. Effects of seasonal variation on the central nervous system activity of *Ocimum gratissimum* L. essential oil. *J Ethnopharmacol.* 2006;105:161–6.

135. Bowie MW, Slattum PW. Pharmacodynamics in older adults: a review. *Am J Geriatr Pharmacother.* 2007;5(3):263–303.
136. Bigos KL, Pollock BG, Stankevich BA, Bies RR. Sex differences in the pharmacokinetics and pharmacodynamics of antidepressants: an updated review. *Gend Med.* 2009;6(4):522–43.
137. Oksenberg D, Marsters S, O'Dowd BF, Jin H, Havlik S, Peroutka SJ, et al. A single amino-acid difference confers major pharmacological variation between human and rodent 5-HT1B receptors. *Nature.* 1992;360:161–3.
138. Stevens J, Ploeger BA, van der Graaf PH, Danhof M, de Lange ECM. Systemic and direct nose-to-brain transport pharmacokinetic model for remoxipride after intravenous and intranasal administration. *Drug Metab Dispos.* 2011;39(12):2275–82.
139. Grandison MK, Boudinot FD. Age-related changes in protein binding of drugs: implications for therapy. *ClinPharmacokinet.* 2000;38(3):271–90.
140. Greenblatt D. Reduced serum albumin concentration in the elderly: a report from the Boston Collaborative Drug Surveillance Program. *J Am Geriatr Soc.* 1979;27(1):20–2.
141. Piafsky KM, Borgå O, Odar-Cederlöf I, Johansson C, Sjöqvist F. Increased Plasma Protein Binding of Propranolol and Chlorpromazine Mediated by Disease-Induced Elevations of Plasma  $\alpha$ 1 Acid Glycoprotein. *new Engl Juornal pf Med.* 1978;299:1435–9.
142. Shaw TG, Mortel KF, Meyer JS, Rogers RL, Hardenberg J, Cutaia MM. Cerebral blood flow changes in benign aging and cerebrovascular disease. *Neurology.* 1984;34(7):855.
143. Kim SG. Quantification of Relative Cerebral Blood-Flow Change By Flow- Sensitive Alternating Inversion-Recovery (Fair) Technique - Application To Functional Mapping. *Magn Reson Med.* 1995;34(3):293–301.
144. Braun AR, Balkin TJ, Wesensten NJ, Carson RE, Varga M, Baldwin P, et al. Regional cerebral blood flow throughout the sleep-wake cycle An H2 15O PET study. *Brain.* 1997;120:1173–97.
145. Giusto NM, Salvador GA, Castagnet PI, Pasquaré SJ, Ilincheta de Boschero MG. Age-Associated Changes in Central Nervous System Glycerolipid Composition and Metabolism. *Neurochem Res.* 2002;27(11):1513–23.
146. Yehuda S, Rabinovitz S, Carasso RL, Mostofsky DI. The role of polyunsaturated fatty acids in restoring the aging neuronal membrane. *Neurobiol Aging.* 2002;23:843–53.
147. Uauy R, Dangour AD. Nutrition in Brain Development and Aging: Role of Essential Fatty Acids. *Nutr Rev.* 2006;64(5):24–33.
148. Yehuda S, Rabinovitz S, Mostofsky DI. Essential fatty acids and the brain: From infancy to aging. *Neurobiol Aging.* 2005;26:98–102.
149. Assies J, Lieverse R, Vreken P, Wanders RJA, Dingemans PMJA, Linszen DH. Significantly reduced docosahexaenoic and docosapentaenoic acid concentrations in erythrocyte membranes from schizophrenic patients compared with a carefully matched control group. *Biol Psychiatry.* 2001;49(6):510–22.

150. Mark KS, Davis TP. Cerebral microvascular changes in permeability and tight junctions induced by hypoxia-reoxygenation. *Am J Physiol Heart Circ Physiol.* 2002;282(4):1485–94.
151. Sandoval KE, Witt KA. Blood-brain barrier tight junction permeability and ischemic stroke. *Neurobiol Dis.* 2008;32(2):200–19.
152. Marco S, Skaper SD. Amyloid beta-peptide1-42 alters tight junction protein distribution and expression in brain microvessel endothelial cells. *Neurosci Lett.* 2006;401:219–24.
153. Plumb J, McQuaid S, Mirakhur M, Kirk J. Abnormal endothelial tight junctions in active lesions and normal-appearing white matter in multiple sclerosis. *Brain Pathol.* 2002;12:154–69.
154. Blomqvist G, Hospital K, Brain M. Facilitated transport of glucose from blood to brain in man on cerebral glucose utilization. *Eur J Nucl Med.* 1991;18:834–7.
155. Miyagawa T, Oku T, Uehara H, Desai R, Beattie B, Tjuvajev J, et al. “Facilitated” amino acid transport is upregulated in brain tumors. *J Cereb blood flow Metab.* 1998;18(5):500–9.
156. Claudio L, Kress Y, Norton WT, Brosnan CF. Increased vesicular transport and decreased mitochondrial content in blood-brain barrier endothelial cells during experimental autoimmune encephalomyelitis. *Am J Pathol.* 1989;135(6):1157–68.
157. Reulen HJ, Graham R, Spatz M, Klatzo I. Role of pressure gradients and bulk flow in dynamics of vasogenic brain edema. *J Neurosurg.* 1977;46(1):24–35.
158. Resnick SM, Pham DL, Kraut MA, Zonderman AB, Davatzikos C. Longitudinal Magnetic Resonance Imaging Studies of Older Adults : A Shrinking Brain. *J Neurosci.* 2003;23(8):3295–301.
159. Serot JM, Béné MC, Foliguet B, Faure GC. Altered choroid plexus basement membrane and epithelium in late-onset Alzheimer’s disease: An ultrastructural study. *Ann N Y Acad Sci.* 1997;826:507–9.
160. May C, Kaye JA, Atack JR, Schapiro MB, Friedland RP, Rapoport SI. Cerebrospinal fluid production is reduced in healthy aging. *Neurology.* 1990;40:500–3.
161. Silverberg GD, Mayo M, Saul T, Rubenstein E, McGuire D. Alzheimer’s disease, normal-pressure hydrocephalus, and senescent changes in CSF circulatory physiology: a hypothesis. *Lancet Neurol.* 2003;2:506–11.
162. Johanson CE, Duncan JA, Klinge PM, Brinker T, Stopa EG, Silverberg GD. Multiplicity of cerebrospinal fluid functions: New challenges in health and disease. *Cerebrospinal Fluid Res.* 2008 Jan;5(10):1–32.
163. Mann A, Miksys SL, Gaedigk A, Kish SJ, Mash DC, Tyndale RF. The neuroprotective enzyme CYP2D6 increases in the brain with age and is lower in Parkinson’s disease patients. *Neurobiol Aging.* 2012;33(9):2160–71.
164. Orelund L, Gottfries C-G. Brain and brain monoamine oxidase in aging and in dementia of Alzheimer’s type. *Prog Neuro-Psychopharmacology Biol Psychiatry.* 1986;10:533–40.

165. Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, et al. Functional Analysis of Genetic Variation in Catechol-O-Methyltransferase (COMT): Effects on mRNA, Protein, and Enzyme Activity in Postmortem Human Brain. *Am J Hum Genet.* 2004;75:807–21.
166. Meyer-Lindenberg A, Nichols T, Callicott JH, Ding J, Kolachana B, Buckholtz J, et al. Impact of complex genetic variation in COMT on human brain function. *Mol Psychiatry.* 2006;11(9):867–77.
167. Howard LA, Miksys S, Hoffmann E, Mash D, Tyndale RF. Brain CYP2E1 is induced by nicotine and ethanol in rat and is higher in smokers and alcoholics. *Br J Pharmacol.* 2003;138(7):1376–86.
168. Toornvliet R, van Berckel BNM, Luurtsema G, Lubberink M, Geldof AA, Bosch TM, et al. Effect of age on functional P-glycoprotein in the blood-brain barrier measured by use of (R)-[<sup>11</sup>C] verapamil and positron emission tomography. *Clin Pharmacol Ther.* 2006;79(6):540–8.
169. Vorbrodth AW, Dobrogowska DH, Meeker HC, Carp RI. Immunogold study of regional differences in the distribution of glucose transporter (GLUT-1) in mouse brain associated with physiological and accelerated aging and scrapie infection. *J Neurocytol.* 1999;28:711–9.
170. Aronica E, Gorter JA, Jansen GH, van Veelen CW, van Rijen PC, Leenstra S, et al. Expression and cellular distribution of multidrug transporter proteins in two major causes of medically intractable epilepsy: focal cortical dysplasia and glioneuronal tumors. *Neuroscience.* 2003;118(2):417–29.
171. Spiegl-Kreinecker S, Buchroithner J, Elbling L, Steiner E, Wurm G, Bodenteich A, et al. Expression and functional activity of the ABC-transporter proteins P-glycoprotein and multidrug-resistance protein 1 in human brain tumor cells and astrocytes. *J Neurooncol.* 2002;57(1):27–36.
172. Danbolt NC. Glutamate uptake. *Prog Neurobiol.* 2001;65(1):1–105.
173. Holmes GL. Seizure-induced neuronal injury. *Neurology.* 2002;59(9):3–6.
174. Kervezee L, Hartman R, van den Berg D-J, Shimizu S, Emoto-Yamamoto Y, Meijer JH, et al. Diurnal variation in P-glycoprotein-mediated transport and cerebrospinal fluid turnover in the brain. *AAPS J.* 2014;16(5):1029–37.
175. Collins JM, Dedrick RL. Distributed model for drug delivery to CSF and brain tissue. *Am J Physiol.* 1983;245(3):303–10.
176. Ooie T, Terasaki T, Suzuki H, Sugiyama Y. Kinetic Evidence for Active Efflux Transport across the Blood-Brain Barrier of Quinolone Antibiotics. *J Pharmacol Exp Ther.* 1997;283(1):293–304.
177. Takasawa K, Terasaki T, Suzuki H, Ooie T, Sugiyama Y. Distributed model analysis of 3'-azido-3'-deoxythymidine and 2,3'-dideoxyinosine distribution in brain tissue and cerebrospinal fluid. *J Pharmacol Exp Ther.* 1997;282(3):1509–17.
178. Hansen DK, Scott DO, Otis KW, Lunte SM. Comparison of in vitro BBMEC permeability and in vivo CNS uptake by microdialysis sampling. *J Pharm Biomed Anal.* 2002;27:945–58.

179. Bourasset F, Scherrmann JM. Carrier-mediated processes at several rat brain interfaces determine the neuropharmacokinetics of morphine and morphine-6-beta-D-glucuronide. *Life Sci.* 2006;78(20):2302–14.
180. Liu X, Smith BJ, Chen C, Callegari E, Becker SL, Chen X, et al. Use of a Physiologically Based Pharmacokinetic Model to Study the Time to Reach Brain Equilibrium: An Experimental Analysis of the Role of Blood-Brain Barrier Permeability, Plasma Protein Binding, and Brain Tissue Binding. *J Pharmacol Exp Ther.* 2005;313(3):1254–62.
181. Kielbasa W, Stratford RE. Exploratory Translational Modeling Approach in Drug Development to Predict Human Brain Pharmacokinetics and Pharmacologically Relevant Clinical Doses. *Drug Metab Dispos.* 2012;40(5):877–83.
182. Fenneteau F, Turgeon J, Couture L, Michaud V, Li J, Nekka F. Assessing drug distribution in tissues expressing P-glycoprotein through physiologically based pharmacokinetic modeling: model structure and parameters determination. *Theor Biol Med Model.* 2009;36:495–522.
183. Ball K, Bouzom F, Scherrmann J-M, Walther B, Declèves X. Physiologically Based Pharmacokinetic Modelling of Drug Penetration Across the Blood-Brain Barrier--Towards a Mechanistic IVIVE-Based Approach. *AAPS.* 2013;15(4):913–32.
184. Badhan RKS, Chenel M, Penny JI. Development of a Physiologically-Based Pharmacokinetic Model of the Rat Central Nervous System. *Pharmaceutics.* 2014;6(1):97–136.
185. Wagner C, Zhao P, Pan Y, Hsu V, Grillo J, Huang SM, et al. Application of Physiologically Based Pharmacokinetic (PBPK) Modeling to Support Dose Selection : Report of an FDA Public Workshop on PBPK. *Clin Pharmacol Ther.* 2015;4(4):226–30.
186. Shepard T, Scott G, Cole S, Nordmark A, Bouzom F. Physiologically Based Models in Regulatory Submissions: Output From the ABPI/MHRA Forum on Physiologically Based Modeling and Simulation. *CPT Pharmacometrics Syst Pharmacol.* 2015;4:221–5.
187. European Medicines Agency. Concept paper on qualification and reporting of physiologically-based pharmacokinetic (PBPK) modelling and analyses. 2014; Available from: [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Scientific\\_guideline/2014/06/WC500169452.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2014/06/WC500169452.pdf)
188. Erdő F, Denes L, de Lange ECM. Age-associated physiological and pathological changes at the blood-brain barrier. *J Cereb Blood Flow Metab.* 2017;37(1):4–24.
189. Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG. Animal Research: Reporting In Vivo Experiments: The ARRIVE Guidelines. *J Gene Med.* 2010;12(8):561–3.
190. de Witte WEA, Danhof M, van der Graaf PH, de Lange ECM. In vivo Target Residence Time and Kinetic Selectivity: The Association Rate Constant as Determinant. *Trends Pharmacol Sci.* 2016;37(10):831–42.
191. de Witte WEA, Wong YC, Nederpelt I, Heitman LH, Danhof M, van der Graaf PH, et al. Mechanistic models enable the rational use of in vitro drug-target binding kinetics for better drug effects in patients. *Expert Opin Drug Discov.* 2016;11(1):45–63.

