REVIEW

Addressing Central Nervous System (CNS) Penetration in Drug Discovery: Basics and Implications of the Evolving New Concept

by **Andreas Reichel**

Research Pharmacokinetics, Global Drug Discovery, *Bayer Schering Pharma*, Müllerstrasse 178, D-13342 Berlin

(phone: +49 30 4681 1384; fax: +49 30 4689 1384; e-mail: andreas.reichel@bayerhealthcare.com)

Despite enormous efforts, achieving a safe and efficacious concentration profile in the brain remains one of the big challenges in central nervous system (CNS) drug discovery and development. Although there are multiple reasons, many failures are due to underestimating the complexity of the brain, also in terms of pharmacokinetics (PK).

To this day, PK support of CNS drug discovery heavily relies on improving the blood–brain barrier (BBB) permeability *in vitro* and/or the brain/plasma ratio (K_p) *in vivo*, even though neither parameter can be reliably linked to pharmacodynamic (PD) and efficacy readouts. While increasing BBB permeability may shorten the onset of drug action, an increase in the total amount in brain may not necessarily increase the relevant drug concentration at the pharmacological target. Since the traditional K_p ratio is based on a crude homogenization of brain tissue, it ignores the compartmentalization of the brain and an increase favors non-specific binding to brain lipids rather than free drug levels.

To better link exposure/PK to efficacy/PD and to delineate key parameters, an integrated approach to CNS drug discovery is emerging which distinguishes total from unbound brain concentrations. As the complex nature of the brain requires different compartments to be considered when trying to understand and improve new compounds, several complementary parameters need to be measured *in vitro* and *in vivo*, and integrated into a coherent model of brain penetration and distribution.

The new paradigm thus concentrates on finding drug candidates with the right balance between free fraction in plasma and brain, and between rate and extent of CNS penetration. Integrating this data into a coherent model of CNS distribution which can be linked to efficacy will allow it to design compounds with an optimal mix in physicochemical, pharmacologic, and pharmacokinetic properties, ultimately mitigating the risk for failures in the clinic.

Abbreviations

A_{brain}	amount of compound in brain, corrected for intravascular content [μg/g brain]
ADME	absorption, distribution, metabolism, excretion
AUC	area under the concentration time curve, *e.g.*, in plasma or in brain
BBB	blood-brain barrier
CNS	central nervous system
CSF	cerebrospinal fluid
$C_{u,brain}$	unbound concenration in brain, surrogate for brain ISF levels [ng/ml]
DMPK	drug metabolism and pharmacokinetics
ER	b-a/a-b efflux ratio, *e.g.*, in the MDCK-MDR1 permeation assay
$f_{u,brain}$	fraction unbound in brain
$f_{u,plasma}$	fraction unbound in plasma

ISF	interstitial fluid
ICF	intracellular fluid
K_{in}	influx clearance into brain [μl/min/g brain]
K_{out}	efflux clearance out of brain [μl/min/g brain]
K_p	total brain to total plasma concentration ratio
$K_{p,uu}$	unbound brain to unbound plasma concentration ratio
M6G	morphine-6-*O*-glucuronide
MDCK	*Madin–Darby* Canine Kidney cell line
MDR1	human multidrug resistance-1 efflux protein, also called P-glycoprotein (P-gp)
PD	pharmacodynamics
PET	positron emission tomography
PK	pharmacokinetics
PS	permeability surface area product [μl/min/g brain]
P_{app}	permeabiliy coefficient [nm/s]
Q_{br}	cerebral blood flow [μl/min/g brain]
$V_{u,brain}$	unbound volume of distribution in brain [ml/g brain]

1. Introduction. – The discovery and development of new medicines to treat diseases of the CNS is one of the most challenging undertakings of today's pharmaceutical industry, with the rate of attrition being higher than in any other therapeutic area [1]. This high risk of failure for new CNS drugs is linked to the extraordinary complexity of the anatomy and physiology of the human brain, and its pathologies. Accordingly, the reasons for failure are as well very complex.

CNS Diseases are traditionally defined by clinical symptoms rather than pharmacological mechanisms, hence there often is a large fraction of non-responders to mechanistic drug candidates in the patient population in the clinic, masking the effect in a potentially responding sub-population. Part of the problem resides in the still insufficient understanding of the underlying pathophysiology of the diseases. As seen, for example, in the many clinical trials of stroke, candidate drugs often have no effects in human despite showing significant effects in the laboratory questioning the clinical relevance of animal disease models [2]. Also, short-term favorable effects may reverse over longer-term application, demonstrating that results after chronic treatment may differ from acute effects, as the CNS may respond to treatment in a very complex mode [3]. Another persisting problem is to identify an appropriate dose and schedule in the clinical trial which is both efficacious and safe, this difficulty being further aggravated if the drug candidate has a very narrow therapeutic window, and validated biomarkers are not at hand [4][5]. Last but not least, the vasculature of the brain which forms the so-called blood-brain barrier (BBB) may serve as formidable obstacle to the entry of drugs into the brain causing CNS exposure to be insufficient for efficacy [6–8].

For oral CNS medications, the situation is also complicated by the many pharmacokinetic hurdles a compound has to overcome between oral administration and reaching the target site of action within the CNS, *e.g.*, intestinal absorption, distribution within the body including the brain, and subsequently metabolism and excretion (*Fig. 1*). Consequently, PK scientists have to address many ADME processes from early on in the drug discovery process in order to optimize and balance the various compound properties so to increase the chances of success in the clinic [7]. There is now a wide range of *in vitro* ADME assays and *in vivo* PK study types available alongside

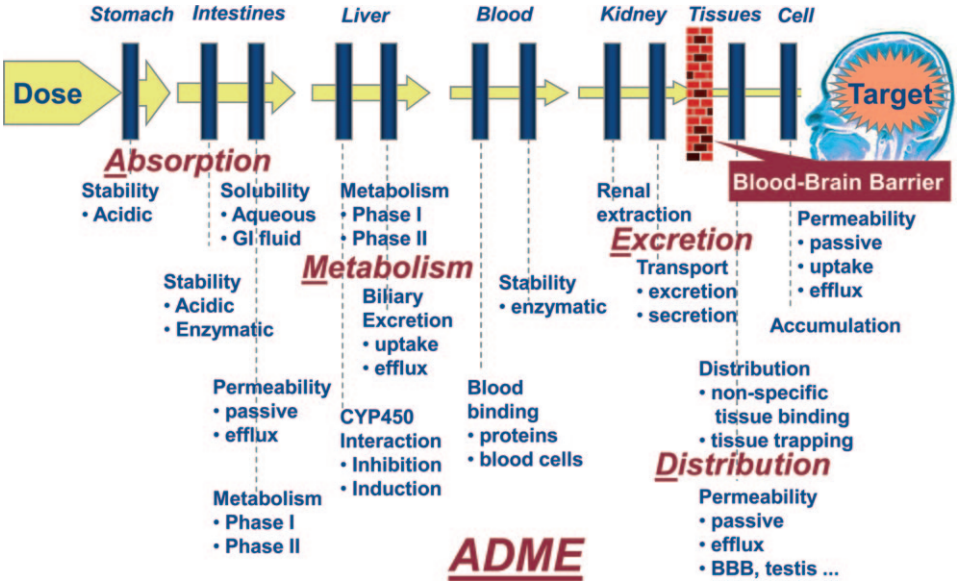

Fig. 1. *Pharmacokinetic barriers between oral dose and the site of the drug target within the CNS.* The figure illustrates tissues, processes, and factors controlling the absorption, distribution, metabolism and elimination of drugs.

well-established approaches to identify and optimize DMPK liabilities in today's drug-discovery process [9–11].

In addition, for CNS drug discovery programs brain penetration of new compounds is a key property to be addressed. However, although there is now a range of tools covering *in silico*, *in vitro*, and *in vivo* methods, our past understanding of brain penetration was based heavily on measuring total brain levels in rodents. As this method is easy and simple, large numbers of compounds have been screened in the past for high total brain levels. Moreover, total brain levels, expressed as brain/plasma ratio, can be predicted *in silico* further pushing compounds towards high total brain levels. However, although more and more compounds with high and higher total brain levels have been generated, there was no corresponding increase in the *in vivo* efficacy of these compounds despite excellent *in vitro* potency at the target mechanism. Indeed, as both *in vitro* potency and total brain levels are driven mainly by lipophilicity [12], many CNS drug discovery programs have ended up on a 'lipid escalator' which, however, led them to nowhere [7b][13].

The failure of the traditional approach has led to the emergence of a new concept for examining brain penetration in drug discovery [14–18]. This approach better takes into account the complexity of the brain by considering the brain as having separate PK compartments, by more clearly differentiating brain distribution in terms of total and unbound drug levels, and by more rigorously discriminating between rate and extent of brain penetration. The aim of this review is to describe the new concept and to derive implications for CNS drug discovery and development.

2. Barriers and Compartments of the Brain. – 2.1. *Barriers within the Brain.* The CNS is separated from the peripheral blood circulation by physiological barriers which provide a fully autonomous milieu for the cells within the CNS while maintaining a constant supply with nutrients and removing waste. As CNS functions fundamentally rely on a highly regulated flow of ions across and along neurons, the brain ISF environment requires a very tight control of its composition. The brain ISF is, therefore, effectively separated from the highly fluctuating fluid compartment of circulating blood [19][20]. It is the existence of these barriers between the blood and the CNS which impede the accessibility to their cerebral targets for many drug molecules circulating in the blood stream [21].

The two most important barriers within the CNS are the BBB and the blood-CSF barrier (BCSFB). The BBB is formed by endothelial cells lining the brain capillaries (*Fig. 2*). Because brain endothelial cells are sealed together by a very complex network of tight junctions, all traffic of any material being it ions, solutes, nutrients, hormones, larger molecules, or even cells has to occur *via* the brain endothelial cells. As the BBB has a very complex multicellular organisation consisting not only of brain endothelial cells, but also of pericytes, astrocytes, neurons, and other cell types, this traffic is under close, presumably even local control of the CNS. To better account for this key feature of the BBB and its implications, the term neurovascular unit has been coined recently [23].

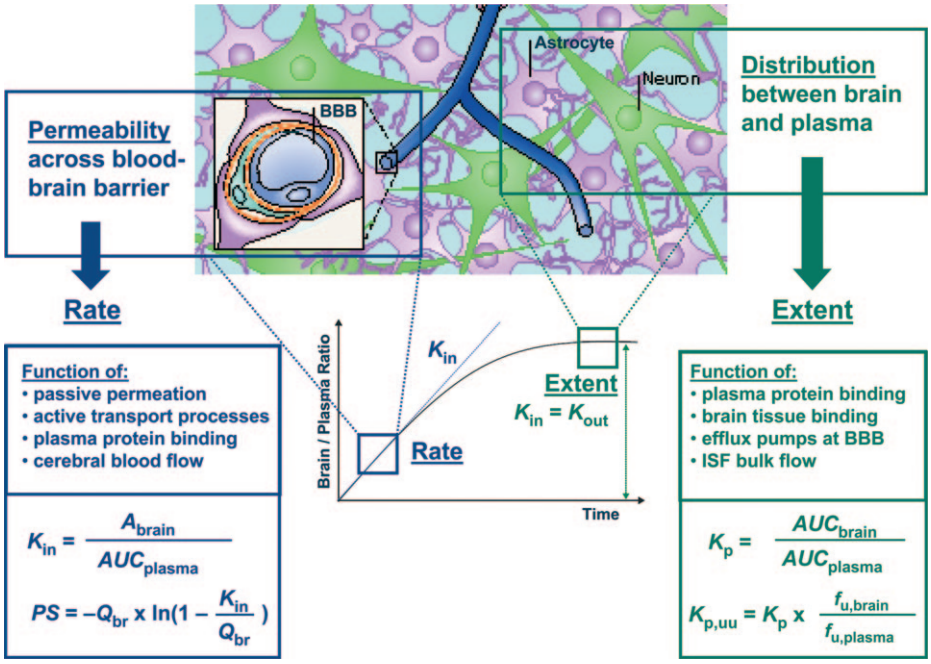

Fig. 2. *Schematic illustration of the rate of permeation and the extent of brain distribution of a drug as independent parameters of CNS drug penetration.* Shown are also factors controlling both parameters and principal equations for their determination. See also [17][18][22].

Besides the BBB, there is another barrier which separates blood from CSF, *i.e.*, the BCSFB. This barrier, which is located at the level of the choroid plexus, differs from the BBB in that its barrier function originates from the tight epithelium lining the ventricle rather than the endothelium as in the cerebral capillaries.

From a PK point of view, the brain vasculature has the following physiological parameters: in human, the capillary length is 650 km, the capillary volume is 1 ml, the luminal diameter is 3 μm, the mean distance is 40 μm, and the surface area is *ca.* 12 m^2 approximating 100–150 cm^2/g brain [24]. Cerebral blood flow is *ca.* 0.5–2 ml/min/g brain in rat, resulting in transit time through the brain of only 5 s. The capillary volume is *ca.* 11 μl/g brain, which is less than 1‰. In contrast, the fluid compartment of the brain interstitial fluid amounts to *ca.* 20% of the brain parenchyma [17]. In the rat, ISF flows with a bulk flow rate of *ca.* 0.2 μl/min/g towards the CSF. The volume of CSF is *ca.* 160 ml in human and 250 μl in rat, with the rate of CSF secretion being *ca.* 350 and 2.1 μl/min, respectively [25]. The relative surface areas between BBB and BCSFB are estimated to be *ca.* 5000 : 1, and the density of the capillaries within the brain parenchyma is so high ($< 8–20$ μm) that virtually every neuron is supplied by its own capillary. Therefore, the BBB is generally viewed as having a much greater role than the BCSFB in the delivery of CNS medications to the brain [21][26]. The impact of the BBB will be predominantly on the rate of CNS penetration, while the extent of brain penetration into and the distribution of a drug within the brain depends on other factors as discussed in the following section (*Figs. 2* and *3*).

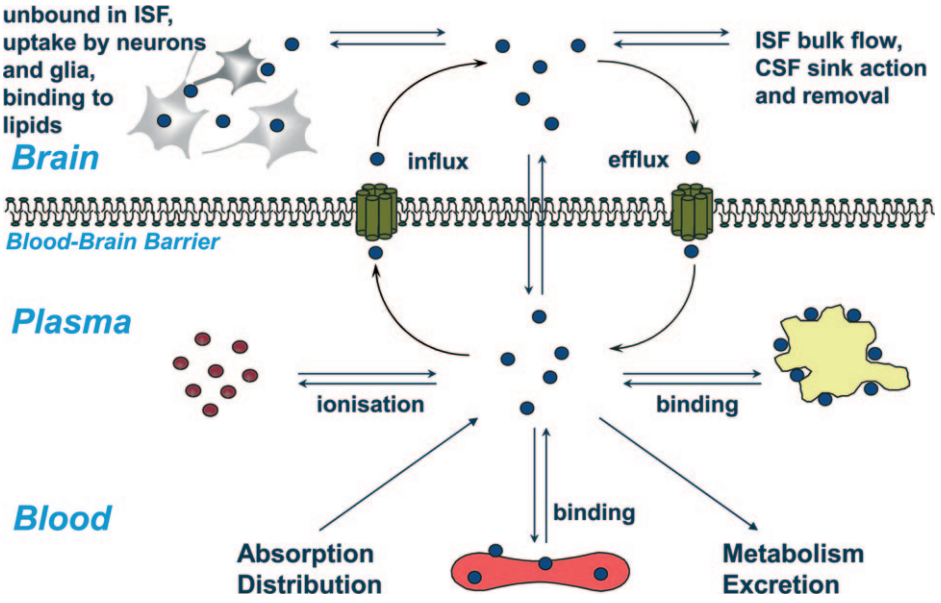

Fig. 3. *Multitude of factors illustrating the complexity of the processes which in sum contribute to the penetration of a drug into the CNS, its distribution within the CNS, and its removal from the CNS.*

2.2. *Compartments within the Brain.* The parenchyma of the brain has an extremely complex morphological structure, which also varies among the different brain regions.

The conception of brain distribution, however, benefits greatly from even considering just a few key PK compartments within the brain (*Fig. 4*), even though this seems overly simplistic from a physiological point of view. Nevertheless, the concept of separate but interrelated PK compartments within the brain has helped tremendously in rationalizing brain penetration and distribution in relation to drug efficacy, as briefly illustrated with the example of morphine and its equipotent glucuronidated metabolites (*Table 1*).

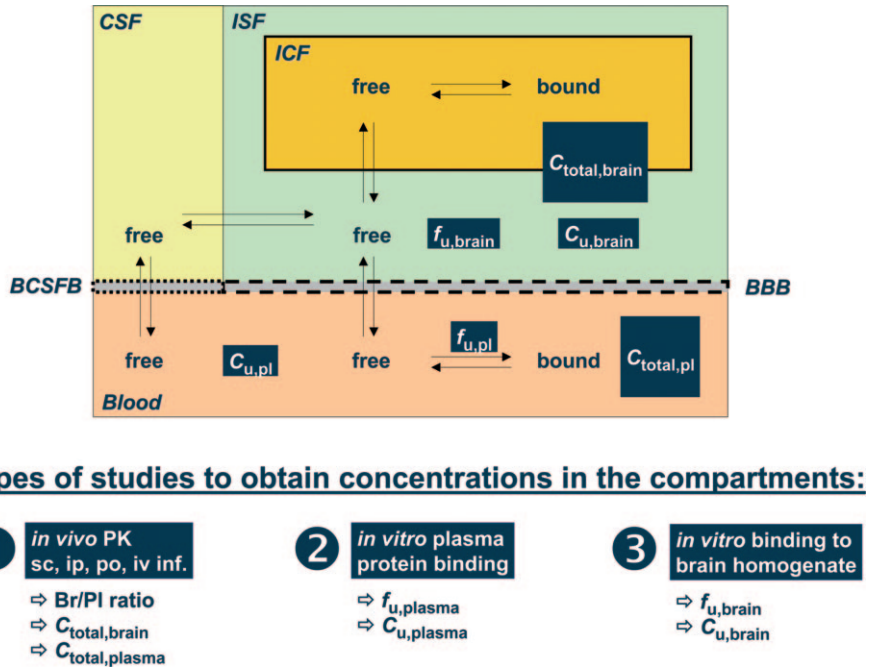

Fig. 4. *Principal pharmacokinetic compartments of the CNS, and the relation between bound and unbound concentrations in the compartments.* The dark boxes illustrate parameters which can be measured *in vitro* and *in vivo*, and their relation to the concentration in these PK compartments. The three boxes at the bottom summarize the methods by which the parameters shown can be obtained: *1*) *in vivo* determination of the total brain to total plasma ratio K_p, the total amount of drug in brain $A_{total,brain}$, and total plasma concentrations $C_{total,plasma}$; *2*) and *3*) equilibrium dialysis of blood plasma and brain homogenate giving the fraction unbound in plasma and brain, from which the unbound concentrations in plasma $C_{u,plasma}$ and brain $C_{u,brain}$ from the *in vivo* study (*1*) can be derived.

Although morphine-6-*O*-glucuronide (M6G) has a much lower extent and slower rate of brain penetration than morphine itself, it shows a similarly high central analgesic efficacy in rat [27][31]. This is unexplained by the classic concept of CNS penetration, considering both the poor brain/plasma ratio of M6G of 0.069 as opposed to 0.54 for morphine [17], and the much lower rate of BBB permeation, expressed as permeability surface area (PS) product with values for M6G of 0.11 as opposed to 3.5 µl/min/g brain for morphine [28]. In spite of having similar affinity for the pharmacological target

Table 1. *CNS and Peripheral PK Parameters Describing the Pharmacokinetics of Morphine and Morphine-6-*O*-Glucuronide in Terms of Rate of Permeation across the BBB, Extent of Brain Penetration, and Distribution within the CNS, in Addition to Some of the Classic PK Parameters.* Data compiled from several sources [17][27–30].

	Morphine	Morphine-6-*O*-glucuronide
PS [μl/min/g brain]	3.5	0.11
K_p	0.54	0.07
$K_{p,u}$	0.65	0.08
$K_{p,uu}$	0.29	0.29
$V_{u,brain}$ [ml/g brain]	2.1	0.2
AUC (ISF) [μM min]	79	336
Conc. ratio [ISF]/[ICF]	1:4	125:1
AUC_{plasma} [μM min]	252	945
$V_{D,plasma}$ [l/kg]	2.2	0.33
CL_{plasma} [ml/min/kg]	30	13
$F_{u,plasma}$	0.83	0.86

[32], according to the classic concept of brain penetration, M6G should actually show no *in vivo* efficacy at all. The contradiction can be dissolved, however, by delineating the unbound fraction of the drug in brain from total brain levels [27]. As the action of both morphine and M6G are driven through binding to μ-opioid receptors located on neuronal cell surfaces, it is the free concentration of the drug in the brain ISF compartment which elicits the drug action rather than the total amount of drug in the brain. Indeed, although total brain levels of M6G are much lower than those of morphine, the exposure at site of the target receptor, *i.e.*, the levels within the ISF, is even higher for M6G (four-times those of morphine; see *Table 1*). Hence, the relevant effect compartment is brain ISF and its drug levels are independent of and not captured by total brain levels as indeed can be seen for many other CNS drugs as well (*Fig. 5*).

The delineation of compartments constituting total and free brain concentrations is a key to rationalize the efficacy of any drug binding to extracellular receptors, but is also applicable to drugs binding to intracellular targets. As shown in *Fig. 3*, the concentration in the brain ISF is regulated by at least five independent factors: *1*) the plasma exposure which depends on total clearance and volume of distribution, *2*) plasma protein binding which determines the unbound fraction of the drug available for brain penetration, *3*) BBB transport rates which determine the transfer of the drug between plasma and ISF resulting from passive diffusion, active uptake, or efflux transport processes, *4*) brain cellular *vs.* brain ISF partitioning and binding to receptors, and *5*) elimination from CNS through brain metabolism and/or clearance of the drug by the CSF.

It is thus obvious that the simple ratio of total brain to total plasma is too crude as to be able to provide useful information as a single parameter. Indeed, the complexity of the processes controlling the drug concentrations in the PK compartments of the brain requires input of several methods, each providing a defined piece of information required to compile a more differentiated picture of the whereabouts of the drug molecule within the CNS. Only if the concentrations in the effect compartment of the

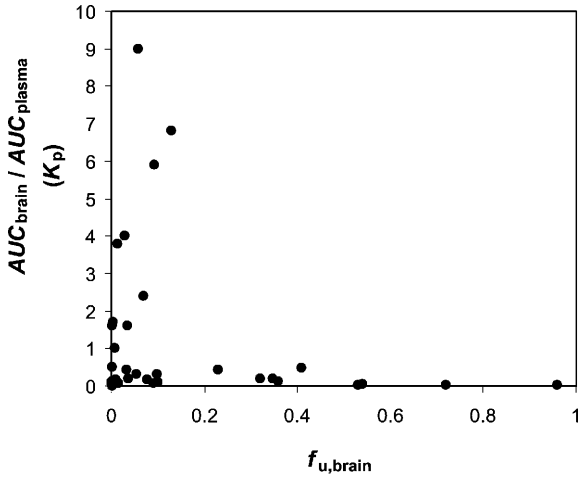

Fig. 5. *Relationship between the ratio of total brain concentration to total plasma concentration, and the free fraction in brain, suggesting that the free fraction is highest when the ratio is low.* Data of 34 drugs taken and re-plotted from [33].

brain can be approximated, a predictive link to the efficacy of a drug can be established [34].

3. Methods of Study in CNS Drug Discovery. – 3.1. *Traditional Methods to Study Brain Penetration.* The question of brain penetration is thus addressed early on in CNS drug discovery projects using a cascade of tools of increasing complexity ranging from *in silico* predictions, to *in vitro* assays and *in vivo* animal studies [7]. The present article does not attempt to give a full account of the large range of models and techniques available today, but instead will focus on the assays and study types which are currently being used broadly in CNS drug discovery programmes across the pharmaceutical industry and the new methods which are important in the emerging new concept. More detailed information of a wider range of methods can be found in [7][15][22][26][35][36].

3.1.1. In vitro *Assays of Brain Penetration – Permeability as Information on the* Rate *of Penetration across the BBB.* As the accessibility of a drug from blood into brain is controlled by the rate of permeation across the tight layer of brain endothelial cells, cell culture models have been established to reconstitute the BBB *in vitro* to provide a rapid measure of the permeability for newly synthesized compounds.

Despite three decades of experiences in culturing brain endothelial cells, there is still no generally satisfying *in vitro* model of the BBB available today [26][37][38]. While primary brain endothelial cell cultures are too cumbersome to be applied routinely, all immortalized brain endothelial cell lines generated thus far lack the tightness required to differentiate between and hence rank poorly permeable compounds. Therefore, most CNS discovery groups in industry prefer to use the MDCK cell line to screen compounds for BBB permeability [39–41]. Although MDCK cells are neither endothelial cells nor do they originate from brain, the high

tightness of the monolayer results in permeability values which correlate well with *in vivo* brain permeation, especially when transfected with the MDR1 efflux pump which is highly active at the BBB *in vivo* [41][42]. In addition to the permeability measurement, a so-called efflux ratio (ER; ratio of the permeability of both directions) is also determined allowing to identify and reject compounds with strong recognition by MDR1 [43].

Permeability assays using MDCK cells can be automated thus producing permeability data in a throughput, speed, and resolution sufficient to guide chemical synthesis cycles in lead optimization. Permeability data are suitable to rank compounds for permeability and to exclude very poorly permeable compounds from *in vivo* studies as they are unlikely to make progress. They also provide first hints on the involvement of active transport processes. Permeability data do not, however, provide any information on the free concentrations to be achieved within the brain, as this is determined by the binding properties of the compound in brain relative to blood and the magnitude of the circulating levels in blood. Permeability data serve only one aspect of CNS penetration, *i.e.*, rate, and thus must be supplemented by data containing information on the *extent* of brain penetration.

3.1.2. In vivo *Study of Brain Penetration – Brain/Plasma Ratio as Information on the* Extent *of Penetration into Whole Brain*. The most common method to study brain penetration *in vivo* is the determination of the brain/plasma ratio in rodents. Typically, the test compound is dosed intraperitoneally (ip), subcutaneously (sc), or per os (po), and both plasma and brain are sampled at *ca.* 3–5 time points. Alternatively, the compound is infused intravenously until steady state is assumed, and plasma and brain are sampled at one time point only, thereby reducing animal numbers. In all setups, brain tissue is homogenized and precipitated, and the total brain concentration of the compound is determined (mostly by LC/MS) and related to its concentration in plasma.

While the measurement of brain concentrations allows to rank compounds according to total brain levels (related to dose) and general CNS penetrability (related to plasma), these data do not provide reliable information on the concentration at the target site. Since the homogenization of the brain tissue destroys all tissue compartments, this method cannot provide information on compound levels in any specific effect compartment, *e.g.*, brain ISF or ICF. Furthermore, brain/plasma ratios are generally invariant to dose and thus cannot be related to dose–response data.

To circumvent this key limitation of the crude measurement of total levels in brain, sampling of CSF [44][45] and/or brain microdialysis of ISF can be used [46]. However, both methods have their drawbacks, in particular practicability (microdialysis) and reliability (CSF sampling) which weaken their applicability in routine drug discovery [7][18][26].

3.2. *New* in vitro *Methods to Complement the Traditional Study Repertory.* Although *in vivo* techniques such as CSF sampling and brain microdialysis have been around for many years, it was not until *in vitro* methods have become available that the free fraction of compound in brain has begun to be studied more routinely in CNS drug discovery.

3.2.1. In vitro *Binding Studies in Brain Homogenate or Brain Slices – Unbound Concentration in Brain as Surrogate Information on Brain* Distribution. *Maurer et al.* [47] introduced a simple and elegant *in vitro* method to determine the free fraction in

brain based on the equilibrium dialysis of the compound between buffer and brain homogenate. The method can be performed in parallel with the estimation of the free fraction in plasma and is able to rapidly produce data on $f_{u,brain}$ and $f_{u,plasma}$ for a large number of compounds. These data allow it to assess the distribution behavior of compounds within the brain *in vivo*. As the brain ISF contains only a very low amount of proteins, $f_{u,brain}$ can be used directly to estimate unbound levels of a compound in brain ISF. Noteworthy, there is a very poor correlation between $f_{u,plasma}$ and $f_{u,brain}$ (*Fig. 6*), hence the free fraction in plasma is not a suitable surrogate for unbound brain concentrations [48], the most likely reason being the very different lipid and protein, composition of plasma and brain, with plasma having twice as much protein, while brain has 20-fold more lipids [49].

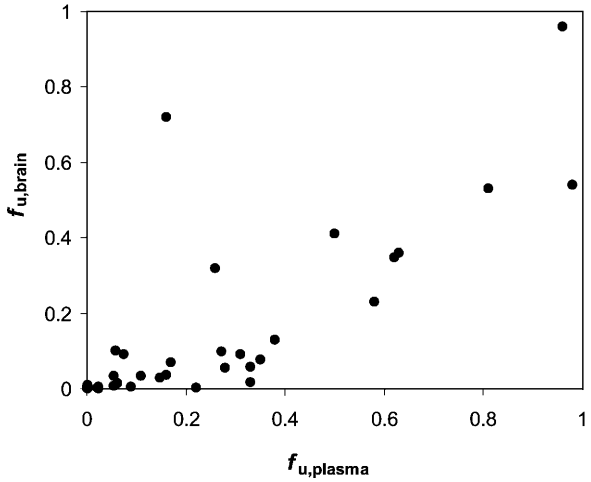

Fig. 6. *Relationship between the free fraction in plasma,* $f_{u,plasma}$*, and the free fraction in brain,* $f_{u,brain}$*, illustrating the differences in the extent of binding to plasma and brain tissue.* Data of 34 drugs taken and re-plotted from [33].

Becker and *Liu* [50], and *Fridén et al.* [51] developed an alternative *in vitro* method to determine the brain free fraction by using a slice technique which, in contrast to the brain homogenization method, maintains the cellular structure of the brain tissue throughout the *in vitro* distribution study. The advantage of this more elaborate method is that any differences between ISF and ICF concentrations can be captured in the resulting $f_{u,brain}$ values, while the more simple brain homogenate method is unable to distinguish between ISF and ICF levels. Fortunately, this assumption applies to the majority of cases so that the more cumbersome brain slice technique may be used only for those compounds where transport processes at the level of brain cells move the equilibrium between ISF and ICF markedly away from unity, *e.g.*, by strong active cellular uptake as seen for gabapentin [17][18]. This kind of information can often be derived from circumstantial evidence residing in normal routine data. Recently, a high-throughput method for rapid screening of *in vitro* drug–brain homogenate binding has been presented [52].

The above methods, which readily provide information on the free fraction of many compounds in brain tissue *in vitro*, have filled an important gap in our understanding of brain penetration and distribution. They have been the essential missing link which now allows routine access to the concentrations in a pharmacologically more relevant effect compartment in the brain. Indeed, they have been the breakthrough needed to pave the way to routinely apply also for the drug discovery of CNS diseases the free drug hypothesis which is most widely used to establish quantitative PK/exposure–PD/effect relationships [53].

4. Integrated Approach to Study CNS Penetration in Drug Discovery. – The wealth of experiences gained especially during the last decade, together with the introduction of higher throughput *in vitro* equilibrium dialysis methods, has now led to significant advances in our understanding of CNS penetration. This has culminated in a new concept for rationalizing brain penetration which may not fall short of triggering a shift in paradigm for CNS drug discovery.

The central component of the new concept is the clear differentiation between rate (of BBB permeation), extent (of brain penetration), and distribution (within the CNS). These three distinct but interdependent aspects of CNS penetration all have to be examined and integrated into one coherent concept (*Fig. 4* and later *Fig. 9*). A number of excellent articles have recently laid down the fundamental basis for this new, coherent approach to CNS penetration [14–18] which is briefly outlined in the following.

It is a big asset of the new paradigm that it can be readily flanged onto current practices in drug discovery. Indeed, a number of currently used assays and studies are simply complemented by some additional methods. The more radical change is the way of how the data are being looked at, and how decision making is being guided.

4.1. *First Tier:* in vitro *Assays* (*Fig. 7*). In line with regular procedures at the stage of lead compound characterization and optimization, a panel of *in vitro* ADME assays is run, *e.g.*, metabolic stability in liver microsomes, Caco-2 permeability, CYP interactions. In relation to CNS penetration, this *in vitro* panel is supplemented by three assays: *1*) permeability in MDCK-MDR1 cells (bidirectional format), and *2*) equilibrium dialysis of blood plasma, and *3*) of brain homogenate (from the pharmacological animal species).

The MDR1-MDCK cell data will be used to assess *i*) the general permeation behavior of the compound in a cellular setup (P_{app} value), and *ii*) its susceptibility to drug efflux by MDR1 (ER). The first read-out can be taken as surrogate for the rate of permeation across the BBB and should ideally have a P_{app} value of >150 nm/s. The second read-out is indicative of potential limitations in the brain penetration by drug efflux at the BBB and should ideally have an ER <3 [15][39]. However, it needs to be kept in mind that these thresholds are not rigid and may well be modified in a running project, as a poor P_{app} or a high ER value may both be compensated for by other properties of the compound as shown below. For practical reasons, information on ER may be taken alternatively from the bidirectional Caco-2 permeation assay, as in many instances this data is being determined on a routine basis in all drug discovery projects.

4.2. *Second Tier:* in vivo *Studies* (*Fig. 8*). Once a compound has demonstrated favorable *in vitro* data, *in vivo* studies will follow to examine the behavior of the

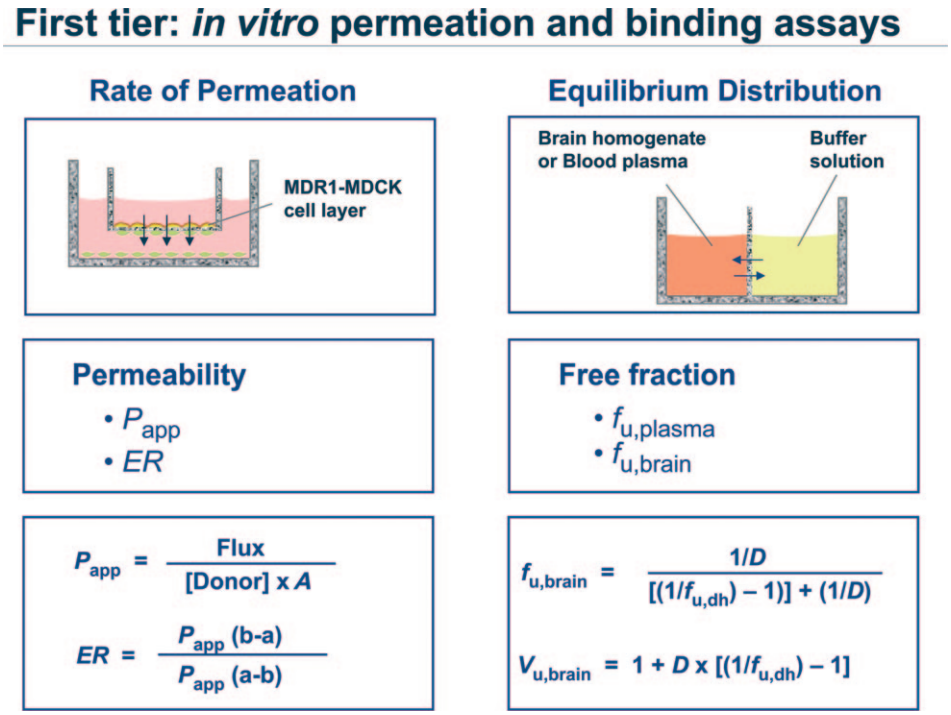

Fig. 7. *Schematic illustration of the first tier of* in vitro *assays, and parameters to characterize the rate of permeation and the binding characteristics of the compound to blood plasma and brain tissue* (homogenate). Uptake by brain tissue slices may be taken as alternative to the equilibrium dialysis of brain homogenate [18][51]. Abbreviations: *A:* surface area of filter; *D:* dilution of brain homogenate; $f_{u,dh}$: concentration ratio between buffer and diluted homogenate. For references, see [33][51][54].

compound in the whole animal. To be able to link the results with animal efficacy studies, the same species/strain should be taken.

Besides the regular single-dose PK study which determines the disposition PK and the oral PK of the compound (*e.g.*, total blood clearance, volume of distribution at steady-state, *AUC*, half-life, and oral bioavailability), the 'classic' total brain concentration *vs.* total plasma concentration ratio (K_p) should be determined either at several time points after ip, sc, or po dosing, or after iv infusion at a single time point where near steady-state is assumed. For the latter, the time period can be shortened by applying a bolus (loading dose) at the start of infusion.

The resulting concentration data will now be expressed in several ways, acknowledging the lessons learned from the 'free concept' of brain penetration. First, the classic K_p is being calculated. Using $f_{u,brain}$ and $f_{u,plasma}$ data from the first tier, K_p is then transformed into the unbound $K_{p,uu}$. Furthermore, the unbound brain concentrations as well as the unbound volume of distribution in brain can be calculated. The total brain concentration can either be taken separately from each time point or as $C_{average}$ from $AUC_{(0\text{-}yh)}$ divided by the time y over which the *AUC* was recorded.

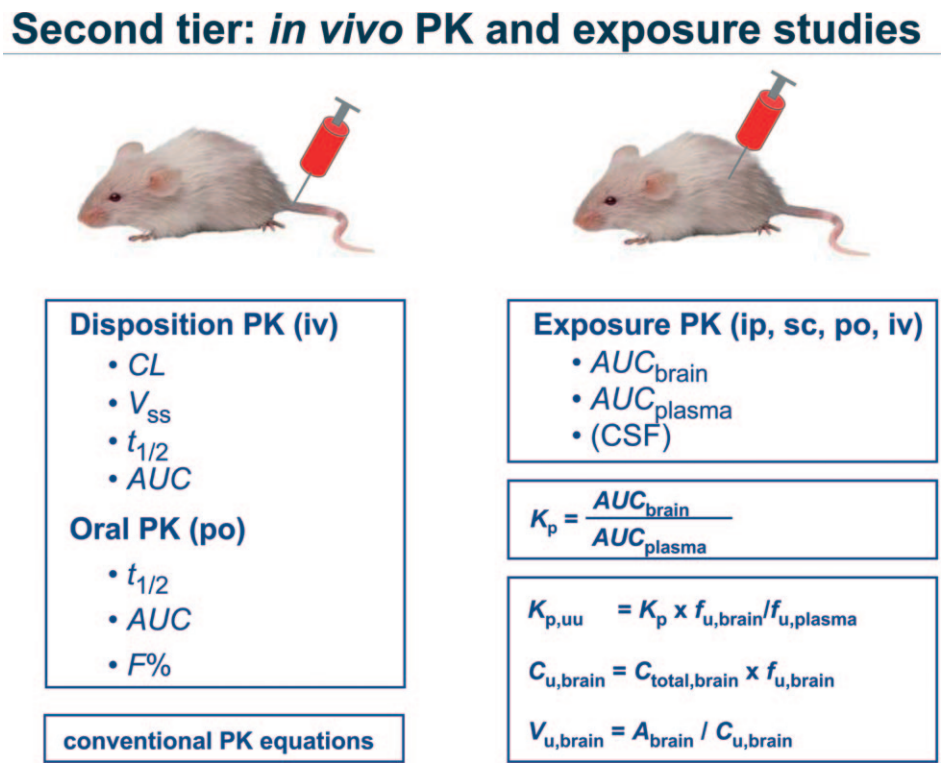

Fig. 8. *Schematic illustration of the second tier of the* in vivo *PK and brain exposure studies in rodents to determine the principal PK parameters of a compound and its exposure in the brain.* For calculation of peripheral PK parameters, see, for example, [55]. Abbreviations: *CL:* clearance; V_{ss}: volume of distribution at steady state; $t_{1/2}$: elimination half-life; *AUC:* area under the plasma concentration time curve; *F*%: oral bioavailability.

4.3. *Interpretation, Use, and Implication of Results* (*Fig. 9*). While the classic K_p value seems to be driven predominantly by nonspecific binding to brain lipids and hence may just be an *in vivo* measure of lipophilicity [56], its unbound relative, $K_{p,uu}$, is much more useful. It is a measure of the extent of the distribution equilibrium of a compound between the unbound fractions in brain and in blood plasma. If the value is close to unity, passive diffusion across the BBB can be assumed (or any influx being offset by efflux). This interpretation may be further supported by the *in vitro* permeability and efflux ratio data from the MDR1-MCDK or Caco-2 assay if available. In case the distribution between blood and brain is mainly determined by passive diffusion (*i.e.*, Caco-2 ER $<2-3$), brain concentrations may be approximated directly from plasma levels as illustrated in *Fig. 10*.

The unbound volume of distribution, $V_{u,brain}$, is an indicator for the distribution behavior of a compound within the brain and is irrespective of the extent of equilibrium between brain and plasma, *i.e.*, K_p and $K_{p,uu}$. $V_{u,brain}$ is an apparent volume term which is calculated by dividing the total amount of drug in brain (corrected for the amount in the

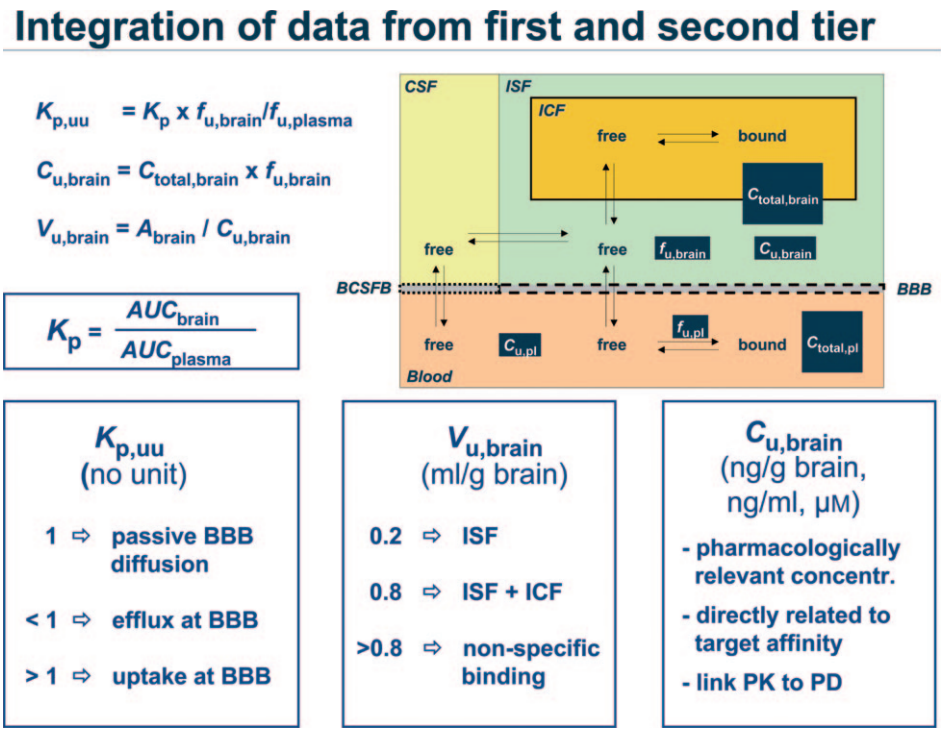

Fig. 9. *Interpretation and use of the brain PK parameters derived from both the first and second tier of* in vitro *assays and* in vivo *studies.* For further information, see also the excellent reviews of *Hammarlund-Udenaes et al.* [17][18].

cerebral vasculature) by the unbound concentration of the drug in brain (*Fig. 9*). Hence, $V_{u,brain}$ does not describe a 'real' volume and is interpreted in analogy to the 'classic' V_{ss} which is calculated from plasma concentration–time profiles obtained after iv dosing. $V_{u,brain}$ indicates whether a compound is distributed merely in ISF (*e.g.*, M6G whose $V_{u,brain}$ value corresponds to the volume of the ISF of *ca.* 0.2 ml/g brain), throughout the brain water space, *i.e.*, into both ISF and ICF ($V_{u,brain}$ *ca.* 0.8 ml/g brain), or has a tendency to nonspecifically bind to brain tissue ($V_{u,brain} > 0.8$ ml/g brain).

As a third result, $C_{u,brain}$ can be calculated for every time point where brain levels have been determined by correcting the total concentration in brain with $f_{u,brain}$ [58]. $C_{u,brain}$ is often a measure of the pharmacologically relevant exposure within the brain and hence gives a key piece of information for the PD effect compartment. In contrast to K_p values which are dose-static, $C_{u,brain}$ values can be used to interpret dose–response results from efficacy studies and hence to establish dose/exposure/effect, *i.e.*, PK/PD relationships [59], as exemplified for opioids including morphine and M6G by *Kalvass et al.* [34], and *Boström et al.* [60]. $C_{u,brain}$ Values not only depend on the free fractions in brain and plasma but also on the compound's peripheral PK profile, *i.e.*, plasma *AUC* and in turn *CL*, V_{ss}, and *F*%. $C_{u,brain}$ can thus also be improved by optimizing these basic

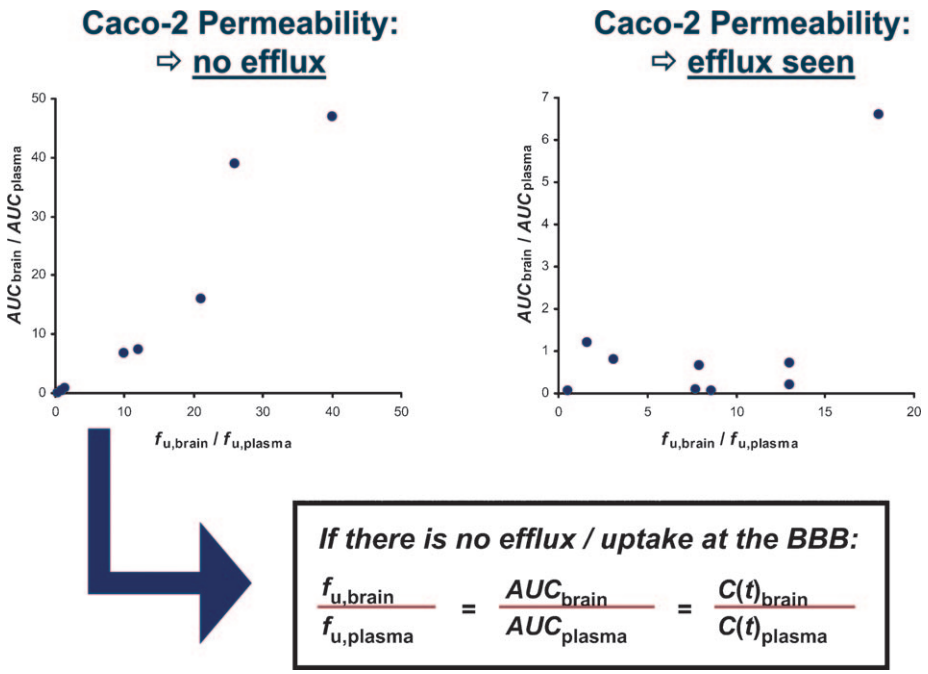

Fig. 10. *Correlation between the ratio of* AUC_{brain} *to* AUC_{plasma} *with the ratio* $f_{u,brain}$ *to* $f_{u,plasma}$ *for two sets of* n = *9 compounds, each with the data taken and re-plotted from* [57]. The left set of compounds was shown to permeate passively across Caco-2 cells, while the right set of compounds showed a significant drug efflux in the Caco-2 permeation assay. Once compounds can be assumed to permeate the BBB predominantly by passive diffusion, the equation shown is valid, allowing us to estimate total and free brain levels simply based on *in vivo* plasma concentrations, and the *in vitro* unbound fractions in plasma and brain homogenate.

PK properties, for example, by reducing total *CL* and/or V_{ss} which concomitantly may also increase $C_{u,brain}$.

All three parameters, $K_{p,uu}$, $V_{u,brain}$, and $C_{u,brain}$, are very useful to help design *in vivo* PD and efficacy studies in terms of dose size and dosing schedule to increase the likelihood to achieve efficacious concentrations in brain ISF or ICF as PD effect compartments. In addition, these parameters also contain information on the time needed to achieve efficacious concentrations (*Table 2*) and on transporter-mediated drug–drug interactions at the BBB (*Fig. 9*).

The time needed to achieve efficacious concentrations in the brain will depend on the rate of permeation as well as $V_{u,brain}$, the more rapid the first and the lower the last, the more rapid the extent of equilibrium will be achieved [17][18][61][62]. Hence, if a short onset of drug action is required, as, for example, in the acute treatment of epilepsy or stroke, the drug should combine a high P_{app} value (>150 nm) with little/no ER, and a high $f_{u,brain}$ value (>70–80%), with the $V_{u,brain}$ value not too much in excess of 0.8 ml/g brain. It has to be kept in mind that, for chronic treatments, time to achieve equilibrium is less important, hence the above parameters may be balanced in a very different way.

Because a $K_{p,uu}$ value <1 hints for active efflux at the BBB, *Hammarlund-Udenaes et al.* [17][18] suggest to use this parameter also as indicator for potential transporter-mediated drug–drug interactions and inter-patient variability. For example, the $K_{p,uu}$ value of 0.48 of loperamide indicates the potential of a twofold increase in brain ISF after blockade of efflux at the BBB [17], while total levels in brain may increase even more as suggested by the tenfold increase in K_p in P-gp knock-out mice [63]. This contradiction is just an apparent one, because it is exactly this disconnection between unbound ISF concentrations and total brain levels which is central to the free concept of brain penetration. This difference may also be at least part of the reason why clinically relevant drug–drug interactions with MDR1 at the BBB are seen so sparsely [64][65].

A very pragmatic but effective approach to elucidate the nature of potential efflux at the BBB has been proposed recently by *Kalvass et al.* [33], and *Jeffrey* and *Summerfield* [13]. The authors used a simple quadrant plot of ER *vs.* $K_{p,uu}$ to suggest whether passive diffusion or active efflux are dominating, and if significant efflux is seen, if P-gp or another efflux pump is likely to be involved in the brain penetration of a compound.

From a CNS project point of view, it is important to find compounds with the right balance of physicochemical, pharmacological, pharmacokinetic, and toxicological properties. The presented concept may assist in finding an optimal balance of general PK and relevant target exposure in relation to target affinity. For medicinal chemists, the working ranges of the CNS PK parameters, and their impact on the brain penetration and distribution when changing them, are of particular interest during lead optimization.

Looking at the data available thus far suggests that compounds seem to differ much more in terms of rate than in terms of extent of brain penetration. While permeability varies as much as 20,000-fold, K_p and $K_{p,uu}$ values vary only up to 400-fold and 150-fold, respectively [17]. $V_{u,brain}$ Data obtained thus far range from near 0.2 (*i.e.*, volume of brain ISF) to *ca.* 375 ml/g brain [17][18].

Because permeability is a principal prerequisite for CNS penetration and varies most, it is tempting to optimize compounds for a very high permeability using the rapid *in vitro* screens as described above. However, a normal-to-good permeability may suffice well, and chemistry efforts should rather be focused on improving $f_{u,brain}$, and hence $K_{p,uu}$ and $C_{u,brain}$, as these two parameters have a much greater impact on the desired pharmacological efficacy, even though the dynamic range of $K_{p,uu}$ is the smallest of the CNS PK parameters. As stated above, permeability may be of lesser importance during chronic administration schedules, and even poor permeants are able elicit significant pharmacological effects in human as seen for M6G. Indeed, high specific binding at the pharmacological target in the CNS and greater free fractions in brain can counterbalance poor BBB permeation and/or extensive plasma protein binding.

As a very general guidance for an optimal PK target profile of CNS drugs, $K_{p,uu}$ should be close to 1, $P_{app} > 150$ nm with little ER, and $f_{u,brain}$ should be such as to allow the unbound concentrations *in vivo* to exceed the target affinity as determined *in vitro*. K_p Values may be recorded but should not be taken for decision making. Total *CL* and V_{ss} should be kept low enough as to provide a high plasma exposure. Note, increasing

V_{ss} does not necessarily improve brain penetration in terms of levels in the PD effect compartment.

The *in vitro* assays in the first tier are both capable and sufficient to guide structural modifications, to select compounds for further *in vivo* studies, and to diagnose *in vivo* results. This advantage surely will have a very positive impact on the speed by which the new concept of brain penetration is leveraged by many CNS drug discovery projects.

5. Summary and Outlook. – The complex structure of the CNS makes brain penetration a very complex feature which cannot be rationalized on the basis of any single parameter. Although total brain concentrations are still the most common measure of CNS exposure, it is now emerging that they are more an indication of high nonspecific binding to brain tissue rather than being pharmacologically relevant concentrations. Neither total brain levels nor BBB permeability can be taken without considering the binding capacity of the brain tissue, when a link between exposure and efficacy is needed. The current paradigm of brain penetration is, therefore, changing towards a more compartmentalized view which allows a better rationalization of the distribution of compounds within the brain and makes use of brain compartments which are more relevant pharmacological effects.

Central to the emerging new paradigm is a clear differentiation between rate and extent of brain penetration, and between total and unbound drug levels as parameters for drug distribution within the brain. In attempting of a more holistic view, the concept is able to resolve a number of apparently contradicting observations, *e.g.*, why CNS effects can be seen in the clinic, even though a CNS drug poorly permeates the BBB, is substrate of efflux pumps, has low total brain levels, is poorly bound to brain tissue, or has a very high plasma protein binding.

The new paradigm does not require to abandon traditional assays, but supplements them and views results in a more integrated fashion. The proposed methods and combined interpretation of parameters is very much in analogy to conventional PK (*Table 2*) which will make it easy for the new paradigm to be accepted in running CNS drug discovery programmes. However, it does ban the isolated, sole use of the popular but misleading total brain-to-total plasma ratio from decision making. This is also an important change which may make obsolete many *in silico* models, which predict this parameter (often expressed as LogBB), and may ultimately shift computation chemistry efforts towards more structure-based modifications, which attempt to improve those particular properties of a compound which show a clear link to the desired effects [66].

The holistic concept of CNS penetration considers rate of permeation across the BBB, extent of brain penetration, and the intra-brain distribution of a CNS drug as distinct but interrelated properties of a CNS drug, which have to be determined by different *in vitro* and *in vivo* methods. Therefore, the integration of data from various assays and studies is becoming a central part of the evolving paradigm in order to develop quantitative relationships between dose, exposure, and efficacy.

The inclusion of data on receptor occupancy and biomarkers will enable better study design and dose predictions, ultimately mitigating the risks associated with poor clinical efficacy and drug safety which are currently the main reasons why new drug candidates fail in clinical trials [67]. Currently, there are also attempts to merge the

Table 2. *Conceptual Analogy of 'Classic' PK and CNS PK of the New Concept of CNS Penetration and Distribution*. The analogy relates to the principal considerations of rate, extent, and distribution, and is based on the concept of unbound concentrations as being the driver for pharmacological drug actions.

	'Classic' PK	CNS PK
1) Rate	'Elimination' clearance	'Uptake' clearance
	$CL = \frac{\text{Dose}}{AUC_{\text{plasma}}}$	$K_{\text{in}} = \frac{A_{\text{brain}}}{AUC_{\text{plasma}}}$
2) Extent	Extent of oral bioavailability	Extent of brain uptake
	$F = \frac{AUC_{\text{po}}}{AUC_{\text{iv}}}$	$K_{\text{p}} = \frac{AUC_{\text{brain}}}{AUC_{\text{plasma}}}$
3) Distribution	Concept of total *vs.* unbound concentrations	
	V_{ss}, $f_{\text{u,plasma}}$, $c_{\text{u,plasma}}$	$V_{\text{u,brain}}$, $f_{\text{u,brain}}$, $c_{\text{u,brain}}$
4) Half-life	Half-life of elimination	Half-life to equilibrium
	$T_{1/2,\text{el}} = \frac{\ln 2 \times V_{\text{ss}}}{CL}$	$T_{1/2,\text{equ}} = \frac{\ln 2 \times V_{\text{u,brain}}}{\text{PS} \times f_{\text{u,brain}}}$

presented concept of CNS penetration with the field of preclinical drug development and clinical PET [68–70] to make extrapolations from animal to human more realistic [71].

By integrating these data and technologies into PK/PD modeling and simulations, it will be possible to generate a working understanding of the PK and PD of potential drug candidates in the human CNS [72]. Such an understanding will be paramount to define PK parameters which are favorable for the desired indication, to guide the preclinical development of the compound (*e.g.*, dose selection for toxicity testing in higher species, as well as first-in-man and therapeutic doses) and ultimately to increase the chances for a successful phase-II study in human patients.

The growing emphasis on translational medicine to bridge the gap between CNS research and drug development, and the concerted application of new technologies, in particular brain imaging, may not only accelerate the discovery and improve the success rate of new CNS drugs, but may also give a fresh boost to the motivation for CNS drug development which is still hampered by too many uncertainties.

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