BLOOD–BRAIN BARRIER IN DRUG DISCOVERY
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Optimizing Brain Exposure of CNS Drugs and Minimizing Brain Side Effects for Peripheral Drugs

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Innovation of treatments for human disease is an engaging endeavor that inspires
the intellects, skills, risks and ethics of medical caregivers, researchers, business,
and government to prolong and improve the quality of human life. In particular,
central nervous system (CNS) disorders are a major area of undertreated medical
need. Many researchers aspire to discover successful new treatments for these
devastating CNS diseases. Pharmaceuticals are a major contributor to CNS and
peripheral disease treatment. They emerge from a long and complex process of drug
discovery and development. The science and process of drug discovery and
development is an ever-evolving and challenging venture that is attaining steady
improvements in understanding the underlying mechanisms affecting pharmaceuticals
and innovating new approaches that lead to enhanced quality of patient care.

Past improvements in knowledge regarding the blood–brain barrier (BBB) have
contributed to the development of CNS drugs. In recent years, the depth and breadth
of knowledge of the BBB and drug interactions in the brain have accelerated. They
are yielding innovative drugs that improve on current CNS disease treatments and,
excitingly, treat previously intractable CNS diseases. In the broader pharmaceutical
field, BBB knowledge is reducing unwanted CNS side effects of drugs that treat
peripheral diseases. Improvements in both CNS and peripheral drugs, based on this
knowledge, are highly beneficial for patients.

The chapters in this book were written by researchers that are actively involved in
increasing the understanding of the BBB and drug interactions in the brain and
applying this to more quickly discover and develop better drugs. All of us who work
toward better human disease treatment can really appreciate the contributions of
these authors in sharing their knowledge and insights. Furthermore, they offer highly
valuable guidance for researchers for successful drug discovery and development.
Early chapters provide an overview of the unique pharmacokinetics of brain exposure. The fundamentals of drug binding to CNS tissue and plasma are described, with emphasis on the primary role of free drug concentration in determining in vivo efficacy. Free drug concepts have recently been widely accepted in the field and are crucial for researchers to understand and apply in practice.

The extraordinary mechanisms affecting drug permeation through the BBB are discussed in four chapters (Chapters 5–8). These describe the BBB tight junctions that limit access of some drugs to the brain, the constraints on BBB transcellular passive diffusion, the efflux transport that reduces brain exposure, and the uptake transporters that offer intriguing opportunities for enhancing brain penetration.

Furthermore, ground-breaking research is discussed, which uses BBB receptors to enable uptake of biological molecule constructs. These biologics would otherwise not be able to cross the BBB and have advantages for treatment of certain CNS diseases not treatable by small molecule drugs.

Predictions and measured data are important in discovering new drugs. These indicate the behavior of drug candidates at various barriers that limit CNS exposure. A series of chapters discuss state-of-the-art approaches for in silico prediction, in vitro data measurement of specific barriers, and in vivo methods for measuring the free drug concentration and imaging compound locations in the CNS tissues. Recent advancements in physiologically based pharmacokinetic and pharmacokinetic/pharmacodynamic (PK/PD) tools are effective new approaches to predict PK and efficacy in preclinical and clinical space.

Medicinal chemists and project leaders will benefit from the CNS drug design strategies that use current insights on the BBB and CNS barriers to achieve enhanced CNS drug exposure. Case studies examine how integration of the data and design strategies advanced successful new drugs to the market. Nanotechnology and nasal CNS drug delivery techniques are also discussed as other alternative approaches to enhance brain access.

The editors greatly thank the individual chapter authors for kindly sharing their knowledge, strategies, and experience. It was a great pleasure to collaborate with them on development of this book. We admire the outstanding and heart-felt work they contributed so that all drug researchers could benefit and achieve increased success in development of the drugs of the future. We wish for you success in achieving your goals for new disease treatments for the benefit of the patients.

June, 2014

Li Di
Edward H. Kerns
Brain exposure can affect drug development success for all diseases. For neuroscience therapeutics, a leading area of pharmaceutical research, development, and product portfolios in pharmaceutical companies and research institutions, insufficient brain exposure leaves many central nervous system (CNS) diseases untreated or without optimum drugs, despite the vast resources applied to the problem. Researchers working to treat CNS diseases were stymied by the blood–brain barrier (BBB), but, in recent years, experience led to improved drug exposure at brain targets. Conversely, researchers working on peripheral diseases encountered CNS side effects owing to brain exposure at unintended CNS targets, but they are increasingly successful at reducing brain exposure. These advances on brain exposure came as pharmaceutical science uncovered the intricacies of drug molecule interactions at the BBB and within brain tissue. Newly discovered interactions provide an opportunity to overcome previous project disappointments, understand previously unexplained observations, and enable new tools for successful drug development.

This book comprises the contributions of experts regarding the complex interactions encountered by drug molecules that affect brain exposure and their successful solution in drug discovery, development, and clinical studies, including the following:

- Complexities of brain physiology and anatomy
- Designing CNS drug candidates to reduce transporter BBB efflux or increase BBB uptake
• Designing peripheral drugs to increase BBB efflux
• Focus on brain free drug concentration for efficacy
• Constructing novel biologics to deliver therapeutic molecules to the CNS
• Building pharmacokinetic–pharmacodynamic (PK/PD) and physiologically-based pharmacokinetic (PBPK) models for CNS therapy
• Projecting in vivo CNS exposure
• Nanotechnology and nasal dosing for CNS delivery
• In silico, in vitro, and in vivo methods of predicting and measuring CNS barriers, exposure, and free drug concentration
• Imaging for CNS therapy
• Case studies of successful recent drug product advances in brain delivery enhancement or reduction

RESTRICTED BRAIN EXPOSURE REDUCES CNS DRUG EFFICACY

A primary cause of the disappointment in developing CNS disease treatments is that the brain is a difficult organ for drug therapy. In past years, a high percentage of promising CNS drug candidates have failed. A major cause of this failure is the restricted access of many drug candidates circulating in the blood to penetrate into the brain owing to the BBB. Chapters 2 and 4 discuss the physiology of the BBB and differences among species and disease states. For most organs, drug molecules freely move between the blood and tissue via open junctions between capillary cells, but the BBB presents greater restrictions via tight junctions that reduce drug molecule access to brain tissue. Thus, molecules that do not have facile passive transcellular diffusion (e.g., acids, biologics) are restricted. In addition, efflux transporters (e.g., Pgp, BCRP), actively pump the molecules of some compounds out of the brain. These barriers to BBB permeation and the general characteristics of compounds that are efflux substrates are detailed in Chapters 5 and 6. These barriers effectively reduce the concentration, and therefore the efficacy, of some potentially therapeutic drug molecules to brain cells.

Another component of brain exposure restriction is binding of drug molecules to blood and brain tissue components. This restricts the free drug concentration that is available to bind to the therapeutic target protein molecules. In past years, the concentration of drug molecules that are available to bind to the brain target was assumed to be the total concentration measured in the brain tissue. However, in recent years, there has been a major shift in acceptance and application of the Free Drug Hypothesis, which states that only the unbound drug molecules are available to bind to the target to produce efficacy. Binding varies with the structure and physicochemical properties of each compound. This recognition has solved many previously unexplained failures in translation from in vitro activity to in vivo efficacy. The primary role of free drug concentration in determining in vivo efficacy is now being widely applied to CNS research and is reviewed in Chapter 3.
PERMITTED CNS ACCESS INCREASES SIDE EFFECTS OF PERIPHERAL DRUGS

Many drug candidates for peripheral therapeutic targets have minimal restrictions in penetrating the BBB and affecting brain targets. For example, they may have high passive diffusion through the BBB endothelial cells and not be efflux substrates. These drugs penetrate into the brain and may interact with CNS targets to cause difficult side effects for patients. Such effects lead to research project cancelation, regulatory rejection, drug product use restrictions, reduction of patient administration compliance, and long-term toxicities. For these reasons, drug researchers and developers need to investigate whether a new drug candidate causes unfavorable CNS effects \textit{in vivo}. Chapters 20 and 21 explain this issue for peripheral drugs and how it may be overcome.

A NEW GENERATION OF CNS EXPOSURE TOOLS

As the interactions affecting brain exposure are elucidated, in silico, \textit{in vitro}, and \textit{in vivo} methods for these interactions are developed. In addition, these interactions are included in methods for \textit{in vivo} projection. Such methods allow drug researchers to screen for potential problems, measure specific interactions (e.g., Pgp efflux), and quantify how they affect drug tissue concentrations \textit{in vivo}. These tools provide reliable information for lead selection and optimization to benefit drug research projects throughout their progress. Chapter 9 discusses the development and state of the art of in silico BBB predictions. BBB permeability is often predicted using \textit{in vitro} artificial and cell membrane assays (Chapters 10 and 11). Another component of brain exposure assessment is \textit{in vitro} assays for brain binding, as discussed in Chapter 12. This information is typically used in combination with \textit{in vivo} brain exposure studies (Chapter 13) to determine the free drug concentration in brain tissue. Direct measurement of free drug concentration using microdialysis is reviewed in Chapter 16. Another important advance in the field of brain exposure is the replacement of the Log BB and B/P parameters by the more valuable $K_{puu}$, the free drug distribution coefficient between brain and plasma, as discussed in Chapters 2, 3, 4, and 18. There is an increasing sophistication in PBPK modeling for the BBB (Chapter 14) and PK/PD model building (Chapter 15) for CNS drug candidates, which improve interpretations of biological efficacy, as well as projections for higher animals and human clinical studies. Recent advances in imaging techniques for CNS discovery research are discussed in Chapter 17.

Drug design advancements for brain exposure enhancement have taken advantage of the growing knowledge of drug interactions at the BBB. Small molecule design to optimize exposure (Chapter 18) and case study examples (Chapter 19) report successful strategies in CNS drug discovery. Concepts for the enhancement of brain exposure by designing drugs as substrates for BBB uptake transporters are advancing and are reviewed in Chapter 7. Biological drugs (antibodies, proteins) of higher-molecular weight generally do not pass the BBB. However, recent success in
delivering biologics was achieved by making constructs that contain the biological drug and a group that binds to a BBB receptor that promotes transport across the BBB. Chapter 8 explains the exciting progress in this promising field.

Researchers working on peripheral drugs will benefit from insights into the design suggestions for minimizing brain exposure in Chapter 20 and the successful case studies on nonsedative antihistamines in Chapter 21 that have efficacy at peripheral targets but are restricted by the BBB from producing effects at the same or closely related receptors in the brain.

For more compounds that are very recalcitrant to CNS exposure, researchers are developing new concepts for CNS delivery. Technologies using nanotechnology have the possibility to enhance delivery across the BBB (Chapter 24). Concepts and evidence for CNS drug delivery using the nasal route is also reviewed in Chapter 25.

CASE STUDIES OF DRUG DEVELOPMENT SUCCESSES

We often find guidance from successful case study examples. Thus, colleagues have kindly provided successful CNS exposure case studies for fycompa, an AMPA receptor antagonist (Chapter 22) and for vortioxetine, a serotonin modulator and simulator (Chapter 23). The inspiration and enlightenment of these experienced examples provide encouragement and direction for our research projects.

CONCLUSION

This book was prepared with the purpose of benefiting drug researchers in the following areas:

• Fundamental knowledge about the BBB and drug binding
• Implications of these restrictions for brain pharmacokinetics (PK) and pharmacodynamics (PD)
• Drug structure design elements that overcome BBB barriers for CNS drugs
• Drug design principles that enhance these brain exposure barriers for peripheral drugs
• Methods for assessing compound restrictions at the BBB
• Case studies from CNS drug discovery

Valuable perspectives on the future of BBB research and CNS drug development are provided by distinguished BBB researcher Dr. Joan Abbott in Chapter 26. The sharing of valuable insights about brain exposure by the chapter authors in this volume is intended to advance the fundamental knowledge about the BBB and brain exposure throughout all areas of drug development. Practical real-world information and examples are emphasized for the purpose of developing therapies for underserved diseases and of developing improved drugs.
PART 1

PHARMACOKINETICS OF BRAIN EXPOSURE
INTRODUCTION

Drugs are likely to exert their pharmacological effects only if they have a proper chance to engage with their molecular targets at the site of action in the body. This is true for all drug targets, including those that reside within the central nervous system (CNS). A prerequisite for drug target engagement, that is, binding of a drug to its molecular target protein, is the exposure of the target at concentrations in excess of the pharmacological potency of the compound for a sufficient period of time. Adequate CNS exposure of a drug at the site of its pharmacological target is, therefore, paramount for a drug to be able to elicit CNS activity [1]. The concept of active target site exposure has now become a central tenet for the pharmacokinetic (PK) optimization in drug discovery projects focusing on optimizing unbound rather than total drug concentrations [2–4]. Without sufficient exposure of the drug target, the likelihood is very low that a drug will be able to express target-mediated pharmacology and, ultimately, the desired effects on the course of the disease.

Both target exposure and target engagement have been identified as two out of three “pillars of success” of drug discovery programs during a retrospective analysis of about 40 clinical Phase II programs running at Pfizer between 2005 and 2009 [5]. The third pillar of success is the demonstration of the relevance of the expression of the pharmacology for the intended therapeutic intervention (Fig. 2.1). This holds particularly true for CNS drug discovery and development, which are suffering from
Pharmacokinetics of CNS Penetration

Dauntingly low clinical trial success rate and the lack of a clear understanding of underlying reasons for the failures [6, 7]. Very often it remains unclear whether a failure was due to the pharmacological target hypothesis being wrong or the target exposure being insufficient to exert the desired pharmacological effects. Some authors [8–10] suggest that not only will the development of new CNS medications benefit from a better understanding of target exposure and engagement, but applying these principals may also help redefine dose and dosing regimens of already existing “old” medications, for example, many classical antipsychotics which have never undergone rational dose-finding studies. The authors suggest applying positron emission tomography (PET) occupancy studies in patients as a basis to readjust the currently recommended doses in order to make their use more efficient and safer compared to the traditional doses and, hopefully, also improve their often poor response rates within the patient population. PET studies are ideal as they allow addressing several key questions directly in patients [11]: Does the drug reach the target site? Does the drug interact with the desired target? Is the concentration of the drug at the target site sufficient to elicit an effect? What is the temporal nature of such an interaction? What is the relationship between the target site concentration and the administered dose and plasma concentration?

Although PET studies, which can also be carried out in animals, are able to answer many of these key questions directly, PET technology is not applicable in most CNS drug discovery projects due to the absence of suitable PET tracers for novel targets. Therefore, and due to the inherent difficulty to directly measure the active site concentrations at the CNS target, alternative methodologies and surrogate approaches that are compatible with modern-day drug discovery and development have been developed [12–14].

**FIGURE 2.1** Schematic presentation of the key processes and the link between pharmacokinetics and pharmacodynamics of CNS drugs, which ultimately translate a drug dose into a drug response.
This chapter summarises the key processes that control the drug concentrations at the site of the CNS target, in particular the pharmacokinetics of CNS penetration and distribution.

CNS PENETRATION

Unlike most other organs in the mammalian body, the brain is separated from the blood circulation by the existence of physiological barriers. In order to get access to the brain tissue, a drug needs to be able to cross these barriers.

Barriers within the Brain

There are two important barriers between the CNS and the blood circulation: the blood–brain barrier (BBB) and the blood–cerebrospinal fluid (CSF) barrier (BCSFB), which are introduced here only very briefly. Although the BBB is highly complex and formed by multiple cell types (Fig. 2.3, left), the gatekeeper function is essentially a result of the endothelial cells lining the brain capillaries as they are very tightly sealed together by an intricate network of tight junctions [15]. Since these effectively prevent paracellular transport between the cells, movement of any material can only occur through the endothelial cells, thereby allowing the brain to control all traffic including that of ions, solutes, nutrients, hormones, larger molecules, or even cells (e.g., immune cells).

Besides the BBB, which separates the blood circulation from the brain’s parenchyma, there is also a barrier between the blood circulation and the CSF. This barrier, the BCSFB, 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 choroid plexus of the ventricles of the brain, which are supplied by leaky capillaries [16].

From a PK point of view, the following anatomical and physiological parameters of the BBB are of relevance: brain capillary length and volume in humans are about 650 km and 1 ml, respectively, with the area of the luminal capillary surface approximately 12 m², which is equivalent to 100–240 cm²/g brain depending on the brain region [15, 17]. The thickness of the BBB is between 200 and 500 nm. The luminal diameter of brain capillaries is about 4 μm in rats and 7 μm in humans, with a mean distance between two capillaries of about 40 μm and the transit time of blood of about 5 s. The capillary volume of 11 μl/g brain is very low, that is, less than 1% of the brain. In contrast, the compartment of the brain interstitial fluid (ISF) amounts to about 20% of the brain parenchyma [18, 19]. In rats, ISF flows with a bulk flow rate of approximately 0.15–0.29 μl/min/g toward the CSF [20]. The volume of CSF is approximately 250 μl in rats and 160 ml in humans, with the rate of CSF secretion being approximately 2.1 and 350 μl/min, respectively [21, 22].

Because the ratio of the surface areas between the BBB and the BCSFB is in the range of 5000:1, and the density of the capillaries within the brain parenchyma is so high that virtually every neuron can be supplied by its own capillary, the BBB
Pharmacokinetics of CNS Penetration is generally considered to play the major role in the transfer of CNS drugs to the brain [17, 19, 23].

Understanding the BBB is therefore an essential element for optimizing CNS penetration in drug discovery. The BBB impacts both the rate and the extent of CNS penetration (Fig. 2.2), which is discussed in more detail in the following sections.

**Rate of CNS Penetration**

The rate of CNS penetration relates to the speed at which a compound enters the CNS, independent of how much drug will enter the brain or to the degree of CNS penetration. The rate of CNS penetration is controlled by two factors: the cerebral blood flow (CBF), which controls the amount of drug delivered to the brain, and the permeability of the compound across the BBB. According to the classical principles of PK, either of these two factors can become the rate-limiting step in the process of tissue penetration [24].

**Cerebral Blood Flow (CBF, F)** In rats, blood flow through brain capillaries is about 0.5–2 ml/min/g brain, which varies between brain regions, neuronal activity, and CNS diseases [18, 25]. In humans, CBF is slower than in rats, with values of
CNS Penetration

0.15 and 0.6 ml/min/g brain for white and gray matter, respectively [26]. The CBF delivers the maximum amount of drug the brain is exposed to and, thus, constitutes the upper limit of the rate of brain penetration in vivo. For drugs whose rate of CNS penetration is perfusion-limited, changes in CBF will thus affect their CNS penetration, for example, under the influence of anesthetics, which often decrease CBF [27].

Permeability ($P$) The permeability relates to the speed of crossing the BBB of a drug and depends on the membrane properties of the BBB and the physicochemical properties of the crossing compound. There are several mechanisms by which a compound can pass through the BBB (Fig. 2.3): passive transcellular diffusion, which may be limited by efflux pumps, facilitated by carrier-mediated uptake, and adsorptive or receptor-mediated transcytosis, which is more relevant for large molecules [28]. Paracellular diffusion, which is an important mechanism for drug penetration of peripheral tissues, is virtually nonexistent in the CNS, due to the complex network of tight junctions between the brain endothelial cells.

The BBB permeability can be examined in vivo and in vitro. Since permeability ($P$) and surface area ($S$) cannot be easily distinguished in vivo, the permeability surface (PS) area product is most often given readout [29, 30]. The PS product is equivalent to the net influx clearance ($CL_{in}$) and both are measured in units of flow: μl/min/g brain [31]. PS products may span a range of about 10,000-fold [32, 33] and cannot easily be compared across studies or with the CBF, as such, since the results

![Schematic illustration of the blood–brain barrier and typical cell types constituting it (left) and principal pathways available to drugs, in order to gain access to the brain parenchyma: passive diffusion, which may be restricted by active efflux, carrier-mediated uptake via transporters expressed on the brain endothelium, and endocytosis, which may be mediated by specific receptors on the luminal endothelial cell surface or less specifically triggered by adsorption to the endothelial cell membrane.](image)
depend on the exact conditions of the \textit{in situ} brain perfusion method used, in particular, the rate and the duration of the perfusion and the composition of the perfusion fluid (e.g., the amount of plasma protein). The \textit{in situ} brain perfusion technique requires high technical skills, is very labor-intensive, and, hence, not suitable for routine drug discovery screening. An alternative \textit{in vivo} method to determine the rate of CNS penetration is to determine the amount of compound in the brain after oral or systemic administration as $K_{in}$ value, which relates the amount of compound in the brain (homogenate) at time $t$ ($A_{brain}(t)$) to the plasma exposure up to this time point ($AUC_{plasma}(0-t)$). To be more exact, $A_{brain}(t)$ should be corrected for the amount of drug remaining in the cerebral vasculature at the end of the experiment [25, 29]. The experimental setup to generate $K_{in}$ data follows that of regular \textit{in vivo} PK studies, making $K_{in}$ a more popular \textit{in vivo} estimate than the PS product.

$$K_{in} = \frac{A_{brain}(t)}{AUC_{plasma}(0-t)}$$

(2.1)

The Renkin–Crone equation relates $K_{in}$ and PS based on the basic principles of capillary flow, with $F$ being the flow in the system in question, that is, either CBF or the rate of perfusion in the experiment [34]. $K_{in}$ may be correct for the unbound fraction in plasma or the perfusate (see Fig. 2.2) [25]. The classical equation without protein-binding correction is

$$K_{in} = F^* (1 - e^{PS/F})$$

(2.2)

The capillary flow model underlying this equation ensures that the rate of CNS penetration cannot be higher than the CBF, even if the intrinsic permeability is very high. Hence, the upper limit of $K_{in}$ is the CBF (for high-permeability compounds) and the lower limit is PS (for low-permeability compounds). It has been estimated that for a drug to be permeability-limited the PS product has to be 10% of the CBF or less, resulting in a tissue extraction of less than 20% compared to blood. PS products in the range of, or greater than, the CBF make the tissue penetration of the drug perfusion-limited. The available data on PS products suggest that CNS drugs typically do not belong in the category of permeability-limited compounds [22]. This may well be a result of the availability of high-throughput \textit{in vitro} permeability models in drug discovery and the successful use of in silico tools to predict PS products based on the physicochemical properties of the drug. Very good results have been made with the following equation, which predicts the passive PS product expressed as log PS [35, 36]:

$$\log PS = 0.123*\log D - 0.00656*TPSA + 0.0588*Vbase - 1.76$$

(2.3)

where $\log D$ is the partition coefficient in octanol/water at pH 7.4, TPSA is the topological van der Waals polar surface area, and Vbase is the van der Waals surface area of the basic atoms.

Today, \textit{in vitro} assays have become the method of choice to assess permeability as they reflect both the passive diffusion and the transporter-mediated component (in particular, efflux). Typically, the rate of transport is measured across a tight
monolayer of cells, which resemble most of the critical barrier properties of the BBB [23, 28, 37]. The permeability is expressed as apparent permeability coefficient ($P_{app}$):

$$P_{app} = \frac{dCr}{dt} \frac{Vr}{A \times C0}$$  \hspace{1cm} (2.4)

where $dCr/dt$ is the slope of the cumulative concentration in the receiver compartment versus time, $Vr$ is the volume of the receiver compartment, $A$ is the surface area of the monolayer, and $C0$ is the initial concentration in the donor compartment. The assay is often run in both directions in order to assess the susceptibility of the test compound toward drug efflux using the efflux ratio (ER):

$$ER = \frac{P_{app} (B-A)}{P_{app} (A-B)}$$ \hspace{1cm} (2.5)

While there is still no one in vitro BBB model available that resembles all key aspects of the BBB, mDCK-mDR1 cells have become the most widely used cell line to determine the in vitro permeability in CNS drug discovery [28, 38–42]. Typically, $P_{app}$ values at approximately, or greater than, 100 nm/s are taken as evidence for high permeability, with ER values ideally around 1, or below 2–3.

While in the past there has been too much emphasis on optimizing permeability, that is, the rate of CNS penetration, it is now being accepted that, in particular for chronic treatment of CNS disorders, the extent is the more important parameter to be examined. Although low permeability may delay the time to equilibrium, it will not affect the level of the drug equilibrium between blood and brain.

**Extent of CNS Penetration**

While rate is an important parameter to describe CNS penetration, it does not determine the extent (i.e., degree) to which a compound will enter brain tissue (Fig. 2.2). This has sometimes been confused in the past, leading to overemphasis of in vitro permeability assays in drug discovery programs, whose actual purpose was to increase the extent of brain penetration. It was the seminal review by Hammarlund-Udenaes that made very clear the distinction between rate and extent of CNS penetration [18]. Another source of confusion was the misleading assessment of CNS penetration based on the ratio of total brain/plasma concentrations [13, 43].

**Total Brain/Plasma Ratio (Kp)** Traditionally, a typical in vivo study to assess brain penetration involved the measurement of brain and plasma sample concentrations at 3–4 time points after ip, sc, iv, or oral administration to rodents. At selected time points, plasma samples were drawn and brain tissue was removed and subsequently homogenized for quantification by liquid chromatography–mass spectrometry (LCMS) analysis [14, 44, 45]. The method was amenable to cassette dosing, thereby
allowing significant reduction of the number of animals to be used [46]. The extent of brain penetration was expressed as follows:

\[ \text{Kp} = \frac{\text{AUC}_{\text{tot,brain}}}{\text{AUC}_{\text{tot,plasma}}} \quad (2.6) \]

Whenever a project team went on to improve brain penetration by increasing Kp, however, they ran into the problem that compounds with higher Kps, despite an often improved potency, did not result in better efficacy in animal models [43, 47]. On examining the brain’s unbound concentrations it was found that increasing total concentrations does not necessarily lead to higher unbound concentrations, which most closely relate to the active site concentrations [1, 14, 48–51]. Since the brain has a relatively high lipid content [52], increasing Kp was often a simple consequence of increasing the lipophilicity of the drug, thereby steering project teams into a drug lipidization trap [10, 13, 43, 49]. Computational methods aimed at predicting Kp are therefore of highly questionable value as guiding tools for CNS drug discovery.

The key caveat of Kp is its composite nature of three independent factors: nonspecific drug binding to brain tissue, nonspecific drug binding to plasma proteins, and specific drug transport across the BBB [18]. For CNS drugs which often are very lipophilic, Kp is dominated by nonspecific drug binding, masking the transport properties of the drug.

**Unbound Brain/Plasma Ratio (Kp,uu)** Correction for nonspecific binding both to brain tissue and plasma proteins [43, 53, 54] is therefore an essential element which led to the concept of Kp,uu [18]. Kp,uu is not confounded by nonspecific binding of the drug and is thus a parameter that directly reflects the transport equilibrium across the BBB. Kp,uu is calculated from Kp, the fraction unbound in plasma (fu,plasma) and the fraction unbound in brain tissue (fu,brain):

\[ \text{Kp,uu} = \text{Kp} \times \frac{\text{fu,brain}}{\text{fu,plasma}} \quad (2.7) \]

Hence, Kp,uu can therefore also be expressed as

\[ \text{Kp,uu} = \frac{\text{AUC}_{\text{tot,brain}}}{\text{AUC}_{\text{tot,plasma}}} \times \frac{\text{fu,brain}}{\text{fu,plasma}} \quad (2.8) \]

Both fu,plasma and fu,brain can be measured readily *in vitro* by equilibrium dialysis [53, 55]. See also Chapter 12 for more details on the method. Since the ratio of fu,plasma and fu,tissue can be regarded as an *in vitro* estimate of Kp [24], the following equation can be used as an alternative:

\[ \text{Kp,uu} = \frac{\text{Kp(in vivo)}}{\text{Kp(in vitro)}} \quad (2.9) \]