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Novel CNS drug discovery and development approach: model-based integration to predict neuro-pharmacokinetics and pharmacodynamics

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ABSTRACT

Introduction: CNS drug development has been hampered by inadequate consideration of CNS pharmacokinetic (PK), pharmacodynamics (PD) and disease complexity (reductionist approach). Improvement is required via integrative model-based approaches.

Areas covered: The authors summarize factors that have played a role in the high attrition rate of CNS compounds. Recent advances in CNS research and drug discovery are presented, especially with regard to assessment of relevant neuro-PK parameters. Suggestions for further improvements are also discussed.

Expert opinion: Understanding time- and condition dependent interrelationships between neuro-PK and neuro-PD processes is key to predictions in different conditions. As a first screen, it is suggested to use *in silico/in vitro* derived molecular properties of candidate compounds and predict concentration-time profiles of compounds in multiple compartments of the human CNS, using time-course based physiology-based (PB) PK models. Then, for selected compounds, one can include *in vitro* drug-target binding kinetics to predict target occupancy (TO)-time profiles in humans. This will improve neuro-PD prediction. Furthermore, a pharmaco-omics approach is suggested, providing multilevel and paralleled data on systems processes from individuals in a systems-wide manner. Thus, clinical trials will be better informed, using fewer animals, while also, needing fewer individuals and samples per individual for proof of concept in humans.

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1. Introduction

The aim of drug discovery and development is to find compounds able to modify body processes by interaction with a target that is related to a disease. It typically consists of a number of phases. These include:

Target identification: aiming to find a molecular target that is involved in the disease (progression);

Target validation: investigating if the target is 'druggable';

Lead discovery: identification of small molecules that modulate the target, and transformation of the obtained information into a high content lead series;

Drug candidate selection: identification of a few promising compounds, using *in silico* and preclinical *in vitro* and *in vivo* testing to further understand the compound properties with regard to its pharmacokinetics (PK), pharmacodynamics (PD, the therapeutic effects and side effects), mechanisms of action, and the best dosage and route of administration;

Clinical studies (phase 1–4): investigation of the PK and safety in human, followed by the analysis of the therapeutic effects in healthy subjects and in patients and, if not 'first in class,' comparison with similar drugs to obtain a drug that is 'best in class.'

CNS drug discovery has been hampered by inadequate understanding or consideration of a number of factors. These include the complexity of brain anatomy and function; the neuro-PK with regard to blood–brain barrier (BBB) transport

and intra-brain distribution as well as the measures to study these processes; adequate biomarkers of CNS drug effects (neuro-PD), and the complex nature of CNS diseases [1]. Also, the reductionist view and thereby lack of understanding of the interaction and interdependencies of all these factors have contributed to the high attrition rates of CNS drugs. These factors are discussed below, while recent improvements in techniques and approaches to understand neuro-PK and PD will be discussed in the next section.

1.1. The CNS is an organ with complex anatomy, structure, and function

While in many tissues in the body a drug is relatively free to exchange between blood and the extracellular space in the tissues, this is not the case for CNS tissue. The CNS is separated from the blood by BBB and other barriers that have highly specialized properties [2], which has a huge impact on the relationship between plasma PK and neuro-PK. Moreover, the CNS is far from being a homogenous tissue. It has many different tissue structures and fluid cavities (ventricles), while target expression may vary substantially among the different locations [3] and, also, the same target may have distinct functions in different locations. Furthermore, there is fluid flow. The main fluid is the cerebrospinal fluid (CSF) that is produced by the choroid plexus cells of the blood–CSF barrier

Article highlights

- Factors that govern neuro-PK and CNS target exposure profiles plasma PK, BBB transport, brain extracellular-intracellular and brain-intracellular-subcellular distribution, as well as brainECF bulk flow and CSF turnover. Information on these factors should include unbound and bound drug concentrations, and integration of these factors is needed for understanding neuro-PK, as the underlying processes occur simultaneously and are interdependent.
- Experimental approaches should be such that a distinction can be made between drug specific and system specific properties. With such information the recently developed CNS PBPK model can be further informed, to allow for scaling between drugs and/or scaling between species to predict CNS target exposure profiles.
- CNS target exposure profiles can be used to predict CNS TO profiles, by incorporation of drug-target binding kinetics, endogenous ligand-target binding kinetic and target turnover.
- Typically, multiple biological pathways are involved in a single CNS disease, and single pathways are associated with multiple diseases. This indicates the need for a systems approach for identification and validation of targets for (potential) CNS drugs

This box summarizes key points contained in the article.

(BSCFB). Then there is the brain extracellular fluid (brain ECF) produced by the BBB, which flows into the direction of the ventricle (called brain ECF bulk flow). Brain ECF merges with the CSF, and the CSF is eliminated via the arachnoid villi into the blood stream. CSF production and elimination rates determine the CSF turnover rate [4]. Moreover, very recently, it became apparent that also the CNS contains lymphatic vessels, thus also lymph flow needs to be taken into account [5].

All these processes have an impact on (local) neuro-PK. Furthermore, CNS functionality is complicated by the networks of interacting neurotransmission pathways. Neurotransmitter receptors have been the typical target for many (classical) CNS drugs, while ‘single target’ pharmacological intervention and/or the impact of a disease on one target will often influence another one. Last but not least, neuro-PD is typically not directly quantifiable and needs to be assessed in an indirect way, using biomarkers that may be more or less adequate in reflecting the real CNS effect. This makes neuro-PD difficult to measure and to predict.

1.2. The inaccessibility of the human brain for sampling

Since the driving force of CNS drug action is the concentration-time profile of the drug at the target site, it is important for pharmaceutical companies to have effective and cost-efficient tools to measure and predict human brain target site exposure before proceeding to more expensive clinical trials. However, the possibility of direct measurement of human brain concentrations is highly limited. Since information on CNS drug distribution in human brain typically cannot be obtained directly, it must be inferred from *in silico*, *in vitro*, and *in vivo* preclinical experimental approaches.

1.3. Inadequate knowledge on neuro-PK

Neuro-PK results from transport across the BBB and BSCFB, intra-brain distribution, and target interaction. The free drug

hypothesis has been around for many years stating that the unbound drug concentration is available for membrane transport and target interaction. Wang and Welty [6] were the first to show the importance of using *unbound* drug concentrations in proper calculations of BBB transport and intra-brain distribution, and to introduce the term ‘volume of distribution in brain’ as the extent of drug distribution between brain unbound concentrations to total amount of the compound in the brain tissue. However, in CNS drug discovery, the measurement of unbound drug concentrations is typically not applied, not just because of the lack of quick and easy assay methods, but also because of a lack of understanding of the drivers in neuro-PK processes. Instead, total brain drug concentrations are used to determine BBB permeability (rate) of a drug as well as its *total* brain over *total* blood concentration ratio (K_p , brain, logBB; extent). The values of these parameters were considered as good indicators of brain penetration and thereby target exposure (the higher the better), often without proper understanding of the difference between rate and extent of BBB transport. This has appeared to be misleading in understanding rate versus extent of BBB transport and intra-brain distribution. With that, also the link to target exposure and interaction could not be made appropriately.

1.4. Incomplete information on and understanding of the complex CNS diseases

CNS diseases are typically multifactorial and complex. They often have genetic, physiological, neurochemical, degenerative, and inflammatory components, which display variation within the patient group [7,8]. For example, for Alzheimer’s disease, Parkinson’s disease, and other neurodegenerative disorders, multiple- and in many cases divergent-disease etiologies are involved. Moreover, CNS diseases often get diagnosed in a late stage of the disease, where the chance of curing the disease is virtually zero, where at best disease progression can be halted and often symptom suppression is the only possibility left. Altogether, this makes that defining a CNS disease, and its state and stage, is very difficult.

1.5. Problems in identification and validation of targets for (potential) CNS drugs

Target validation aims to determine whether a biological compound (e.g. receptor, enzyme, DNA/RNA) is directly involved in the disease of interest, and whether it can be modified by a drug or other interventions. For example, if a human disease is the result of a single gene mutation, and that mutation can be corrected therapeutically, the target is validated. An example of this is Huntington’s disease, for which the causative gene huntingtin was identified many years ago [9]. However, to date, no successful therapeutic approach has been demonstrated on the basis of that knowledge, indicating that a valid target does not per se guarantee the development of a drug [10]. Moreover, in many cases the etiology of CNS diseases is multifactorial, making target identification and validation difficult if not impossible when focusing on single targets. Indeed, satisfactory treatment of the disease via a single target might be an illusion.

1.6. Reductionist view and fragmented information

Then last, but certainly not least, there has been the tendency to oversimplify the relevant factors underlying disease and drug effects. These factors were evaluated in isolation, neglecting the complex interactions and interrelationships. Parameter values for a very high number of compounds have been assessed, often in high-throughput mode in different systems, without taking into account the context dependency of these values. Such 'fragmented' and 'stand-alone' data did not lead to increased understanding. Apparently, for the CNS, with its complex PK, PD and disease processes, there has not been an easy way out. To paraphrase Einstein: 'Everything should be made as simple as possible, but no simpler.'

2. Current advances in approaches and techniques

For a proper CNS effect, the drug should have the ability to access the CNS 'at the right place, at the right time, and at the right concentration.' Research advances in chemistry, drug metabolism, pharmacology, and toxicology have provided much insight into understanding the pitfalls of the drug discovery and development, and a number of advances in CNS drug discovery are currently embraced.

2.1. Identification and validation of targets for (potential) CNS drugs

The 'omics' and 'profiling' research area clearly indicates that neurodegenerative diseases and their associated neuropsychiatric comorbidities are multifactorial in origin. Genomics has provided many molecular targets that provide both opportunities and challenges in the discovery and development of novel medicines for the treatment of human CNS disorders [11–13]. In addition, more is learned about the biology of CNS diseases, and syndromes may be subdivided into more specific categories that are better understood in terms of pathophysiology and patho-etiology, also with regard to early signs of the CNS disease. This is likely to lead to development of more targeted treatments, being focused on the underlying causes of the disease as well as its prevention (life-style, diet, etc.).

In general, the research focus has shifted from single target toward target networks (systems pharmacology), as networks include the compensatory factors that impact the neuro-PD [7,10,11]. These networks are constructed on basis of integrated genomics, proteomics, and metabolomics data, narrowing down the large number of potential genomics-informed targets into a fewer number of relevant disease-causing targets [14,15]. Metabolomics has the advantage that the measured metabolites are accessible from body fluids such as plasma and urine, which provides opportunities for biomarker discovery for clinical application. CNS diseases are often associated with energy substrates, amino acids, neurotransmitters, neurochemicals and structural lipids. Typically, multiple pathways are involved in a single disease, and single pathways are associated with multiple diseases, underlining the need for a systems approach in CNS drug development [15].

Although not being an example in the CNS area, Cho et al. [16] suggested a useful approach that is different from conventional screening systems: the phenotypic screening in combination with multi-omics-based target identification and validation (MOTIV). The phenotypic screening provides information on the effect of small molecule compounds in the cell or at organism level, since small molecules not only affect a single target but the entire cellular mechanism within a cell or organism. The MOTIV approach provides a systematic approach to discover the target protein of a bioactive small molecule. Then, network analysis and validations of these candidates result in identifying the biologically relevant target protein and cellular mechanism. The combination of phenotypic screening and MOTIV may provide an effective approach to discover new bioactive small molecule and their target protein and mechanism of action.

Target identification and validation may be further informed by pharmacometabolomics, which is a new approach to identify changes in the metabolome upon drug action, revealing potentially perturbed biological pathways. A lipidomics analysis revealed a broad range of lipids changing upon treatment with the antipsychotics risperidone and olanzapine, but not aripiprazole [17]. These changes are associated with weight gain as a side effect. Furthermore, an analysis of multiple hormones and biogenic amines after treatment with the dopamine D2 receptor antagonist remoxipride revealed diverse response profiles. With integrated PK–PD analyses, multiple *in vivo* potencies for these responses were determined, associated with several biological pathways. These included the dopamine metabolism, the adrenaline metabolism, the serine–glycine–threonine metabolism, and the serotonin metabolism [18,19].

2.2. Understanding neuro-PK

Drug distribution into and within the brain is governed by many processes, including plasma PK, plasma protein binding, passive and active transport across the BBB and BCSFB [2], and once within the brain, brain ECF bulk flow, diffusion, passive and active extracellular–intracellular exchange, and CSF turnover. In the preclinical setting, there are several *in vitro*, *ex vivo*, and *in vivo* techniques that provide information on brain target site exposure. Such information can provide either direct or indirect information on bound and/or unbound concentrations, with or without temporal resolution, with or without spatial resolution, and with or without a clear distinction between rate and extent of the processes involved. It is of great importance to understand the mechanisms involved in uptake into and efflux from the brain, on one hand being governed by BBB functionality in terms of passive (paracellular and transcellular) diffusion, facilitated diffusion, active influx, active efflux, and absorptive or receptor-mediated endocytosis, and, on the other hand, by drug physicochemical properties and structure. As only the unbound drug is able to pass through the membranes, it is the unbound concentration difference between brain and plasma that drives BBB transport. Likewise, it is the unbound concentration difference between brain ECF and the cellular cytosol that drives extra-

intracellular transport. Also, for drug–target interaction the unbound concentration is the driving factor [20].

2.2.1. Neuro-PK measurement

Recently, a number of important improvements have been made in the understanding of drug distribution into and within the brain. This has been brought about especially by new approaches which have been developed, that allow a relatively rapid and easy assessment of unbound concentrations in brain tissue (brain homogenate dialysis equilibration and brain slice method) [21,22]. Thus, it is possible to determine the brain over plasma ratio of *unbound* concentrations (extent of brain equilibration; $K_{puu,brain}$), and extra-intracellular unbound concentration ratios ($K_{puu,cell}$). Furthermore, based on the pH partitioning theory, subcellular distribution into lysosomes can be simulated. With the new Combinatory Mapping Approach [23], the relationship between plasma PK and neuro-PK can be obtained in a more high-throughput mode, which makes it very useful for drug discovery (Figure 1). These approaches, however, still the focus on (assumed) steady-state conditions.

2.2.2. In silico prediction of BBB transport and brain distribution

In silico approaches to predict BBB permeability in earlier days were typically based on K_p , brain (logBB) relationships and found lipophilicity to be the main driver [24]. As discussed earlier, BBB permeability, which is the rate of BBB transport, was often confused with the extent of brain distribution, which confounded the search for good predictors of brain exposure. Other *in silico* approaches have focused on quantitative structure–property relationships (QSPR), using the physicochemical properties of a drug as predictors of the rate and extent of BBB transport and brain distribution [25–29].

With unbound concentrations taken into account, it was reported that the main drug property governing the extent of

brain distribution is the number of hydrogen bond acceptors. Thus, higher lipophilicity is a helpful property for faster rate of transport across the BBB, while a low number of hydrogen bond acceptors helps in a larger extent of brain distribution. Nevertheless, higher lipophilicity may increase nonspecific binding to brain tissue, while also highly lipophilic compounds have a tendency to be substrates for efflux transport, such as by P-glycoprotein, which leads to a net decrease of brain distribution. Thus, in contrast to earlier guidelines, drugs aimed for the CNS should not be too lipophilic, and have a low number of hydrogen bond acceptors for a more extensive brain distribution of the unbound drug.

It should be noted that in many academic investigations and CNS drug discovery programs, the focus is still on (assumed) steady-state conditions. The argument is that with aiming for a repeated/chronic dosing, a steady-state condition will be reached, so that only steady-state conditions are relevant to investigate. However, fluctuations in drug levels may have important consequences for target occupancy (TO), etc. This will be discussed in the target binding kinetics section below.

2.2.3. In silico prediction of neuro-PK

A very useful approach to predict neuro-PK is to make use of physiology-based pharmacokinetic (PBPK) modeling. As in PBPK modeling, the explicit separation is made between drug and systems properties, it is a great translational tool. To date, many more or less complex (semi-)PBPK models have been published for CNS drug distribution [30–34]. Gaohua et al. [33] published a human PBPK model with a few physiological CNS compartments. Other (semi-) PBPK models have been developed using extensive information obtained from preclinical species, with explicit inclusion of unbound drug concentrations [31,32]. To that end, the microdialysis technique [35] provided valuable data that were obtained in parallel in the multiple CNS compartments, to further pave the way

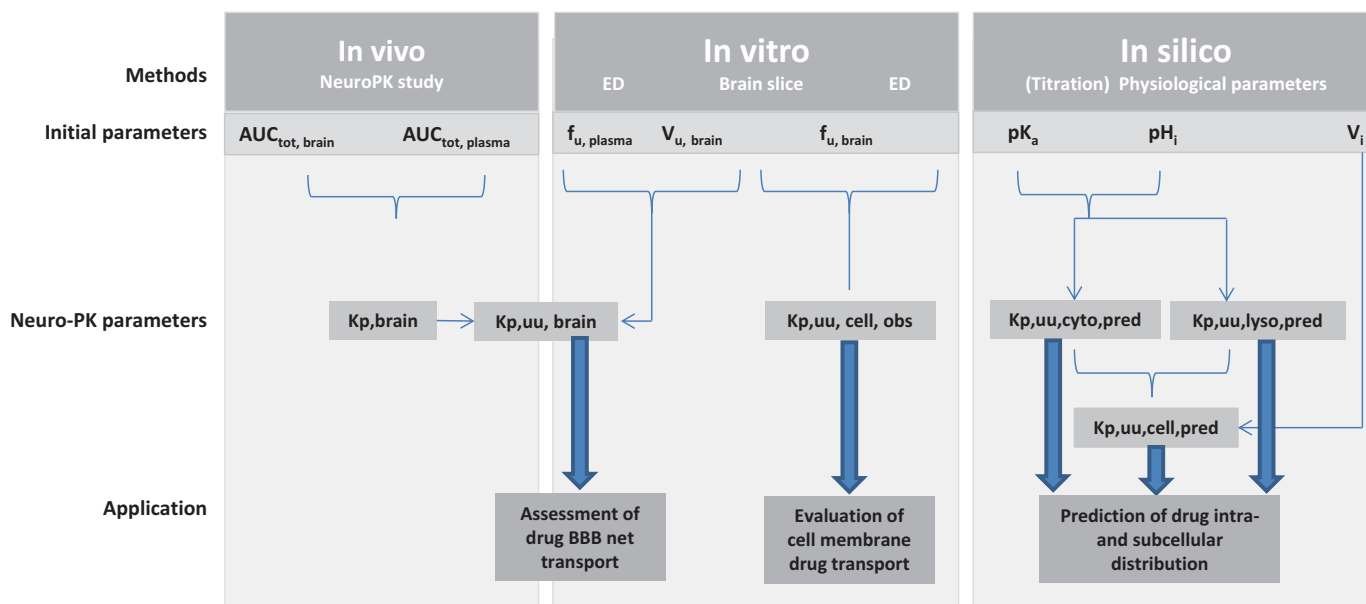


Figure 1. The combinatory mapping approach. (Redrawn from [23] with permission of Springer).

toward the generic semi-PBPK CNS drug distribution model. This model has been applied to nine compounds with highly different physicochemical properties and demonstrated excellent description of the rat data for all these compounds, and an adequate prediction of human CNS data that were available for acetaminophen and morphine [34]. Here, it should be noted that one microdialysis experiment in a single freely moving animal can provide lots of serial data points, obtained under the same experimental condition of the animal, and thereby revealing the interrelationships of neuro-PK processes [36,37], which in addition is important also for refinement, reduction, and replacement of animal experiments [38]. Advanced multilevel experiments and mathematical modeling are needed to reveal interrelationships between processes that occur in parallel in the body, and to reduce the use of animals. The ultimate goal is to develop a generic PBPK model by which prediction of CNS drug distribution can be made without the need for animal data. Such a model has recently been developed [39] (Figure 2) and shows good agreement with the observed *in vivo* data (Figure 3), and therefore seems suitable to be used as an '*in silico*' screening method for adequate CNS drug distribution of new chemical entities.

2.3. Target binding kinetics

Target binding kinetics and the resulting induction of signal transduction processes will ultimately lead to a biological

effect. Therefore, in current CNS discovery programs, TO is often measured, for which equilibrium is often assumed between free and target-bound drug concentrations [40]. However, this equilibrium is not always reached quickly or maintained continuously after it has been reached [41–43]. The rate of drug-target equilibration (the binding kinetics) is determined by the drug-target association rate constant (k_{on}) and the drug-target dissociation rate constant (k_{off}). Drug-target binding kinetics not only influences the time course of TO, but also affects the local drug concentration around the target for drugs with a high affinity or a high local target concentration [42,44]. This is important as target concentrations (e.g. receptor density) may differ substantially between different CNS regions (Figure 4). If target concentrations or drug target affinities are high enough, the influence of target binding on drug concentrations can also be observed from plasma concentrations, as illustrated in Figure 5. Recently, there have been efforts from both academic and industrial research communities to advance the *in silico* and *in vitro* assessment of binding kinetics, and the translation of this knowledge into *in vivo* settings. One of these efforts is the Kinetics for Drug Discovery Consortium, a 5-year public-private partnership which aimed to define the role of binding kinetics in drug discovery [46].

For understanding the influence of target binding kinetics on *in vivo* drug action, it is therefore important to have adequate information on the unbound concentration of the

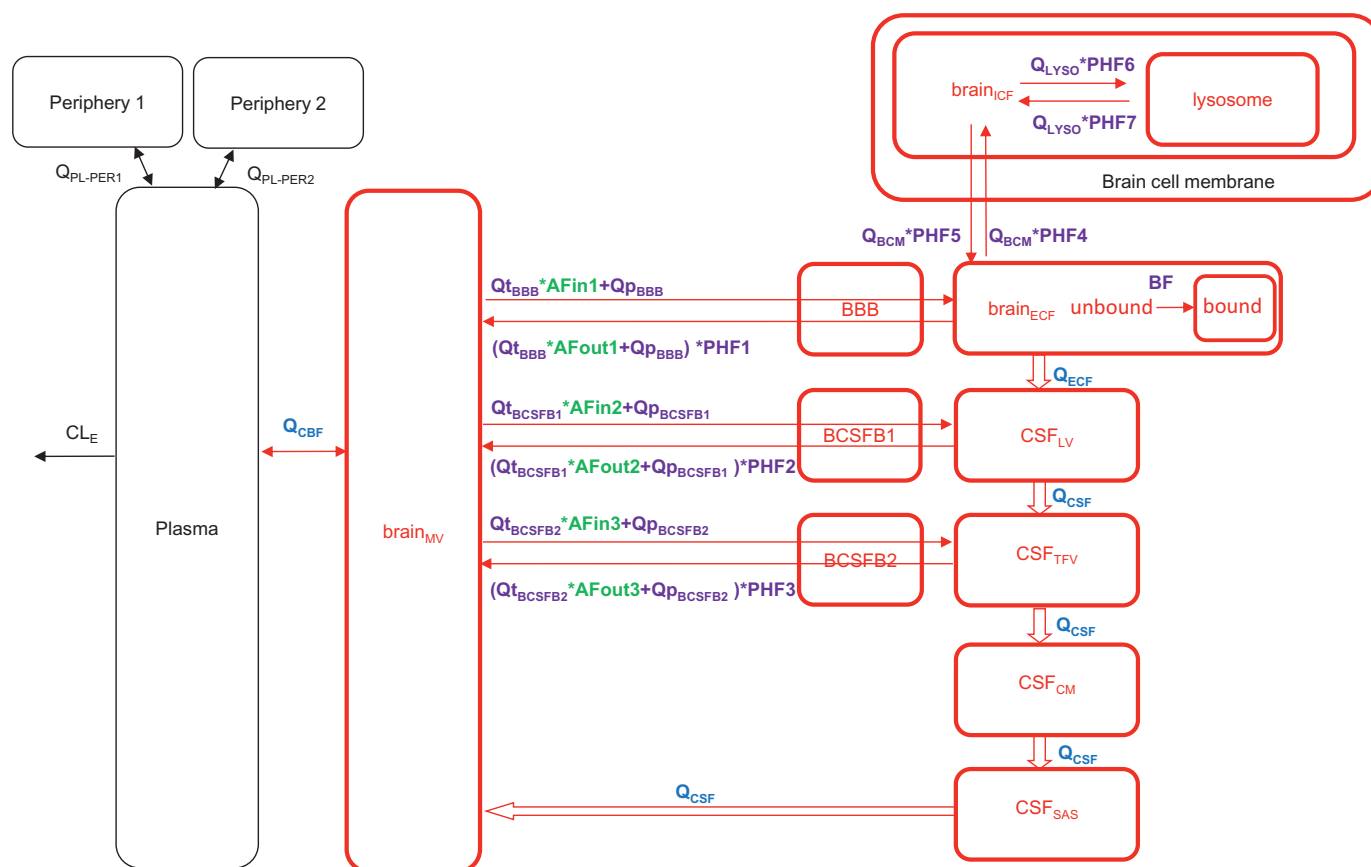


Figure 2. The structure of the generic CNS PBPK model Structure: Black: Plasma PK model, Red: CNS PBPK model. Parameters: Black: estimated plasma PK parameters; Blue: system-specific parameters; Green: drug-specific parameters; Purple: combination of system-specific and drug-specific parameters. (Redrawn from [39] with permission of Wiley & Sons).

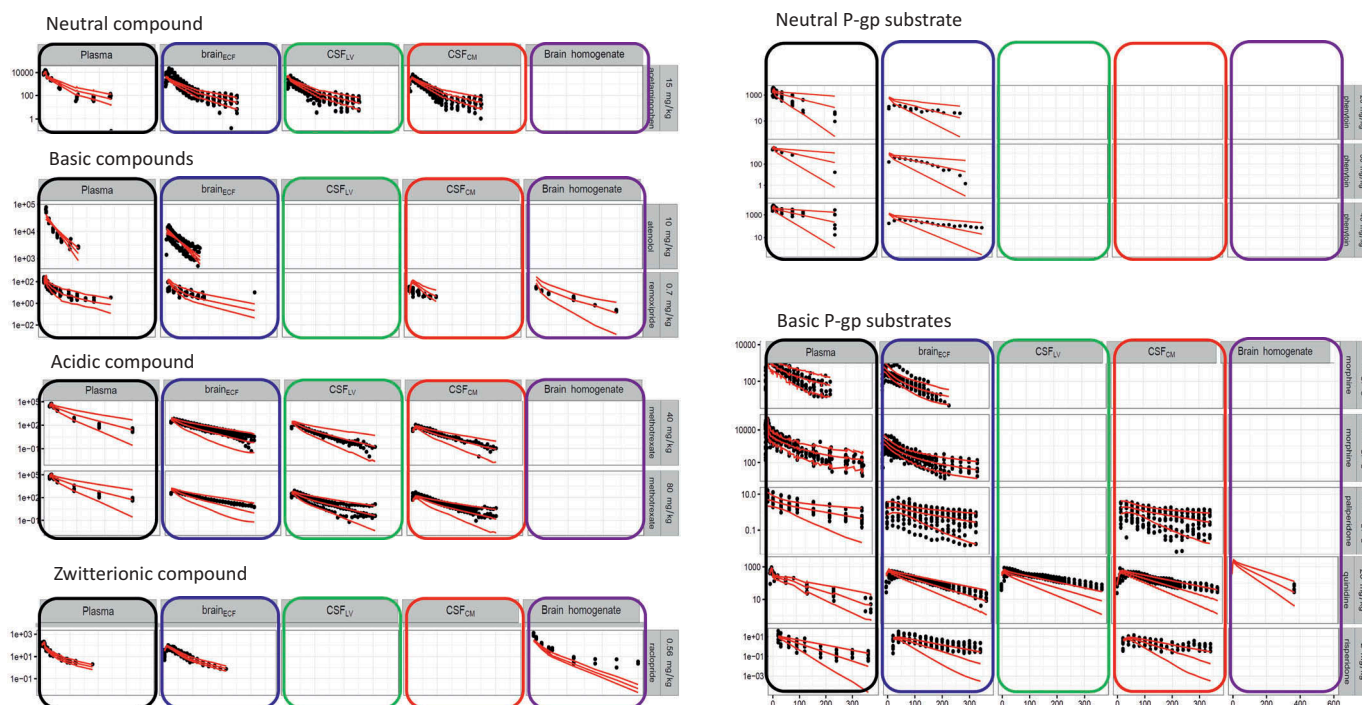


Figure 3. BPPK model prediction (red lines for median and 95% percentiles) and observed data (black dots) obtained in the five color-filled compartments.

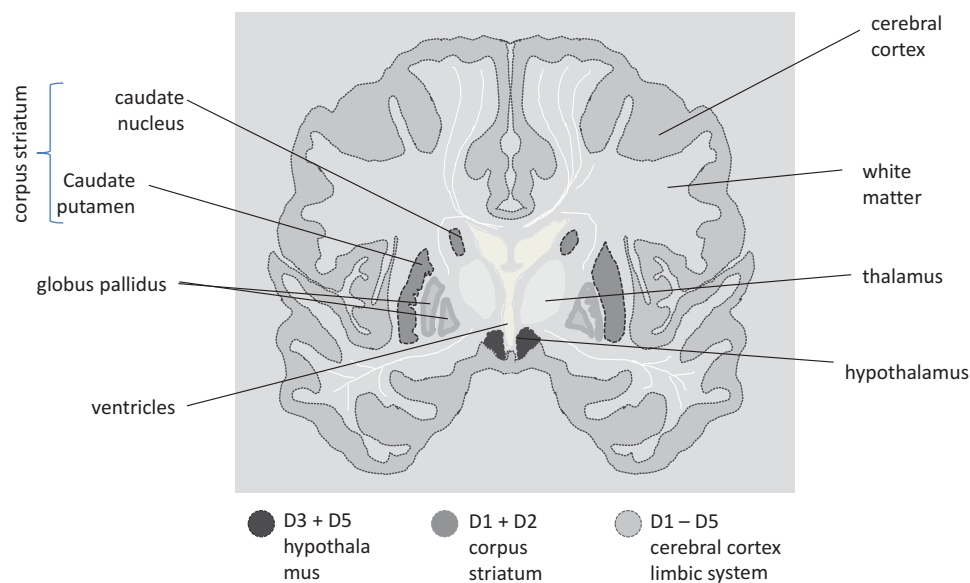


Figure 4. Cartoon of transversal cross section of human brain with the dopaminergic receptor areas indicated. Receptor density is region specific and receptor subtype specific.

compound in plasma and in brain regions where the target resides. Both *ex vivo* and *in vivo* TO of drugs can be evaluated in experimental animals using both invasive and noninvasive methods [47]

The *ex vivo* approach uses tissue slice autoradiography, or biochemical measures of TO, wherein the fraction of total target binding sites occupied by the drug is inferred from the residual binding capacity of a radiotracer added to the postmortem tissue *ex vivo*. The *in vivo* approach, on the other hand, measures the displacement of the radiotracer from the

target tissue when both the drug and the radiotracer are administered to the living animal. In the past decade, the use of non-radiolabeled tracers is increasingly adopted in *in vivo* studies, in which the tissue concentration of the administered non-radiolabeled tracer is quantified by liquid chromatography-mass spectrometry (LC-MS) instead of radioactivity counting [48]. The main advantages of this method are that the parent tracer can be differentiated from its metabolites, and it allows simultaneous quantification of the tracer displacement (TO) and the neuro-PK [49]. The measurement of TO

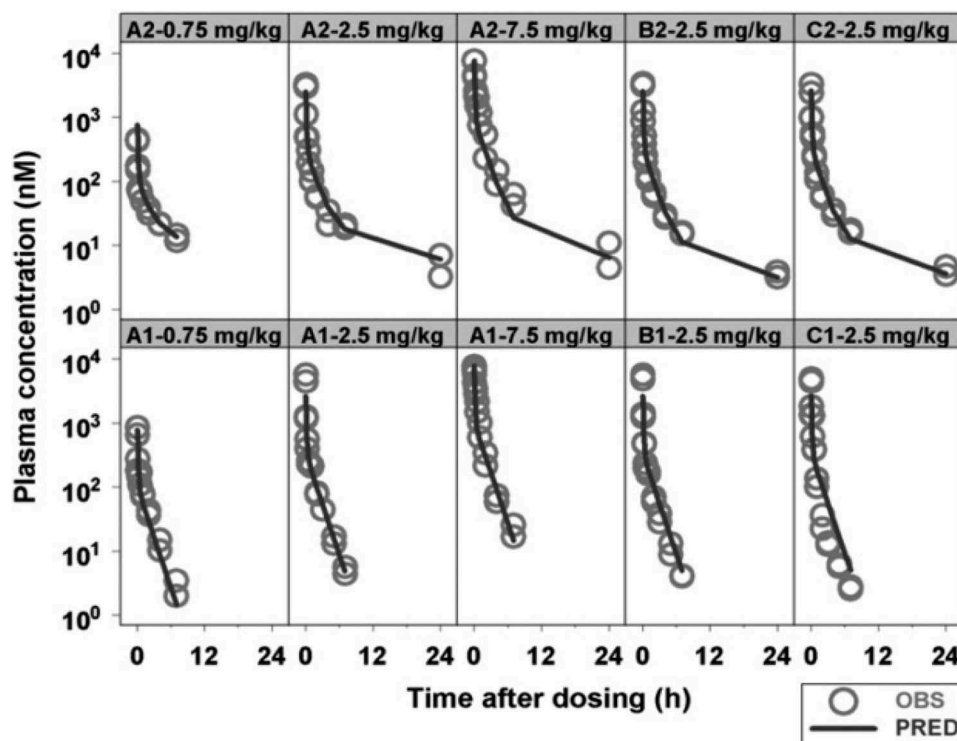


Figure 5. Compared plasma pharmacokinetics of high affinity compounds (top row) and their low affinity analogues (bottom row). The circles represent observed plasma concentrations in rats, the lines represent model predictions. (Reprinted from [45] with permission of ASPET).

and target site PK can provide valuable insight into the driving factors for the time course of drug action. However, additional factors such as target turnover/desensitization, endogenous ligand binding, and signal transduction can also influence the time course of drug action and need to be taken into account [47].

In humans and animals, TO can be evaluated noninvasively using positron emission tomography (PET) or single-photon emission computed tomography (SPECT). These imaging techniques are powerful because they provide high temporal and spatial resolutions. PET radioligands are now available for more than 40 CNS targets [50]. PET/SPECT is most useful when no easily measurable clinical PD markers are available, or when substantial time is required before an indication of efficacy can be observed clinically. In this respect, PET/SPECT can be used to demonstrate that the drug candidates reach their target and bind to their target, as a proof of concept enabling effective treatment. Moreover, PET/SPECT imaging can also be used to identify whether a delay between plasma unbound drug concentrations and CNS effect is caused by slow target equilibration or by slow transduction processes. Dose (or plasma concentration)-TO curves can be obtained to guide the dosing in clinical trials [50–52].

Drug-target interactions are increasingly incorporated in mechanism-based PK–PD modeling to support drug development. These models allow quantitative assessment of the relation between neuro-PK and TO. As already discussed, PBPK modeling is particularly useful in translating the obtained information from animals to humans by using the available knowledge on species-dependent physiological characteristics such as blood flow and organ volumes [53–55].

Based on the exposure–response relationship in the animal species and using TO as a translational biomarker, the efficacy of a drug candidate in humans could be predicted in early stage development. Systematic implementation of translational modeling can therefore bolster confidence of successfully evaluating proof of mechanism in humans and ultimately improve the success rate in Phase II, which is currently only around 10% [56]. Thus, to predict drug effects based on TO, integration of all determining factors is required, including drug transport between blood and CNS, CNS fluid flows, target site PK profile, drug-target binding kinetics, endogenous ligand-target binding kinetic, and target turnover.

2.4. CNS drug effects

Many times neuro-PK, and/or TO, is studied without measuring associated (biomarkers of the) drug effects. Actually, it would be of great added value if neuro-PK and associated PD would be obtained in a single experimental subject or at least single experimental context, as rate and extent of body processes are context dependent (according to the Mastermind Research Approach [36]). To link (neuro-)PK to the physiologic response, PK–PD modeling is often applied. Depending on the PK, as well as the type of response (inhibition or stimulation), different PK–PD models can be used. With that, it is possible to learn more about factors that play a role in target activation and signal transduction to the ultimate effect or biomarker of the effect [57–61], interspecies differences in concentration–effect relationships [62], tolerance and sensitization [63], and intra- and interindividual variability. Moreover, for studies with only PK and PD observations but not TO data, a delay between

the PK and PD is often be explained by a biophase (or effect compartment) distribution model. However, incorporating drug-target binding into the model might explain the same delay and could be more mechanistic [64]. On the other hand, when solving the shortcomings in knowledge on target site distribution of drugs, the principles of the operational model of agonism (receptor theory) will provide the basis for future developments in drug development by classifying drugs and predicting their mechanism of action in pharmacology [65–68]. PK–PD approaches have typically focused on anticipated drug effects. As drug probably has additional, unknown effects, quantitative insights in CNS drug effects should better be obtained for the whole biological system, including the unknown mechanisms of action [69,70].

2.5. Biomarkers of drug effects and disease

To predict the drug effects in human on the basis of translational animal and mathematical models, specific expressions are needed to quantitatively characterize the processes on the causal path between drug administration and effect. These include target site distribution, target binding and activation, transduction, PD interactions, and homeostatic feedback mechanisms. Ultimately also the effects on and of disease processes and disease progression have to be considered. These can be characterized by biomarkers according to the biomarker classification system [71] (Table 1).

Obtaining combined information on a number of biomarker types (preferable in parallel, within a single biological

system) will allow the development of better models, with increased accuracy and predictability. The better we will be able to develop predictive models in preclinical studies, the more the number of often extremely costly clinical studies can be reduced. The focus should therefore be on the design of quantitative *in vivo* animal studies such that translational pharmacology approaches can be applied [25,36,37,61,89], which will be discussed below. In refined animal models, the biomarkers of the effect that can be measured in both animals and human will be particularly useful.

Multimodal neuroimaging that combines PET/SPECT with techniques like magnetic resonance imaging, computed tomography, and electroencephalography can simultaneously provide anatomic, functional, biochemical, and metabolic information alongside TO assessment. These techniques are increasingly employed in assessing the pathophysiological changes in different disease states, such as target expression, release of endogenous ligands that bind the drug target (e.g. neurotransmitters), neuroinflammation/glia activation, cerebral blood flow, and BBB integrity, all of which could alter the drug PK, TO, and biomarker profiles [50–52].

To obtain insight in the multiple processes in the biological system, increasing efforts are made to show the utility of a multi-biomarker approach, both in disease conditions and upon drug administration [17,90]. With that, the system-wide pathophysiological and pharmacological influences are reflected by a multi-biomarker response. To that end, it is important to connect such data to information on drug distribution to target sites, target binding kinetics, signal trans-

Table 1. Biomarker classification [71] and approaches to assess quantitative information.

Biomarker	Description	Approaches	References
Type 0	Genotype or phenotype	Genotype and phenotype are determinants of the drug response, that influences target site exposure or response due to variation in the expression of e.g. enzymes or receptors. High quality gene expression data have significantly impacted the direction of investigation by allowing for a better molecular understanding of BBB development, function, and dysfunction. Genome-wide microarray expression data sets for the BBB have become available. Also changes in disease conditions have been investigated. Information on protein expression levels of transporters and receptors can be obtained using quantitative targeted absolute proteomics, whereas information on enzyme conversion rates can be identified using quantitative targeted metabolomics.	[72–77]
Type 1	Drug concentrations in general and at the target site in particular.	Quantitative biomarkers that represent the target site distribution of drugs and metabolites for compounds that act in the CNS are difficult to obtain in man, but are readily available <i>in vivo</i> in animals.	[3,6,31,32,34,75]
Type 2	Degree of target occupancy	In theory, effects may occur at different degrees of target occupancy and may be species dependent. The relationship between target occupancy and effect is therefore important for the understanding of inter- and intraindividual variability. Information on target occupancy is available by bioassays <i>in vitro</i> , tracer displacement in postmortem tissue <i>in vivo</i> and noninvasive PET/SPECT imaging.	[42,44,47,48,78–80]
Type 3	Quantification of the target site activation	By means of <i>in vitro</i> bioassays, information can be obtained on receptor activation in animal and man. Techniques like quantitative electroencephalograms (EEG) and functional-magnetic resonance imaging (fMRI) can obtain specific receptor activation data in preclinical and clinical <i>in vivo</i> setting.	[51,58,81–85]
Type 4	Physiological measures	Physiological measures should be measured in the integral biological system, as they are often controlled by homeostatic feedback mechanisms. Such measures can, for example, be on pituitary hormones that play a very important role in communication between CNS and the periphery. Also for physiological measurements quantitative EEG, PET scanning, and functional MRI techniques are very useful.	[18,51,62,82,84–89]
Type 5	Disease processes	Characterization of disease processes which are particularly useful in clinical settings.	[37,85–87]
Type 6	Clinical endpoints	Clinical endpoints such as occurrence of a disease, symptom, sign, or laboratory abnormality that links to target outcomes.	[88]

duction, and homeostatic feedback mechanisms. Such insight is obtained by integration of data obtained from multilevel studies, that is, measurement of different biomarker types in a time-dependent manner [19,71,91,92,93]. In all cases, the experimental approach should be such that a distinction can be made between drug-specific and system-specific properties, to allow for scaling between drugs and/or scaling between species [61].

3. Conclusion

For a proper CNS effect, the drug should have the ability to access the CNS 'at the right place, at the right time, and at the right concentration.' To develop treatments with improved safety and efficacy, one of the scientific challenges is to understand the biological mechanisms underlying the PK–PD relationships of CNS drugs. The currently applied simplistic approach to produce data on multiple processes in isolation is not informative as processes are context dependent and interdependent. The knowledge on heterogeneity (variability) in rate and extent of processes between drug dosing and CNS effects is needed to predict the impact of drug-induced and disease-induced perturbations in the biological system. To that end, the integrative 'Mastermind Research Approach' [36] is needed to decipher the interrelationships of processes that govern plasma PK, BBB transport, intra-brain distribution, as well as CNS effects in different conditions.

4. Expert opinion

One of the major weaknesses in CNS drug discovery and development has been the tendency to oversimplify relevant factors underlying CNS disease and drug effects. Typically, parameter values of PK, PD, and disease processes were obtained in isolation, in different systems, thereby ignoring their interrelationships and systems dependencies. Simple decision trees based on such isolated parameter values were used to drive decisions on taking compounds further in development or not. However, this has not led to knowledge and understanding the system, in terms of interdependencies and condition dependent rate and extent of multiple processes between drug dosing, neuro-PK, and neuro-PD, as needed for predictions.

In the relationship between plasma PK and neuro-PK, a first improvement was by the notion for the need for unbound drug concentration-time profiles in plasma and brain to inform on rate and extent of BBB transport, which can only be provided by the microdialysis technique. A second improvement was on understanding of extra-intracellular drug distribution within the brain (at presumed steady-state conditions), using the brain slice technique. Then, knowledge on (steady state) distribution of bound and unbound drug between plasma, brain ECF, brain intracellular space, and cellular compartments, was integrated in the Combinatory Mapping Approach [22]. This approach has recently been embraced by a number of pharmaceutical companies.

A first left-over issue is, however, to understand *time-dependency*. Even in chronic dosing, fluctuations in plasma and CNS drug levels may occur and may have important consequences

for CNS TO [44]. Also, relationships between concentration–time profiles in brainECF and intracellular space (drug target sites) and between those target-site concentration–time profiles and the ones in CSF compartments are important, as mostly only CSF can be obtained in the clinical setting and should be relied on for having information about drug concentrations at the target site [4]. A number of CNS PBPK models have been developed, however, only addressing steady-state conditions [30,33]. The use of extensive series data sets on unbound drug concentrations, obtained in parallel in brainECF and different CSF locations in the rat, a generic semi-PBPK brain rat model has been developed [34] followed by the full-PBPK brain rat model [39]. This model can scale between species, and allows prediction of *concentration–time profiles* of compounds in multiple compartments of the human brain on the basis of solely physicochemical properties of compounds [39].

This indicates the following future perspective for prediction of neuro PK and TO profiles: In a very early stage of drug development, physicochemical properties of drugs can be measured *in vitro* and/or predicted by *in silico* models. Also, plasma PK profiles can be predicted by currently available full PBPK models. This information on plasma PK and physicochemical drug properties can inform the CNS PBPK model to predict neuro-PK of individual candidate compounds, in either rat or human. Then, *in vitro* drug-target binding kinetics can be included in the model to predict TO-time profiles in the specific species selected. TO predictions could be validated by actual measurements. With this approach fewer animals are needed (replacement by using *in silico* models). Also, clinical trials will be better informed, needing fewer individuals and samples per individual for first-in human studies.

A next, important challenge is prediction of drug effects, especially in disease states. Currently, to link (neuro-)PK to the systems response, PK–PD modeling is often applied. These models can include target activation (receptor theory [65–68]), signal transduction [57–61], interspecies differences [62], tolerance and sensitization [63], and intra- and interindividual variability. Also here, it should be noted that parameter values of PK, PD, and disease processes should not be obtained in isolation, and in different systems, because in such manner interrelationships and systems dependencies of processes cannot be assessed. Moreover, so far, PK–PD approaches have typically focused on a single anticipated drug effects. As a drug probably has additional, unknown effects, quantitative insights in CNS drug effects should better be obtained for the whole biological system (systems-wide approach), thereby including the unknown mechanisms of action [69,70]. Here, also, time-course data, obtained at different biomarker levels (Table 1) under multiple conditions, is key to be able to apply mathematical modeling for unraveling interrelationships and condition dependencies. Where it concerns body fluids, serial sampling, and where possible microdialysis, is very useful. Such time-course samples can be subjected to 'omics' approaches that will broaden our understanding of (changes in) the networks that compose the biological system, in health and disease. Especially, a pharmacometabolomics approach is suggested for potentially multi-target neuropharmacodynamics. In addition, the use of imaging techniques should be considered, as those can be

applied to animals as well as human subjects, providing translational insights. Though 'omic' approaches are expensive, it is the only way to go if we wish to have better insights into condition-dependency of biological systems processes.

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