Conventional Behavioral Models and Readouts in Drug Discovery: The Importance of Improving Translation

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R. Schreiber (ed.), *Modern CNS Drug Discovery*, https://doi.org/10.1007/978-3-030-62351-7_5

This chapter describes the relevance and importance of preclinical models and readouts in the drug development process. Recently, animal behavioral models have been criticized on their role in the poor translation into novel pharmacotherapies. The first section addresses the importance of validity, ethics, and model/readout selection in preclinical behavioral pharmacology. As an example, models and readouts in the preclinical development of analgesic drugs for inflammatory and neuropathic pain are described. Furthermore, evoked and non-evoked pain readouts are reviewed. In the second section, the shortcomings of conventional preclinical models are discussed, and the necessity of improving translation in CNS drug discovery and development are also discussed. Several steps are proposed that could enhance translation from animal data to clinical efficacy. In this regard, neuroimaging and PK/PD modeling strategies are posed as crucial aspects in generating more valid, robust, reliable preclinical data resulting in more effective and translatable therapies. In conclusion, applying classical pharmacology to problems of translational medicine will aid us in improving the way we think about and use animal models. Closer collaboration and cross-over between clinical, pathological, and pharmacological research are paramount in optimizing the success of preclinical translation into novel medicines for patients in need worldwide.

5

Learning Objectives

- The necessity of preclinical behavioral models and readouts in drug discovery
- The importance of translational medicine for preclinical research
- The value of neuroimaging approaches and pharmacokinetic-pharmacodynamic (PK/PD) modeling strategies in preclinical drug discovery and development

5.1 General Introduction in Behavioral Models and Readouts

A crucial step in the discovery of novel drugs is proof of in vivo activity and efficacy. As described in the previous chapters, the drug discovery process is long and complicated starting from target identification and validation, followed by high throughput screening, virtual screening, numerous rounds of iterative structure activity relationship (*SAR*) modifications until a series of molecules is identified with drug-like properties. These molecules have demonstrated potency and selectivity at the desired target, favorable physicochemical properties, efficacy in in vitro and ex vivo model systems, and acceptable pharmacokinetic profiles. Next, target engagement, efficacy, and safety/tolerability need to be confirmed in an intact living organism, to further advance these molecules toward clinical trials and putative novel therapies. For this pivotal step, the use of animal studies is fundamentally required and inevitable.

5.1.1 Model Versus Readout and Validity

Pivotal in understanding behavioral preclinical research is to differentiate between an animal *model* and a *readout* (or test). *Animal models* or animal models of disease used in research may have an existing, inbred, or induced disease or injury that is similar to a human condition. The use of animal models allows researchers to investigate disease states in ways which would be not possible in patients. Procedures can be performed on the non-human animal that imply a level of harm that would not be considered ethical to

inflict on a human. The best models of disease are similar in *etiology* and *phenotype* to the human equivalent. However, complex human diseases can often be better understood in a simplified system in which individual parts of the disease process are isolated and examined. Once an appropriate animal model of the disease or underlying part/mechanism of the disease has been established and validated, the model can be used to investigate specific hypotheses. Examples of animal models are a genetic modification in mice that mimic Aβ plaque formation in the brain of human Alzheimer's disease or a spinal nerve lesion model that mimics human neuropathic pain conditions. To test specific hypotheses in an animal model, accurate and robust *readouts* (or tests) are required, for example, a Morris water maze test for assessment of spatial learning or von Frey filaments for assessment of mechanical hypersensitivity. Thus, the model represents the implied changes to the animal to represent the disease state or mechanism of interest, whereas the readout represents the method of quantifying the effects of a (e.g., genetic or pharmacological) manipulation in that model.

Definition

Animal models are representations of human disease or conditions in a non-human species, etiology is the mechanism of cause of the disease, and phenotype stands for the respective signs and symptoms of the disease.

Preclinical development of novel drugs requires both robust models and readouts to assess disease-like behavior, underlying mechanisms, and efficacy of drug treatment. To be useful in predicting efficacy, the model and readout need to demonstrate *sensitivity*, *specificity,* and *predictivity* (Rice et al. 2008; Rutten et al. 2014).

Definition

Sensitivity is the ability to detect a true positive control, specificity stands for the ability to detect a true negative, and predictivity is the ability to predict the outcome in other model/species. Face validity assures that the biology and symptoms as seen in humans are similar in the animal model, and construct validity assures that the target exerts the same biological processes in both organisms.

Two further aspects are important in the validation of animal models, namely, *face validity* and *construct validity* (Denayer et al. 2014). A crucial aspect in preclinical development is the translation of preclinical findings into clinical efficacy. As such, *translational medicine* is the area of research that aims to improve human health by determining the relevance of novel discoveries in biological sciences to human disease. Translational medicine seeks to coordinate the use of new knowledge in clinical practice and to incorporate clinical observations and questions into scientific hypotheses in the laboratory. Thus, it is a bidirectional concept, encompassing so-called *bench-to-bedside* factors, which aim to increase the efficiency by which new therapeutic strategies developed through preclinical research are tested clinically, and *bedside-to-bench* factors, which provide feedback about the applications of new treatments and how they can be improved.

For many diseases that do not involve the central nervous system (CNS), animal models can be straightforward and clear readouts can be identified to investigate drug efficacy. For example, when developing novel anti-inflammatory drugs, a rodent model of inflammation by injection of liposaccharide (LPS) is employed and readouts such as edema or swelling can be assessed accurately and objectively (e.g., by caliper or plethysmography) and biomarkers such as the release of pro-inflam-

matory cytokines (e.g., TNF-alpha, or interleukins) can be measured. Both readouts can be directly inhibited by anti-inflammatory drugs. Furthermore, the same readouts (reduction of swelling and cytokine release) are employed in preclinical assays as well as in clinical trials which contributes to better translation of preclinical findings. For CNS drugs, the use of animal models and readouts is in most cases far less straightforward and several issues need to be taken into account. These will be discussed in this chapter. Indeed, for many CNS disorders the underlying mechanisms are poorly understood, the pathology is difficult to model in animals, and the readouts in animals are different from those in humans.

Because entire books have been written about all the different models and readouts for CNS disorders (e.g., McArthur and Borsini 2008), we focus here on animal models and readouts used in chronic pain and analgesic drug discovery research as an example. The drug discovery rationale, challenges, shortcomings, and discussions on translation described for the indication "pain" also apply in general to other CNS disease areas. Comprehensive reviews on animal models for specific indications have been published elsewhere (e.g., see Whiteside et al. (2013) for Pain; Puzzo et al. (2015) for Alzheimer's Disease; Soderlund and Lindskog (2018) for Depression; Harro (2018) for Anxiety).

3R Principles for Animal Experimentation

The use of animals in science is inevitable and required to unravel the underlying biology and pathology of diseases, or the mechanism of action and safety of putative novel therapies. In addition, animal studies on efficacy and safety are legally required by the regulatory agencies [e.g., the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA)] that approve novel drugs to market. Importantly, all efforts must be made to restrict animal use and suffering. The *3Rs* are the guiding principles for animal experimentation, and they are adopted by ethical committees and governments across the world (first described by Russell and Burch (1992)). The 3Rs stand for Replace, Reduce, Refine and represent a responsible approach to animal testing. The goal is to replace animal experiments whenever possible. In addition, the aim is to keep the number of animal experiments as low as possible and to only use the necessary number of animals. Finally, it is vital to ensure that the distress inflicted upon the animals is as low as possible.

- *Replace*: Replacing an animal experiment to the greatest possible extent, as long as adequate alternatives are available.
- *Reduce*: The reduction of animal experiments and the number of laboratory animals to the greatest possible extent. *Statistical power calculations* must be performed in advance to the experiments to ensure that sufficient (but not more than that) animals are used to meet the criteria for obtaining a statistically significant outcome.
- *Refine*: The methods and treatment of the animals during the experiments, and with regard to the way they are kept, should ensure that the distress caused to them is minimized to the greatest possible extent and that their well-being is taken into account as far as possible.

All three pillars are of equal importance in the 3R principles. Anyone conducting research with laboratory animals is obliged to comply with the 3R principles and regulations by local ethical authorities/government. Clearly many people object to animal research on principle (Regan 2007), and these objections have been discussed in detail elsewhere (Cohen 1986; Derbyshire 2002, 2006). This chapter will focus on animal models and readouts in CNS drug discovery, the difficulties and pitfalls, and the possible ways to improve translational medicine in the drug discovery process. A scientific rationale for the use of animal research as an important mechanism in advancing drug discovery is provided. Those that object to animal research on principle will, understandably, be unmoved by the scientific advances animal research provides.

5.2 Models and Readouts in Pain Drug Development

Experiments investigating pain in human subjects have intrinsic practical difficulties; accordingly, early analgesic development relies on animal models (Mogil 2009). Modeling human pain in experimental animals is inherently challenging for several reasons. First, pain is defined as an unpleasant sensory and emotional experience (definition on website of the International Association for Studies against Pain (IASP)), and is thus subjective, existing only in the person who experiences it (first-person perspective). Importantly, humans communicate their pain experience verbally, and pain is quantifiable via numerical or visual analogue scales (Barrot 2012). The absence of verbal communication in animals is undoubtedly a challenge to the evaluation of pain, and therefore indirect behavioral readouts must be used as *surrogate markers*. Thus, preclinical pain research must rely on stimulus-evoked responses or alterations in the behavior of an animal as readout (Barrot 2012; Deuis et al. 2017). Obviously the same issue with lack of verbal communication holds true for the evaluation of other CNS disorders such as memory-loss, anxiety, and depression (◘ Fig. 5.1). Second, pain is not merely a somatosensory experience due to a noxious stimulus, but is influenced by a number of factors including affective, attentional, and cognitive states, all of which are dynamic in nature and difficult to model. Third, the suffering aspects of clinically relevant pain cannot be fully modeled in animals, as suffering must be minimized in animal research for ethical reasons and follow the 3R policies. Furthermore, rodents are prey animals that live in social groups and will therefore try and hide any sign of weakness, including pain, as this would make them more vulnerable to predators or lose their rank in the group hierarchy (Deacon 2006). Finally, human pain cannot be modeled as a single disease, but rather as a syndrome brought on by a wide variety or conditions (Jensen 2010).

◘ **Fig. 5.1** Cartoon of a rat suffering from chronic pain. It is an impossible quest to completely mimic chronic pain, and other CNS, disorders in rodents. (Modified from: Cryan et al. (2002). Copyright license obtained from Elsevier)

5.2.1 Models of Neuropathic and Inflammatory Pain

Neuropathic pain is defined as "pain initiated or caused by a primary lesion or dysfunction in the nervous system" (Merskey and Bogduk 1994). *Inflammatory pain* refers to increased sensitivity due to the inflammatory response associated with tissue damage. Under these sensitized conditions for both neuropathic and inflammatory pain, an innocuous stimulus can be perceived as painful—this is known as *allodynia,* and the pain evoked by a noxious stimulus is exaggerated in both amplitude and duration—this is known as *hyperalgesia* (Sandkuhler 2009).

Over the years, many animal models for inflammatory pain using irritant agents and surgical and non-surgical animal models for neuropathic pain have been developed and used for preclinical testing. The most common models are summarized in ◘ Fig. 5.2. Briefly, to model inflammatory pain, substances that result in an immune response are injected directly into the peritoneum, paw, or into the joint, respectively, #1, #2, and #3 in ◘ Fig. 5.2. The most commonly used irritant substances are phenylbenzoquinone and other acidic compounds in writhing tests (Eckhardt et al. 1958; Vander Wende and Margolin

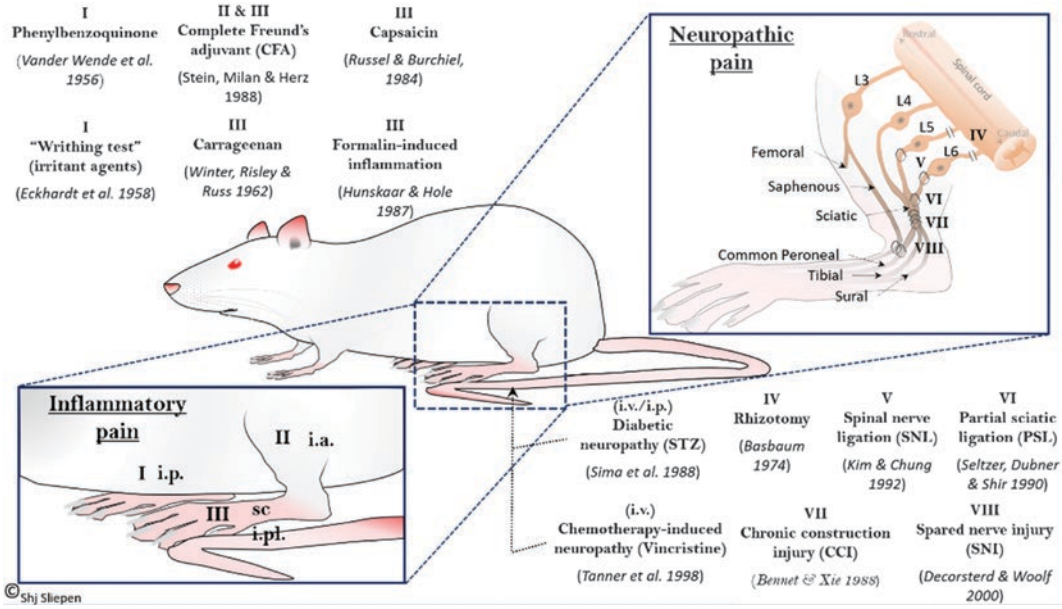

Fig. 5.2 A schematic illustration of well-known inflammatory and neuropathic pain models. i.p. intraperitoneal, i.a. intraarticular, sc subcutaneous, i.pl intraplantar, i.v. intravenously, STZ streptozotocin (From: Sliepen (2019). *Reuse permission obtained from SHJ Sliepen*)

1956). For local administration into limbs or joints heat-killed mycobacterium butyricum/tuberculosum, i.e., complete Freund's adjuvant (CFA) (Stein et al. 1988), formalin (Hunskaar et al. 1986), hot chilly pepper extract, i.e., capsaicin (Russell and Burchiel 1984), or sulphated polysaccharides from seaweed, i.e., carrageenan (Winter et al. 1962) are used. Injection of CFA into a paw, ankle, or knee joint results in local inflammation and serves as a model for human arthritic pain (Neugebauer et al. 2007). The use of genomic data is changing to a significant degree how we understand human disease. In the area of inflammation, the ability to build new genomic models in mice using information from genomic, proteomic, and metabolomic studies is growing ever more.

The majority of the neuropathic pain models are induced by unilateral surgical damage to a specific nerve (mono-neuropathic) in order to study the effects of pain-like behavior in a controlled manner. The most commonly used methods (see Fig. 5.2) are chronic constriction injury (CCI) of the sciatic nerve (#VII; (Bennett and Xie 1988)), spared nerve injury (SNI: #VIII, (Decosterd and Woolf 2000)), and spinal nerve ligation (SNL; #V, (Kim and Chung 1992)) models as these generate the most reproducible phenotypes. In the rhyzotomy model (#IV, (Basbaum 1974)), one spinal nerve (L5) is transected, whereas in the SNL model two spinal nerves (L5 and L6) coming from the dorsal root of the spinal cord are tightly ligated. In the partial sciatic nerve ligation (PSL; #VI; (Seltzer et al. 1990)) model, a portion of the sciatic nerve is tightly ligated, whereas the CCI model involves placement of four loose chromic-gut ligatures around the sciatic nerve. In the spared nerve injury (SNI) model, the common peroneal and tibial nerves are cut, sparing the sural nerve. Alternatively, models have been designed to study poly-neuropathic pain where multiple nerves in the body are affected, such as, the streptozotocin (STZ) models for diabetic polyneuropathy (Sima et al. 1988) or vincristine models for chemotherapy-induced peripheral neuropathy (Tanner et al. 1998).

5.2.2 Readouts for Assessment of Pain-Like Behavior

Preclinical pain models may be associated with spontaneous pain-related behavior as well as allodynia and hyperalgesia, which result in enhanced responses to mechanical, heat, and/or cooling stimuli (Campbell and Meyer 2006). There are various ways to measure pain-like behavior in animals, either via a response to an applied stimulus (stimulus-evoked readout) or assessment of behavior independent of an applied stimulus (non-stimulus-evoked readout). For stimulus-evoked readouts, an external stimulus (mechanical, thermal, or chemical) is applied to a specific site on the test subjects' body to elicit a behavioral response. The nature of this response is frequently a withdrawal response to the stimulus, and the readout is either the force at which the response occurs, the latency until the response occurs, or the number of responses to the specific stimulus. Subsequently, mechanical hyperalgesia can be assessed by applying increased pressure to the paws (Randall and Selitto 1957) and mechanical allodynia via application of von Frey filaments to the plantar surface of the paw (Dixon 1980). Thermal stimuli are divided into heat stimuli, and responses are measured in a tail-flick, hot plate, or Hargreaves test, and cold stimuli, where responses are measured in a cold plate test. A main criticism of stimulus-evoked readouts is that they often rely on thresholds or latencies which do not adequately reflect clinical pain (Klinck et al. 2017). Furthermore, they are often induced by an experimenter, possibly resulting in a bias.

Thus far, translation of preclinical findings into clinical studies has been difficult and numerous examples exist where preclinically efficacious analgesic compounds did not show an effect in Phase 2 proof-of-concept clinical trials (see below). Part of this challenging translation may be due to inappropriate and unpredictable animal models and readouts. Therefore, a great effort has been made to improve alternative non-stimulus-evoked behavioral tests (Percie du Sert and Rice 2014); to standardize animal models and readouts; and to increase experimental rigidity to reduce bias in preclinical research (Knopp et al. 2015). A promising example of these alternative readouts is the burrowing test (Andrews et al. 2011; Deacon 2006), in which animals are allowed to exhibit their innate behavior of digging tunnels and burrows in a laboratory setting. Several inflammatory and neuropathic pain models result in reduced burrowing behavior, which was reversed by analgesics (Andrews et al. 2012; Huang et al. 2013; Rutten et al. 2018). A major advantage for preclinical analgesic drug development is that burrowing is less prone to generate false positives due to impaired motor skills or sedation, as opposed to traditional stimulus-evoked tests (Rutten et al. 2014).

5.2.3 An Example of Failed Translation from Animal Data to Clinical Trials of NK-1 Antagonist as Putative Novel Analgesic Drugs

Neurokin receptor 1 (NK-1) antagonists block the receptor for the neurotransmitter Substance P and boost activation of serotonin 5-HT3 receptors in order to prevent nausea and vomiting. The discovery of NK1 receptor antagonists was a turning point in the prevention of nausea and vomiting associated with cancer chemotherapy. Scientists believed that NK-1 antagonism would be a promising target for treatment of chronic pain. Unfortunately, NK-1 antagonists have become an infamous example of where preclinical efficacy did not translate into clinical efficacy in Phase 2 trials for chronic pain. Indeed, NK 1 receptor antagonists have failed to exhibit efficacy in clinical trials of a variety of clinical pain states. By contrast, there were sufficient well-conducted animal studies in which an NK1 receptor antagonist attenuated the behavioral or electrophysiological response to a noxious stimulus to justify performing clinical trials for analgesia (Hill 2000). The profile of the compounds across the behavioral tests was actually comparable to that of non-steroid anti-inflammatory drugs (NSAIDs), which

are analgesic in humans. Thus, NK 1 receptor antagonists seem to be able to block behavioral responses to noxious and other stressful sensory stimuli at a level detectable in animal tests, but the translation to achieve the level of sensory blockade required to produce clinical analgesia in humans failed. The importance of supraspinal targets of analgesics has been underestimated, and most preclinical studies of analgesia are focused on the spinal dorsal horn, despite the fact that many substances elicit their analgesic effects primarily at the supraspinal level (Hill 2000). Finally, it is relevant to ask whether the failure to predict the presence or absence of analgesic properties in humans in the case of NK1 receptor antagonists has implications for the discovery and clinical evaluation of other putative analgesics. On the one hand, many examples exist of a substance exhibiting analgesia in animal models and clinical analgesia in humans. For example, ketamine is an antagonist of NMDA receptors, which are widely distributed in the CNS, and is analgesic in both animals and humans. On the other hand, the enkephalinase inhibitors, which increase the concentrations of endogenous opioid peptides, possess antinociceptive effects in animals but lack analgesic effects in humans (Villanueva 2000).

What preclinical criteria should be used to determine whether clinical trials of a new analgesic are likely to be successful? Perhaps one of the main challenges for preclinical studies of pain and other CNS diseases today is to employ holistic and integrative approaches to improve our preclinical disease understanding and to enable the building of bridges between scientists and clinicians interested in discovering novel treatment options for CNS diseases.

5.3 Improving Translation in CNS Drug Discovery and Development

Evaluating brain function by means of imaging technology in patients and respective animal models has the potential to characterize mechanisms associated with the disorder or disorder-related phenotypes and could provide a means of better *bench-to-bedside* and *bedside-to-bench* translation.

5.3.1 Neuroimaging

As discussed above, translation from behavioral models and readouts in rodents toward clinical patient–reported outcome measures is troublesome in drug discovery for pain and other CNS disorders. Figure 5.3 gives a clear representation of the difference in phenotype and CNS properties between clinical and preclinical settings in the field of pain research.

Functional magnetic resonance imaging (fMRI) is an excellent tool to study the effect of manipulations of brain function in a non-invasive and longitudinal manner. Several MRI techniques permit the assessment of functional connectivity during rest as well as brain activation triggered by sensory stimulation and/or a pharmacological challenge in both rodents and humans. Stimulation with a drug and in combination with MRI is called *pharmacological MRI (PhMRI)*, and it has a number of interesting possibilities compared to conventional fMRI. Using selective pharmacological tools, the neurotransmitter-specific brain circuitry, neurotransmitter release, and binding associated with the pharmacokinetics and/or the pharmacodynamics of drugs can be investigated (Jenkins 2012). As such, PhMRI can be characterized as a molecular imaging technique using the natural hemodynamic transduction related to neuro-receptor stimuli.

Although differences in brain size, structure, and function exist between rodents and humans, a preservation of CNS networks across species has been observed using functional brain imaging (Gozzi et al. 2006). Furthermore, using phMRI-consistent pharmacodynamic responses have been observed across species for opioids (See Fig. 5.4, (Becerra et al. 2013)) and other analgesic drugs (Borsook and Becerra 2011).

It is currently believed that neuroimaging may describe the central representation of pain or pain phenotypes and yields a basis for the development and selection of clini-

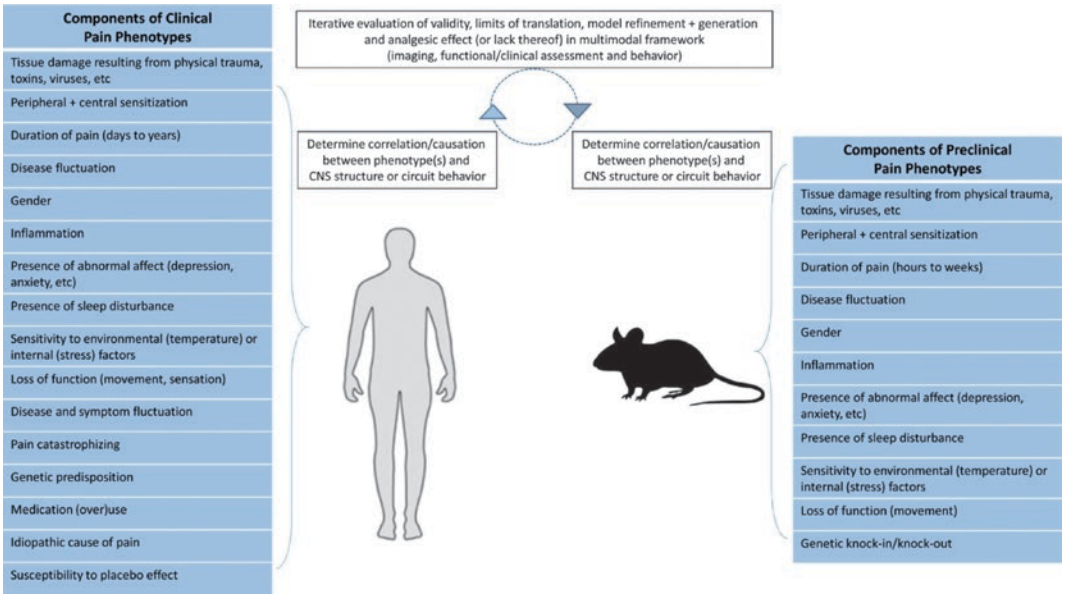

Fig. 5.3 Convergence of phenotypes and CNS properties in clinical and preclinical settings. A model of clinical and preclinical pain experimentation considers the use of pain-related phenotypes in conjunction with CNS function to assess and improve the overall validity of preclinical pain investigations (From: Upadhyay et al. (2018). Copyright license obtained from Elsevier)

cally relevant animal assays (For review see: Upadhyay et al. (2018)). The large numbers of molecules available, which do not require a radio-label, means that phMRI has become a very useful tool for performing drug discovery. Translational phMRI approaches may increase the probability of finding meaningful novel drugs that can help satisfy the significant unmet medical needs of patients suffering from CNS disorders.

5.3.2 PK/PD-Modeling

A crucial step in the development of novel drugs is to generate a growing understanding of the relationship between the *pharmacokinetic* (*PK*) profile and the *pharmacodynamic* (*PD*) profile. As such, *PK/PD modeling* refers to a data (PK and PD)-driven exploratory analysis, based on mathematical or statistical models. In other words, the objective of pharmacokinetic-pharmacodynamic (PK/PD) modeling is the development and application of mathematical models to describe and/or predict the time course of dose-to-concentration (PK) and concentration-to-effect (PD) of pharmacological active agents in health and disease (Martini et al. 2011). Clinically, the rationale for measuring drug concentration is that the relationship between concentration and effect should be less variable than the relationship between dose and effect (Atkinson et al. 2007). Therefore, accurately measuring the concentration will allow for better predictions of drug effect than dose information alone.

Definition

The *pharmacokinetic* (*PK*) profile represents how the organism affects the drug by means of absorption, distribution, metabolism and excretion, and which concentrations of the drug reach the target organ. The *pharmacodynamic* (*PD*) profile represents how the drug affects the organism, and what dose causes which (side) effect.

This allows the observed drug effect to be related directly to the time after a given dose. Therefore, the combined PK/PD model provides a means of understanding

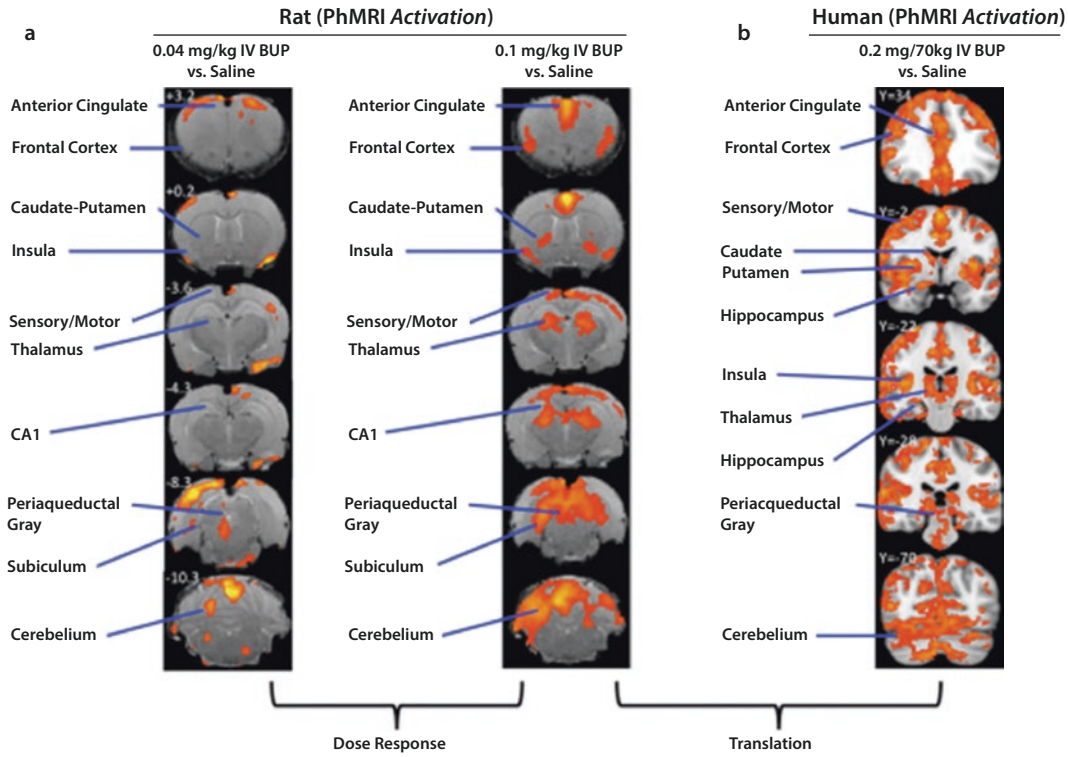

Fig. 5.4 PhMRI activation after i.v. buprenorphine administration. **a** PhMRI of 0.04 and 0.1 mg/kg i.v. buprenorphine yielded dose-dependent phMRI activation (drug. saline) in the conscious rat. **b** phMRI activation was observed in the human buprenorphine phMRI dataset with 0.2 mg/kg i.v. buprenorphine administered. The labeled brain structures highlight regions where phMRI activation was induced at the higher doses of buprenorphine tested in both species (From: Becerra et al. (2013). Copyright license obtained from ASPET Springer publishing group)

the time course of drug effect, namely, the extent, onset, and duration of drug action (Wright et al. 2011). Kinetic-dynamic reasoning should, whenever possible, be based on in vitro and in vivo concentration-time, response-time, and concentration-response relationships, with an underlying ambition to couple this to the disease state. The discipline of modeling is always data-driven, and it relies on multiple analyses of the same dataset in an iterative mode with successive and/or competing models.

PK/PD modeling and simulation can add value in all stages of the drug development process starting from the preclinical development stage up to late stage clinical development. To utilize PK/PD modeling and simulation in its optimal potential for drug development, models should be developed early in drug discovery, preferably during the preclinical phase. Such models are continuously updated and refined as more data become available. Their validation is necessary during development, and they will then provide valuable support to make important decisions, with an increased confidence level around the analyzed data.

During the preclinical phase of drug development, various in vitro and in vivo studies have been used to screen compounds for efficacy. From in vivo efficacy models, the *EC_{50} concentration* is determined, which is the average plasma concentration at which half of the subjects show a pharmacological effect of 50%. Of note, more often than not the dose-response curves in in vivo efficacy models lack dose-dependency, and EC_{50} cannot be determined (e.g., inverted U-shaped curves),

in those cases the minimal effective dose/concentration (MED/MEC) will be calculated.

Further along the way, in vivo safety pharmacology assessments will be performed, often in parallel to efficacy testing, to examine side-effect profiles, and to determine the lowest dose/concentration at which the compound demonstrated no adverse effects (*NOAEL*). The efficacy EC_{50} values from the different in vivo animal models are then compared to the NOAEL levels from different safety and toxicology studies to determine *safety margins*, or *therapeutic index*.

Definition

The *EC_{50} concentration* stands for the average plasma concentration at which half of the subjects show a pharmacological effect of 50%. *NOAEL* stands for the lowest dose/concentration at which the drug demonstrated no adverse effects. The *safety margin* or *therapeutic index* describes the distance in order of magnitude between wanted effects, i.e., efficacy and unwanted effects, i.e., aversive side effects.

These values in combination with the PK/PD models are crucial in ranking compounds from a chemical series, and they are helpful techniques in understanding the complex behavior of specific drugs, especially with respect to estimation of clinical dosing protocols and assessment of therapeutic indices and safety margins based on preclinical in vitro and in vivo data. By appropriate use of PK/PD modeling the EC_{50}, MEC and safety margins are inter- or extrapolated and used to predict and determine whether a compound may proceed into further development, i.e., testing in higher species: dog, non-human primate, and eventually human clinical trials, or whether it will be stopped from further development. PK/PD modeling offers the greatest value if preclinical data can be modeled in combination with existing clinical data on related compounds (internal or competitors data) (Lesko et al. 2000).

In the later clinical stages of drug development, the PK/PD models are complemented with clinical efficacy, safety, and biomarker data in order to improve the model and enhance its predictive power. Recently, promising efforts emerge in which public domain medical knowledge about the relationship between biomarker responses and clinical outcomes for different diseases are used to build extensive PK/PD models (Pirisi 2003; Schlessinger and Eddy 2002). Large and structured databases with clinical findings are required for building such disease models and pooling patients' data from different databases that exist across the pharmaceutical industry would provide an invaluable source of information for disease modeling. If pharmaceutical companies were to collaborate on a precompetitive level to generate clinical databases and validate the disease models this would greatly benefit Phase 3 design and target population selection across the industry.

In conclusion, PK/PD modeling and simulation is an invaluable tool aiding crucial decision-making in drug development. Decisions on compound and dose selection, study design, or patient population, all of which can lead to a considerable reduction in cost of development. Thus, better implementation of PK/PD modeling throughout the drug discovery and development process could enhance translational success and result in less failed clinical trials and eventually better drugs entering the market (Gabrielsson and Weiner 2006).

5.4 Discussion

In general, preclinical CNS models are most often highly simplified representations of clinical features that are common across multiple conditions, such as tactile allodynia for both diabetic neuropathy and chemotherapy-induced pain or memory impairment for both Alzheimer's disease and schizophrenia. Of note, any combination of model and readout reflects a limited set of these clinical signs and their underlying pathophysiological mecha-

nisms, and therefore the choice of model and readout from the battery of available assays is an important consideration (Soderlund and Lindskog 2018). A single model should not be expected to represent all aspects of the clinical conditions, but data generated in preclinical efficacy models are nevertheless useful in predicting drug efficacy when used in conjunction with other methods, ranging from drug metabolism and pharmacokinetic analysis to electrophysiology and functional imaging, biomarkers, safety margins, and PK/PD modeling.

However, recently, animal behavioral models have been increasingly scrutinized and criticized for their role in the poor translation of novel pharmacotherapies. Indeed the number of failed clinical trials and the paucity of novel market approvals for CNS disorders such as Alzheimer's disease, pain, and major depressive disorder blatantly underscore this (Bazzari et al. 2019; Mogil 2019; Soderlund and Lindskog 2018). What is important is that efforts are being made to improve the translation of preclinical findings into clinical efficacy. Recently, several proposals were made to improve translation from animal models into human clinical situations.

First, of course, better translational models are required. Employing disease models in species more relevant to humans than rodents, such as non-human primates and the implication of new technologies such as *Clustered Regularly Interspaced Short Palindromic Repeats* (*CRISPR*) genome DNA manipulation are progressing rapidly (See King (2018)) and may increase translational success of drug development in Alzheimer's disease and other CNS indications. Second, issues of internal validity and reproducibility of animal models must be improved. Many preclinical studies suffer from poor methodological design, lack of statistical power, and bias induced by lack of blinding and randomization (see Knopp et al. (2015)). Ideally preclinical experiments should be conducted with the same experimental rigidity and standardization as clinical studies, and strict guidelines (e.g., ARRIVE guidelines) for preclinical animal studies must always be implemented and enforced (Kilkenny et al. 2010; Rice et al. 2013). Additionally, more efforts should be made to standardize models and readouts to allow for comparison and meta-analysis of preclinical data (See Wodarski et al. (2016)).

Third, to enhance the interaction between the clinic and neurobiology, the National Institute of Health has proposed to use Research Domain Criteria (RDoC) as a novel approach to categorizing psychiatric conditions (see ► http://www.nimh.nih.gov/research-priorities/rdoc/constructs/rdoc-matrix.shtml, ► Chap. 14) as opposed to classic diagnostic classification systems such as DSM or ICD. As such, future diagnostic systems cannot reflect ongoing advances in genetics, neuroscience, and cognitive science until a literature organized around these disciplines is available. The goal of the RDoC project is to provide a framework for research to transform the approach to the nosology of mental disorders (Cuthbert and Insel 2013). Thus, a system based on well-defined neurobiological constructs that will facilitate better communication between research and clinic should be created (Soderlund and Lindskog 2018). This could be useful not only for mental disorders but also other CNS disorders, such as pain.

Fourth, the industry has typically worked on a target and then tried to fit it to a patient population (often the prescribed regulatory patient groups). As such, conventional drug therapy typically considers large patient populations to be relatively homogeneous (the one-drug-fits-all approach). Only recently genetically based differences in response to a single-drug or multiple-drug treatment have been adopted and accepted (Vogenberg et al. 2010). *Personalized medicine* approaches stipulate that any given drug can be therapeutic in some individuals but ineffective in others, and some individuals experience adverse drug effects whereas others are unaffected. These findings should be back-translated into preclinical responder and non-responder analysis that could be helpful in better understanding efficacy.

Finally, an important step toward better translation is to create networks to learn from each other and collaborate on a non-competitive level. To be more successful in drug discovery, pharmaceutical industry,

academic institutions, and healthcare practitioners need to accept failures and learn from them to find new solutions for the many patients suffering from diseases of the CNS. Initiatives such as the innovative medicines initiative (IMI) Europain (► www.imieuropain.org) and IMI Paincare (► www.imi-paincare.eu) connect scientists from clinic and preclinic as well as from academia and industry to jointly improve their research and strive for better translation and analgesic drug development. Similar initiatives exist for other disease indications of the CNS (see ► www.imi.europa.eu).

Conclusion

In conclusion, this chapter has focused on conventional behavioral (animal) models and their usefulness and shortcomings in the drug discovery process. The need for greater understanding of the fundamental physiology underlying CNS diseases will persist at least as long as treatment of patients suffering from these diseases remains suboptimal. *From a scientific perspective, there are no short-to-medium term solutions that would lead to true advances in drug discovery, which would render animal studies obsolete. Nevertheless, the combination of human phMRI imaging (and other human) studies along with appropriate PK/PD modeling and more valid, robust, reliable animal studies will lead to far more effective and translatable science and ultimately novel drugs than has been the case thus far.* Furthermore, what used to be termed pharmacology is increasingly being labeled translational medicine and there are hopeful signs that some universities and medical schools are beginning to rethink how biomedical scientists ought to be trained (Webb 2014). Applying the sound principles of classical pharmacology to problems of translational medicine will aid us all in improving the way we think about and use animal models based on the careful cross fertilization from clinical, pathological, and pharmacological research.

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