Review

Back to the future of psychopharmacology: A perspective on animal models in drug discovery

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ABSTRACT

Psychopharmacology has had some bad publicity lately. Frankly, there have been some major problems along the way in developing new effective drugs for psychiatric disorders. After a prolonged period of high investments but low success rates, big pharmaceutical companies seem to retract their activities in the psychopharmacology field. Yet, the burden of mental disorders is likely to keep on growing in the next decades. In this position paper, we focus on drug development for depression and anxiety disorders, to narrow the scope of the assay. We describe the current situation of the psychopharmacology field, and analyse some of the methods and paradigms that have brought us here, but which should perhaps change to bring us even further. In addition, some of the factors contributing to the current stagnation in psychopharmacology are discussed. Finally, we suggest a number of changes that could lead to a more rational strategy for central nervous system drug development and which may circumvent some of the pitfalls leading to "me too" approaches. Central to the suggested changes, is the notion that mental disorders do not lead to several symptoms, but a network of causally related symptoms convolutes into a mental disorder. We call upon academia to put these changes in the early phases of drug development into effect.

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1. Introduction

1.1. Problems in the drug development pipeline

In drug development for central nervous system (CNS) disorders, the failure of compounds in clinical phase III trials is typically 20% higher than in other disease areas (Kaitin, 2010). Selection of candidate drugs in the preclinical drug discovery phase is apparently not optimal in the CNS field. Considering the large number of people affected and the high associated costs, there is much to gain with the development of drugs with improved efficacy. The more so since the burden of mental disorders continues to grow (WHO, 2013). Focussing on anxiety and depression, a large study performed in 30 European countries in 2010 estimated the one-year prevalence of anxiety disorders at 69.1 million people, and the one-year prevalence of mood disorders at 33.3 million. Total annual costs for these disorders were estimated at 74.4 and 113.4 billion euros, respectively (Olesen

et al., 2012). Importantly, the number of patients with depression and anxiety is likely to even further increase (Olesen et al., 2012).

In this position paper, we will address issues that may have contributed to the low success rate in CNS drug discovery in the last decade (Section 2). For this, we take anxiety and depressive disorders as an example. We will then present approaches that may help to break the logjam we are facing (Section 3). This paper is not intended to provide the reader with a complete overview. We would rather address key issues that may help to rethink currently used approaches and stimulate further discussion.

The drug development pipeline involves distinct phases and decision points at which promising leads are selected. This de novo drug development is a 10–17 year process, starting off with target discovery and validation in the preclinical phase, followed by clinical phases I–V, in which drug safety and efficacy have to be established (Ginsburg and McCarthy, 2001; Nwaka and Ridley, 2003; Fishburn, 2013). In general, only a small percentage (15%) of candidate drugs will make it to actual clinical use. For CNS drugs, this percentage is with 8% drastically lower. The identification of valid targets at the beginning of the pipeline is crucial for the quality of the following steps (Kola and Landis, 2004). Target discovery and validation involve genomic research, cell-based assays and animal research. This is followed by lead identification and optimization, in order to identify compounds that can modify

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target activity in a way that would improve the disease state. These first pre-clinical steps suffer from problems typical for neuropsychiatric disorders. As outlined in Section 2, these problems may well contribute to the higher rate of failure of drugs in CNS research. To overcome these problems, we feel that academia can and should play an important role, especially in the early phases of drug development (Kaitin, 2010).

1.2. The role of academia

There are several players along the drug development pipeline such as academia, small pharmaceutical and biotechnology companies, contract research organizations and large pharmaceutical companies. Especially in the early phases of drug development (target discovery and validation, and development of suitable tests for lead optimization), there is room for further improvement. Much can be gained by eliminating molecules with little potential early in the discovery process (Dimitri, 2011). And academia may well be the player best suited to take the lead. One of the core activities of academia is gathering knowledge through fundamental research. Unravelling pathological mechanisms, discovery of new therapeutic targets and development of valid in vivo and in vitro test models are activities classically befitting academia. Therefore, the improvement of the initial steps in the drug development pipeline is in the hands of universities and research institutes (Kaitin, 2010; Fishburn, 2013). Academia should re-evaluate the models that were used in the past for target discovery and validation, and refresh some of the "old" paradigms. Now that large pharmaceutical companies restrict their CNS drug development because of the low chance of success (Miller, 2010), maybe academia can be the throttle letting the drug pipeline roar again.

2. Trouble shooting the target discovery process

We will set the stage with a reflection on the current state of affairs and the problems that are particularly relevant for CNS drug discovery, focusing on anxiety and depression.

2.1. Complex disorders, what is the fabric of the mind?

Emotions, memory and behaviour emerge from the brain in a complex manner. Human behaviour is formed by interplay between genetic factors, past experience and the current environmental settings. Because of this complexity, dysfunctional behaviours are difficult to superimpose on brain function. While we believe that the brain is the substrate of thoughts and emotions, we are often not able to directly relate a specific behaviour to the causative neurobiological substrate that it emerges from on a one-to-one basis (Feltman Barrett, 2009). This gap in our knowledge, linking biological processes directly to behavioural outcome, prevents us from finding molecular targets that are casually related to specific behaviours and their dysfunctions.

2.1.1. Multi-genetic disorders

The most elementary neurobiological functions like propagation of action potentials, chemical signal transduction or synaptogenesis involve many proteins and therefore, a large number of genes. Thus, the malfunctioning of a certain neurobiological feature may be caused by many different factors. The mechanisms underlying psychological functions consist of many neurobiological features. It is not surprising that psychiatric disorders like anxiety or depressive disorders are complex and the aetiology is poorly understood (Tsankova et al., 2007; Nestler and Hyman, 2010). This is a main concern for the process of target discovery,

the strategy of which is based on knowledge of the pathological mechanism.

2.1.2. Environmental factors

Environmental factors can influence behaviour by directly changing the emotional status or cognitive processes, like memory formation or retrieval. Particular experiences combined with a genetic predisposition may lead to anxiety or depressive disorders (Caspi et al., 2003, 2010; van Winkel et al., 2014). Such experiences are often difficult to quantify. The perceived severity of a typical may differ between different individuals according to past experiences. For example, early life stress but also temperament or coping style may influence the perceived burden of a stressor for an individual and thus the chance of developing psychopathology (McEwen and Stellar, 1993). This means that environmental factors leading to depression or anxiety disorders can be variable in nature and have a sizeable inter-individual effect. Interactions between environmental and genetic (risk) factors are therefore difficult to predict. However, understanding this interplay between nature and nurture is important for our knowledge of the aetiology of depression and anxiety disorders and the distinction between causative epigenetic changes and bystander effects (Tsankova et al., 2007). This complicates the characterization of pathways involved in the neuropathology underlying anxiety and depressive disorders.

2.1.3. Diagnostic criteria

Diagnosis of anxiety or depressive disorders using either the Diagnostic and Statistical Manual of Mental Disorders (DSM) (APA, 2013) or the International Classification of Diseases (ICD) (Chapter V) (CDC, 2010) is based upon symptomatology. The criteria for a major depressive disorder for instance involve a wide range of symptoms, leading to large heterogeneity in the population of patients diagnosed with a major depressive disorder. Moreover, the DSM based diagnosis for depression has a poor reliability (Clarke et al., 2013; Friedman et al., 2013; Narrow et al., 2013; Regier et al., 2013). Furthermore, it does not take into account the severity of the depression or comorbidity with anxiety, which are both important predictors for the outcome of pharmacotherapy (Fournier et al., 2010). Unrecognized misdiagnosis can lead to erroneous prescription of medication that will result in a lower estimate of the efficacy of the drug used. Reliable tools for diagnosis including the severity of depression may result in a better prediction of the efficacy of antidepressants and fewer patients that will be qualified as pharmacoresistant.

2.2. Preclinical research approach

In preclinical research, experimental animals are used for the discovery and validation of therapeutic targets. The purpose of these animal models is to mimic aspects of a disease in a non-human animal in order to perform experimental research without harming humans. To be representative of the human condition, animal models should satisfy certain validating criteria, first introduced by Willner (1984). Although the exact definitions have shifted over the years and differ between researchers, the generally held standard is that a good animal model should have construct, face, and predictive validity. In the case of animal disease models for psychiatric disorders however, we encounter several problems in trying to fulfil these criteria.

2.2.1. Validity of animal models
2.2.1.1. Construct validity. Construct validity assumes that the human disease and the animal model share a common pathological mechanism. Since our knowledge of the underlying neurobiological

mechanisms of these diseases is inadequate (see Section 2), the concept of construct validity is hard to fulfil in psychopharmacology. In Section 3, we offer some suggestions on approaches that may help improve our understanding of the pathophysiology and with that construct validity of animal models. Often, the term "model" is restricted to animal models that have construct validity, whereas the term "test" is used for all other approaches. For ease of writing we have not applied this distinction consistently in this paper.

2.2.1.2. Face validity. For face validity, the apparent similarity between disease and model is evaluated, as is the absence of features that are not seen clinically (Willner, 1984). In psychopharmacology however, face validity is a subjective measure based on anthropomorphism. Whether the human features we attribute to the experimental animal really match the expressed animal behaviour, always remains a matter of interpretation. The forced swimming test for example was introduced by Porsolt et al. (1977) as a model of behavioural despair: "*The method is based on the observation that a rat, when forced to swim in a situation from which there is no escape, will, after an initial period of vigorous activity, eventually cease to move altogether making only those movements necessary to keep its head above water. We think that this characteristic and readily identifiable behavioural immobility, indicates a state of despair in which the rat has learned that escape is impossible and resigns itself to the experimental conditions*" (Porsolt et al., 1978).

As reviewed by Borsini and Meli (1988) however, subsequent studies showed that the observed immobility is more likely to reflect an adaptive response than despair, since immobility was not associated with inescapability during water immersion, forced swimming was not associated with behavioural deficits as induced by learned helplessness procedures and lower fear levels were observed upon re-exposure of rats to the water (Willner, 1984; Borsini and Meli, 1988). Yet, until today the test is frequently referred to as measuring behavioural despair, demonstrating the different interpretations researcher hold on animal behaviour (Bouwknecht, 2014; Wang et al., 2014; Jindal et al., 2015). Irrespective of the debate about its relevance to depression, the forced swimming test is a widely used screen for antidepressant drug action because of its high predictive validity.

2.2.1.3. Predictive validity. Over the years, an animal model has been considered to have predictive validity if it detects compounds that are in clinical use for the disorder under study, irrespective of their molecular structure and mechanism of action. Furthermore, the model should show specificity (no false-positive hits) and sensitivity (no false-negative findings). The high rate of failure of drugs in clinical phase III trials however suggests that currently used models have modest selectivity at best. A recent meta-analysis on drugs tested in the isolation-induced distress vocalization test in guinea pigs indeed showed that of the 56 experimental drugs tested for anxiolytic properties, 45 drugs were reported active, including three neurokinin (NK_1) receptor antagonists which were later reported inactive in clinical trials (Groenink et al., 2014). The case of the development of neurokinin (NK_1) receptor antagonists is further elaborated upon in Fig. 1.

A factor that may contribute to moderate specificity is the fact that often the read-out of a model is not a symptom of anxiety or depression at all, for example locomotor activity of olfactory-bulbectomized rats in an open field test (Hendriksen et al., 2014) or temperature in the stress-induced hyperthermia test (Olivier et al., 2003). Other substances may modulate such a read-out, and be found effective in the model, without having anxiolytic or antidepressant properties (Olivier et al., 2003).

More importantly though, is the vicious circle we seem to have created around the predictive validity criterion. Currently available treatment is not beneficial in all patients (e.g. 25% non-responders in case of anxiety disorders) and has considerable side effects. Therefore, we aim to develop novel drugs with improved efficacy. To achieve this, we validate models based on registered drugs with limited efficacy, and then we rely on these models to identify novel drugs with improved efficacy. As a consequence, currently used models are likely to detect compounds with an already known and comparable mechanism of action (*me-too* drugs). More problematic however is the fact that the current approach is prone to false-negative findings. The ability to identify the efficacy of known drugs does not mean that the model will also detect novel drugs with other mechanisms of action. In case of animals of anxiety, almost all models detect the anxiolytic actions of benzodiazepines, but only few respond to selective serotonin re-uptake inhibitors (SSRIs), which are equally (or more) beneficial in the treatment of anxiety disorders in humans (Chessick et al., 2006; Baldwin et al., 2014).

2.2.1.3.1. Problems defining clinical efficacy of drugs. A further limitation to defining predictive validity of an animal model lies in the assessment of clinical efficacy. The design and conduct of clinical studies present several problems, including large placebo responses, patient selection and arbitrary diagnosis (Zimmerman et al., 2002; Brody et al., 2011). In addition, in clinical practice, beneficial effects of psycho-active drugs often appear limited to subgroups of patients. If clinical efficacy may vary from patient to patient, it becomes hard to objectively define the predictive value of an animal model based on clinical efficacy.

In all, the importance of predictive validity may have been overrated. In addition to the issues addressed above, the emphasis on predictive validity of models may also have hampered the introduction and acceptance of novel, improved animal models. Models that may tap on different neurobiological mechanisms, and may well have improved translational value (Ennaceur, 2014).

2.2.2. Drug regimens

In our view, treating the different symptoms may eventually result in curing the disorder (see Section 3.2.2). Improved relieve of symptoms may be achieved through augmentation of cognitive therapy, or augmentation of currently applied pharmacotherapy of anxiety and depression. In addition, assuming that neuroplasticity is an important factor in the pathology of these diseases, the time span needed to relieve symptoms is more likely in the range of days or weeks, than in hours.

2.2.2.1. Acute versus chronic drug treatment. To optimize validity of data obtained in animal models, both treatment duration and time that treatment is started after disease induction should be realistic in terms of what is clinically relevant. Currently, that would imply a drug treatment period of two to six months for anxiety and depression. Treatment effects in animals should also improve upon prolonged administration, or be at least similar to acute effects. Yet, the majority of animal studies are limited to studying acute treatment effects. The emphasis on animal models designed to detect acute treatment effects may have reduced clinical predictability. Drugs that only exert beneficial effects upon prolonged treatment may be discarded, whereas drugs that are found active in acute models may tap on mechanisms less relevant to the actual disorder.

2.2.2.2. Monotherapy, multi-target drugs and behavioural therapy. We have come a long way since the discovery of chlorpromazine and Librium in the 1950–60s. Over the years,

A

Neurokinin (NK)$_1$ receptors as target for anxiety disorders

As discussed in this paper, the fact that NK$_1$ receptor antagonists did not exert beneficial effects in patients with anxiety disorders may lie in selecting the wrong target, in limited predictive value of the animal models used, as well as issues concerning the outcome of clinical trials. The selection of NK$_1$ receptors as target for the treatment of anxiety disorders, was primarily based on neuroanatomical location and the involvement of the substance P/tachykinin system in the regulation of emotional regulation (for reviews see Ebner et al., 2009; Herpfer and Lieb, 2005). A considerable number of NK$_1$ Receptor antagonists has been developed and tested for their antidepressant and anxiolytic action. With regard to anxiety disorder, four of these NK$_1$ Receptor antagonists have been tested in patients suffering from anxiety disorders. The NK$_1$ receptor antagonists aprepitant (also known as MK-689, (Kwako et al., 2015)) and GR205171 (also known as vofopitant (Mathew et al., 2011)) had no effect on PTSD symptoms, whereas effects of LY686017 (also known as tradipitant or VLY-686 (Tauscher et al., 2010)) did not differ from placebo in patients with social anxiety disorder. To our knowledge, study results of the two clinical studies performed with vestipitant on social anxiety disorder have not been reported (NCT00343707, NCT00403962).

Target selection

☛NK1 receptors located in brain areas involved in emotional regulation

☛Stressful situations associated with increased substance P levels, in both humans and animals

☛Upregulation of tachykinin transmission in individuals suffering from stress-related disorders

☛Administration of substance P induces anxiety-like behaviours in animals

☛Spatial overlap of substance P/neurokinin system with monoamine systems, that are involved in anxiety and depression

Below, we have summarized the effects of NK$_1$ receptor antagonists in models of anxiety. The overview is limited to compounds that have been tested in clinical trials, and of which the effects in patients have been reported (for a complete overview of the effects of NK$_1$ receptor antagonists in animal models see Griebel and Holmes, 2013). Of the 26 experiments performed, 20 studies reported anxiolytic-like effects. In the remaining 6 studies, drug treatment did not differ from vehicle control. Although the number of positive tests does not inform about the actual effect size induced, these results do suggest that the compounds exert anxiolytic-like effects in these tests. All tests however, are based on suppression of an adequate behavioural response, not on the suppression of excessive or pathological anxiety. Among other things, this may explain why the drugs had positive effects in animal models but lacked effect in patients. It appears also difficult to relate the choice for social anxiety disorder rand PTSD to results in particular tests. For instance, NK$_1$ receptor antagonist do not appear particularly effective in vocalization and social interaction tests. Finally, it may well be that not all studies performed have also been reported. For instance, we have only found one research paper reporting on the effects of LY686017 in an animal test supposed to be related to anxiety (Taucher et al., 2010). In the behavioural assay used, the inhibition of NK$_1$ agonist-induced foot tapping in gerbils is used to assess central nervous system (CNS) activity of tachykinin NK$_1$ receptor antagonists. It remains unclear whether patient selection for clinical trials or limited value of animal models used, are at fault here. It is clear however, that the encouraging preclinical results did not fulfill their promise.

Fig. 1. Neurokinin (NK)1 receptors as target for anxiety disorders (Ebner et al., 2009; Griebel and Holmes, 2013; Herpfer and Lieb, 2005; Kwako et al., 2015; Mathew et al., 2011; Tauscher et al., 2010).

new discoveries in psychopharmacology have improved the quality of life of many patients. It is well feasible that the approaches used in preclinical research were sufficient to identify the low hanging fruit: drug classes targeting the most prominent pathways suited to improve symptoms of less complicated disease states.

B

Drug evaluation in animal models			
Animal model	*reduced anxiety*	*inactive*	*species*
Conflict test	-	GR205171	hamster
Distress vocalization	Aprepitant	-	guinea pig
	GR205171 (2x)	-	guinea pig
	GR205171 (3x)	-	rat
	GR205171	-	mouse
Elevated plus maze	Aprepitant	-	gerbil
	GR205171	-	gerbil
	-	GR205171	rat
	-	GR205171	hamster
Fear potentiated startle	GR205171	-	gerbil
Foot tapping (NK$_1$ R agonist –induced)	Aprepitant	-	gerbil
	LY686017	-	gerbil
Foot tapping (stress-induced)	Aprepitant	-	gerbil
	GR205171	-	gerbil
Marble burying	Aprepitant	-	mouse
	GR205171 (2x)	-	mouse
Social interaction	Aprepitant	-	gerbil
	GR205171 (2x)	GR205171	gerbil
	-	GR205171	rat
T-tube test	-	GR205171	hamster
Vogel conflict	Aprepitant	-	rat
	GR205171	-	rat
Drug evaluation in clinical trials. Reported effects of NK$_1$ receptor antagonists			
Disorder	*reduced anxiety*	*inactive*	
Post-traumatic stress disorder	-	*Aprepitant*	
	-	*GR205171*	
Social anxiety disorder	-	*LY686017*	

Fig. 1. Continued.

From this perspective, we may want to briefly touch upon target strategy when discussing success rate in CNS drug discovery. Is it reasonable to expect that we can cure these complex disorders with just a single drug? Multi-target agents may be better suited to improve core and co-morbid symptoms of certain subgroups of patients than selective drugs (Millan, 2006, 2009).

Multi-target approach: battling mental disorders symptom by symptom

Multi target therapy is one of the logical forthcoming strategies when mental disorders are dissected into different symptoms for research purposes. The multiple target therapies for mental disorders can be divided into two different approaches, multiple targets that focus on the same symptom, or different targets that each address different symptoms of the same mental disorder. Ultimately, this can be achieved within one drug acting on different targets or, by combination of drugs. The use of multiple targets for the same symptom may allow for lower dosing for each drug and thereby reducing the risk of side effects. The ultimate goal for this would be to achieve a synergistic effect of these drugs on the reduction of the symptom. Examples for this approach are the triple reuptake inhibitors, which enhance the levels of serotonin, dopamine and noradrenalin by blocking transporter-mediated reuptake.This combined effect on three monoamine systems result in long lasting effects on the brain reward system (Prins et al., 2012). However, the clinical advantage still has to be established.

When the molecular players of a functional neuronal mechanism underlying a certain symptom are identified, it becomes possible to select two or more targets to adjust the mechanism in the desired direction. A typical example for this can be found in the amygdala. The neuropeptides corticotropin releasing factor (CRF) and neuropeptide Y (NPY) have opposite functions in the regulation of anxiety. The balance between the activity of both neuropeptides in the amygdala appears to determine the sensitivity to stressors and the level of anxiety (Heilig et al., 1994; Sajdyk et al., 2006; Hendriksen et al., 2012). Figure A (modified after Heilig et al., 1994) shows a scheme of the balance between CRF and NPY and the resulting effect on anxiety. The value of this basal neurobiological knowledge   lies in the translation to pharmacology. By combining the two mechanisms (CRF receptor antagonist and NPY receptor agonists), beneficial effects may be enhanced while the side effects accompanying either CRF antagonism or NPY agonism may be reduced, since lower dosages of the two individual drugs can suffice.
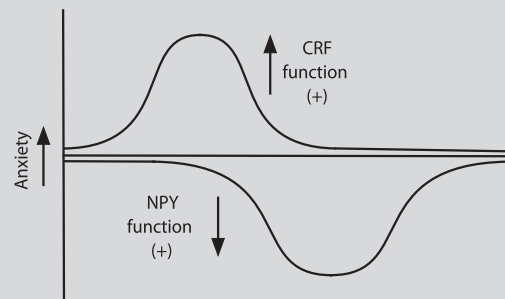
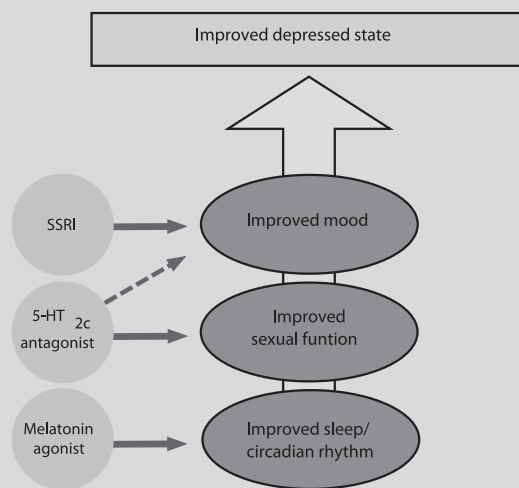Figure A. Regulation of anxiety by amygdalar CRF and NPY functionality

On the level of the disorder it might also be possible to obtain a synergistic effect by acting on different targets for different symptoms. For example, the "sad mood" symptom of depression can be improved by blocking the serotonin reuptake transporter (figure B). $5-HT_{2C}$ antagonists is reported to improve sexual function (Millan, 2005; Rosenzweig-Lipson et al., 2007) and melatonin agonist agomelatine helps to reschedule disrupted circadian rhythms (Lemoine et al., 2007; Guardiola-Lemaitre et al., 2014). Interestingly, agomelatine not only acts on melatonine receptors but also acts as an antagonist for $5-HT_{2C}$ receptors. Therapeutic effectiveness was shown for agomelatine in patients with major depression (Loo et al., 2002; Millan, 2005). As different depressed patients may suffer from different symptoms, depending on the "type of depression" the relevance of a multi target strategy becomes more and more clear (Korte et al., 2015)

Figure B. Multiple targets for multiple symptoms: symptom by symtom improvment

Fig. 2. Multi-target approach: battling mental disorders symptom by symptom (Prins et al., 2012; Heilig et al., 1994; Sajdyk et al., 2006; Hendriksen et al., 2012; Millan, 2005; Rosenzweig-Lipson et al., 2007; Lemoine et al., 2007; Guardiola-Lemaitre et al., 2014; Loo et al., 2002; Korte et al., 2015.

For an extended explanation on multi-target approach and some typical examples see Fig. 2.

The development of add-on therapy, drugs that augment the effects of cognitive-behavioural therapy may proof another fruitful

Table 1

PubMed search on original research papers studying depression and/or antidepressant effects using forced swimming test or intracranial self-stimulation in rodents.

Depression model used	1985–2015	Total (since 1964)	Search string Pubmed (January 15, 2015)
Research papers on depression not limited to a particular model	18,518	21,750	(rats [tiab] or mice [tiab]) AND (antidepress∗ [tiab] OR depressi∗ [tiab] OR anhedoni∗ [tiab]) NOT (addicti∗ [tiab] OR pain [tiab]) NOT review
Research papers on depression reporting on forced swimming test (FST)	3083	3095	(forced swimming [tiab] OR forced swimming test OR forced swim [tiab] OR forced swim test [tiab]) AND (rats [tiab] or mice [tiab]) AND (antidepress∗ [tiab] OR depressi∗ [tiab] OR anhedoni∗ [tiab]) NOT (addicti∗ [tiab] OR pain [tiab]) NOT review
Research papers on depression reporting on intracranial self-stimulation (ICSS)	75	84	intracranial self-stimulation [tiab] AND (rats [tiab] or mice [tiab]) AND (antidepress∗ [tiab] OR depressi∗ [tiab] OR anhedoni∗ [tiab]) NOT (addicti∗ [tiab] OR pain [tiab]) NOT review

[tiab] words in title or abstract. Papers studying addiction (addicti*) or pain were excluded from the search.
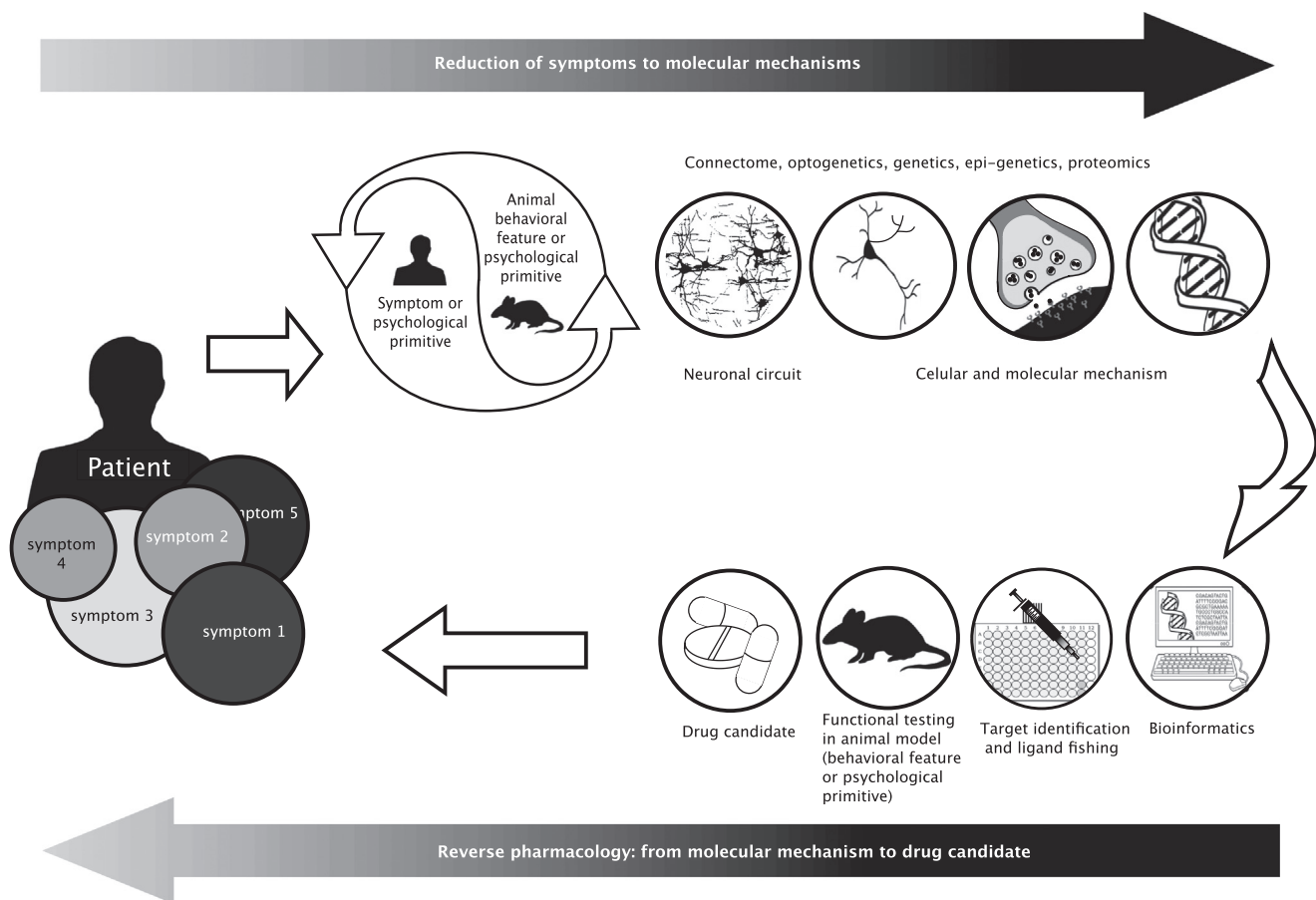

Fig. 3. This still leaves us with the problem of translating symptoms to animal behaviour. Technical advances of the last decade such as the extensive knowledge about the genome of rodents and humans, mapping of neural pathways underlying human brain functions in the connectome project, proteomics and optogenetics, might provide us with new possibilities to accomplish the task of behavioural reductionism and get us on track with reversed pharmacology for psychiatric diseases.

approach. Although cognitive behavioural therapy is a generally effective treatment for anxiety disorders, a large group of patients experiences meaningful residual symptoms. Recent advances in our understanding of extinction learning have paved the way to novel clinical strategies to augment exposure-based treatments, for example with D-cycloserine (Hofmann et al., 2015).

2.2.3. "Nothing is more expensive than a missed opportunity" (H. Jackson Brown Jr.)

Clearly, we are not the first to address these issues. Back in 1984, after reviewing 18 animal models of depression, Willner concluded, that intra-cranial self-stimulation was the model with the strongest overall validity for depression (Willner, 1984). Yet, a

recent PubMed search showed that since then, only 75 original research papers have been published studying antidepressant mechanisms and drug properties in the intracranial self-stimulation model in rodents, on a total of 18,518 from 1985 until January 2015 (Table 1). Forced swimming test on the other hand, which received similar scores on predictive and face validity, but lacked construct validity according to Willners criteria, has been reported upon 3083 times since then (Table 1). The trouble with opportunity is that it always comes disguised as hard work.

Current state of affairs suggest that by investing in easy and fast tests for high throughput screening in the early stages of drug development, we may have missed truly novel mechanisms of action and important breakthroughs.

2.2.4. Quality of research

Another issue that may have hampered identification of relevant targets and drugs is the quality of research. By now it is clear that experimental animal studies are often performed without random allocation to treatment groups, and without blind outcome assessment (Ioannidis, 2005; Landis et al., 2012; Hirst et al., 2014). Both could, unintentionally, result in overestimation of drug effects and false positive findings. In addition, sample size calculations are hardly reported. Many studies are likely underpowered (Button et al., 2013). On one hand, this could lead us to discard an active drug, which is not identified as such, due to lack of power. On the other hand, underpowered studies may give rise to an overestimation of drug efficacy, as a result of the winners curse (Nuzzo, 2014). In either case, the improper conduct of studies may contribute to selecting the wrong drugs.

A related issue is publication bias. The trend to publish positive but not negative findings, and the lack of replication studies may cloud our judgement on the importance of targets and mechanisms under study (Sena et al., 2010). Although less well studied, there is no reason to assume that issues regarding quality of research are different in psychopharmacology than in other fields of CNS research (Wahlsten et al., 2006; Button et al., 2013; Groenink et al., 2014).

2.2.5. Typical human diseases

A more general problem with animal models for psychiatric disease is the typical human nature of some of the symptoms that amount to a certain mental disorder. Many of the features of psychiatric disorders are typically human traits or the lack thereof. Animals used in experiments are often not capable of the same cognitive, emotional, and behavioural expressions as humans. Some cortical areas in the human brain, such as a region in the temporo-parietal junction, are involved in typical human abilities like reasoning about the contents of mental states, such as beliefs (Saxe, 2006). It is generally believed rodents not able to have the sense of a foreshortened future or the wish to end its own life. Other symptoms like nightmares or feelings of sadness are impossible to measure in an animal. This means that it will be impossible for most psychiatric diseases to be modelled in an animal in such a way that all clinical features are represented.

3. Future directives for preclinical research

3.1. Back to the future of psychopharmacology

In the nineteen sixties, CNS drug development flourished because of advanced techniques in chemistry, a better understanding of psychology and physiology and the progressive improvement of diagnostic tools thanks to the development of the Diagnostic and Statistical Manual of Mental Disorders (DSM) (APA, 2013). The advantages back then gave CNS drug development a boost that has now come to a halt. CNS drug discovery and especially development of new antidepressants has stagnated (Riordan and Cutler, 2012; Hendrie et al., 2013). However, there might be some light shining at the horizon. A combination of new techniques like optogenetics (Deisseroth et al., 2006), the extensive knowledge about the genome of rodents and humans (Genome, 2001; Gibbs et al., 2004; DOE, 2015), and the mapping of neural pathways underlying human brain functions in the connectome project (Bardin, 2012; Toga et al., 2015) may provide us with tools to give preclinical psychopharmacology research another boost. Be it as it may, development of new techniques in itself will not be enough. A shift in the in vivo paradigms used may also be mandatory.

In pursuit of rationale processes for the discovery of new therapeutic targets, the gap in our understanding of the neuronal substrate that is causally related to a specific behaviour must be bridged. Only then we can rationally modify the neuronal substrate that alters the brain state that is responsible for a corresponding emotion or behaviour.

3.2. How to accomplish this? The value of endophenotypes

First of all, we have to abandon the idea that an anxiety disorder or depression can be modelled in a rodent. The realization that we are unable to translate all aspects of a psychiatric disorder into one animal model must lead to an approach that uses simple, measurable behaviours as markers for the more complex human pathology. To accomplish this, the concept of endophenotypes was introduced by Gottesman (Gottesman and Gould, 2003; Gould and Gottesman, 2006). The endophenotype approach aims to separate the complex human disease into simpler, heritable, measurable features in animal models. These markers must have translational value for the human condition. Although one such a measurable feature may be meagre by itself, together with other endophenotypic features, a more comprehensive picture of the human disorder may be composed. Whether the features that are studied have to be hereditary can be questioned. It is generally agreed upon that genetic predisposition may increase the risk for the development of psychiatric disorders. However, in case of anxiety disorders and depression there is also a major contribution of environmental factors. Therefore, the features we study in animal models may not need to be hereditary at all. Models using environment factors (e.g. chronic stress) to induce a certain measurable behavioural feature may be just as valid in this respect.

3.2.1. How to capture construct validity?

As described in Section 2.2.1.1, animal models used in psychopharmacology most often lack construct validity. Because of this, rational target discovery and validation is very problematic. A solution for this may be to deconstruct disorders to the symptoms they consist of. The key element in the strategy to split up the human disorder in different, symptom-like behaviours in animal models is the choice of a "measurable feature". The real leap forward here is to choose measurable behaviours that show elementary functions that can be identified as distinct neurobiological actions. This means that if a specific neurobiological substrate is activated, the associated specific behaviour should be executed obligatorily and exclusively. Vice versa, if we observe that specific behaviour, we can be certain of the cellular network and biochemical pathways it arose from in the brain. This reductionism of behaviour to activities of neurobiological processes requires that these measurable behavioural features are simple and unambiguous. More complex behaviours will not be governed consistently by the same neurobiological substrate. For instance, behaviour elicited by touching an electrified shock probe in the shock-probe burying test, will be diffuse. Except for the actual burying, it will also elicit freezing, stretched attend postures and exploration (Groenink et al., 1995). In the fear potentiated startle test on the other hand, the eliciting startling noise will generate one specific and well-defined reflex (Lee et al., 1996). The magnitude of this reflex however may be modulated by the level of fear elicited by additional (conditioned) stimuli (Veening et al., 2009).

This microreduction of distinct behavioural features into brain functions will not only enable us to explain the behaviour in terms of brain states and biochemical processes, but will also give us the means to make valid predictions on how changes at the molecular

level cause distinct behavioural changes. This brings us closer to the next step in which we can rationally select putative targets that have a fair chance of affecting the selected behaviour. The fact that in this way only parts of the human disorder will be addressed and the human disorder composed of several symptoms will probably shift treatment towards multi-target approaches and poly-therapy.

The rational design of drugs aimed at augmenting outcome of behavioural therapy may also benefit from a better understanding of molecular systems underlying certain behavioural processes. For example, the use of D-cycloserine for the facilitation of extinction therapy for phobias (Hofmann et al., 2015) or the use of MDMA, hydrocortisone or beta-blockers (de Kleine et al., 2013) in the treatment of posttraumatic stress disorder (PTSD) show the potential of these add-on drugs. The drug itself does not impact on the disease, but it facilitates the behavioural therapy. These examples illustrate how knowledge of the neurobiological substrate involved in extinction and reconsolidation processes enabled the rational selection of drug-able targets that facilitate the actions of this particular neurobiological substrate.

3.2.2. Symptoms building up to a disorder

One of the essential questions that should be addressed is which behaviours or behavioural features are elementary enough to be represented in the brain by clear and consistently identifiable neurobiological processes? Can and should these elementary behavioural features be similar to human symptoms of psychiatric disorders? Or do we have to deconstruct behaviour further to even more fundamental characteristics? The approach of taking symptoms of human disorders as basis for animal models has a rational base. Mental disorders can be conceptualized as clusters of causally related symptoms (Cramer et al., 2010). Importantly, the principle idea of this approach is that mental disorders do not lead to several symptoms, but a network of causally related symptoms convolutes into a mental disorder. Network models of symptoms of mental disorders taken from the DSM IV (APA, 2013) agree with this idea. In addition, comorbidity can also be explained in these network models, as the association of symptoms that are expressed in two disorders. The strength of the connections between different symptoms predicts the real life rate of comorbidity (Borsboom et al., 2011; Borsboom and Cramer, 2013). In this view, symptoms are the real building blocks of psychiatric disorders, and as such they would be the ideal behavioural features to study in animal models. This still leaves us with the problem of translating symptoms to animal behaviour but this is much less problematic than modelling the whole mental disorder (in fact all symptoms together) in one animal model.

3.2.3. Symptoms or psychological primitives?

But can behaviours that represent symptoms be reduced to causal neurobiological actions? Because only then, we can infer realistic estimates of how features at the molecular level may influence behaviour, the symptom in this case. Do we need basal behavioural components that cannot be further deconstructed into smaller parts, the so-called "psychological primitives"? Lisa Feldman Barrett makes an interesting case for these elementary features of behaviour. She advocates reducing behaviours or emotions into basic, psychological primitives (Feltman Barrett, 2009). If we want to improve the baking of bread it is not helpful to make slices of bread to study how we can improve it. We need the recipe, and knowledge of the function of the ingredients, if we want to make educated changes to the bread.

In other words: if the behaviours we want to change are deconstructed into "chunks" of behaviour or emotions, but those chunks are not the essential building blocks, we cannot expect to gain information from these chunks about the behaviour they amount to. Here the fields of neurobiology and psychology should collide and fill this gap that keeps us from rational target discovery.

3.2.4. Reverse pharmacology

Because the classical (forward) pharmacological approach, where compounds are screened in animal disease models to find beneficial effects, has failed to produce new therapeutic targets in the last 15 years and the low hanging fruit in drug discovery has already been picked, we have to tackle the elephant in the room. A more rational approach based on knowledge of underlying disease mechanisms is crucial. Reverse- or target-based pharmacology uses the understanding of molecular mechanisms to develop new targets (Takenaka, 2001). It is probably fair to say that only if the scientific community succeeds in the reduction of behaviour to causally related (molecular) brain mechanisms, a reverse pharmacology approach can be applied to the field of psychiatric disorders (see Fig. 3). One of the foremost important things for academia is gaining and spreading of knowledge. So this tremendous task should be primarily a duty for academia.

3.3. New techniques and projects

Technical advances of the last decade might provide us with just the right tools to get reversed pharmacology for psychiatric diseases on track. The human genome project (DOE, 2015) provides us with the essential genomic information and makes every gene potentially identifiable. The information coming forth from the human, rat and mouse genome projects (Bouck et al., 2000; Genome, 2001; Venter et al., 2001; Jacob and Kwitek, 2002; Waterston et al., 2002; Gibbs et al., 2004) does not directly provide us with a better understanding of brain mechanisms underlying behavioural features, but it does provide knowledge that can be used in techniques as optogenetics and building of the connectome (Deisseroth et al., 2006; Bardin, 2012; Toga et al., 2015). Optogenetics uses light to activate a precise, genetically targeted neuronal circuit (Deisseroth et al., 2006). The possibility to specifically switch neurons on and off is a valuable tool in the identification of neuronal circuits involved in for instance cocaine addiction (Witten et al., 2010), or to identify the pathways of the amygdalar complex involved in fear conditioning (Haubensak et al., 2010; Johansen et al., 2012). Combined with the knowledge of the genome, this technique provides a huge support for mapping neuronal substrates that are causally related to basal behavioural features. Integration data of behavioural functional brain circuit and the genetic makeup of this circuit is not enough though. Brain circuits do not stand alone, but are integrated in other circuits receiving input from other areas and generating output to other brain areas or to the periphery. It will be essential to include the connections of a behavioural functional circuit with "the rest of the brain", to enable educated assumptions about the effects alterations in this circuit may induce (Fig. 3).

3.3.1. Other promising strategies

While fundamental research suits universities and research institutions, the pharmaceutical industry may benefit more from a pragmatic and readily applicable approach, which does not necessarily build on fundamental knowledge about the pathophysiology. By screening the effects of a large number of compounds on a more or less fixed set of behaviours, a database can be build with drug–behaviour profiles. The behavioural profile of unknown compounds can be determined and screened against this drug–behaviour profile database. In this way, behavioural profiles similar to that of known psychoactive drugs can be recognized.

This pragmatic method further increases our knowledge about the drug–behaviour interactions, while circumventing the problems with animal models. PsychoGenics Inc for instance implemented this strategy for CNS drug research. Besides several research solutions making use of the more traditional animal models, PsychoGenics Inc also developed a box in which several behavioural features of mice can be recorded (SmartCubes®). These behaviours are not directly disease-related, but the strength of this strategy lies in the drug–behaviour profile database that they have generated (Alexandrov et al., 2015). While this is a smart and innovative strategy that will be advantageous for the immediate problems of pharmaceutical companies, it will not elucidate the underlying pathological mechanisms of psychiatric disorders. Again here the distinct roles for the industry and academia are shown.

4. Conclusion

Over the past sixty years, experimental animal studies have contributed considerably to new discoveries in psychopharmacology. The resulting introduction of psychotropic drugs into the clinic has improved the quality of life of many patients. Innovation however seems to have come to a halt, leaving the more complex disease states untreated. As outlined in this paper, academic institutions could play an important role in a successful second beginning, by focussing their research on basal behavioural features, reverse pharmacology and novel techniques. This however will be a long-term endeavour. For the short and medium term, opportunities may lie in behavioural drug profile databases, augmentation of behavioural therapy, as well as in multi-target therapy.

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