

# Mouse Models of Depression

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## 1. Introduction

Mood disorders such as depression are the most prevalent diseases amongst psychiatric disorders and a leading cause for disability worldwide. Modelling mood disorders in animals is a major challenge considering the complex nature of the diseases. Nevertheless, existing models and paradigms have proven extremely useful not only with respect to the identification and improvement of therapeutic substances, but also regarding the validation of neurobiological underpinnings.

In this chapter we introduce some basic concepts with respect to the questions what and how animal models are able to contribute to our understanding of mood disorders. The chapter centers on the mouse as the key model organism in neuropsychiatric research and gives an overview on the most popular behavioural tests and models with a particular focus on major depression. This chapter is not meant to be fully exhaustive considering the comprehensive literature on this topic, but rather intends to point out general concepts and controversies in the field, for instance to clarify the differences between animal models, behavioural tests and antidepressant screening paradigms. Moreover, the chapter will call the attention to some novel strategies and technologies that are envisioned to significantly impact the future development and application of animal models of mood disorders. Finally, the chapter will introduce latest views on the importance of introducing gene  $\times$  environment interactions into animal models of etiologic relevance.

### 1.1 Epidemiology of depression

Depression, officially termed major depressive disorder (MDD) ranks among the most prevalent diseases worldwide. According to the estimations of the World Health Organization, depression will be the second leading cause of disability in 2020 (Murray and Lopez, 1997). Recent epidemiological studies indicate that severe forms of depression affect 2-5% of the population worldwide, and up to 20% are affected by milder forms of the disease (Kessler et al., 2003). Moreover, depressive patients have a 2-4 fold increased risk of developing cardiovascular diseases and 10-15% of individuals with major depression commit suicide (Keck, 2006).

Up to now, depression is diagnosed according to criteria in the Diagnostic Manual of Mental Disorders (DSM-IV), which characterizes a major depressive episode by at least five of the following symptoms: (1) depressed or irritable mood, (2) decreased interest or loss of pleasure, (3) weight gain or loss, (4) insomnia or hypersomnia, (5) psychomotor retardation or agitation, (6) fatigue or loss of energy, (7) feelings of worthlessness or inappropriate guilt,

(8) diminished ability to think or concentrate, (9) recurrent thoughts of death and suicide. The symptoms must be evident almost daily for at least 2 weeks. Additionally, symptoms of anxiety are also often seen in depressed individuals (Berton and Nestler, 2006).

The genetic risk to develop depression is 40–50% (Levinson, 2006), but there are also several environmental risk factors for MDD. These include gender, stressful life events, adverse childhood experiences and certain personality traits (Fava and Kendler, 2000). Many recent studies support the hypothesis that stressful events correlate with an increased vulnerability for depression in a way that stressful situations often precede the onset of illness and are also associated with the severity of depression (Brown et al., 1987; Dunner et al., 1979; Holsboer, 2001; Nemeroff, 1988). Such stressors can lead to a transient hyperactivation of the hypothalamic-pituitary-adrenocortical (HPA) axis, resulting in increased glucocorticoid secretion. In this regard, depression is often associated with a dysregulation of the HPA axis under chronic stress conditions (Holsboer and Barden, 1996). Additionally, treatment of depressed patients with antidepressants can restore the homeostasis of the HPA axis and thereby contributes to clinical improvement (Holsboer, 2000; Holsboer and Barden, 1996).

In contrast to the epidemiological magnitude of the disease, the progress of pharmacological therapy for depression is still limited (Nestler et al., 2002). All available antidepressants act via monoamine neurotransmitter systems, but only 50–70% of the patients exhibit acceptable responses to treatment (Morilak and Frazer, 2004). Additionally, slow onset of clinical effects as well as severe side effects associated with antidepressant therapy frequently lead to discontinuation of treatment. Although the latest generation of drugs has fewer side effects, they still exhibit similar effectiveness rates and show a substantial delay of 4–6 weeks between onset of treatment and clinical improvement. Beside that, 25–35% of the patients remain resistant to the treatment even after 6 weeks of therapy (Berton and Nestler, 2006; Holsboer, 2005). Apart from monoamines, many other targets have been analyzed, including glucocorticoid receptor and corticotropin-releasing hormone receptor antagonists (Berton and Nestler, 2006; Grigoriadis, 2005) as well as histone deacetylase inhibitors (Covington, et al., 2009). However, so far none of these studies has resulted in a new adequate treatment of the disease. Besides these aspects, the molecular mechanisms underlying depression still remain largely unknown.

All these arguments raise the necessity of finding novel approaches to discover different targets for antidepressant treatment and of developing new mouse models to study the effects of monoamine and non-monoamine-related molecules.

## **1.2 The endophenotype concept – modelling symptoms of depression in mice**

### **1.2.1 The endophenotype concept**

The highly variable composition of symptoms, course of illness and response to treatment renders it very difficult to accurately define and consequently diagnose depression. The so-called psychopathological endophenotypes represent key symptoms of major depression. An endophenotype represents a trait that is intermediate between genotype and disease, not necessarily beholden to the diagnostic criteria for single illness, but in many cases useful to simplify our understanding of complex or heterogeneous disorders (Shyn and Hamilton, 2010). An endophenotype is associated with illness in the population, is heritable and is primarily state-independent. Furthermore, endophenotype and illness co-segregate within families and the endophenotype found in affected family members is found in nonaffected family members at a higher rate than in the general population (Gottesman and Gould,

2003;Gould and Gottesman, 2006). The methods available to identify endophenotypes include neuropsychological, cognitive, neurophysiological, neuroanatomical, and biochemical measures (Hasler et al., 2004).

### 1.2.2 Depression-associated endophenotypes in mice

Mimicking any human behavioural trait in a mouse is extremely difficult, which makes the undertaking to try and model a multifactorial disease such as depression nearly impossible. How do we recapitulate all aspects of depression in an animal, when the criteria used to classify a major depression are of extreme heterogeneous and sometimes even of opposite nature (e.g., substantial weight gain or loss, insomnia or hypersomnia)? On top of that, animals not only lack consciousness of self, self-reflection and consideration of others but also aspects of the disorder such as depressed mood, low self-esteem or suicidality (Deussing JM, 2006). This does not mean that it is impossible to develop useful animal models, but rather highlights the unlikelihood of generating a model that will mirror the full extent of a given human neuropsychiatric disorder (Nestler and Hyman, 2010). Nonetheless, in depression, as well as other mood disorders, certain endophenotypes can be reproduced independently and evaluated in animals (Table 1). As mentioned above, these include physiological, endocrinological, neuroanatomical and behavioural alterations, many of which can be measured in mice.

So far a variety of different mouse models have been established to improve our understanding of the pathophysiology of a wide spectrum of psychiatric diseases. However, a full consensus regarding the prerequisites of a valid animal model is still lacking in the scientific community. Up to now, the three criteria set up by McKinney and Bunney (McKinney and Bunney, 1969) are still widely accepted; they include construct validity, face validity, and predictive validity.

*Construct (or etiologic) validity*, the most complex of the three terms, requires that the symptoms produced in the animal model are based on the same underlying neurobiological mechanisms as in humans. Thus, one tries to recreate mechanisms/processes in the animal which would also initiate the disease in humans (Nestler and Hyman, 2010). The ideal way would be to introduce a known human disease-causing gene variant into a mouse and thereby alter intracellular mechanisms, which in the end lead to the disease (Chadman et al., 2009). Unfortunately this is currently far from being realistic, since most disease-causing genetic alterations have not been established with certainty and the probability that a single gene is solely responsible for the disease is highly unlikely. In addition, an animal model does not have to be based on a genetic change, but can also be subject to an environmental challenge or a combination of both.

*Face validity* is achieved when the animal exhibits specific symptoms of the disease which are similar in the human condition. These can be of biochemical, anatomical, neuropathological or behavioural nature. Thus the concept of face validity can also be regarded as the attempt to reproduce certain endophenotypes which can be accurately measured in the animal.

Finally, *predictive validity* refers to the ability of the animal to correctly respond to pharmacological treatment, which should correlate with results from clinical trials.

In this context, it is important to note that the more criteria the proposed model meets, the more compelling it will be (Malkesman et al., 2010). Simply put, researchers are faced with the challenge of 1) constructing a model with similarity in disease progression and symptomatology to humans, 2) detecting these phenotypes with the appropriate behavioural tests and 3) reverting them with treatment modalities that are also effective in humans.

DSM-IV symptoms	Endophenotypes in mice	Appropriate test/analysis
Depressed or irritable mood	Cannot be modeled	
Decreased interest or loss of pleasure	Anhedonia	Sucrose preference Intracranial self-stimulation Conditioned place-preference Female urine sniffing
Weight gain or loss	Can easily be measured	Abnormal loss or gain of weight after stress
Insomnia or hypersomnia	Abnormal sleep architecture	Assessed by electroencephalogram recordings
Psychomotor retardation or agitation	Alterations in locomotion	open field alterations of homecage activity treadmill running Gate analysis
Fatigue or loss of energy	Alterations in locomotion	Reduced homecage activity Treadmill running Nest building
Feelings of worthlessness or inappropriate guilt	Cannot be modeled	
Diminished ability to think or concentrate	Deficits in working and spatial memory	Morris water maze Y-maze fear conditioning Attentional set-shifting
Recurrent thoughts of death and suicide	Cannot be modeled	
	<b>Additional endophenotypes</b>	
	Anxiety-related behavior	Open field Elevated plus maze Dark-light box Novelty-induced hypophagia Novel object exploration Modified hole board Marble burying
	Changes in social behavior	Social interaction/avoidance Sociability
	Behavioral despair	Forced swim test Tail suspension test Learned helplessness
	Neuroendocrine disturbances	Alterations in corticosterone secretion Dexamethasone suppression Combined dexamethasone/CRH challenge
	Changes in neuroanatomy	Analysis of hippocampal volume by resonance imaging (often reduced in depressed patients)

Table 1. Depression-associated endophenotypes that can be modelled in mice.

Although progress has been made to identify the underlying molecular mechanism of depression, the lack of specific markers and biomarkers still represents a major drawback. In contrast to many other illnesses such as cancer, infectious diseases, cardiovascular arrest, diabetes, stroke, etc., depression cannot simply be diagnosed by making use of the usual methods, such as assessing blood pressure, glucose levels, inflammatory agents, heart rate and others. Clinicians still fully rely on observations and verbal communication. Therefore, behavioural tests that are used to assess phenotypic alterations relevant to depression in mice are of extreme importance and will be discussed in more detail.

## **2. Behavioural tests – tools used to assess phenotypic alterations in animal models**

First, we want to point out the importance of discriminating between an animal model, a behavioural test and an antidepressant screening paradigm. Animal models, as discussed above, are expected to show sufficient construct, face and predictive validity. Behavioural tests on the other hand are used to assess phenotypic alterations relevant to the disease and should be regarded as a technical tool and not a model (Table 1). Likewise, antidepressant screening paradigms, such as the forced swim test, also do not represent a model of depression but should rather be regarded as drug-screening assays.

Nowadays researchers are discordant when it comes to the term “depression-related test”. Given the fact that there is no consensus as to what can be regarded as “depression-like” behaviour in animals it has become increasingly popular to look at, and talk of behavioural endophenotypes or sub-categories, which in some cases may, and in others may not represent aspects of depression. These include anxiety-, reward-, social and despair-based behaviour as well as alterations in general locomotion, sleep, food and liquid intake.

### **2.1 Tests assessing despair-based / stress-coping behaviour**

In earlier days, the Porsolt forced swim test (FST) and the tail suspension test (TST) were regarded as typical depression-like paradigms, given the fact that both were developed to screen monoamine-based antidepressant drugs. This is currently a matter of debate, since both assess the response to an acute inescapable stressor, provoking despair-based behaviour/immobility or stress-coping behaviour rather than depression-like behaviour. The FST makes use of the fact that rodents eventually develop immobility when being placed in a cylinder of water after they have stopped active escape behaviours, such as climbing or swimming (Cryan and Holmes, 2005). A related task is the TST, which relies on similar assumptions and interpretations as the FST. Here the mice are hung upside down by their tails and the time spent immobile is assessed. One major advantage of the TST is that it is not confounded by stressful hypothermia as is the case in the FST. However, the TST is restricted to strains that do not tend to climb their tail which otherwise confuses the interpretation of behavioural measures (Mayorga and Lucki, 2001). In either case the underlying principles measuring the lack of active coping behaviour are identical. However, the question whether immobility should be interpreted as passive stress-coping, behavioural despair or even depression-like behaviour remains controversial. One aspect favouring the stress-coping rather than depression-like aspect of the FST are the outcomes when manipulating the corticotropin-releasing hormone (CRH) system. CRH overexpressing animals as well as central application of CRH result in decreased immobility (Butler et al., 1990; van Gaalen et al., 2002; Lu et al., 2008). However, it is widely believed that high levels of CRH are rather pro-

depressive (Holsboer and Ising, 2008) and it was shown that CRHR1 antagonists have an effect similar to paroxetine and other antidepressants (Holsboer and Ising, 2008). Thus it is likely that the CRH/CRHR1 stress system also accounts for regulating and/or triggering responses to the test (Refojo and Deussing, 2011). In that regard, Gardier already pointed out that the FST assesses stress-induced anxiety rather than depression (Gardier and Bourin, 2001). In addition, it is very unlikely that such a short period of inescapable stress is able to induce a depression-like state in a wild-type animal. Nevertheless, the FST and TST have proved reliable across many laboratories by demonstrating their ability to detect a broad spectrum of substances with antidepressant efficacy, all of which reduce immobility. However, most effects are already observed after acute treatment, which contrasts the human situation, where chronic application of antidepressants is necessary to achieve clinical responses (Nestler and Hyman, 2010). For this reason, we believe that the FST and TST should rather be viewed as paradigms designed to assess strategies of stress-coping behaviour and monoamine-based antidepressant action (Lucki, 1997), and not as models of depression (Refojo and Deussing, 2011).

In addition to the FST and TST, the learned helplessness paradigm also makes use of stressor uncontrollability and passive vs. active coping responses (Cryan and Holmes, 2005). The paradigm is based on the observation that animals exposed to uncontrolled or unpredictable aversive events (e.g. electrical shocks) for a sufficient period of time will develop long-lasting deficits in escape performance (Seligman and Maier, 1967). Short-term treatment with antidepressants as well as anxiolytics has shown to reverse the enforced behavioural phenotype, which doesn't make the paradigm particularly selective (Cryan and Mombereau, 2004). In addition, only some of the animals develop signs of helplessness and those are usually short-lived. Similar to the FST and TST, the learned helplessness paradigm does not parallel clinical settings with regard to the slow onset of antidepressant action, but remains a good tool for the assessment of stress-coping behaviour (Deussing, 2006).

## 2.2 Anxiety-based tests

The lack of clear a distinction between depression and anxiety amongst researchers poses a major issue in the interpretation of behavioural tests. Depression, by definition, is considered a pathological mental condition. Anxiety, however, is a normal state of cognitive and behavioural preparedness that an organism mobilizes in response to a future or distant potential threat (Leonardo and Hen, 2008). Although anxiety is often necessary and even protective, excessive anxiety can trigger disabling responses that, in time, lead to anxiety disorders (e.g., generalized anxiety disorder, social phobia, simple phobia, panic disorder, posttraumatic stress disorder (PTSD), and obsessive compulsive disorder) (Bienvenu et al., 2009). Anxiety can often emerge as part of a depressive syndrome, but this does not hold true for all patients and certainly not for all animal models (Krishnan et al., 2007;Wallace et al., 2009). It is also important to note that the underlying neural circuitries are believed to be different in depression and anxiety (Nestler and Hyman, 2010). However, in animal models anxiety is considered a core endophenotype of depression, which is mainly due to the availability of a wide range of standardized tests, all of which assess anxiety-like behaviour. Most of these assays are based on approach-avoidance conflicts and were developed and validated using classical benzodiazepine-like anxiolytic compounds. Mice generally display high levels of exploration of a novel environment but also have an innate aversion to enter exposed, well-lit areas. The elevated plus-maze (EPM) and elevated zero-maze present the subject mouse with the choice of spending time exploring the open areas of a plus-shaped or

circular runway, versus spending time exploring the enclosed arms and arcs (Handley and Mithani, 1984; Chadman et al., 2009). Other exploration-based tasks, founded on similar conflicting tendencies to approach versus avoid a potentially dangerous area are the dark-light box (DaLi) and open field (OF) test. In the latter the aversive area is represented by the central zone of a brightly lit open field. In the dark-light box test averseness is achieved by a highly illuminated compartment (Lister, 1990; Belzung and Griebel, 2001). The novel object exploration test makes use of similar principles, the only difference being that mice are first habituated to an environment and then exposed to novelty (novel object). Explorative paradigms such as the EPM, DaLi and brightly lit OF have been suggested to measure state anxiety (Belzung and Le, 1994; van Gaalen and Steckler, 2000). In contrast, exploration of novelty in an area known to be safe has been suggested to fundamentally differ from the exploration of a totally new environment and thus claimed to reflect trait rather than state anxiety (Griebel et al., 1993; van Gaalen and Steckler, 2000). Other tests are the modified hole board the mirrored chamber test, the staircase test and the marble burying test (Broekkamp et al., 1986; Holmes, 2001; Belzung and Griebel, 2001; Ohl et al., 2003). Another emerging paradigm is the novelty-induced suppression of feeding test, which measures the latency until food consumption in a novel environment. Here, rodents have shown to respond to chronic but not acute antidepressant treatment, resulting in decreased latency to feed (Dulawa and Hen, 2005). This goes along with the fact that anxiety in humans can in many cases be treated with chronic antidepressant administration (Nestler and Hyman, 2010). Even though approach-avoidance tests are critical in the assessment of anxiety-related behaviour in mice, they still have to be interpreted with caution. In most of the tests it is not possible to distinguish between an anxiety response and other phenotypes such as motivation, novelty-seeking, impulsivity or arousal. Thus increased time spent in an aversive area can be interpreted as both, decreased anxiety or increased motivation or arousal. Different exploratory and even coping strategies can also be misinterpreted as alterations in anxiety-like behaviour. Additionally, tests based on exploration can be strongly influenced by differences in basal locomotion and cognition (Refojo and Deussing, 2011). As is the case with the FST and TST, the above mentioned anxiety-based assays represent useful initial screens, but should never be used as definite evidence of a depression-like phenotype. This emphasizes the importance of parallel use of several slightly different anxiety tests, as well as the utilization of internal controls with respect to locomotion. Most importantly, additional non-anxiety based tests should be considered (see below).

### **2.3 Reward-related and anhedonic behaviour**

Anhedonia, a hallmark of depression is defined as the inability to experience pleasure from activities formally found enjoyable. Dopamine neuronal functioning is essential in sustaining a wide variety of pleasurable and rewarding experiences (Wise and Bozarth, 1985; Wise, 2002). Especially dopaminergic neurons, projecting from the ventral tegmental area to the prefrontal cortex, basolateral amygdala, and nucleus accumbens are essential in reward processes (Wise, 2002). The most widely accepted approach to assess reward-seeking behaviour is via the sucrose consumption and preference tests. Decreased intake of palatable solutions, such as sucrose is regarded as a behavioural measure of hedonic deficit/depressive-like state (Willner, 2005). One drawback, however, is that one cannot rule out appetitive, metabolic or sensorial influences, which may be altered in genetically

modified animals. In addition, enhanced hedonic drive and motivation, which are hallmarks of manic symptoms in bipolar disorder, may often be misinterpreted as decreased depression-like behaviour (Hasler et al., 2004). Operant paradigms, such as the conditioned place-preference (CPP), are also widely applied to assess anhedonic behaviour. Although mainly used to determine the addiction potential of drugs, CPP can also be employed to test animals in a drug-free state. Even though methodological details differ among laboratories, a typical CPP experiment includes differential pairing of two distinct sets of environmental (contextual) cues with a stimulus (e.g., drug, food, copulatory opportunity) (Bardo and Bevins, 2000). When tested later on in the absence of the stimulus, the approaches and the amount of time spent in the compartments previously associated with the positive stimulus serve as an indicator of preference and a measure of reward learning. Obvious drawbacks include the rather elaborate testing procedure as well as the fact that the paradigm is heavily dependent on learning, memory and motor activity. Operant self-administration, using the so-called operant box (or Skinner box), represent additional methods in anhedonic research (Sanchis-Segura and Spanagel, 2006). In contrast, intracranial self-stimulation-based procedures make use of the phenomenon that direct stimulation of distinct brain regions through electrical or chemical means can activate the reward system and serve as an operant reinforcer (Sanchis-Segura and Spanagel, 2006). However, the use of brain stimulation reward techniques requires surgery and sometimes extensive periods of training that often exceed the recovery time from the surgery (Malkesman et al., 2010). Alternatives are for instance the recently developed female urine sniffing test (FUST). The FUST is a nonoperant test, designed to measure reward-seeking behaviour in rodents based on the interest in pheromonal odors from the opposite sex (Malkesman et al., 2010). The duration of female urine sniffing was significantly decreased after foot-shock stress and could be reverted upon antidepressant treatment (Malkesman et al., 2010). As with other tests, the FUST faces limitations when working with transgenic animals, which in some cases may suffer from olfactory system dysfunction (Hull and Dominguez, 2007). Furthermore, the preference for estrus female odour might also be related to social and not just sexual behaviour (Wersinger et al., 2004). As with anxiety tests, multiple tasks that evaluate different aspects of reward sensitivity should be considered, and are likely to provide more insights into the behavioural and neurobiological processes of mood disorders such as depression.

#### **2.4 Cognition-based tests**

A majority of depressed patients, especially older adults, show pronounced cognitive deficits typically consisting of memory impairments, poor attention, and executive dysfunction (Butters et al., 2004; Crocco et al., 2010). Hence, it is of great interest to model altered cognitive behaviours in mice. However, this undertaking faces many challenges, given the fact that the rodent cortex is much more primitive than the human, which makes it extremely difficult to address many aspects of cognitive processing in mice (Cryan and Holmes, 2005). Therefore, many of the applied cognition tasks, such as the Morris water maze (MWM) and Y-maze test, focus on general cognitive function mediated by the hippocampal region. Developed by Morris in 1984, the MWM test assesses spatial learning in mice and rats and is strongly reflective of hippocampal synaptic plasticity and NMDA receptor function. The test relies on distal, visual cues that can help the animal to locate and navigate to a submerged escape platform from different

starting locations within an open swimming arena. Spatial learning is evaluated across repeated trials and reference memory is assessed by preference for the platform area once the platform is absent (Morris, 1984; D'Hooze and De Deyn, 2001). Similarly, the Y-maze also assesses spatial memory and hippocampal integrity. It is based on the animal's natural curiosity to discover novel environments (Conrad et al., 1996; Dellu et al., 2000), but is not confounded by possible effects of hypothermia. Incidents of chronic social stress, a precondition of many depressed patients, have shown to alter cognitive performance in a battery of tests including the MWM and Y-maze task (McEwen and Sapolsky, 1995; Song et al., 2006; Wang et al., 2011).

It is important to note that many cognitive deficits, such as misappraisal and over-attention to threatening stimuli, are also observed in panic disorder, generalized anxiety disorders and phobias (Cryan and Holmes, 2005). Thus, researchers often speak of emotional cognition, which can be analysed in rodents with certain well established concepts such as Pavlovian fear-conditioning (Davis, 1990; Fendt and Fanselow, 1999; Maren, 2001). Such contextual and cued fear conditioning tasks represent additional methodologies to investigate memory, as they require that the animals learn the association of a non-aversive context or cue with an aversive stimulus. The ability to learn this association is measured by the amount of freezing exhibited in response to the cue or context alone (Amann et al., 2010). Many variations of the paradigm exist, including altering the type of cue and stimulus, and, once learned, testing the rate at which learning is extinguished (Fanselow, 1980). The failure to extinguish learned fear responses is one key feature of post-traumatic stress disorder, phobias and other anxiety disorders (El-Ghundi et al., 2001).

Another recently validated test in rats is the attentional set-shifting test (Birrell and Brown, 2000; Bondi et al., 2008). The animals are trained to dig for food in bowls which are presented in pairs, only one being baited. The rat has to select the bowl to dig in based on the texture that covers the bowl's surface or the odor of the medium with which it is filled. Once the training procedure is completed, the animals perform a series of discrimination tasks, including reversal, an intradimensional shift, and an extradimensional shift. The number of trials required to perform six consecutive correct responses in different stages is scored. Rats subjected to chronic unpredictable stress exhibited impairments in the attentional set-shifting test, which were prevented by desipramine or escitalopram treatment (Bondi et al., 2008). In addition, lesions of the medial prefrontal cortex selectively disrupted extradimensional set shifting (Birrell and Brown, 2000). Although this paradigm is quite labour intensive and not yet validated for the mouse, it represents a very interesting means to evaluate prefrontal cortex function as a cognitive trait relevant for depression.

Although cognitive tests are not used as standard tools when assessing depression-like behaviour, they certainly provide insights into the cognitive aspects of the emotional state. Thus, it is strongly recommended to include cognitive tasks in schedules for testing emotional behaviour especially in combination with some of the well-established anxiety tests described above.

## **2.5 Assessing behaviour via social tests**

Social behaviour can be defined as any behaviour that influences, or is influenced by, other members of the same species. A variety of neuropsychiatric disorders, including depression,

are characterized by disruptions of social behaviour (Nestler and Hyman, 2010). Many of the social test paradigms were originally developed to study schizophrenia and autism-like behaviour, in which alterations in social behaviour represent core symptoms of the disease. However, social withdrawal also represents a common symptom in psychiatric conditions such as depression, social phobia, and PTSD (Berton and Nestler, 2006). Exposure to chronic social stress, after which rodents show signs of anhedonia and increased anxiety-like behaviour, is also effective in inducing social withdrawal (Krishnan et al., 2007). Although most social tasks in mice are employed after repeated exposure to a stressor, it should be considered to employ these tests also under basal conditions especially when evaluating transgenic mouse lines.

The classical social interaction paradigm encompasses the free exploration of an unfamiliar congener by the experimental mouse. Social interaction is measured by the time spent in close proximity to the unfamiliar mouse as well as the amount and duration of additional behaviour including sniffing, following, grooming, biting, mounting etc. In many cases, social avoidance behaviour is associated with anxiety- and depression-like behaviour.

The social avoidance paradigm represents a similar but faster and more systematic approach to assess social avoidance. In contrast to the standard tests, social approach towards an unfamiliar mouse enclosed in a wire mesh cage is measured (Berton et al., 2006). This excludes subject bias from the observer, since only the time spent in close proximity to the target is assessed. In addition, aggressive behaviours such as biting and fighting can be excluded. Other paradigms, including the sociability test can be used to assess the social preference between a stranger/conspecific and an object (Moy et al., 2004). In addition, social novelty can be evaluated by introducing a second unfamiliar mouse. In rats, it was shown that social behaviour is individually stable and that sociability is related to 5-HT metabolism in the prefrontal cortex (Tonissaaar et al., 2004;Tonissaaar et al., 2008b). Additionally, sociability and anxiety were shown to be closely related domains (Tonissaaar et al., 2004;Tonissaaar et al., 2008a). Even though preclinical research has clearly favoured the rat for the assessment of social behaviour, the propagation of transgenic and gene targeting technologies in the mouse has established it as a unique model in psychiatric research. Thus, an ever increasing number of social tasks is established in mice and should definitely be considered when assessing animal models of depression.

### **3. Outline of existing mouse models of depression**

Most animal models of depression are either based on environmental challenges or on manipulation of sensory and integrative functions of the brain. By means of a variety of stressful conditions, certain symptoms that are inferred to be “depression-like” can be evoked in animals. In contrast, molecular and cellular tools, which allow the development of targeted genetic manipulation strategies in embryonic stem cells, are also widely applied to generate so called “depression models”. Below, the most prominent and widely used animal models of depression will be considered. In addition, innovative strategies and state-of-the-art technologies used to construct novel genetic mouse models of depression will be discussed.

#### **3.1 Lesion models**

Patients exhibiting depression or other psychiatric syndromes often show alterations in structures related to olfactory function. Olfactory performance was shown to be reduced in

depressed patients (Pause et al., 2001) together with morphological differences in olfactory projection areas, noticeably in the amygdala (Nestler et al., 2002). Thus, the bulbectomized rat or mouse has been considered a model of agitated depression (Leonard and Tuite, 1981; Kelly et al., 1997). After ablation of the olfactory bulb, mice demonstrate typical loss of smell but also disruptions of the limbic-hypothalamic axis with the consequence of behavioural, neurochemical, neuroendocrine and neuroimmune alterations, all of which may resemble changes in depressed patients (Song and Leonard, 2005). The behavioural outcome of olfactory bulbectomy is largely thought to result from compensatory mechanisms of neuronal reorganisation. Thus, most of the literature indicates that the olfactory bulb is not a mere sensory area, suggesting that it could have non-olfactory functions relevant for modulation of behaviour (Edwards et al., 1972; Cain and Paxinos, 1974; Mucignat-Caretta et al., 2004). In addition, peripheral anosmia fails to produce the observed behavioural changes in mice, indicating once more that loss of smell alone is not the sole cause for the observed syndromes (Mar et al., 2000). Underlying changes are thought to involve alterations in synaptic strength and/or loss of spine density in various limbic regions including the amygdala and hippocampus (Kelly and Leonard, 1999). Marked changes in major neurotransmitter systems have also been observed in bulbectomized rodents (Kelly, 1999). However, the most consistent behavioural phenotype of bulbectomized animals is a hyperactive response in the open field paradigm, which is reversible upon antidepressant treatment (Cryan et al., 1999). This model of depression shows high face validity as it mimics the slow onset of antidepressant action reported in clinical studies (Willner and Mitchell, 2002). However, high construct validity is not achieved as the model fails to recapitulate the etiology of disease progression.

### 3.2 Pharmacological models

Pharmacological models in the ideal sense should induce behavioural changes and treatment responses partly similar to depression. Reserpine, a sympatholytic drug that depletes catecholamines in the brain (Eranco and Hopsu, 1958; Mascorro and Yates, 1971), is believed to chemically evoke a depression-like phenotype in animals. The drug was shown to induce a syndrome of locomotor hypomotility and reduced body temperature in rodents. In addition, reserpine exhibits effects on the adrenal glands which resemble those of physiological stress (Joh et al., 1973). This model is based on the capability of antidepressants to reverse the inhibitory effects caused by reserpine on motility in rats and mice (Nutt, 2006). The psychostimulant withdrawal paradigm, another widely applied pharmacological model, displays both, responsiveness to antidepressants and induces characteristic symptoms of depression. In humans, withdrawal from chronic psychostimulants generates symptoms that have strong behavioural and psychological parallels to depression including diminished interest in pleasure. Withdrawal from chronic amphetamine treatment results in behavioural changes that were shown to be analogous to some aspects of depression. These included reward deficits and increased immobility in the FST (Kokkinidis et al., 1986; Cryan et al., 2003). Therefore, examination of the behavioural effects of drug withdrawal may provide insights into the underlying neurobiological mechanisms and aid in the development of animal models of depression that are sensitive to antidepressant agents. Although many of these models show robust predictive and even face validity, most of them fail to mimic disease etiology. In that regard, pharmacological models such as those mentioned above, significantly contributed to the strengthening of the

monoamine theory of depression, which assumes that an elevation of serotonin and norepinephrine levels will improve depressive symptomatology. However, they are limited in their value as effective models of depression given the ever expanding impact of genetic and environmental models.

### 3.3 Genetic mouse models

Genetically engineered mice represent a powerful tool to study candidate genes thought to participate in a particular disease. In general, scientists discriminate between forward and reverse genetics. Forward genetics allows the identification of relevant genes without any prior knowledge of genetic or mechanistic underpinnings of a phenotype of interest. Classically, forward genetics involves larger scale random mutagenesis screens such as ENU-(N-ethyl-N-nitrosourea) or gene-trap-based approaches which have resulted in a great number of mutants displaying aspects of depression-like behaviour. On the other hand, reverse genetics involves genetic manipulations that result in loss- or gain-of-function mutants. These include classical transgenic mice that have additional copies of certain genes in their genome, which results in a gain-of-function. Similarly, knock-in techniques are frequently applied to generate gain-of-function animals. However, transgenes can also be used to induce a loss-of-function if the inserted transgene produces an antisense mRNA of the target gene. Similarly, short hairpin RNAs directed against the gene of interest have also been widely used (Kleinhammer et al., 2010). However, disruption of specific target genes is most commonly achieved via generation of knockout mice. Embryonic stem (ES) cell technology has been widely used to produce null mutants or 'conventional' knockouts (Capecchi, 2005). In that case, the targeting vector is constructed to allow the precise disruption of a gene resulting in the complete ablation of protein and/or mRNA production within every cell. Such conventional knockout mice were of immense importance in identifying candidate genes involved in depression and other mood disorders (see below). However, they were limited in their ability to further uncover specific brain regions and neural circuitries involved in disease etiology. In many cases, homozygous knockouts were not viable or induced developmental and peripheral changes such as reduced weight or organ dysfunction. Since then, technologies in this field have expanded rapidly, introducing sophisticated conditional strategies (Branda and Dymecki, 2004). This progress has allowed an increasingly refined control of spatial and temporal gene expression. In particular, the propagation of site-specific recombinases makes it possible to address gene function in a spatially and temporally restricted manner. For example, mouse lines expressing Cre recombinases selectively in neurons of a specific neurotransmitter type allow for gene targeting of specific populations of neurons. Moreover, increasing availability of mouse lines expressing the tamoxifen-inducible Cre recombinase variant CreERT2 (Branda and Dymecki, 2004) offers additional temporal control and avoids obscurities due to developmental functions of targeted genes. In addition, conditional strategies such as RNAi technology or virus-mediated genetic manipulation also enable the control over spatial and temporal gene expression, and are thus becoming increasingly important.

Currently, three main theories try to conceptualize the molecular mechanisms underlying depression. These include the monoamine and neurotrophic hypothesis, and the HPA system. Thus, most genetic approaches aim at altering the expression of genes that are

involved in these systems and thereby analyse their respective role in animal behaviour, neuroendocrine and molecular parameters (Urani et al., 2005). The monoamine hypothesis postulates that depression is caused by an impairment of serotonergic, noradrenergic or dopaminergic neurotransmission. The monoaminergic deficiency can be due to several factors including decreased synthesis or early degradation of neurotransmitters, altered expression or function of neurotransmitter receptors and impairment of signal transduction systems activated by post-synaptic neurotransmitter receptors (Berton and Nestler, 2006).

The neurotrophic hypothesis of depression assumes that the cAMP responsive element-binding protein (CREB) - brain derived neurotrophic factor (BDNF) - tyrosine kinase B receptor (TRKB) pathway is involved in the pathophysiology of depression and action of antidepressants. Originally the theory was based on findings in rodents, demonstrating that acute or chronic stress decreases BDNF expression in the hippocampus and that diverse classes of antidepressants produce the opposite effect and can prevent the actions of stress (Berton and Nestler, 2006).

A dysregulation of the HPA axis, the major neuroendocrine stress system in mammals, has also been postulated to play a role in human depression. Hyperactivity of the HPA axis is observed in the majority of patients with depression, as manifested by increased expression of CRH in the hypothalamus, increased levels of CRH in the cerebrospinal fluid (CSF) and reduced feedback inhibition of the axis by CRH and glucocorticoids (Sapolsky, 2000; Barden, 2004; de Kloet et al., 2005; Deussing and Wurst, 2005; Muller and Holsboer, 2006).

Although the above mentioned systems are commonly accepted, other neuromodulatory systems have also been implicated in depression, e.g. substance P, neuropeptide Y, aquaporines, and the immune system, in particular the activation of cytokines (Rosenkranz, 2007; Kong et al., 2009; Morales-Medina et al., 2010; Blume et al., 2011). Most likely, neither of the theories will ever hold true on its own in explaining all underlying mechanisms of depression. An interaction of these systems, combining dysregulations of more than one neuronal circuit with environmental factors, is probably more likely to explain the etiology of depression. Nevertheless, these theories represented initial starting points for the generation of possibly valid "depression-models". Among many of the generated mouse mutants based on the mentioned hypothesis, only very few, if any, can be considered valid genetic depression models, but rather represent models of predisposition to depression (Cryan and Mombereau, 2004). Some of the seminal genetic models will be discussed in more detail below.

### 3.3.1 Models based on the monoamine hypothesis of depression

Several knockout mice of candidate genes related to the monoamine hypothesis were generated in the past. The main ones included the serotonin-(SERT), noradrenaline (NAT)-, and dopamine (DAT) transporters, which represent major targets of antidepressants and psychostimulants such as cocaine and 3,4-methylenedioxy-N-methylamphetamine (MDMA/ecstasy). The monoamine transporters (MATs) are localized at the presynaptic membranes of monoaminergic neurons where they modulate the fate and restrict the lifetime of the released monoamines (Haenisch and Bonisch, 2011). Most classical antidepressants act via MAT inhibition thereby increasing the availability of monoamines in the synaptic cleft. Thus one would speculate that MAT knockouts would show a similar phenotype to that observed after antidepressant treatment. Interestingly, SERT knockout mice show a strong anxiety-like phenotype and are not

resistant to depression. Although they display an excess of extracellular 5HT during embryonic development, marked depletion of 5HT was shown in several regions of the adult mouse brain (Bengel et al., 1998). This favoured the assumption that life-long absence of SERT can lead to chronic serotonin depletion resulting in depression-like behaviour (Gross et al., 2000;Gross et al., 2002).

In order to study the endophenotypes of dopaminergic dysregulation Caron and co-workers (Giros et al., 1996) developed conventional DAT knockout (DAT-KO) mice. These animals show persistent hyperdopaminergia, resulting in a downregulation of pre- and postsynaptic dopamine receptors (Gainetdinov et al., 1999;Jones et al., 1999). Concerning the behavioural phenotype, DAT-KO mice show very pronounced hyperlocomotion (Giros et al., 1996;Gainetdinov et al., 1999;Pogorelov et al., 2005), decreased immobility in the FST (Spielewoy et al., 2000), increased sucrose preference (Perona et al., 2008), impairments in cognitive function (Gainetdinov et al., 1999) and deficits in reversal learning (Morice et al., 2007). These observations support a certain role of the dopamine transporter in mediating an antidepressant-like phenotype.

In contrast, the phenotype of conventional NAT knockouts fits the profile of antidepressant efficacy of drugs that antagonize the noradrenalin transporter (Xu et al., 2000). These mice behave like antidepressant-treated animals, exhibiting decreased immobility in the FST and TST (Xu et al., 2000) as well as increased sucrose consumption (Haenisch et al., 2009). In addition, NAT knockout mice show less susceptibility towards acute and chronic stress (Haenisch et al., 2009). Thus, NAT knockout animals seem to be a good model to obtain more in-depth knowledge on the pathophysiology of depression and antidepressant action.

MAT deficient mice represent interesting tools to study human genetic conditions in which these transporters are downregulated, but render them less useful for investigating their normal role in the adult brain. It should also be noted that monoamine transporters can often compensate for each other if they are completely knocked out (Haenisch and Bonisch, 2011). For example, noradrenaline can be taken up and stored in striatal dopaminergic neurons (Gobert et al., 2004). These compensatory processes represent an additional difficulty in understanding the transporter role in the adult brain. In this regard, the conventional SERT and DAT knockout mice rendered insights into the brain serotonergic and dopaminergic systems, but could not fully address the involvement of these monoamines in a depression-like state. A conditional knockout of SERT and DAT within the CNS serotonergic and dopaminergic neurons, respectively, would greatly aid in this undertaking. Unfortunately, until now, no tissue-specific conditional knockouts of the SERT and DAT have been generated. However, SERT knockdown via siRNA led to a reduction of SERT mRNA levels in the dorsal and medial raphe nuclei as well as immobility in the FST, which was identical to the response obtained from wildtype mice treated with the antidepressant citalopram (Hoyer et al., 2006). Knockdown of the dopamine transporter within the ventral tegmental area led to behavioural alterations also found in patients with bipolar disorder (Young et al., 2010;Young et al., 2011). These models represent interesting and valuable pharmacological tools and stress the importance of developing conditional monoamine-transporter knockouts in the future.

In addition to MATs, conventional monoamine receptor mutant mice also mimic some aspects of depression-like behaviour. Fourteen subtypes of 5HT receptors have been identified so far (Hoyer and Martin, 1997) many of which have been targeted genetically

(Holmes, 2001). However, it is far from clear which receptors are mediating specific aspects of emotional behaviour. So far, the 5HT<sub>1A</sub> receptor (5HT<sub>1A</sub>-R) has been investigated in greater detail. 5HT<sub>1A</sub>-R knockouts display increased anxiety-related behaviour (Heisler et al., 1998; Ramboz et al., 1998; Sibille et al., 2000) and HPA axis dysregulation (Gross et al., 2000). Most of the other serotonin receptor knockout mice show no behavioural alterations. Although the behavioural effects in 5HT<sub>1A</sub>-R knockout mice were rather small, they still represent a valuable pharmacological model to study pharmacodynamical, biochemical and behavioural characteristics of serotonergic antidepressants (Urani et al., 2005). Similarly, there are hardly any pharmacological tools available that are selective enough to discriminate between the different subtypes of  $\alpha_2$  adrenergic receptors. Thus, mice carrying deletions for these receptors could help to better understand receptor function and improve drug specificity. Mutant  $\alpha_{2A}$  adrenergic receptor mice show an increase in anxiety-like behaviour (Lahdesmaki et al., 2002), increased immobility in the FST and have a disrupted circadian rhythm (Schramm et al., 2001), a symptom often observed in human depression. Although monoamine receptor KOs represent valuable pharmacological tools, they are limited in their value as an animal model of depression. This is mostly due to the fact that deletion of a signal receptor is probably not sufficient to induce reliable depression-like phenotypes. In addition, compensatory mechanisms in early embryogenesis can lead to misinterpretations of receptor function pointing out once more the importance of conditional KOs.

### 3.3.2 Models based on the neurotrophic factor-related hypothesis of depression

The neurotrophin hypothesis of depression has also been addressed in the mouse by targeting neurotrophic factors such as BDNF and the respective TRKB receptors. However, conventional BDNF and TRKB receptor KOs are not viable (Conover et al., 1995). Thus, most of the earlier studies were conducted with heterozygous knockout mice. These were behaviourally indistinguishable from control littermates, consequently not representing a genetic model of depression (MacQueen et al., 2001). In contrast, forebrain-specific BDNF deletion leads to hyperactivity, obesity and increased anxiety-like behaviour (Rios et al., 2001). In addition, inducible knockout of BDNF from the hippocampus and other forebrain regions prevented the antidepressant effects of certain reuptake inhibitors (Monteggia et al., 2004). These animal models were of great value in linking the role of BDNF in the adult brain with antidepressant-like activity of certain drugs. However, complications arise from the fact that BDNF seems to exert quite opposite effects in different neural circuits. Although it shows antidepressant-like effects at the level of the hippocampus, BDNF infusion into the ventral tegmental area induces a prodepression-like effect (Eisch et al., 2003; Berton and Nestler, 2006). Conversely, conditional forebrain deletion of the BDNF receptor TRKB induced only a few behavioural changes, many of which are inconsistent (Zorner et al., 2003). Nevertheless, the conditional BDNF knockouts significantly contributed to the dissection of the role of BDNF in depression-related behaviours and responses to antidepressant drugs.

### 3.3.3 Models based on alterations of the HPA axis

#### 3.3.3.1 The CRH/CRHR1 System

The generation and analysis of numerous constitutive mouse mutants affecting different parts of the HPA axis is another example where genetically modified mice have

demonstrated their enormous potential. The genetic dissection of the organism's major stress-integrating system in the mouse has confirmed a major role of corticosteroid receptors and of the CRH system in the pathogenesis of affective disorders including depression (Deussing and Würst, 2005). Therefore it seemed obvious to target the major player of the system, CRH. However, homozygous CRH knockout mice are viable and show no behavioural abnormalities compared to control littermates (Muglia et al., 1995). Basal pituitary ACTH secretion is compensated by an increased expression of the co-secretagogue neuropeptide vasopressin (AVP), thus CRH deficiency impairs but does not fully block pituitary-adrenal responses to diverse stressors (Venihaki and Majzoub, 1999). In contrast, conventional CRH receptor 1 knockouts display a severe impairment of stress-induced HPA axis activation and marked glucocorticoid deficiency (Muller and Holsboer, 2006). In addition, they demonstrate increased locomotor activity and decreased anxiety-like behaviour, both under basal conditions and after ethanol withdrawal (Timpl et al., 1998; Sillaber et al., 2002). As a result, conventional CRHR1 deletion was shown to affect behaviour as well as neuroendocrine regulation, which obstructs the analyses of the role of CRH as a neuromodulator independent of HPA axis activation. By generating forebrain-specific CRHR1 knockouts, this problem was overcome (Muller et al., 2003). These animals demonstrated a marked decrease in anxiety-related behaviour, which was not influenced by central nervous system effects of circulating stress hormones. In addition, forebrain CRHR1 deficiency has been shown to attenuate chronic stress-induced cognitive deficits and dendritic remodelling (Wang et al., 2011). However, it remains a matter of debate which brain structures and circuits are mediating anxiety-like behaviour in mice. So far, most of the results provide evidence that amygdalar CRHR1 is responsible for the observed phenotypes (Liebsch et al., 1995; Sztainberg et al., 2010). Thus it would be of great importance to further dissect the origin of these effects by generating mice with deletions of CRHR1 in certain neuronal subpopulations and/or specific brain regions.

Taking into account that depression is often accompanied with excessive glucocorticoids and elevated CRH levels in the cerebrospinal fluid, the generation of mice overexpressing CRH was of utter importance. Different lines of CRH-overexpressing mice consistently display an increase in anxiety-related behaviour (Stenzel-Poore et al., 1996; Kolber et al., 2010). However, in all cases unrestricted CRH overexpression resulted in elevated corticosterone levels accompanied by symptoms of Cushing-like syndrome, complicating the interpretation of stress-related behavioural results. This problem was circumvented by designing mutants overexpressing CRH under the CNS-specific Nestin and the forebrain-specific Camk2a promoters (Lu et al., 2008). In both lines, the basal HPA axis activity remained unaltered. CRH overexpression in the whole CNS, but not when expressed in specific forebrain regions, resulted in stress-induced hypersecretion of corticosterone and decreased immobility in the FST and TST (Lu et al., 2008). These changes were probably due to acute effects of overexpressed CRH as they were normalized by CRH-R1 antagonist treatment. However, forebrain-specific CRH overexpression during postnatal development was shown to cause long-lasting anxiogenic and despair-like phenotypes (Kolber et al., 2010). This supports the hypothesis that CRH in limbic regions such as the amygdala, hippocampus and prefrontal cortex can induce anxiety-like changes. This further coincides with results obtained from conditional forebrain-specific CRHR1 knockout animals, which demonstrate decreased anxiety-like behaviour. However, the problems concerning ectopic expression cannot be neglected and thus it remains unclear whether CRH overexpressing

mouse mutants show sufficient, if any, construct validity. The best way to address this issue would be to generate a CRH overexpressing mouse under the control of its endogenous promoter, thereby restricting the overexpression to its actual expression sites.

### 3.3.3.2 Glucocorticoids and glucocorticoid receptors

Excessive stimulation of the HPA axis, implicated in depression, is mostly reflected by excessive glucocorticoids. Conventional glucocorticoid receptor knockouts have thus been developed in order to address the function of the GR in depression. Initially, a GR-antisense transgenic mouse was developed (Pepin et al., 1992), which demonstrated behavioural and neuroendocrine signs and symptoms common among depressed patients. These included reduced negative feedback sensitivity to dexamethasone (Stec et al., 1994) and enhanced stress hormone response (Pepin et al., 1992). Most importantly, this model showed good predictive validity evident in an antidepressant response which induced numerous changes including increased GR mRNA, reduced HPA axis activity (Montkowski et al., 1995) and enhanced hippocampal LTP (Steckler et al., 2001). A few years later, conventional GR knockouts were developed (Cole et al., 1995). These showed a similar phenotype to that observed in GR-antisense transgenic mice. In addition, they exhibited increased helplessness after stress exposure and reduced hippocampal BDNF content (Ridder et al., 2005). Forebrain-specific GR deletion produces non-suppression of corticosterone following dexamethasone administration, altered circadian HPA axis activity, and increased hypothalamic vasopressin expression, which are all hallmarks of depression. Interestingly, they also exhibited a robust despair-like phenotype, which was reverted by antidepressant treatment (Boyle et al., 2005). Taking into account the observations from the conventional GR knockouts, it does not seem surprising that GR antagonists are being considered and currently tested as possible non-monoamine-based antidepressants.

Conditional, forebrain-restricted overexpressing GR mutants were also developed in order to address the function of the receptor in the pathophysiology of depression. However, increased GR expression specifically in the forebrain is unlikely to occur under normal circumstances (Muller and Holsboer, 2006). Nevertheless, some important insights into how the GR modulates emotional responsiveness have been obtained (Wei et al., 2004). Increased anxiety-related behaviour was observed in conditional GR overexpressing mice, which was reverted by desipramine treatment. However, the HPA axis remained unchanged, rendering this line a model of "increased emotional lability". In contrast, when GR overexpression was achieved by means of inserting two additional copies of the gene using a yeast artificial chromosome, the animals demonstrated a stress-resistant HPA system and showed reduced helplessness after stress exposure (Reichardt et al., 2000). However, it should be noted that in this case the GR overexpression was not restricted to the central nervous system. In this regard, both transgenic lines provided some insights concerning stress responsiveness, but are not very suitable as a model for depression.

It becomes quite clear that no single model fulfils all the criteria necessary to be coined "depression-model". Future technologies, such as constitutive and conditional RNAi transgenesis, zinc-finger and optogenetic approaches will have a great impact on the development of more suitable models for depression and other psychiatric disorders.

## 4. Next generation of genetic models – new techniques and strategies

### 4.1 Models based on Genome-Wide Association Studies (GWAS)

In a genome-wide association study (GWAS) a large number of genetic polymorphisms across the whole genome is examined to identify genetic associations with an observable trait or disease. The power of a GWAS is restricted by the sample size and the technical properties of the genotyping platform used with regard to the coverage of genomic locations. GWASs can be used to detect case-control- or family-based associations (Craddock et al., 2008). The great advantage of this method compared to classical candidate gene studies is that it allows genetic investigation of a disease in a non-hypothesis-driven manner. Using this unbiased approach increases the possibility to find new and even unexpected genes associated with a certain disease. A key factor for this kind of study is a preferably large sample size in order to detect even small effect sizes. There is evidence that for psychiatric disorders most of the heritable risk is due to interactions of combinations of genetic risk variants each with a relatively small effect on the general outcome (Cichon et al., 2009). An obvious challenge concerning the genetic investigation of psychiatric disorders in comparison to non-psychiatric diseases is that the phenotype and the clinical picture of mood disorders are more difficult to define. Therefore, the patient samples used for a GWAS are often rather heterogeneous with regard to sex, age, symptoms, environmental factors and other issues. To increase the statistical power of GWAS studies a so-called meta-analysis can be performed by pooling all GWAS data available for a certain disease and subsequent statistical evaluation in order to increase the sample size. Associations for several candidate genes have been identified in a meta-analysis of genetic studies on major depression, including apolipoprotein E (*APOE*), dopamine receptor D4 (*DRD4*), guanine nucleotide-binding protein (*GNB3*), methylenetetrahydrofolate reductase (*MTHFR*), dopamine transporter (*SLC6A3*) and serotonin transporter (*SLC6A4*) (Lopez-Leon et al., 2008). Additionally, several associations with immune-related genes have been reported, for instance *P2RX7* which is a purinergic ATP-gated calcium channel that modulates macrophage-induced inflammatory responses and is also expressed in neurons and glia cells in the brain. A non-synonymous coding SNP in the *P2RX7* gene (rs2230912) leading to an amino acid substitution (Gln460Arg) has been found to be associated with major depression and bipolar disorder in several independent studies (Barden et al., 2006; Lucae et al., 2006; McQuillin et al., 2009; Hejjas et al., 2009; Soronen et al., 2011). These data highlight *P2RX7* as a new interesting candidate gene for mood disorders even though these findings need further investigation. The proper way to validate the biological significance of such implicated risk variations is the generation of an appropriate *in vivo* model. Using knock-in mouse models, human association data can be validated and pharmacological compounds can be tested *in vivo*. In a study of Chen et al (Chen et al., 2006) a common single-nucleotide polymorphism (SNP) in the brain-derived neurotrophic factor (BDNF) gene, leading to a substitution of methionine for valine at codon 66 (Val66Met) was investigated in a mouse model carrying the BDNF variant. This SNP was shown to be associated with alterations in brain anatomy and memory, but its relevance for psychiatric disorders has been unclear. In response to stress these mutant mice exhibited increased anxiety-related behaviour suggesting that this genetic predisposition combined with an environmental challenge may increase the risk to develop anxiety and depressive disorders (Chen et al., 2006).

## 4.2 Generating models using optogenetics

The use of traditional electrophysiological, pharmacological and genetic methods goes along with considerable deficits, which make them partly unsuitable to study neural circuits with fine spatial and temporal resolution *in vivo* (Carter and de, 2011). To overcome these limitations, a new technology termed optogenetics (Deisseroth et al., 2006) has been developed to precisely stimulate, inhibit or alter the activity of specific cells and their processes with high temporal accuracy and rapid reversibility. The special feature of this approach are effectors which can be activated by light and are genetically encoded allowing direct control of specific cell populations *in vitro* and *in vivo*. The most commonly used optogenetic effectors are genetically engineered variants of natural opsins, light-sensitive ion channels that can be stimulated in response to specific wavelengths of light leading either to membrane depolarisation, hyperpolarisation or change in intracellular signalling (Carter and de, 2011). The first class includes channelrhodopsin-2 (ChR2), isolated from the green algae *Chlamydomonas reinhardtii* which is sensitive to blue light and has already successfully been used in transgenic mice (Arenkiel et al., 2007; Tsai et al., 2009; Huber et al., 2008). Photons are absorbed by the all-*trans*-retinal cofactor of ChR2 that is endogenously expressed at sufficient levels in the central nervous system of vertebrates (Li et al., 2005; Bi et al., 2006; Ishizuka et al., 2006; Zhang et al., 2006). ChR2 can be activated and closed very rapidly upon light on- and offset, respectively, allowing stimulation of neurons within milliseconds (Boyden et al., 2005; Li et al., 2005). The inhibitory counterpart of ChR2, the chloride pump halorhodopsin (NpHR), was isolated from the bacteria *Natronomonas pharaoni* and possesses an activation spectrum in the yellow range, complementary to that of channelrhodopsin. Similarly to ChR2, NpHR uses all-*trans* retinal as chromophore and can therefore be applied in vertebrate organisms without exogenous cofactors. By expressing both proteins in the same cell, one can either activate or silence it by illumination with different wavelengths (Fiala et al., 2010). A third strategy is to design artificial rhodopsin-GPCR chimeras that combine the light responsive elements of rhodopsin with the biochemical signalling functionality of specific GPCRs termed Opto-XRs (Kramer et al., 2009; Airan et al., 2009). Recently, this method was used to develop an Opto-XR that controls serotonin signalling via the 5-HT<sub>1A</sub> receptor (Oh et al., 2010).

Several studies have used optogenetic techniques to investigate the neural circuitries and molecular mechanisms underlying mammalian behaviour and the etiology of neurological disorders (Carter and de, 2011). For instance, recent studies used optogenetics to dissect the neural circuitry within basal ganglia underlying Parkinson's disease (Gradinaru et al., 2009; Kravitz et al., 2010). It was also shown that optogenetic stimulation of the *locus coeruleus* (LC) leads to an immediate shift from sleep to wakefulness whereas optogenetic inhibition causes a decrease in wakefulness (Carter and de, 2011). However, sustained stimulation of the LC produces a behavioural state resembling cataplexy, a transient loss of muscle tone which is a hallmark of narcolepsy. These results suggest that overstimulation can cause behavioural arrests similar to those seen in neuropsychiatric disorders. In addition, using optogenetics, selective phasic photostimulation of dopaminergic neurons in the ventral tegmental area (VTA) was able to trigger behavioural conditioning whereas tonic activity was not (Tsai et al., 2009). Stimulation of dopaminergic neurons in the VTA led to secretion of glutamate into the nucleus accumbens in addition to dopamine, suggesting that mesolimbic reward signalling may involve glutamatergic transmission (Tecuapetla et al., 2010; Stuber et al., 2010). Many other studies using optogenetic probes have been performed,

including associative fear memory (Ciocchi et al., 2010; Johansen et al., 2010; Haubensak et al., 2010), epilepsy (Tonnesen et al., 2009) and others. Thus, optogenetics represents an uprising and promising technique in molecular brain research, which will certainly aid in the development and analysis of new mouse models of depression.

### 4.3 Mouse engineering by means of zinc finger nucleases

Another new promising technique called genome editing enables efficient and precise genetic modification by induction of a double-strand break in a specific target sequence in the genome using zinc finger nucleases (ZFN), followed by the generation of genetic modifications during subsequent DNA repair. These zinc finger nucleases are sequence-specific endonucleases that can be modified to cleave a desired DNA target. This method was initially applied to *Drosophila melanogaster* (Bibikova et al., 2002) and has already been used to disrupt endogenous loci in rats (Geurts and Moreno, 2010), by using this technique, basically any eukaryote can be genetically modified.

Genetic disruption using this technology is achieved by taking advantage of errors introduced during DNA repair to destroy the function of a gene or genomic region in a single step without selection for the desired event (Urnov et al., 2010). This process is called non-homologous end-joining, a template-independent and therefore imperfect repair mechanism resulting in deletions or insertions. For gene disruption in rats, engineered zinc finger nucleases with extended recognition sites were used to produce knockout animals for two different endogenous genes, and transmission of the disrupted alleles occurred at a frequency of 10–100% (Geurts and Moreno, 2010). The great advantage of this ZFN-driven knockout approach is that only one generation is needed, and compares favourably with others strategies such as classical gene targeting in mouse embryonic stem cells considering duration and screening effort.

A second, more complex approach using a ZFN-induced double-strand break which is recombinogenic in higher eukaryotes is called homology-based genome editing. This technique requires the simultaneous provision of an appropriately designed, homology-containing donor DNA molecule along with the locus-specific ZFNs. This enables the study of gene function and modelling of disease-causing mutations through the creation of a point mutation that is characteristic of a known disease-predisposing allele or that disables a motif that is thought to be crucial for function (Urnov et al., 2010). This method was applied to three different genes in *D. melanogaster*, and in up to 90% the offspring of treated animals carried the donor-specific alleles of the target gene (Beumer et al., 2006).

Meyer and colleagues have recently explored whether gene targeting can be directly performed in murine zygotes by the use of zinc-finger nucleases. They reported that gene targeting could be successfully achieved in murine one-cell embryos upon the coinjection of targeting vectors with zinc-finger nucleases, without preselection (Meyer et al., 2010).

Using the ZFN technology will enable to identify and validate genes involved in complex diseases such as depression. Especially for the validation of candidate genes for disease susceptibility identified in linkage and association studies this method could provide a powerful tool in the future (Geurts and Moreno, 2010).

Furthermore, other types of nucleases based on engineered transcription activator-like effectors (TALEs) are currently under development (Christian et al., 2010). TAL effector nucleases have the advantage that they are very simple to engineer and have already been used to target endogenous loci in human cells (Miller et al., 2011). Up to now, no transgenic

organisms based on TALE nuclease technology have been reported yet, but this certainly is just a matter of time.

## 5. Mouse models based on environmental challenges

Besides genetic risk factors, many studies have implicated environmental alterations including stressful life events with the development of affective disorders (Pezawas et al., 2005; Ising and Holsboer, 2006). Exposure to stress or to traumatic life experiences has a strong impact on the manifestation of depression, suggesting an impairment of proper stress-coping strategies in depressed patients (Kessler, 1997; de Kloet et al., 2005). Therefore, depression is also regarded as a stress-related disorder, and, accordingly, many of the animal models of depression are based on the exposure to various types of acute or chronic stressors. However, up to now little consensus exists on the definition of stress. Many studies interpret the presence of a stress response, evident in a sudden increase of corticosterone, as an indicator of stress exposure. However, appetitive and rewarding situations such as sexual behaviour and winning a social interaction elicit HPA axis responses that are similar in magnitude as highly aversive situations like social defeat (Koolhaas et al., 2011). This points out that the physiological response per se does not necessarily indicate a state of stress. In other words, when is a stimulus a stressor and what makes a response a stress response? Koolhaas and colleagues agreed on the view that stress should be considered as a cognitive perception of uncontrollability and/or unpredictability that is expressed in a physiological and behavioural response. Hence, an unpredictable situation should be characterized by the absence of an anticipatory response, whereas uncontrollability can be defined as a reduced recovery of the neuroendocrine reaction (Koolhaas et al., 2011). In that regard the most prominent models will be explained below.

### 5.1 Chronic stress models

The chronic character of stressors is generally considered an important factor in the development of various forms of stress-related pathologies. Several chronic stress procedures have been employed in the past, trying to achieve a measure of construct and face validity. Chronic mild or chronic unpredictable stress involves exposing rodents to a series of repeated physical stressors, including foot shock, restraint, low temperatures, loud music etc, over a period of weeks or longer (Willner, 2005; Nestler and Hyman, 2010). Towards the end of the stress procedure the animals develop signs of anhedonia, which can be reverted by chronic, but not acute, administration of antidepressants. Although this model shows aspects of construct and face validity, it is not easily reproduced across laboratories. This is probably caused by repeated exposure to a certain stimulus, which eventually renders it predictable. Thus, stimuli which have been perceived as stressors at the beginning cease to be perceived as such after a while. This holds especially true for repeated restraint (immobilization) stress in view of the strong decline of the physiological response upon its repetition (Grissom et al., 2008). In this regard, some commonly used animal models of chronic stress may represent models of adaptation rather than models of stress-related pathology (Koolhaas et al., 2011). A means of circumventing this problem is to use stressors with a certain degree of ecological validity. These stressors should be unpredictable, uncontrollable and challenge the natural defence mechanisms of the animal. So far only a few models seem to meet these criteria including chronic social defeat stress. Here, an animal is repeatedly exposed to an aggressive dominant animal, leading to social

subordination, after which the mice show a range of depression-like symptoms, including anhedonia, social withdrawal and cognitive impairments (Berton et al., 2006; Wang et al., 2011). Most of these are reversible by chronic, but not acute, antidepressant treatment (Berton et al., 2006). In addition, chronic social defeat was shown to induce a metabolic syndrome in mice characterized by weight gain, insulin and leptin resistance (Chuang et al., 2010), consistent with homeostatic abnormalities observed in depressed patients. In addition, experience of social defeat leads to changes in the state of the serotonergic and noradrenergic systems of various parts of the brain (Berton et al., 1998). A further advantage of chronic social defeat is the potential to segregate defeated subjects into susceptible and unsusceptible populations on the basis of considerable individual variance to social defeat behavioural outcomes (Krishnan et al., 2007). Thus, the social defeat procedure exhibits features of construct, face and predictive validity, although the intensity of the stress used is more severe than that seen in humans. The main drawback of chronic stress paradigms is that the evoked phenotypes often “resemble social anxiety” and not depression. It is difficult to identify the required stress duration which induces depression-like symptoms rather than sole anxiety responses. Kudryavtseva and colleagues propose that longer sessions of social stress (at least 20 days) are required to induce depression-like phenotypes, whereas Nestler and colleagues claim to observe such behavioural alterations already after 10 days (Berton et al., 2006). Additional studies will help to further elaborate on this aspect and possibly pave the road for improved strategies in modelling depression.

## 5.2 Early life stress models

Similar validity compared to the chronic social defeat model was achieved for early life stress, including prenatal stress, early postnatal handling, and most of all maternal separation (Francis et al., 1996; Ladd et al., 2000; Caldji et al., 2000; Meaney, 2001). Traumatic life events in childhood have been shown to result in an increased sensitivity to the effects of stress later in life and alter the individual's vulnerability to stress-related psychiatric disorders such as depression (Graham et al., 1999; Heim and Nemeroff, 2001). Early life stress in mice produces neuroendocrine and behavioural changes that persist into adulthood, some of which can be reverted by antidepressants (Meaney, 2001). The most widely used model is the maternal separation paradigm of early life deprivation, in which pups are separated from the dam for 1-24 h per day during the first two postnatal weeks. This results in increased anxiety-like and despair-based behaviour as well as increased HPA axis response, all of which can be observed in adulthood (de Kloet et al., 2005). It is important to mention that shorter periods of separation tend to produce opposite effects. Thus early life challenges may conversely induce changes that prepare an individual for life in a more hostile environment and therefore be predominantly beneficial. Hence, it has been proposed that adult diseases such as depression might not be promoted by early life adversity per se, but by a mismatch of the programmed and the later actual environment in combination with a more vulnerable or resilient genetic predisposition (Schmidt, 2011). Although the exact physiological nature of the effects of postnatal maternal separation is not fully understood, the paradigm demonstrated its value for studying the neurobiological basis of the impact of early life stress on emotional behaviour (Cryan and Holmes, 2005). More recently, a new early life stress model based on similar principles has been developed. The main difference is that the new model omits the separation from the mother and thereby avoids metabolic disturbances, exhaustion, or hypothermia of the pups. This is

evoked by means of fragmented maternal care, generated by reducing the amount of nesting and bedding material available to the dam (Rice et al., 2008).

## 6. Gene-environment interactions

Although chronic stress has been implicated in the onset of psychiatric disorders, it has to be kept in mind that not all individuals exposed to severe stress will progress to disease. In that sense, it is also quite unlikely that a single genetic variant is responsible for a specific disorder. Therefore, it is of great necessity to understand the cause of individual differences and the consequences of variation in vulnerability, with regard to disease progression. It is clear that major efforts should be directed towards the combination of genetic modifications and environmental challenges in the same subject. Such stimulation of gene-environment interactions is more likely to reflect the pathophysiological mechanisms of depression. Many studies have already applied this concept by subjecting transgenic lines to chronic social stress procedures (Berton et al., 2006; Wagner et al., 2010; Wang et al., 2011). These studies provide further evidence that disease-associated genetic alterations do not have to be pathological/beneficial under normal conditions, but in combination with chronic stress can either cause vulnerability or resilience towards the development of depression-like phenotypes.

## 7. Classical antidepressants – limitations and future prospects

The majority of antidepressant drug discovery efforts during the past decades have focused on finding more selective serotonin- or noradrenaline-based agonists or antagonists having modes of action similar to already available drugs, only with the ability to act more quickly and safely. However, until now this approach has not lead to improved treatment. There are some novel drugs known as atypical antidepressants which have ascribed monoamine-based mechanisms, but there is only weak evidence that their implied mechanisms actually account for their clinical efficacy. In parallel, non-monoamine systems that might contribute to the pathophysiology of depression have been analyzed in the past, revealing potential biomarkers for depression, such as CRH, P2RX7, BDNF, etc. (see above). However, none of these discoveries has been translated into a new bona fide treatment for depression so far. Ironically, the search for non-monoamine-based antidepressants has often relied on the actions of monoamine-based drugs. This highlights the necessity to develop improved animal models of depression. Applying the techniques and approaches mentioned above should aid in this undertaking and hopefully translate into the development of new treatment modalities apart from classical antidepressants.

## 8. Conclusion

The major problem in the establishment of a suitable animal model of depression is that the development of such a model requires a better understanding of the etiology of the disorders. The current state of clinical knowledge lacks objective diagnostic tests and validated biomarkers of such a highly heterogeneous illness. However, such models are of great necessity for understanding disease pathophysiology and for hastening the development of treatments based on new molecular targets. We have given a general overview of the current mouse models of depression and outlined some of the difficulties in the generation and

validation of such models. In addition, new strategies and technologies have been discussed, which will greatly contribute to our understanding of disease pathology. Nevertheless, it is very unlikely that mice will ever recapitulate all of the salient features of a human mental illness. Above all, models are meant to serve as investigative tools. In that regard, researchers should critically judge construct, face and predictive validity and not simply assume that behavioural alterations in one or more tests are sufficient to render a model of depression. In addition, differences between males and females are often not addressed in preclinical research. Extensive literature reports that mood disorders are more frequent in women than in men, but the great majority of basic research has focussed on male rodents as animal models (Palanza, 2001). This emphasizes the need for reliable depression models in females. In addition other endophenotypes of depression, such as alterations in sleep, should also be addressed more consistently in mice. To accomplish the goal of creating more appropriate animal models of depression, it will be necessary to implement and combine all recent advances in genetics, pharmacology and electrical stimulation with environmental challenges (Fig. 1). This will hopefully initiate the development of new treatment modalities which are based on knowledge and not serendipity.

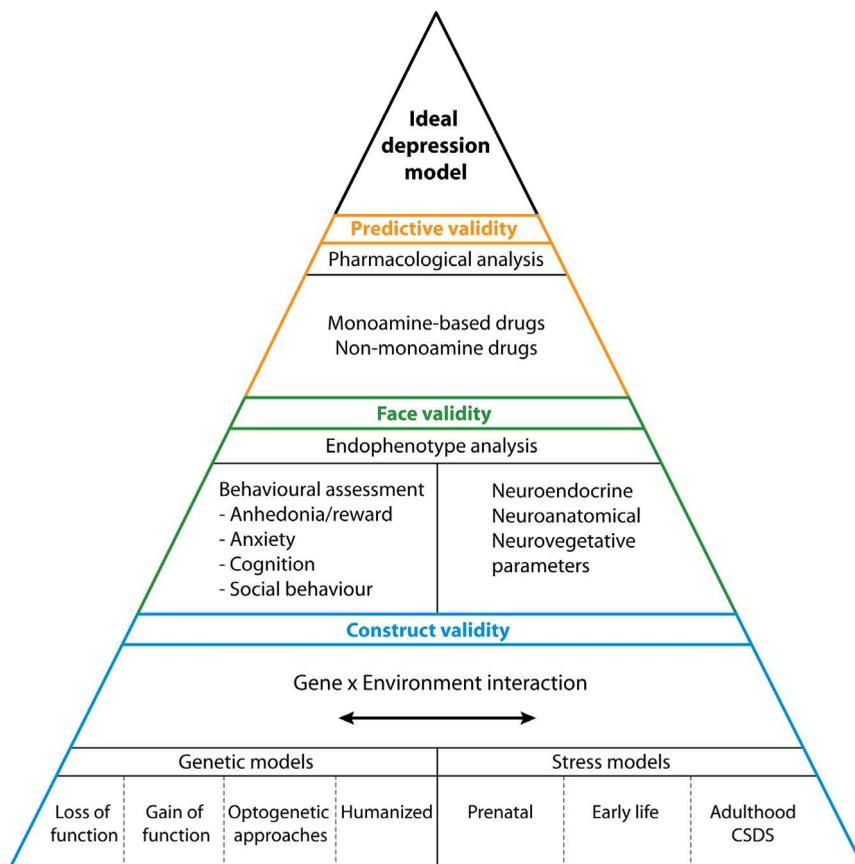


Fig. 1. Towards an ideal mouse model of depression.

In order to generate more suitable depression models with strong construct validity, major efforts should be directed towards the combination of genetic modification and environmental challenges in the same subject. This would simulate gene-environment interactions that more plausibly reflect the pathophysiological mechanisms of depression. Such models should show sufficient face validity, as assessed by behavioural and/or physiological parameters and respond to classical and/or novel drugs (predictive validity).

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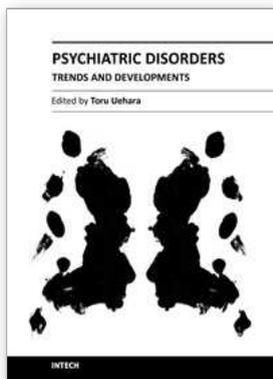
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## Psychiatric Disorders - Trends and Developments

Edited by Dr. Toru Uehara

ISBN 978-953-307-745-1

Hard cover, 514 pages

**Publisher** InTech

**Published online** 26, October, 2011

**Published in print edition** October, 2011

Due to their prevalence, pervasiveness and burden inflicted on men and women of today, psychiatric disorders are considered as one of the most important, severe and painful illnesses. This impairment of cognitive, emotional, or behavioural functioning is in some cases tragic. Aside from knowing the physical organic factors, such as infections, endocrinal illnesses or head injuries, the aetiology of psychiatric disorders has remained a mystery. However, recent advances in psychiatry and neuroscience have been successful in discovering subsequent pathophysiology and reaching associated bio-psycho-social factors. This book consists of recent trends and developments in psychiatry from all over the world, presented in the form of multifarious and comprehensive articles. The first two sections of the book are reserved for articles on schizophrenia and depression, two major illnesses present in this field. The third section of the book is reserved for addiction psychiatry, related not only to socio-cultural but also biological alterations. The last section of the book, titled Biological Neuropsychiatry, consists of three topics - updated molecular biology, fundamental neuroscience and clinical neuropsychiatric conditions. Doubtlessly, this book will be fruitful for future developments and collaboration in world psychiatry.

### How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Nina Dedic, Sandra M. Walser and Jan M. Deussing (2011). Mouse Models of Depression, *Psychiatric Disorders - Trends and Developments*, Dr. Toru Uehara (Ed.), ISBN: 978-953-307-745-1, InTech, Available from: <http://www.intechopen.com/books/psychiatric-disorders-trends-and-developments/mouse-models-of-depression>

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