A translational research framework for enhanced validity of mouse models of psychopathological states in depression

Pryce, C R; Seifritz, E

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Abstract

Depression presents as a disorder of feelings and thoughts that debilitate daily functioning and can be life threatening. Increased understanding of these specific emotional-cognitive pathological states and their underlying pathophysiologies and neuropathologies is fundamental to an increased understanding of the disorder and, therefore, to development of much-needed improved therapies. Despite this, there is a current lack of emphasis on development and application of translational (i.e. valid) neuropsychological measures in depression research. The appropriate strategy is neuropsychological research translated, bi-directionally, between epidemiological and clinical human research and in vivo - ex vivo preclinical research conducted, primarily, with mice. This paper presents a translational framework to stimulate and inform such research, in four inter-dependent sections. (1) A depression systems-model describes the pathway between human environment-gene (E-G) epidemiology, pathophysiology, psycho- and neuropathology, symptoms, and diagnosis. This model indicates that G-->emotional-cognitive endophenotypes and E-G/endophenotype-->emotional-cognitive state markers are central to experimental and translational depression research. (2) Human neuropsychological tests with (potential) translational value for the quantitative study of these endophenotypes and state markers are presented. (3) The analogous rodent behavioural tests are presented and their translational validity in terms of providing analogue emotional-cognitive endophenotypes and state markers are discussed. (4) The need for aetiological validity of mouse models in terms of G-->endophenotypes and E-G-->state markers is presented. We conclude that the informed application of the proposed neuropsychological translational framework will yield mouse models of high face, construct and aetiological validity with respect to emotional-cognitive dysfunction in depression. These models, together with the available technological tools, can then be studied to increase understanding of depression pathophysiology and neuropathology, leading to identification and validation of novel therapeutic targets and the development of effective, personalized antidepressant treatments.
A translational research framework for enhanced validity of mouse models of psychopathological states in depression

Christopher R. Pryce* and Erich Seifritz

Clinic for Affective Disorders & General Psychiatry, Psychiatric University Hospital Zurich, Switzerland

*Correspondence to:

Dr. C. R. Pryce, Preclinical Laboratory for Translational Research into Affective Disorders, Clinic for Affective Disorders & General Psychiatry, Psychiatric University Hospital Zurich, August Forel-Strasse 1, CH-8008 Zurich, Switzerland. Tel.: +41 44 634 8873. E-mail: christopher.pryce@bli.uzh.ch

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Summary

Depression presents as a disorder of feelings and thoughts that debilitating daily functioning and can be life threatening. Increased understanding of these specific emotional-cognitive pathological states and their underlying pathophysiologies and neuropathologies is fundamental to an increased understanding of the disorder and, therefore, to development of much-needed improved therapies. Despite this, there is a current lack of emphasis on development and application of translational (i.e. valid) neuropsychological measures in depression research. The appropriate strategy is neuropsychological research translated, bi-directionally, between epidemiological and clinical human research and in vivo - ex vivo preclinical research conducted, primarily, with mice. This paper presents a translational framework to stimulate and inform such research, in four inter-dependent sections. (1) A depression systems-model describes the pathway between human environment-gene (E-G) epidemiology, pathophysiology, psycho- and neuropathology, symptoms, and diagnosis. This model indicates that G → emotional-cognitive endophenotypes and E-G/endophenotype → emotional-cognitive state markers are central to experimental and translational depression research. (2) Human neuropsychological tests with (potential) translational value for the quantitative study of these endophenotypes and state markers are presented. (3) The analogous rodent behavioural tests are presented and their translational validity in terms of providing analogue emotional-cognitive endophenotypes and state markers are discussed. (4) The need for aetiological validity of mouse models in terms of G → endophenotypes and E-G → state markers is presented. We conclude that the informed application of the proposed neuropsychological translational framework will yield mouse models of high face, construct and aetiological validity with respect to emotional-cognitive dysfunction in depression. These models, together with the available technological tools, can then be studied to increase understanding of depression pathophysiology and neuropathology, leading to identification and validation of novel therapeutic targets and the development of effective, personalized anti-depressant treatments.

**Keywords:** Major depressive disorder, neuropsychology, translational research, environment-gene, stress, endophenotype, state marker, molecular target
1. Introduction

The mental disorder depression is a leading cause of suffering, disability and economic burden worldwide (Murray and Lopez, 1997; Kessler et al., 2005). To-date, the considerable research into depression has failed to identify a single molecular entity, which has then been targeted pharmacologically to lead to the development of a novel antidepressant therapy. The current generation of antidepressant therapies, the selective monoamine reuptake inhibitors, is derived from the tricyclic antidepressants, such as imipramine, and the monoamine oxidase inhibitors, the antidepressant properties of both of which were discovered serendipitously (Pletscher, 1991). The primary mechanism-of-action of tricyclic antidepressants is to inhibit the pre-synaptic reuptake of serotonin and noradrenaline by their respective transporter proteins, and the selective monoamine reuptake inhibitors have this same mechanism-of-action. Whilst the selective monoamine reuptake inhibitors have, relative to tricyclic antidepressants, yielded clinical progress in terms of reduced side effects and increased patient adherence, they are suboptimal in terms of: latency to onset of effect, efficacy relative to placebo in terms of remission and recovery (being ineffective in many patients), and efficacy in terms of prevalence of relapse (Kirsch et al., 2008; Fournier et al., 2010). This suboptimal efficacy of the selective monoamine reuptake inhibitors is perhaps not surprising given the serendipitous discovery of their mechanism-of-action. Accordingly, there is a marked need for one or more novel antidepressant therapies with a mechanism-of-action based on goal-directed research. This situation leads to the central questions of: why have efforts failed to-date, and what changes in research strategy could prove to be critical in turning past and current failure into future successful depression target discovery and antidepressant development?

It is, of course, not novel to propose that the answers to these questions lie within the scope of translational research, defined here as a bi-directional transfer of epidemiological and clinical knowledge on the nature and causes of depression to animal (preclinical) models, and the transfer of mechanistic and neuropharmacological preclinical knowledge back to depressed patients. What is novel in the current paper is the emphasis placed on the need for improved neuropsychological translational validity of preclinical models in depression and antidepressant research. Specifically, the need for improved validity of the tests used to measure animal behaviour/neuropsychology, and of the gene polymorphisms and environmental factors, and their combined effects, which predispose to, trigger and maintain depression-relevant states in these behavioural tests. However, before describing and justifying the advances that are essential at the preclinical level, it is important to describe the current neuropsychological research efforts towards promoting depression understanding at the clinical level. The starting point for this is a summary of the symptoms and the classification/diagnosis of depression.
2. Depression

2.1. Depression symptoms and nosological diagnosis

Depression is a disorder of feelings, thoughts and somatic functions. It presents as a number of heterogeneous emotional, cognitive and motor symptoms, where the clinical assessment of whether and which symptoms pertain is based on the patient’s (and possibly also a relative’s) verbal report to the clinician’s interview as well as on the clinician’s direct observations (DSM-IV, 1994). Depression is diagnosed using nosological classification, i.e. where a disease or disorder is envisaged as a discrete entity characterized by specific symptoms that: are each scored in an all-or-none manner, tend to co-occur together, follow a certain course and have a certain outcome and, in principle but rarely in practise in mental disorders, are underlain by a recognised neuropathology (van Praag et al., 2004). Two nosological classification systems exist for depression disorders, as for the other psychiatric disorders, and these are the Diagnostic and Statistical Manual of Mental Disorders (DSM), 4th edition, text revision (DSM-IV, 1994) and the International Classification of Diseases (ICD), 10th edition, chapter V: Classification of Mental and Behavioural Disorders (ICD-10, 1994). Both of these nosological classification systems are widely accepted and applied, primarily using DSM, as the bases of psychiatric diagnosis. Within “depression” both DSM and ICD recognise several forms of depressive disorder and grades of severity within these forms. According to DSM, major depressive disorder (MDD) is the most common form of depression, and an approximate equivalent of MDD in ICD is (recurrent) moderate depressive episode. The typical symptoms that must (obligate) and common symptoms that can (facultative) pertain to lead to a nosological diagnosis of MDD, according to DSM and its approximate ICD equivalent, are listed in Table 1. Thus, according to DSM, MDD will be diagnosed if the clinical course is one or more major depressive episodes, with each such episode characterised by five (or more) symptoms during a minimum 2-week period, where at least one of the symptoms is either depressed mood (sadness, emptiness) or anhedonia (loss of interest or pleasure in (almost) all activities). According to ICD-10, (recurrent) moderate depressive episode will be diagnosed if the clinical course is one (or more) episodes during a minimum 2-week period of at least two of the three typical symptoms i.e. depressed mood, loss of interest and enjoyment, reduced energy leading to increased fatigability and diminished activity, and at least three (preferably four) of the other, common symptoms (Table 1).

TABLE 1 ABOUT HERE PLEASE

Based on describable and observable symptoms, the DSM and ICD nosologies allow for relatively clear diagnosis of, and unambiguous communication about, MDD. However, it is equally clear that the clinical entity MDD: is a composite of heterogeneous symptoms; these symptoms will
vary in severity within and between patients, but, in the nosology, are depicted as all-or-none; exists without reference to its neuropsychopathology¹; the neuropsychopathology will be different for the different symptoms. Simply put, the heterogeneous nosological entity of MDD is not an entity based on pathology, and because of its heterogeneity and all-or-none nature is not an entity that can be studied in terms of its pathology. As such, there is incompatibility between the current diagnostic system and psychological and neurobiological research that would aim to increase understanding of MDD neuropsychopathology and, based on this, could lead to development of novel, improved treatments. This situation is schematized in Figure 1: a diagnosis of MDD will inform very little about the specific psychological dysfunctions and concomitant neurobiological changes that underlie the patient’s major symptoms. The responses of the individual symptoms and their underlying psychological functions to the chosen course of treatment cannot be monitored. Little will be learned in terms of treatment responses and non-responses of the symptoms/psychological functions that can be used to increase understanding of their aetiology, and therefore could be used to inform research and development into novel treatments.²

FIGURE 1 ABOUT HERE PLEASE

The lack of quantification in the nosological diagnosis of MDD has stimulated the development of rating scales, most prominently the Hamilton Depression Rating Scale (Ham-D) (Hamilton, 1960) and the Beck Depression Inventory (BDI) (Beck et al., 1961). Both Ham-D and BDI are based largely on the same symptoms as those used in DSM and ICD; in addition, in Ham-D, somatic factors, e.g. gastrointestinal complications and diurnal variation, are also added as symptoms, and in BDI, somatic preoccupation and loss of libido are added. In the Ham-D, scores are attributed to each symptom/category by the physician based on the patient’s verbal report and the physician’s observations. In the BDI, scores are attributed to each symptom/category based on the patient’s responses to the structured questions in the inventory. There is no doubt that the Ham-D and BDI are extremely important instruments in the clinic and to some extent at the interface of clinical research and the development of MDD therapies. Thus, they allow for quantitative assessment of therapy efficacy, both for each individual patient and in trials of developmental

¹ Here we use the term neuropsychopathology to describe the changes at the levels of psychology/behaviour, brain circuitry, and inter-cellular and intra-cellular brain functions, which causally underlie the symptoms of MDD.

² Some integration of the focus on specific symptoms and their underlying neuropathology – a dimensional diagnosis approach - is indeed being considered for the upcoming revision of the DSM (Hyman, 2007). Therefore, this aspect of our translational framework is commensurate with current classification efforts in psychiatry.
compounds relative to placebo and existing reference-standard therapies (e.g. (Kirsch et al., 2008; Fournier et al., 2010). However, if a patient is resistant to her/his current therapy then the Ham-D score will, by definition, remain high, meaning that most symptoms were resistant to treatment. No insight will be gained because, as with the DSM and ICD classifications, an overall rating on the scale will not be helpful in terms of identifying the specific psychological dysfunctions and concomitant neurobiological changes that underlie the patient’s major symptoms and treatment resistance.

2.2. From depression symptoms to emotional-cognitive states

The paucity of the epistemological relationship between current depression diagnosis based on DSM or ICD and its neuropsychopathology is exemplified by the fact that one of the major, obligate symptoms of depression is depressed mood. Of course, this tautology raises the question of what, then, is depressed mood? Scientific understanding of mood is derived from emotion theory and, more recently, affective neuroscience. According to emotion theory, emotions e.g. sadness, helplessness, grief, pleasure, are distinct psychological states that vary in intensity and that arise in response to environmental stimuli or events that are either aversive or rewarding as processed by the brain’s punishment system and reward system (Rolls, 2000). Cognitive processes enable the individual in her/his emotional categorization of the environmental stimulus/event. Cognitive processes also allow an environmental stimulus/event to be re-experienced, or ruminated on, in the absence of its physical presence. Environmental stimuli or events are not absolute in their emotional valence; rather, emotional valence is individual-specific. In the case of each individual, the emotional response to a stimulus/event is determined by: his/her alleles for genes that regulate emotional responsiveness; the expression levels of these alleles and their products in the specific brain regions that constitute emotional-cognitive circuits; and his/her life history with respect to prior emotional experiences (Caspi and Moffitt, 2006; Jacobs et al., 2006). Thus, the extent to which an aversive stimulus activates the punishment system and elicits an emotion such as sadness, or the extent to which a rewarding stimulus activates the reward system and elicits an emotion such as pleasure, will depend on current and prior experience as determined by genetic and environmental factors (Berridge and Robinson, 2003; Cools et al., 2007). Given that these same genetic and environmental factors determine personality, then personality is logically also a major predictor of emotional reactivity. Mood or mood state refers to the general emotional experiences, and therefore disposition, of an individual at a typical time point within a certain time period, e.g. day, week, month. It might be that the mood of an individual can be linked to one or more identifiable major emotional events in the past, and that this might be, reactively, dominating emotional processing in the present, but this is not necessarily the case.
As will be clear from the above, the punishment system and reward system of the brain are the bases of emotions, and emotions are the bases of mood. Logically then, the punishment and reward systems are also the bases of mood. Returning to the above question, what is the depression symptom of depressed mood, this can be reformulated as: What dysfunction(s) of the brain’s punishment system result in its chronic hyper-sensitivity leading to the depression symptoms of e.g. sadness, pessimism, helplessness (Elliott et al., 2002)? Using a similar logic, the other typical obligate DSM and ICD symptom of reduced interest and pleasure needs to be viewed as dysfunction in the brain’s reward system, resulting in its chronic hypo-sensitivity to typically rewarding stimuli and events.

Therefore, it is quite straightforward to conduct a discourse between the terminology of psychiatric diagnosis and the corresponding terminology of emotion theory and affective neuroscience. This is extremely important because this interface provides the link between clinical practise with its current limited understanding of and success in treating MDD on the one hand and, on the other, research efforts that aim to increase understanding of and thereby develop improved treatments for MDD. This interface allows for the shift from MDD as an absolute, abnormal emotional-cognitive state, to MDD as several emotional-cognitive states that are each at the relative extreme end of their continuous distribution (Hyman, 2007). By analysing and understanding diagnostic terms such as helplessness, fatigue, absence of pleasure, with respect to their psycho- and neuro-pathology, insights will be gained into: their contribution to MDD; the value of their treatment to the treatment of MDD as a whole; and the neurobiological targets that can mediate successful treatment. The clear implication of this is that MDD research can only be realistically conducted at the level of individual symptoms or states.

So far, this section has summarised the relationship between MDD as a single clinical disorder and MDD as a constellation of dysfunctional emotional-cognitive and associated somatic states. The next sub-section provides a summary of the current major evidence for the aetiology of MDD as a diagnostic entity as well as of certain of its constituent emotional-cognitive symptoms/states specifically. In close conjunction with the evidence for aetiological factors, we introduce a classification at the interface of the aetiology and the neuropsychology/neuropsychopathology of MDD. This classification, namely of endophenotype - state marker - symptom, is central to the framework proposed here for informing translational research in MDD. A systems model depicting these key concepts and their inter-relationships, is given in Figure 2.

FIGURE 2 ABOUT HERE PLEASE
2.3. Genetic and environmental aetiology of depression endophenotypes and state markers

Major depression disorder is associated with specific genetic polymorphisms (G), life stress events (E), and life stress event x genetic polymorphism interactions (ExG) (Caspi and Moffitt, 2006). Integrating this evidence for the complex aetiology of MDD with the evidence, reviewed above, for MDD as a constellation of heterogeneous emotional-cognitive and somatic dysfunctions, indicates that an aetiology-based classification of dysfunction will be heuristically advantageous, if not essential. Accordingly, here we use the term MDD endophenotype to describe a psychological or neurobiological characteristic that is non-pathologic but increases the individual’s susceptibility to MDD. In terms of causality, endophenotypes are associated predictively with a specific gene polymorphism (G) or specific constellation of gene polymorphisms (GxG). Endophenotypes can be affected by environmental factors but, per the definition used here, not by the life stress events that are themselves associated with MDD. We use MDD state marker to describe a pathologic psychological or neurobiological characteristic that underlies or constitutes (an) MDD symptom(s). State markers are associated with life stress events (E) that increase the risk of MDD, and in particular with life stress event x specific gene polymorphism/endophenotype interactions (ExG). This aetiology-pathology heuristic model, also schematised in Figure 2, provides the background for the brief review of the current evidence for MDD aetiology which follows.

The heritability of MDD is estimated to be about 40% and therefore moderate (Sullivan et al., 2000). A number of genetic-epidemiology studies have been undertaken to study for association between specific gene polymorphisms and MDD. These have all been a priori case-control association studies of candidate genes, with candidacy based on polymorphism and an a priori hypothesis, typically relating to monoaminergic or neurotrophic central nervous system (CNS) signalling. Based on recent meta-analyses of these studies, there is evidence for statistically-significant association of a specific polymorphism with MDD for six genes: serotonin transporter (5-HTT, SLC6A4), dopamine transporter (DAT, SLC6A3), dopamine receptor D4 (DRD4), methylenetetrahydrofolate reductase (MTHFR), guanine nucleotide binding protein (G protein) beta polypeptide 3 (GNB3), and apolipoprotein (APOE) (Levinson, 2006; Lopez-Leon et al., 2008). Hypothesis-free study based on genetic linkage has only recently been applied to MDD (Levinson, 2006), and the systematic genome-wide study of association of polymorphisms, including single nucleotides and copy number variants, has not yet been conducted for MDD.

3 “Endophenotype” and “state marker” have been introduced and applied separately as heuristic concepts in medicine/psychiatry by various authors (e.g. Gottesman and Shields, 1973; Van Praag, 1997; Gottesman and Gould, 2003; Caspi and Moffitt, 2006).
Given that MDD is a multi-factorial and heterogeneous entity, then epidemiological genetic evidence for a main-effect association of a specific, single polymorphism with MDD would actually be expected to be rare. From another perspective, the absence of a gene-MDD association could well be misleading with respect to its association with specific MDD endophenotypes or state markers. Clearly, psychological and neurobiological endophenotypes are the appropriate phenotypic level at which to identify novel risk genes and study their functional effects (Casi and Moffitt, 2006; Walters and Owen, 2007) (Figure 2). An interesting example of this is provided by 5-HTT: one polymorphism of this gene is in the form of absence (“short”, s) or presence (“long”, l) of a base-pair segment within the 5-HTT gene-linked polymorphic region (S-HTTLPR) in the gene’s transcriptional control region. The short polymorphism, 5-HTTLPR-s, is associated with reduced 5-HTT expression and resultant reduced 5-HTT presynaptic reuptake (Murphy and Lesch, 2008). Healthy 5-HTTLPR-s individuals exhibit, relative to healthy 5-HTTLPR-l individuals, increased anxiety- or mood-related temperamental traits (neuroticism) (Lesch et al., 1996), increased amygdala reactivity to angry faces, and low connectivity in the negative feedback circuit between perigenual cingulate and amygdala that correlates with slow extinction of negative emotions (Pezawas et al., 2005; Canli and Lesch, 2007) (see next section for description of neuropsychological endophenotypes and state markers). A second reason why a lack of main-effect association of a gene polymorphism with MDD could well be misleading in terms of MDD aetiology, is that MDD state markers and symptoms are associated with ExG. Therefore, longitudinal genetic-epidemiology studies in which genome-wide association analysis is combined with environmental factor analysis are essential, so that ExG/endophenotype interactions that associate with MDD state markers can be identified (Figure 2).

Clearly, the meaningful study of ExG requires not only the identification of risk genes using a G → endophenotype strategy, but also the identification and quantification of the salient characteristics of environmental MDD-risk factors. In broad terms, the E’s most salient to MDD aetiology are stressors. Environmental stressors associated with MDD comprise stressors occurring during development (e.g. childhood abuse, neglect) and adulthood that increase vulnerability to MDD and stressors occurring more proximate to the onset of MDD and that could trigger the disorder (Brown et al., 1995; Kendler et al., 2002; Monroe and Reid, 2008). It is imperative to scientific rigour that, in epidemiological studies, stressors are measured prospectively and objectively. However, it is a substantial challenge to study stressful life events that occur in the contexts of e.g. employment, finance, housing, health, social relationships, with the aims of identifying which of these events are relevant to MDD and, if relevant, what their salient features are (Agid et al., 2000; Monroe and Reid, 2008). The adverse life event categories identified in one major MDD study were: death of a loved one, ending of a romantic relationship, personal failure or abandoned goal, chronic stress (due to work, finances, legal problems, etc), one’s own health
problems, interpersonal conflict between self and other, distress over future events (Keller et al., 2007). It has been proposed that the most MDD-relevant stressful life events: involve threat, loss or humiliation; are very threatening or unpleasant; are experienced by the person directly; and are of distinct onset i.e. acute. If such E’s are associated with MDD, then they have typically occurred within 3-6 months prior to MDD onset (Brown et al., 1995; Monroe and Reid, 2008). Uncontrollability is another important stress factor: experiencing uncontrollable stress can lead to altered activity in stress neurocircuitry and this, depending on genotype → endophenotype, could lead to MDD characterized by the perception of all stressors as uncontrollable, i.e. exposure to uncontrollable stress both causes and characterises MDD (see next section). In the study of Keller et al. (2007), the different adverse life events were significantly associated with different MDD symptoms: for example, death of loved ones and romantic breakups were marked by high levels of sadness, anhedonia, appetite loss; chronic stress and personal failure were associated with fatigue and hypersomnia.

In laboratory research, psycho-stressors have been identified and incorporated into tests for the study of their acute impact in healthy subjects with or without specific risk genotypes, and in depressed patients (see next section). Examples include: uncontrollable painful stimuli (Bolz and Giedke, 1981; Strigo et al., 2008; Diener et al., 2009), uncontrollable cognitive stimuli (Elliott et al., 1996; Michopoulos et al., 2006; Vasic et al., 2008), pictorial facial expressions of anger (Pezawas et al., 2005), unexpected negative feedback (Taylor Tavares et al., 2008), and temporally unpredictable stimuli (Herry et al., 2007). An additional important example is aversive social challenge e.g. the Trier social stress test (Kirschbaum et al., 1993). It is essential to clarify whether these stressors - which do indeed yield increased reactivity in depressed patients relative to healthy controls - share salient features with life stress events that can lead to MDD.

In terms of the current clinical evidence for ExG → MDD association, probably the most striking example to date is the interaction between the 5-HTT gene-linked polymorphic region (5-HTTLPR) and adulthood stressful life events: In a long-term prospective epidemiological study in New Zealand, young adults with one or two copies of the short (s) allele of 5-HTTLPR (5-HTTLPR-s) who experienced ≥3 adult stressful life events (SLEs) were significantly more likely to develop MDD (30% prevalence) than individuals with 5-HTTLPR-s x <3 SLE (10%), 5-HTTLPR-l x ≥3 SLE (15%) or 5-HTTLPR-l x <3 SLE (10%) (Caspi et al., 2003, see also Cervilla et al., 2007). The SLEs taken into account were those experienced at age 21-26 years and could occur within the contexts of employment, finance, housing, health, and social relationships. In a separate analysis of the same study cohort, experiencing severe maltreatment during childhood also increased the prevalence of MDD in s-allele carriers specifically. The longitudinal, prospective nature of this study, together with the careful
methods used to measure stressful life events, render it challenging to replicate. Indeed, several subsequent retrospective studies have failed to do so and there is actually only limited support for the 5-HTTLPR-s x SLE → MDD association from meta-analysis (Munafo et al., 2009; Risch et al., 2009). Suffice to note here that, in the future, the most informative ExG association studies will be prospective, will score and analyse stressors objectively, and will focus on psycho- and neuropathy state markers of MDD rather than on MDD as a (heterogeneous) nosological entity (Figure 2). With respect to psychopathology state markers, automated tests based on nonverbal responses to stimuli of emotional (aversive, rewarding) significance, allow for sensitive, quantitative (dimensional) analysis of emotional-cognitive processes; a number of such tests have been developed that are highly relevant to MDD, as discussed next.

### 2.4 Neuropsychological tests for MDD emotional-cognitive endophenotypes and state markers

Tests of neuropsychological processes have been developed to yield data relevant to a number of neurodegenerative and psychiatric disorders, including MDD. These tests are automated and thereby enable fine control of stimulus presentation and subject responses. Such tests provide valuable state markers for the disorder; they can also be of immense clinical value in terms of assessing disorder course, and even in diagnosis. Probably the best example of the latter to-date is the paired-associates object-in-space memory test for the early detection of Alzheimer’s dementia (O’Connell et al., 2004). For MDD, in order that an automated behavioural test can be regarded as probing a relevant neuropsychological process (endophenotype or state marker), then this process must: (1) have a clear theoretical relationship to one of the MDD symptoms, and (2) have different values, in the predicted direction, in MDD patients exhibiting the related/dependent symptom relative to healthy probands, and within individual patients during an MDD episode versus remission. When such a test has been validated in this way, it can then also be used as (3) a test for the same process, probably at a reduced level of divergence from control values, as an endophenotype, and therefore to assess the association of this psychological measure with candidate MDD-risk genotypes. In addition to their potential to provide important insights into the psychopathology of MDD, (4) automated psychological tests can be combined with molecular imaging methods thereby allowing for the study of the neuropsychology and neuropsychopathology of the states underlying MDD. Another advantage of automated psychological testing, and that which is a major focus of this paper, is that (5) versions of these tests can be developed for the translational study of the analogous psychological processes in laboratory animal species. It is also important to note that some of these tests measure processes that are not relevant to MDD specifically, but also to the anxiety disorders, such as generalised anxiety disorder and post-traumatic stress disorder, with which MDD is frequently co-morbid according to current nosological classifications.
In automated tests for psychological endophenotypes and psychopathological state markers in MDD, the approach will typically be to measure either a proband’s spontaneous responses to emotionally-salient stimuli, or a proband’s operant responses to neutral stimuli associated with emotionally-salient events. The design of such tests needs to be based on and informed by the obligate/core symptoms of depressed mood and loss of interest or pleasure (Table 1) which, as was discussed in section 2.2, are readily amenable to deconstruction in terms of discrete emotional-cognitive processes. Certain of the facultative/common symptoms are also readily amenable to specific emotional-cognitive interpretation. This is particularly so for feelings of worthlessness (DSM) and reduced self-esteem and self-confidence (ICD) (Table 1): these symptoms are highly commensurate with the psychological concepts of learned helplessness, stress uncontrollability and learned irrelevance, for which automated tests have been developed (see below). The same importance of quantitative assessment methods applies to the MDD symptoms that do not, at least directly, reflect emotional dysfunction e.g. insomnia, psychomotor retardation or agitation. We now proceed to present some automated tests of neuropsychological processes that satisfy the five criteria stated above and illustrate the central importance of automated neuropsychological testing to the experimental and translational approach that will enhance understanding and treatment of MDD. These tests, the symptoms to which they are relevant, and, for later discussion, the analogous tests in the mouse, are given in Table 2.

TABLE 2 ABOUT HERE PLEASE

Emotional and motivational reactivity to rewarding stimuli. Neuropsychological tests of individuals’ emotional reactivity to rewarding stimuli are of direct relevance to the core MDD symptom of loss of interest or pleasure/enjoyment i.e. anhedonia (Table 2). Reward is a complex neuropsychological process, the understanding and study of which is facilitated by the recognition of two major sub-processes, namely: the emotional process of recognising and “liking” a stimulus with pleasurable attributes, and; the incentive-motivational process of “wanting” a stimulus with pleasurable attributes and behaving in a corresponding, goal-directed manner to obtain the stimulus (Berridge and Robinson, 2003). Reduced reward liking is primarily synonymous with the loss of (i.e. reduction in) the pleasure/enjoyment aspect of anhedonia, and reduced reward wanting with the loss of (reduction in) the interest aspect. Neuropsychological tests have been developed that allow for quantification of reward liking or reward wanting, and the neuropsychological profiles of healthy probands and, relative to these, MDD patients in these tests have been investigated. Measurement of reward liking has focussed primarily on verbal valence ratings of the pleasantness experienced when presented with pictorial images from an established inventory for which normative ratings are available, e.g. the International Affective Picture System (IAPS) (Lang et al., 1997). MDD patients with
high levels of anhedonia exhibited reduced ratings of positive images, but not of neutral images, relative to control subjects (Sloan et al., 2001; Mitterschiffthaler et al., 2002). A measure related to the liking valence attributed to a positive pictorial image is that of the ability to recognise an image as emotionally positive. Such a test can be based on a series of pictures of faces expressing gradually increasing intensity of a positive emotion e.g. happiness: MDD patients are impaired in their ability to recognise happy faces, requiring a more intense happy facial expression than do healthy controls (Joorman and Gotlib, 2006). Measurement of reward wanting has deployed cognitive tasks (e.g. working memory) with low-level financial pay-off contingencies e.g. a reward condition in which a subject earns $0.10 each time they make a correct response. By using neutral, rewarding and punishment contingencies, it is possible to study whether MDD patients exhibit a deficit in behaving so as to maximise their reinforcement and whether this is specific to the reward contingency and therefore likely to indicate a deficit in incentive motivation. Indeed, a deficit in performance during reward conditions specifically has been observed in MDD patients relative to control subjects (Henriques and Davidson, 2000).

Reactivity to emotionally negative stimuli. MDD patients frequently report feelings of fear and anxiety (DSM-IV, 1994). This suggests that emotional reactivity to discrete aversive stimuli will be increased and will constitute an important (albeit non-specific) state marker for MDD (Table 2). Neuropsychological tests to quantify aversive emotional reactivity have been established using presentation of photographs of anonymous human faces expressing varying intensities of specific negative emotions e.g. sadness, fear (Nowicki and Carton, 1992). MDD patients who were impaired in their sensitivity to happy faces (see above) did not differ from healthy controls in their ability to identify sad or angry faces, suggesting that they were not more sensitive to faces expressing negative emotions (Joormann and Gotlib, 2006). At the neural level, functional magnetic resonance imaging (fMRI) studies have demonstrated increased amygdala reactivity (left-hemisphere specific or bilateral) to sad or fearful faces in MDD patients relative to controls, as well as reduction in amygdala reactivity in MDD patients following a course of antidepressant treatment (Sheline et al., 2001; Fu et al., 2004). A reliable finding in MDD patients is that they exhibit preferential processing of negatively toned stimuli in tests of attention and memory i.e. cognitive bias (Elliott et al., 2002; see Emotional-cognitive reactivity to ambiguous stimuli). Another situation in which MDD patients exhibit increased reactivity to negative stimuli is in emotional-cognitive tasks where negative emotional distracter stimuli have to be ignored (filtered out) in order to perform a cognitive (e.g. memory) task that involves processing of neutral stimuli (e.g. (Gotlib and Krasnoperova, 1998)).

Emotional-cognitive reactivity to aversive uncontrollability. MDD patients frequently report feelings of: hopelessness, helplessness, lack/loss of control over environmental events, inability to
cope (Beck et al., 1974) (Table 2). These psychological states would appear to be closely inter-related and, whilst they are not diagnostic symptoms of MDD, they would appear to be of substantial relevance to this psychiatric condition. Indeed, non-control over aversive stimuli/events could be a major environmental aetiological factor in MDD, and genetically- and/or environmentally-induced perceived non-control over stimuli/events (non-controllable and normally controllable) could be a major psychopathology in MDD. The term learned helplessness was introduced in experimental psychology as an explanation for the observation that animals exposed to inescapable electric shocks (i.e. aversive unconditioned stimuli) exhibit a subsequent deficit in escape or avoidance behaviour, relative to animals exposed to escapable or no electric shock. The learned helplessness test in mouse is described below, but it is important here to briefly outline the theoretical interpretation and MDD-relevance of the phenomenon. Learned helplessness theory posits that animals can learn that environmental events occur independently of their responses; thus, just as operant learning theory proposes that subjects learn that their behaviour controls the stimulus/reinforcement, learned helplessness proposes that subjects learn that their behaviour cannot control the stimulus/reinforcement (Seligman et al., 1971; see Section 3.3.).

Human versions of the learned helplessness test have been developed by fairly direct translation of the aversive unconditioned stimulus pre-exposure stage of the animal learned helplessness test; that is, with subjects being exposed to either avoidable versus yoked unavoidable electroshocks or escapable versus yoked inescapable electroshocks. Human subjects are then assessed and compared in terms of neural, physiological and psychological state. In healthy subjects, exposure to unavoidable electroshocks did not induce, relative to avoidable-shock subjects, any affect on vascular arousal, or subjective feelings of fear or exhaustion. However, it did induce reduced vascular arousal and increased giving up on a follow-on cognitive test, correct performance of which was reinforced with noise-escape. The authors of this study infer a learned helplessness-based cognitive mediating process via which subjects exposed to unavoidable shocks develop a learning set that their behaviour will be ineffective in controlling aversive stimulation (Hokanson and Sacco, 1976). In another learned helplessness study (Diener et al., 2009), this time in MDD versus healthy subjects, two auditory stimuli were presented, with Stimulus 1 preceding Stimulus 2, and Stimulus 2 being a conditioned stimulus for electroshocks. Controllability of the aversive stimulus via operant response differed across three successive conditions: Control, Loss of control (50% of trials); Restitution of control. Subjective ratings of controllability were similar in MDD and healthy subjects, being reduced during the Loss of control condition. Subjective ratings of helplessness and emotional unpleasantness were significantly increased in MDD versus healthy subjects across all three conditions, and were fairly similar across all three conditions within subject groups. MDD subjects exhibited relatively increased post-imperative negative variation of the electroencephalogram
relative to healthy subjects during loss of and restitution of control conditions (Diener et al., 2009). In another recent study, MDD and healthy subjects were compared in terms of their fMRI neural activity during a heat pain-anticipation period: MDD patients exhibited relatively high activity in the dorsal anterior cingulate cortex and the amygdala. In the MDD subjects only, amygdala activity correlated with the self-assessment score of helplessness feelings during the anticipation period, articulated as “There is nothing I can do to reduce the intensity of pain.” (Strigo et al., 2008).

Learned helplessness is specific to situations in which the unconditioned stimulus is (perceived as) aversive and thereby stimulates emotional-stress systems. However, the theory has been extended to account for behaviour relative to rewarding stimuli by studying the effects of pre-exposure to a lack of association between a neutral stimulus and reward on subsequent discriminatory learning of an association between that stimulus and reward. The effect of pre-exposure to lack of association is to impair subsequent acquisition of the association, a phenomenon referred to as learned irrelevance (Maes et al., 2004). It can be hypothesized that MDD patients will exhibit increased learned irrelevance, as well as increased learned helplessness, due to an increased sensitivity to negative events, but this remains to be systematically investigated.

In the original learned helplessness studies, with rats, it was demonstrated that uncontrollable electroshocks had more detrimental effects if delivered on an unpredictable compared with a predictable schedule, where predictability was provided by a tone conditioned stimulus (Weiss, 1971). Recent translational (human-mouse) studies have investigated the effects of exposure to predictably per se, by exposing subjects to neutral auditory stimuli presented on a predictable or unpredictable time schedule. Healthy subjects exposed to neutral sound pulses on a temporally unpredictable schedule exhibited relative amygdala hyper-activity and increased attentiveness to emotional faces (Herry et al., 2007), suggesting that the investigation of unpredictability, and related processes such as uncertainty, with respect to their ability to elicit and identify state markers of MDD, will be important (e.g. (Bach et al., 2010)).

**Emotional-cognitive response to negative feedback.** Negative feedback refers to the subject performing an operant response and not obtaining a reward, perhaps associated with a no-reward (punishment) signal. The valence of any reward would be expected to be reduced in (anhedonic) MDD versus healthy subjects. Despite this confound it has still been possible to design a test of reactivity to negative feedback in which it can be demonstrated that MDD subjects exhibit increased sensitivity to withholding of reward (Cools et al., 2002) (Table 2). Using inherently neutral stimuli (S’s) presented on a touch-sensitive screen, subjects are instructed to maximise the positive feedback they obtain by learning and selecting the positive discriminatory stimulus (S+) i.e. that S which yields the feedback “Correct!”. On a probabilistic reversal learning test, the identity of S+ reverses after a
number of correct responses so that the previous S- now becomes S+, and vice versa. In addition, on some trials, about 20%, the subject receives misleading negative feedback i.e. choosing S+ yields “Incorrect!” feedback. MDD patients are hyper-sensitive to this misleading negative feedback in terms of their increased likelihood to exhibit lose-shift behaviour, i.e. to choose S-, on the next trial (Taylor Tavares et al., 2008).

**Emotional-cognitive reactivity to ambiguous stimuli.** Reduced pleasure and interest and increased aversive reactivity as independent state markers of MDD have already been addressed above. A further marker is high bias to negative expectancy (Table 2), referring to the emotional-cognitive assessment of ambiguous stimuli; that is, stimuli that could potentially be either rewarding or aversive because of the ambiguous sensory information that they relay. Cognitive biases in assessment of emotional stimuli can be grouped into various categories, such as attention bias, memory bias and judgement bias (Elliott et al., 2002; Paul et al., 2005). We would include attention and memory biases as examples of aversive reactivity (see above), and are concerned here with judgement bias with respect to emotional stimuli and the increased negative judgement bias reported for MDD patients. Emotional-cognitive judgement making includes assessment of ambiguous stimuli, expectations about the future, and risk-taking. For example, using subjective probability assessments of ambiguous situations, individuals in a sad mood overestimate the likelihood of negative outcomes and underestimate the likelihood of positive outcomes, relative to controls (Wright and Bower, 1992). On a verbal fluency paradigm to assess the ability of subjects to think of future positive and negative personal experiences, non-clinical subjects who were classified as anxious-depressed on scales derived from the Present State Examination exhibited both greater anticipation of negative experiences and reduced anticipation of positive experiences, relative to non-anxious, non-depressed probands (MacLeod and Byrne, 1996).

**Physical effort required to complete a manual test.** The state of feelings of lack of energy and of fatigue is an important symptom of MDD (Table 1), and is at the interface of somatic, emotional and cognitive pathologies. Fatigue can be divided into components of psychomotor retardation, physical tiredness and mental fatigue (Demyttenaere et al., 2005) (Table 2). Such separation of fatigue into distinct components is important with respect to its quantification, but there have been relatively few attempts to develop specific tests. One test that has been used to quantify the physical tiredness component of fatigue is the measurement of grip strength using a dynamometer: MDD patients exhibit relatively rapid reduction of the grip strength of the right forelimb across trials of maximum grip strength. This co-occurs with a reduction in the right-left asymmetry of grip strength, and is associated with relative hypo-activation of the left anterior cortical region (Emerson et al., 2001).
Following the framework of aetiology → endophenotype/state marker/symptom used above for human MDD research, then any mouse model of an MDD endophenotype or state marker also needs to comprise a combination of aetiological factors and phenotypic readout. Accordingly, a combination of a G, E or ExG manipulation with aetiological validity and a behavioural test with face and construct validity constitutes a translational mouse model of a MDD endophenotype or state marker. In Sections 3 and 4, respectively, some behavioural tests with demonstrated or potential construct/face validity and some genetic and environmental manipulations with demonstrated or potential aetiological validity, are presented.

3. Mouse behavioural tests in translational depression research

3.1. The central importance of valid behavioural tests in mouse models of depression

In the preceding sections the case for the importance of assessment of emotional-cognitive neuropsychological status in depressed patients has been presented. Depression is a disorder of feelings and thoughts, and we have described the inter-relationships (schematized in Figure 2) between the current diagnostic symptoms of MDD, the emotional-cognitive states that describe the psychopathology of MDD, and psychological tests that provide for quantification of these state markers, as well as of their neural correlates using functional imaging procedures. The aim of the current section is to describe the translational links, or “bridges”, that either already exist or can be developed, between these neuropsychological MDD state marker tests and equivalent tests in mice. Figure 3 presents this translational scheme: it takes the diagnosis - symptom - state marker - neuropsycho-pathology scheme presented for MDD (Figure 2) and extends this to mice, proposing that translational state markers with analogous neural and psychological concomitants to those identified in human MDD, exist in mice. The critical step is then to develop tests for these mouse state markers that are valid with respect to the validity criteria that must be met by such tests.

Table 2 lists the mouse tests that would be the equivalent to each of the human tests described above. The validity criteria of the behavioural tests, based on the terminology originally proposed and justified by Willner (Willner, 1984), are: (1) Face validity i.e. there must be a demonstrated emotional or emotional-cognitive phenomenological similarity underlying the respective state marker readouts of the mouse test and the human test to which it corresponds. Furthermore, the direction of the change (increase or decrease) in the readout in the manipulated versus control mice must be the same as that in the MDD versus control human subjects. (2) Construct validity i.e. the mouse-human face similarity must be underlain by similar psychological, physiological and neurobiological changes in the manipulated versus control mice and the MDD versus control human subjects. The additional validity criteria of (3) aetiological validity and (4)
predictive validity are presented in the appropriate sections below. In section 3.3 we describe the mouse tests which do or could, with further validation research, fulfill these validity criteria for the MDD translational state markers of interest, as listed in Table 2. Preceding this, a short summary is provided of some mouse behavioural tests that are currently commonly applied with the aim of measuring psychological states relevant to MDD and their pharmacological modulation.

FIGURE 3 ABOUT HERE PLEASE

3.2. Current mouse behavioural tests: deficits in face and construct validity

Due in part to the lack of emphasis on discrete endophenotypes and state markers in psychiatry it has, until recently, been extremely difficult for preclinical researchers to identify translational state markers and develop corresponding tests with face and construct validity (Willner, 1997; Willner and Mitchell, 2002; Markou et al., 2009). Despite the new emphasis on endophenotypes and state markers there continues to be, however, a large number of mouse studies that are based on behavioural tests that have a proven relevance to MDD in terms of their readout being sensitive to antidepressant compounds rather than in terms of demonstrated face and construct validity. For example, the forced swim test and the tail suspension test in mice are still widely used in depression research to study the effects of compounds in drug discovery (i.e. screening) as well as the effects of genetic (G) and environmental (E) manipulations (for excellent reviews see (Cryan et al., 2005; Cryan et al., 2005)). The common application of the mouse forced swim test and tail suspension test is linked directly to the robust and reproducible effects of the current and previous generations of monoamine reuptake inhibitors on mouse behaviour in these tests. The effect of the existing monoamine reuptake inhibitor drug classes on mouse behaviour in these tests was demonstrated retrospectively (Porsolt et al., 1977). A test with demonstrated retrospective predictive validity is of course markedly different from a test that has demonstrated face and construct validity with respect to an MDD translational state marker, exhibits predictive validity with respect to reference antidepressants, and prospective predictive validity with respect to a new compound class in development that subsequently exhibits antidepressant efficacy with respect to one or more state markers/symptoms in clinical trials. Despite this and, specifically, despite the lack of demonstrated face or construct validity of the forced swim test or tail suspension test, these tests continue to be widely incorporated into studies.

In both the forced swim test and tail suspension test, motor inactivity develops across time in the test, and this increase in inactivity is antagonised by acute or chronic administration of reference antidepressants. The neuropsychological mechanisms underlying both development and pharmacological antagonism of the observed inactivity are unclear. Behavioural despair resulting
from uncontrollable stressor exposure is often cited as the mediating mechanism (Porsolt, 2000). As will be clear from the central theme of this paper, the proposal that despair resulting from an uncontrollable stressor mediates mouse behaviour in the forced swim test and tail suspension test is problematic. Specifically, this is because: (1) (Even) in humans, despair is multi-factorial and difficult to quantify. (2) There is no comparison group exposed to controllable stress. (3) The tests do not yield a measure of despair that is independent of the measure of inactivity, and therefore there is no possibility to separate despair from inactivity and to study their antidepressant responses separately. It would therefore be parsimonious to propose increased inactivity as the mediating mechanism in the test, and inhibition of increased inactivity as the mechanism of action of reference antidepressants on behaviour in the test.

Therefore, mouse tests with potential predictive validity must have demonstrated face and construct validity and must, therefore, be based on translational state markers. Some existing such tests and potential future candidates are discussed in the next section.

3.3. Mouse behavioural tests with face and construct validity for MDD endophenotypes and state markers

For predictive (i.e. translational) research in MDD, mouse behavioural tests are required that deliver quantitative readouts which are unambiguous analogues of psychological endophenotypes and state markers of MDD. As will be clear from the above discussion of the forced swim test and tail suspension test readouts, establishing such face and construct validity demands that the psychological mediating mechanisms are identified and that potential alternatives are excluded. The mouse behavioural tests described here are the analogue tests of the human MDD endophenotype and state marker tests presented above, which were themselves selected because of their translational value or potential (see Table 2). Whilst the focus of this paper is translational psychological and psychopathological MDD research, it is clear that understanding psychological mechanisms is intimately related to and dependent on understanding neurobiological mechanisms. The neurobiological construct validity of the behavioural tests are not reviewed in detail below, but brief summaries are given and key references cited.

Preference for palatable stimulus versus water. This type of test is proposed as an analogue of human tests that measure emotional reactivity to rewarding (visual) stimuli, and therefore as relevant to the obligate symptom of loss of pleasure/enjoyment (Table 2). The test is based on presentation of free consumption (i.e. without separate operant response) of a palatable gustatory stimulus, typically weak sucrose solution, versus neutral water in a discrimination-choice situation, followed by measurement of the relative consumption of the two solutions. The hypothesis is that
the sucrose preference ratio provides a measure of reward sensitivity or hedonic liking, such that a reduced sucrose preference will constitute an analogue endophenotype or state marker of loss of pleasure/enjoyment. The test was originally developed for studying the effects of exposure to chronic unpredictable mild stressors (see section 4.3 and Table 3) on their sucrose preference of rats (Willner, 1997). Rats exposed to chronic unpredictable mild stressors develop a reduction in their sucrose preference and this reduction is reversed gradually by chronic antidepressant administration. The test has been modified for mice and applied to study effects of chronic unpredictable mild stress and social defeat stress on their consummatory preference for sucrose versus water, and antidepressant effects thereon (Monleon et al., 1984; Krishnan et al., 2007). The neurobiology underlying preference for palatable solutions is well-described in rodents, but the changes underlying reduction in this preference less so (Berridge and Robinson, 2003).

Operant responding for palatable stimulus on delayed reinforcement schedule. These tests are proposed as analogues of human tests that measure incentive motivation relative to rewarding stimuli, typically money, and that therefore assess reward wanting. They are therefore relevant to the loss of interest symptom in MDD (Table 2). Again, the unconditioned stimulus is gustatory-palatable e.g. sucrose pellet, sucrose solution. In such tests the mouse is required to execute specific operant responses, e.g. nose pokes. First, the mouse is trained on the association between its operant response and delivery of reward. Then a delayed reinforcement schedule is introduced such that the effort of the delay between onset of responding and delivery of reward provides a measure of incentive motivation or hedonic wanting. Typical reinforcement schedules are the variable-interval schedule, where the interval between the previous reinforcement and the first operant response that will activate the next reinforcement is unpredictable, and the progressive-ratio schedule, where the subject must execute more operant responses for the next reinforcement than it did for the previous reinforcement (Davey, 1981). Such schedules require effort in terms of waiting time, operant effort, and coping with unpredictability. The hypothesis is that responding on such operant schedules provides a measure of incentive motivation/reward wanting; therefore, a relatively low rate of responding on a variable-interval or a progressive-ratio schedule or a relatively rapid giving up of responding on a progressive-ratio schedule, will constitute an analogue endophenotype or state marker of loss of interest. One confounding mediating mechanism that could also account for such readouts, and which would be relevant to MDD but not to anhedonia, would be an increased sensitivity to the negative feedback received during non-reinforced responses (see Emotional-cognitive reactivity to negative feedback, below). In rats, the effects of early life stress on incentive motivation for reward in adulthood has been studied using operant responding for sucrose solution on a progressive-ratio schedule: early life stress as daily deprivation of social contact led to adults that exhibited reduced responding, and this effect was reversed by chronic antidepressant
(fluoxetine) administration (Ruedi-Bettschen et al., 2005; Leventopoulos et al., 2009). In mice, chronic corticosterone exposure has been demonstrated to lead to reduced operant responding for sucrose pellets on a progressive-ratio schedule; this effect was reversed by chronic antidepressant administration (Gourley et al., 2008). As with reward liking, the neurobiology underlying rodent incentive motivation for palatable stimuli is well-described in rodents, but the changes underlying reduction in this preference less so (Berridge and Robinson, 2003).

**Emotional reactivity to discrete stimuli and to contexts associated with innately negative (footshock) stimuli.** When rats and mice are exposed to a novel environment/context and to a neutral discrete stimulus (e.g. tone, light) that predict, via classical conditioning, an aversive unconditioned stimulus in the form of brief inescapable electric foot shocks, they develop freezing behaviour, indicative of context- and discrete stimulus-conditioned fear. Primarily because of the pioneering studies demonstrating that the amygdala, most notably its basolateral and central nuclei, is a major mediator of these fear processes (LeDoux, 2000), fear-conditioned freezing provides an analogue for the human tests that measure aversive reactivity (Table 2). As noted above, the human tests used in MDD research involve subject sensitivity and amygdala reactivity to innate stimuli such as sad or fearful faces, so there is a distinct difference here in that the rodent tests are based on classical conditioning. There is construct validity with respect to MDD in that, certainly in rodents, the amygdala is the area in which conditioning occurs. Nonetheless, fear-conditioned freezing is particularly relevant to affective disorders such as specific phobias and post-traumatic stress disorder, and indeed can be used to study the development, consolidation, recall, extinction and reconsolidation of discrete stimulus- and context-conditioned fear (Blanchard et al., 2001).

**Two-way escape deficit after uncontrollable stress.** This type of “learned helplessness” test is proposed as an analogue for human tests of emotional-cognitive reactivity to stimulus uncontrollability, and therefore as relevant to the MDD state marker of feelings of lack of control or helplessness (Table 2). Indeed the human tests are adapted from the animal tests and inescapable (uncontrollable) (IES) versus escapable (controllable) (ES) electroshock is deployed as the aversive US, or stressor, across species. In rodent tests, repeated pre-exposure to IES versus the same amount of ES and versus no electroshock (NS), leads to a subsequent deficit in escape behaviour, typically in a two-way shuttle arena escape test, in the IES group relative to the other two groups (ES, NS), which exhibit similar, high levels of escape behaviour.⁴ In rats, pre-exposure to electroshock has typically taken the form of tail shocks, which are terminated in the ES group by turning a running wheel with

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⁴ In contrast to the other tests presented in Table 2, this test includes prior exposure to an environmental stressor as an inherent component. According to the terminology used here, the test is therefore a model. Section 4 provides further explanation of this important distinction.
the forelimbs (Weiss, 1971). In mice, pre-exposure to electroshock has typically taken the form of foot shocks in the same environment as used in the escape test (Anisman and Merali, 2001).

The deficient escape behaviour exhibited by the IES group is interpreted as a learned expectation that aversive stimuli occur independently of behaviour (Maier and Seligman, 1976). Nonetheless, the face and construct validity of the test needs to stand up to the same challenges as those used above to demonstrate the lack of validity of the forced swim test and tail suspension test (FST/TST) as tests of despair. A major advance of the learned helplessness test relative to FST/TST is that the deficit exhibited by the IES cohort is clearly due to stressor uncontrollability, this being the only difference between the stressor experienced by this cohort and the ES cohort. Another criticism raised above against FST/TST, and that has also been levelled against learned helplessness tests, is that it is unclear whether the escape deficit readout is due to motor inactivity per se or whether the motor deficit reflects an emotional-cognitive state based on learning that footshock is independent of behaviour. These alternative explanations are depicted in Figure 4A. Also with respect to this challenge of validity, the learned helplessness test has been much more rigorously studied than have the forced swim test and tail suspension test: Using a test of signalled-punishment suppression of operant behaviour, it has been demonstrated for learned helplessness rats that they also exhibit a subsequent deficit in behaviour-outcome expectancy in this test, in that they exhibit more operant responding, reflecting increased motor behaviour, during the conditioned stimulus that signals that operant responding will elicit foot shock as well as reward (Jackson et al., 1978). This triangulation approach - depicted in Figure 4B - provides strong evidence that impaired behaviour-outcome expectancy (i.e. an emotional-cognitive deficit) underlies the learned helplessness effect and that it is not confounded by motor inactivity.

FIGURE 4 ABOUT HERE PLEASE

Regarding neurobiological mediation, rats that have experienced IES appear, relative to ES rats, to exhibit deficient inhibitory input from the ventromedial prefrontal cortex to the dorsal raphé nucleus. This would lead to the increased dorsal raphé serotonin ouput to cortico-limbic-striatal regions which has been demonstrated to occur in IES rats that then go on to exhibit a subsequent escape deficit. This finding suggests that the assessment within the ventromedial prefrontal cortex of the controllability of the stressor is a critical neurobiological mediator of controllability/helplessness (Amat et al., 2005). According to this hypothesis, experiencing uncontrollable stress can lead to reduced activity in the prefrontal cortex which, if this prefrontal cortex hypo-activity became chronic, could lead to a generalized state of perceiving all stressors as uncontrollable, i.e. exposure to uncontrollable stress both causes and characterises helplessness in MDD due to prefrontal cortex hypo-activity.
In the description of tests of human learned helplessness (section 2.4), it also refers to the recent evidence for unpredictably per se having aversive emotional valence. The study reporting this finding (Herry et al., 2007) was in fact translational: It also demonstrated that, in mice, temporally unpredictable sound pulses lead to increased expression of the immediate-early gene c-fos and impaired rapid habituation of neuronal activity in the basolateral amygdala, and to increased anxiety readouts on the approach-avoidance conflict tests of elevated plus maze and passive avoidance. Therefore, effects of auditory unpredictability need now to be studied at the behavioural and neural levels in MDD patients: evidence for increased aversive reactivity would indicate this as a state marker that would be clearly amenable to translational study.

**Emotional-cognitive reactivity to negative feedback.** There is a recent publication of successful application of a probabilistic reversal learning test in rats (Bari et al., 2010), allowing for the translational study of the increased negative feedback sensitivity state marker of MDD (Table 2). So far, to our knowledge, there is no published method for a probabilistic reversal learning test in mice, which would. It will certainly be worthwhile to attempt to develop such a test: For example, mice could be trained to respond for reward at two nose-poke operanda that are placed in separate locations in a Skinner box. Responding to one operandum (“incorrect”) would be punished by a time out at 100% contingency and responding to the other operandum (“correct”) would be rewarded at 80% contingency and misleadingly punished at 20% contingency. Following 8 correct trials out of 10 consecutive trials, the response-reward contingency would be reversed so that the previously incorrect operandum is now “correct” and rewarded at 80% contingency. As in the equivalent human test, major dependent measures would be total number of correct trials, total number of incorrect trials, and total number of erroneous lose-shift trials following misleading punishment.

**Emotional-cognitive reactivity to ambiguous stimuli.** The establishment in rats and mice of operant discrimination tests that require the subject to make a behavioural response that conveys its emotional-cognitive assessment - as rewarding or aversive - of a sensorially-ambiguous stimulus could well provide validity for the translational study of the MDD state marker of high bias to negative expectancy (Table 2). As noted above, the prime concern here is the judgement aspect of cognitive bias (Paul et al., 2005). One such judgement test has been described for rats: Subjects were conditioned on two simultaneous tone-stimulus - operant response schedules, where one tone (S+) signalled reward (food) following an operant response and a second, distinctly different tone (S-) signalled avoidance of punishment (loud noise) by withholding the operant response. Introduction of intermediate - and therefore emotional-cognitive ambiguous - tone stimuli allowed for the probing of whether the subject anticipated a positive or negative event. Rats that had experienced an environment of chronic unpredictable mild stress were less likely to perform an operant response to
the ambiguous stimuli (suggesting that they had been classified to the tone that non-ambiguously signalled 'withhold operant response to avoid punishment'), suggesting that they had developed an emotional-cognitive bias to expect negative events (Harding et al., 2004). This pioneering demonstration that such a test can be acquired by rats and that behaviour in the test is stressor-sensitive, provides incentive for development of such tests in mice, given their validity with respect to an important MDD state marker.

**Effort/reward choice behaviour and physical effort to complete a manual task.** Although reduced energy/increased fatigability/diminished activity is, according to ICD-10, a core symptom of MDD (Table 1), and despite the availability of rodent behavioural tests for measurement of psychomotor retardation, very few studies have focussed on rodent models of this MDD state. With respect to tests, rats and mice can, for example, be trained and tested in a T-maze apparatus where one arm of the maze is consistently baited with a large amount of palatable food (reward) but requires energetic behaviour, e.g. climbing, to retrieve it, and the other arm is consistently baited with a small amount of reward that is retrieved easily. Non-manipulated rats strongly prefer the large, demanding reward (Mott et al., 2009), thereby providing a basis for the study of any psychomotor fatigue induced by MDD-relevant genetic and environmental manipulations. As described above, relatively rapid reduction in grip strength has been demonstrated in MDD patients, and using an operant apparatus analogous to the human dynamometer test combined with positive reinforcement, grip strength can also be measured readily in rodents (Connolly et al., 2001). Of course, tests of psychomotor performance based on appetitive behaviour for reward can be confounded by effects of manipulations on reward sensitivity per se and such effects need to be controlled for as much as possible, so as to achieve valid tests of fatigue.

4. Mouse genetic and environmental manipulations in translational depression research

4.1. The central importance of manipulations with aetiological validity in mouse models of depression

Here we present the fourth and final section of our translational research framework for improved face, construct and aetiological validity of mouse models of psychopathological states in depression, namely the integrative study of genetic and environmental MDD risk factors in mice. Aetiological validity refers to the independent factors that are manipulated in mice and are studied in terms of their effects in the behavioural tests described above and on their neurobiological underpinnings and correlates. For a mouse study to be aetiologically valid there must be a genetic, environmental or environmental x genetic manipulation that is based on epidemiological evidence
from MDD patients. Genetic manipulations with aetiological validity can be used to investigate their effect on analogue MDD endophenotypes, and environmental and ExG manipulations with aetiological validity can be used to investigate their effect on analogue MDD state markers. Therefore, the combination of a G, E or ExG manipulation with aetiological validity and a behavioural test with face and construct validity constitutes a translational mouse model of a MDD endophenotype or state marker. Such models can then be used to study the pathophysiology and neuropathology of these endophenotypes or state markers, as well as to investigate the effects of pharmacological manipulations thereon. Below are presented some relevant examples of studies that have investigated the effects of genotypes, environmental stress factors, or environmental stress factor-genotype combinations with MDD aetiological validity, on emotional behaviour in mice.

4.2. The study of effects of valid genetic manipulations on MDD behavioural endophenotypes in mice

As stated above (section 2.3), for six genes there is significant evidence for association of a specific polymorphism with MDD: 5-HTT (SLC6A4), DAT (SLC6A3), D4 (DRD4), MTHFR, GNB3, and APOE. To-date, mouse studies of these genotypes have yielded equivocal evidence for a direct causal contribution to affective phenotypes; two examples are presented here to illustrate this. In the case of the human 5-HTT gene, the polymorphism associated with depression is the short (s) 5-HTT gene-linked polymorphic region (5-HTTLPR-s), which leads to reduced 5-HTT expression and reduced pre-synaptic serotonin reuptake (Murphy and Lesch, 2008) (section 2.3). In mouse, this neurochemical phenotype is modelled by heterozygous 5-HTT constitutive knockout (5-HTT+/−): relative to wildtype (5-HTT+/-), 5-HTT+/− mice exhibit low 5-HTT levels (Bengel et al., 1998) and high basal extracellular serotonin levels in cortico-limbic-striatal regions (Matthews et al., 2004). However, 5-HTT+/− mice do not differ from wildtype on anxiety measures in the elevated plus maze or light-dark box (Holmes et al., 2003), nor do they differ from wildtype in their behaviour in the forced swim test (Carroll et al., 2007). In the human DAT gene, there is a variable number of tandem repeats polymorphism in the 3′ untranslated region. The 9-repeat (9R) and 10R are the most common forms of the gene, and the 9R form is associated with relatively low DAT expression (Mill et al., 2002) - which would be predicted to lead to low synaptic dopamine clearance - and increased risk for MDD (Lopez-Leon et al., 2008). A putative model of the 9R genotype, heterozygote DAT+/− mice, exhibited reduced immobility in the

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5 Just as dependent variables such as those yielded by behavioural tests must possess face validity with respect to MDD state markers, so it can be argued that genetic and environmental independent variables possess face validity, e.g. does a stressor procedure in mice exhibit similar characteristics to stressors that are risk factors for MDD e.g. uncontrollability? Here we put the emphasis on the fact that independent study variables must have a demonstrated aetiological relevance to MDD. Nonetheless, we also emphasize that the mouse genetic and environmental manipulations used must have face validity.
tail suspension test (Perona et al., 2008): this is the opposite of the depression-analogue phenotype of increased immobility which has been proposed for this test but, as noted above, the validity of the test is questionable.

4.3. The study of effects of valid environmental manipulations on MDD behavioural state markers in mice

As stated above (section 2.3), environmental stressors associated with MDD comprise stressors occurring during development (e.g. childhood abuse, neglect) and adulthood that increase vulnerability to MDD and stressors occurring more proximate to the onset of MDD and that could trigger the disorder. Accordingly, the development of manipulations of environmental factors with MDD aetiological validity in animals is complex. Details of some of the most-studied environmental stressors in rat and/or mouse, together with their salient and putative translational characteristics, the behavioural tests used to assess their effects, and the resultant behavioural readouts and neurobiological concomitants, are presented in Table 3. For example: Chronic unpredictable mild stress has been demonstrated to lead to a reduced preference for palatable sucrose solution relative to water and increased negative emotional-cognitive bias, in rat (Willner, 1997; Harding et al., 2004). Uncontrollable electroshock stress leads, as described above, to an escape deficit in the two-way shuttle box and a general deficit in behaviour-outcome expectancy (stress uncontrollability, helplessness), in rat and mouse (Anisman et al., 1978; Jackson et al., 1978; Amat et al., 2005). Chronic social stress in the form of regular changing of social group composition leads to increased anxiety on the elevated plus maze and increased immobility in the tail suspension test, in mouse (Schmidt et al., 2007; Sterlemann et al., 2008). Chronic social defeat by physical aggression and the threat thereof leads to a reduced preference for palatable sucrose solution and to increased anxiety on the elevated plus maze, in mouse (Berton et al., 2007; Krishnan et al., 2007). Early life stress, typically in the form of daily maternal separation, leads to reduced incentive motivation for sucrose on a progressive ratio schedule and an increased escape deficit following exposure to uncontrollable electroshock, in rat (Ruedi-Bettschen et al., 2005; Ruedi-Bettschen et al., 2006). A further important factor that emerges from these studies is that of inter-individual variability in reactivity (or resilience) to the environmental stressor. Thus, although the mice used for such studies are all wildtype individuals of the same inbred strain and are therefore genetically homogeneous, e.g. C57BL/6, they can be classified into two groups along one measure of stress reactivity and this predicts their behavioural response in subsequent tests. For example, specifically those mice exposed to chronic social defeat that exhibited subsequent social avoidance of a conspecific went on to exhibit a deficit in sucrose preference (Krishnan et al., 2007); and specifically those mice exposed exposed to chronic social stress that exhibited a subsequent chronic elevation in basal corticosterone titres went on to
exhibit increased anxiety (elevated plus maze) and increased immobility in the tail suspension test (Schmidt et al., 2010).

Some of these same environmental stressors have been studied in terms of their neurobiological effects (Table 3). Such approaches not only aim to establish the neurobiological mechanism underlying the observed behavioural effects and thereby yield evidence of construct validity of the model; they also provide potential new insights into MDD pathophysiology and neuropathology. For example, we have already described the evidence that the escape-deficit inducing effects of uncontrollable stressors are mediated by impaired inhibitory glutamate-GABA signaling from the ventromedial prefrontal cortex to the dorsal raphe nucleus (Amat et al., 2005; Robbins, 2005) (section 3.3.). In a mouse study of two-way escape behaviour following inescapable footshock exposure, the phenotype of escape deficit was associated with altered substance P activity, including increased synthesis in the periaqueductual gray and increased release in the nucleus accumbens (Berton et al., 2007). Also in mice, chronic social stress that led to social avoidance and a reduced preference for palatable sucrose, also induced hyperactivity of dopamine neurons in the ventral tegmental area-nucleus accumbens reward pathway (Krishnan et al., 2007).

In addition to the study of the neurobiological effects of these complex exogenous stressors, another approach has been to experimentally manipulate specific stressor-responsive endogenous factors, using delivery via infusion or viral vector. The emphasis with this approach is to focus on a certain aspect of the stress response and its potential involvement in changes in cellular signalling and gene expression leading to MDD. Examples of endogenous factors studied using this approach, and for which there is supportive evidence for aetiological-pathophysiological validity from MDD neuropathology, are: (1) Elevated corticosteroid activity in the central nervous system, involving: decreased glucose transport, increased glutamate signalling, decreased brain-derived neurotrophic factor, and decreased mitogen-activated protein kinase (MAPK) signalling, leading to impaired cellular resilience (Duman et al., 1997; Manji et al., 2001). (2) Elevated proinflammatory cytokine activity in the central nervous system, involving: reduced serotonin activity, increased glutamate signalling, increased microglia, and reduced astrocytes (Müller and Schwarz, 2007). Elevated corticotropin releasing factor activity in the central nervous system, involving: MAPK extracellular signal-related kinase 1/2 signalling in amygdala and hippocampus (Refojo et al., 2005; Todorovic et al., 2009). For the purposes of this review, important points to note are: (1) Such models of pathophysiology have typically attempted to explain MDD in its entirety rather than to focus on specific state markers/symptoms. (2) The behavioural testing in such studies has typically relied on tests of low face and construct validity e.g. forced swim test, tail suspension test. From the viewpoint
developed in this paper, we clearly would advocate that these vitally important studies of exogenous and endogenous stress factors should focus on specific MDD state markers, and that the contribution from animal studies should be based on translational, i.e. valid, behavioural tests of these state markers.

4.4. The study of effects of valid environment-gene manipulations on MDD behavioural state markers in mice

A valid point of criticism of the preclinical MDD research to-date is that the effects of genetic and environmental manipulations have been largely studied in isolation from each other. Given the wide interest in the ExG model in human MDD research and indeed the important evidence for ExG aetiology of MDD (see section 2.3), there is a clear need for more E-G studies using valid mouse models. These studies would enable the study of E-G additive effects (E+G), E-G interaction effects (ExG) and also E-G protective or resilience (E/G) effects. Certainly in other areas of preclinical research, e.g. obesity (which has high co-morbidity with MDD), there is already a significant emphasis on E-G studies, e.g. effects of energy rich diet in mice expressing mutations that predispose to obesity (Bornstein et al., 2006). There are some important examples of ExG animal models for MDD-relevant phenotypes: In the rhesus macaque, for example, the 5-HTT gene exhibits an orthologue of the human 5-HTTLPR polymorphism, and the interaction of the -s and -l genotypes with severe early life stress in the form of maternal privation has been studied. Macaques with the 5-HTTLPR-s genotype that grew up in the absence of the mother and in the presence of peers exhibited a relatively increased stress response to social isolation, compared with each of the other ExG groups (Barr et al., 2004). In mouse, 5-HTT+/- mice that received low levels of maternal care exhibited a reduced latency to immobility in the tail suspension test relative to those that received high maternal care, although not relative to WT mice that received low or high maternal care (Carola et al., 2008). Although these mice findings are not consistent with the ExG interaction that would be predicted by the human study ((Caspi et al., 2003); see above), the approach taken in this study does have aetiological validity. As the epidemiological evidence accumulates (e.g. (Ressler et al., in press)), then more complex and perhaps more realistic interactions, e.g. ExGxG, ExExG, will also need to be studied in mouse models. Furthermore, epigenetic (epiG) events may well also be a major causal mechanism in MDD, i.e. the processes whereby E’s impact on methylation, acetylation and phosphorylation of histones, on methylation of DNA, and on post-transcriptional gene-silencing mechanisms e.g. microRNAs (Mill and Petronis, 2007; Tsankova et al., 2007; Nestler, 2009). Accordingly, E-EpiG studies will be required.
5. Mouse models with face, construct and aetiological validity as the basis for preclinical treatment discovery

This paper has presented a translational framework to stimulate and inform translational research that aims to increase the validity of mouse models for preclinical MDD research and thereby increase understanding of MDD and provide a robust basis for the development of novel effective treatments. The framework comprises four inter-dependent themes: (1) A depression systems-model (Figure 2) presented the causal pathway between human gene-environment (G-E) epidemiology, pathophysiology, psycho- and neuropathology, symptoms, and diagnosis. This model depicts why G → neuro-psychology endophenotypes and G-E → neuropsychopathology state markers are the most valuable currency of experimental and translational depression research. (2) Some of the human neuropsychological tests with translational value/potential that are currently in use for the study of these endophenotypes and state markers are presented (Table 2). (3) The analogous behavioural tests for mice that are currently available or could be developed are presented (Table 2). Emphasis is placed on the fundamental importance of rigorous analysis and demonstration of face and construct validity (Figure 3). (4) The fundamental importance of rigorous attention to genetic and environmental (Table 3) aetiological validity is presented, so that meaningful G → endophenotype and E-G→ state marker (also ExG, ExGxG, etc → state marker) mouse models can be developed and studied (Figure 3). As referred to throughout this paper, there is now a tremendous realization of the need to increase the translational value of both clinical and preclinical research in depression. As schematized in Figure 5, our framework argues that these clinical and preclinical efforts need to be aligned and integrated in order that translational progress can be made.

FIGURE 5 ABOUT HERE PLEASE

By establishing mouse models that are valid in terms of aetiological factors and emotional-cognitive processes, it will then be possible to investigate the neurobiological changes that are mediating the MDD-relevant emotional-cognitive effects observed. One important function served by the analysis of the neurobiological changes is that they allow the construct validity of the models to be tested. That is, by comparing the in vivo and ex vivo findings with the human findings obtained via neuroimaging and neuropathology, respectively, it is possible to assess analogy in terms of brain areas affected and inter- and intra-cellular processes. The second important function of analysis of the neurobiological changes is that they allow for novel discoveries with respect to potential pathophysiological and neuropathological processes. With respect to both of these functions of neurobiological investigation, technological advances in the study of mouse brain function have markedly increased opportunities for translational research. These include high-resolution molecular magnetic resonance imaging (MRI) applied to such methods as pharmacological MRI and MR
spectroscopy and functional MRI (Rudin, 2009). Furthermore, these translational methods can be complemented in mouse studies with in vivo electrophysiology e.g. basal and stimulus-evoked field potential and single cell recording (Herry et al., 2007; Herry et al., 2008). Ex vivo neurobiological studies, including microarray gene expression studies in specific brain regions of interest (e.g. (Krishnan et al., 2007)), allow for the elucidation of the inter- and intra-cellular signalling genes and proteins that underlie the analogue endophenotypes and state markers.

This paper began with the justification that the considerable research into depression to-date has failed to identify a single molecular entity which has then been targeted pharmacologically to lead to the translational development of a novel antidepressant therapy. To rectify this situation is of course the major rationale for proposing a translational research framework. That is, there is a need for mouse models with demonstrated face, construct and aetiological validity because only such models will allow for insights into depression pathophysiology and neuropathology, and will possess sufficient predictive validity to ensure that compound effects on mouse analogue state markers translate successfully to therapeutic effects in patients. As emphasized in the framework presented here, successful anti-depressant treatments are likely to be those that focus on specific but key state markers/symptoms and, in accordance with the personalized therapy approach, the state marker/symptom and the underlying neurobiological process that need to be targeted, are likely to exhibit inter-individual variation.

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Conflict of interest

The authors have nothing to declare
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Figure Legends

Figure 1. Schema of the incompatibility between the nosological diagnosis of MDD and research aimed at increased understanding of MDD pathophysiology and neuropsychopathology. Whilst the epidemiological evidence is that gene polymorphisms, environmental stressors, and their neurobiological interaction, contribute to MDD pathophysiology, there is no aetiological link between these pathophysiological processes and the heterogenous nosological entity of MDD. The dashed lines and question mark signify the weak heuristic relationship between nosological classification and increased understanding of MDD that would support development of improved antidepressant treatments.

Figure 2. A systems model of aetiology-pathophysiology-pathology inter-relationships in MDD, to facilitate neuroscience-psychology-psychiatry dialogue aimed at increased understanding of and development of improved treatments for MDD. Gene polymorphisms, possibly in interaction with environmental factors, are causally associated with psychological and neural processes – endophenotypes - that are non-pathological but do increase the carrier’s vulnerability to develop MDD. Life stress events, particularly in individuals exhibiting MDD endophenotypes, induce pathophysiological changes in the brain. These pathophysiological processes lead to specific emotional-cognitive psychopathologies and underlying neuropathologies i.e. MDD state markers. Each MDD state marker underlies, or even itself constitutes, a MDD symptom as listed in DSM and/or ICD.

Figure 3. A translational framework for improved validity of mouse models of psychopathological states in MDD. Evidence for MDD aetiology (G, E, ExG) needs to inform the aetiological factors studied in mice. As schematized in Figure 2, MDD state markers in terms of pathological psychological and neural processes need to be the central focus of human research. As depicted here, MDD state markers are also essential for translational clinical-preclinical research: Analogue psychological and neural state markers can be identified in mice. Using analogous tests in humans and mice, these psychological state markers can be quantified. Using analogous brain imaging techniques in humans and mice, the underlying neural state markers can also be quantified. The dashed lines between Human Test and Mouse Test and Human Psycho-pathology and Mouse Psycho-changes indicate the levels at which translational research can lead to improved face and construct validity of mouse research. The dashed line between Human Neuro-pathology and Mouse neuro-changes indicates the level at which translational research can lead to improved construct validity of mouse research. The dashed line between Human Pathophysiology and Mouse Pathophysiology indicates the level at which translational research can lead to improved discovery and understanding of pathophysiological processes. The overall outcome of improved face, construct and aetiological
validity and improved pathophysiological understanding is indicated as novel therapies i.e. improved predictive validity of mouse research.

Figure 4. Assessing the face and construct validity of two-way escape deficit after uncontrollable stress as a test for the state marker of loss of control/helplessness. (A) Exposure to inescapable electroshock stress leads to an escape deficit in the two-way shuttle box in rodents. This could be due to development of psychomotor inactivity, as observed in rodents exposed to inescapable foot shock, or to development of an emotional-cognitive deficit in behaviour-outcome expectancy (“helplessness”). Using a triangulation approach with a second test allows for differentiation between these two potential explanations (see B). (B) Combining the two-way escape test and the signalled-punishment suppression of appetitive behaviour test allows for the accurate assessment of the effect of exposure to inescapable electroshock stress. Abbreviations: CS = conditioned stimulus e.g. tone that predicts response-elicited punishment, ES = escapable foot shock, IES = inescapable foot shock, H1 = alternative hypothesis, NS = no foot shock.

Figure 5. Clinical and preclinical contributions to translational MDD research. (A) Schema of the current nosological system with a weak link between aetiology and symptom, and of the current trend in animal-model tests to infer complex states without face, construct and aetiological validation. The outcome is a lack of analogy and therefore of translational validity, resulting in lack of progress with respect to MDD understanding and treatment. (B) Schema for a functional diagnostic system with a strong link between aetiology and state marker, and animal models based on face, construct and aetiological validity. The outcome is bi-directional translational validity, resulting in progress with respect to MDD understanding and target/therapy discovery.
Table 1. Diagnostic symptoms for major depressive disorder (DSM-IV) and recurrent moderate depressive disorder (ICD-10)

<table>
<thead>
<tr>
<th>Symptom type</th>
<th>DSM-IV classification</th>
<th>ICD-10 classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>At least one of:</td>
<td></td>
<td>At least two of:</td>
</tr>
<tr>
<td>Typical/Core</td>
<td>Depressed mood</td>
<td>Depressed mood</td>
</tr>
<tr>
<td>Typical/Core</td>
<td>Loss of interest or pleasure</td>
<td>Loss of interest or enjoyment</td>
</tr>
<tr>
<td>Typical/Core</td>
<td>Reduced energy/increased fatigability/diminished activity</td>
<td></td>
</tr>
<tr>
<td>At least four of:</td>
<td></td>
<td>At least three of:</td>
</tr>
<tr>
<td>Common</td>
<td>Weight loss</td>
<td>Reduced concentration and attention</td>
</tr>
<tr>
<td>Common</td>
<td>Insomnia</td>
<td>Reduced self-esteem and self-confidence</td>
</tr>
<tr>
<td>Common</td>
<td>Psychomotor agitation or retardation</td>
<td>Ideas of guilt and unworthiness</td>
</tr>
<tr>
<td>Common</td>
<td>Fatigue/loss of energy</td>
<td>Bleak and pessimistic views of the future</td>
</tr>
<tr>
<td>Common</td>
<td>Feelings of worthlessness or guilt</td>
<td>Ideas or acts of self-harm or suicide</td>
</tr>
<tr>
<td>Common</td>
<td>Diminished ability to think or concentrate</td>
<td>Disturbed sleep</td>
</tr>
<tr>
<td>Common</td>
<td>Recurrent thoughts of death or suicide</td>
<td>Diminished appetite</td>
</tr>
<tr>
<td>Common</td>
<td>Suicide attempt/plan</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Some neuropsychological tests of state markers of depression and their mouse analogues

<table>
<thead>
<tr>
<th>Symptom or Endophenotype or State marker</th>
<th>Human Test</th>
<th>Mouse Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of pleasure (DSM)/enjoyment (ICD)</td>
<td>Emotional reactivity/sensitivity to positive (visual) stimuli (e.g. photos of happy faces)</td>
<td>Relative reactivity to palatable stimulus vs water</td>
</tr>
<tr>
<td>Loss of interest (DSM, ICD)</td>
<td>Motivational reactivity to rewarding ($) stimuli</td>
<td>Operant responding for palatable stimulus on effortful reinforcement schedule e.g. variable-interval, progressive ratio</td>
</tr>
<tr>
<td>High reactivity to negative stimuli</td>
<td>Emotional reactivity/sensitivity to negative (visual) stimuli (e.g. photos of sad or fearful faces)</td>
<td>Emotional reactivity (e.g. freezing behaviour) to discrete stimuli and contexts associated with innately negative (foot shock) stimuli</td>
</tr>
<tr>
<td>Stress uncontrollability, Helplessness (Depressed mood (DSM, ICD))(^1)</td>
<td>Emotional-cognitive reactivity to aversive uncontrollability</td>
<td>Escape behaviour in two-way shuttle box</td>
</tr>
<tr>
<td>High negative feedback sensitivity (Depressed mood (DSM, ICD))(^1)</td>
<td>Emotional-cognitive response to negative feedback</td>
<td>Emotional-cognitive response to negative feedback (^2)</td>
</tr>
<tr>
<td>High bias to negative expectancy (Depressed mood (DSM, ICD)) (Bleak and pessimistic views of future (ICD))(^1)</td>
<td>Emotional-cognitive reactivity to ambiguous stimuli</td>
<td>Emotional-cognitive reactivity to ambiguous stimuli (^2)</td>
</tr>
<tr>
<td>Fatigue/loss of energy (DSM) Reduced energy/Increased fatigability (ICD)</td>
<td>Physical effort to complete a manual task (grip strength)</td>
<td>Effort-reward choice behaviour; Grip strength and its fatigue</td>
</tr>
</tbody>
</table>

1 The state marker – symptom pairing is a proposed “best-fit” rather than a clear relationship between the diagnostic symptom and the state marker
2 Such a test has been developed for rat and not yet for mouse
<table>
<thead>
<tr>
<th>Stressor/Species/Control</th>
<th>Translational characteristics</th>
<th>Behavioural tests/effects</th>
<th>Neurobiological effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic unpredictable mild stress</td>
<td>Chronic (∊ 3 weeks) Unpredictable</td>
<td>Preference for palatable reward (↓)</td>
<td>Hippocampus Neuropeptide Y (↓)</td>
<td>Willner (1997)</td>
</tr>
<tr>
<td>Rat, Mouse (adult) Home cage, no manipulation</td>
<td>(different stressors x times) Social threat</td>
<td>Intra-cranial self-stimulation (↓)</td>
<td>Amygdala Substance P (↑)</td>
<td>Moreau et al. (1998)</td>
</tr>
<tr>
<td>Inescapable electric foot shocks</td>
<td>Acute (1-2 days) Uncontrollable Painful</td>
<td>Two-way escape behaviour (↓)</td>
<td>Dorsal raphe nucleus Serotonin (↑)</td>
<td>Amat et al. (2005)</td>
</tr>
<tr>
<td>Rat, Mouse (adult) Escapable foot shocks, No foot shocks</td>
<td></td>
<td>Signalled-punishment suppression of operant behaviour (↓)</td>
<td>Locus coerules Noradrenaline (↑)</td>
<td></td>
</tr>
<tr>
<td>Chronic social defeat Mouse (adult)</td>
<td>Chronic (2-3 weeks) Uncontrollable Social subordination Pain</td>
<td>Social avoidance (↑)</td>
<td>Nucleus accumbens Dopamine (↑)</td>
<td>Krishnan et al. (2007)</td>
</tr>
<tr>
<td>Home cage, no manipulation</td>
<td></td>
<td>Preference for palatable reward (↓)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic social stress Mouse (adolescent)</td>
<td>Chronic (∊ 7 weeks) Unpredictable Social instability</td>
<td>Anxiety on elevated plus maze (↑)</td>
<td>Hippocampus Mineralocorticoid receptor (↓)</td>
<td>Schmidt et al. (2007)</td>
</tr>
<tr>
<td>Home cage, no manipulation</td>
<td></td>
<td>Immobility in the tail suspension test (↑)</td>
<td>Hippocampus Glucocorticoid receptor (↓)</td>
<td>Schmidt et al. (2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Basal plasma corticosterone (↑)</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1

Genes → ENVIRONMENT → Brain → Pathophysiology → Symptom → Diagnosis → DSM/ICD

Symptom → Symptom → ?
Figure 3 modified

Diagram showing the relationship between DSM/ICD diagnoses, symptoms, state markers, neuro-pathology, psycho-pathology, aetiology, pathophysiology, and novel therapies in both human and mouse models.

- DSM/ICD diagnoses
- Symptoms
- State markers
- Neuro-pathology
- Psycho-pathology
- Aetiology
- Pathophysiology
- Novel therapies

The diagram illustrates the flow of information and processes from diagnosis to therapy, highlighting the differences and similarities between human and mouse models.

Figure(s)
Figure 4

(A) 2-way escape behaviour
Measures: transfers + distance moved during/between ESs, ES escape/failures

NO YES
Signalled-punishment suppression of appetitive behaviour

H₁: IES reliably induces increased escape failure versus escapable shock and no shock

(B) Pre-exposure to IES, ES or NS
2-way escape behaviour

Measures: transfers + distance moved during/between ESs, ES escape/failures

H₁: IES pre-exposure reliably induces decreased punishment-CS suppression of operant behaviour

Assay measures motor inactivity

Valid assay for reduced behaviour-outcome association
Depression Symptom e.g. Suicidal

Animal assay

G x E aetiology

State marker e.g. Loss of control

Readout (face, construct) e.g. Escape deficit

G x E manipulation

Translational shift

Human

Mouse

Translational shift

Readout (predictive) e.g. Despair

G x E manipulation

Figure 5