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FEATURE REVIEW

# In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice

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**The ability to modify mice genetically has been one of the major breakthroughs in modern medical science affecting every discipline including psychiatry. It is hoped that the application of such technologies will result in the identification of novel targets for the treatment of diseases such as depression and to gain a better understanding of the molecular pathophysiological mechanisms that are regulated by current clinically effective antidepressant medications. The advent of these tools has resulted in the need to adopt, refine and develop mouse-specific models for analyses of depression-like behavior or behavioral patterns modulated by antidepressants. In this review, we will focus on the utility of current models (eg forced swim test, tail suspension test, olfactory bulbectomy, learned helplessness, chronic mild stress, drug-withdrawal-induced anhedonia) and research strategies aimed at investigating novel targets relevant to depression in the mouse. We will focus on key questions that are considered relevant for examining the utility of such models. Further, we describe other avenues of research that may give clues as to whether indeed a genetically modified animal has alterations relevant to clinical depression. We suggest that it is prudent and most appropriate to use convergent tests that draw on different antidepressant-related endophenotypes, and complimentary physiological analyses in order to provide a program of information concerning whether a given phenotype is functionally relevant to depression-related pathology.**
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## Introduction

*But Mousie, thou are no thy-lane,*
*In proving foresight may be vain:*
*The best laid schemes o' Mice an' Men,*
*Gang aft agley,*
*An' lea'e us nought but grief an' pain,*
*For promis'd joy!*

from *'To A Mouse'* by Robert Burns (1785).

Burns' much quoted lines can in some regard summarize the difficulty faced by many researchers who fervently search for the elusive behavioral phenotype of a genetically modified mouse, generated by using knockout, knockdown, point mutated, random mutated or transgenic technology. This is particularly true with regard to complex multisyndromal psychiatric illnesses such as major depression. In this paper, we will focus on the utility of current models and research strategies for investigating novel targets relevant to depression-like symptoms in the mouse. The review is divided broadly into four parts. Firstly, we discuss the need for and thence the utility of using genetically modified mice to study molecular pathways associated with depression and the mechanism of action of antidepressant treatments. Secondly, we discuss some of the more pressing issues associated with studying behavioral models of depression. Thirdly, we describe some of the more widely used murine models of depression, and finally we discuss other relevant issues relevant to modeling depression specific to genetically modified mice.

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## Using genetically modified mice to study depression

### *Depression: still an unmet medical need*

Depression is one of the most serious disorder in today's society.[1] The World Health Organization predicts that unipolar depression will be the second most prevalent cause of illness-induced disability by 2020,[2] and recently published data suggest that the current lifetime prevalence for depression is as high as 16.2% in the US adult population.[3] Further, with the economic burden of depression estimated to be as

high as $44 billion per year in lost productive work time,[4] the imputes has never been greater to gain better understanding of the underlying pathophysiology and to develop superior treatment strategies for depression. The serendipitous discovery and introduction of the first pharmacological antidepressant medications in the 1950s, and the subsequent refinement of these treatment strategies to have mainly a lower side-effect profile, has greatly aided the therapeutic outlook for depressed patients.[5] However, there are still too many patients who do not respond to initial treatments, and in the case of response, there is often a delay in onset of action of therapeutic efficacy and/or partial rather than full remission. These unmet medical needs have greatly increased efforts in finding new, novel and hopefully more efficacious targets for the treatment of affective disorders.

*Advent of genetically modified mice*

Efforts to gain a better understanding of the underlying pathophysiology of affective disorders are most recently driven by technical advances in the field of molecular genetics.[6] Indeed, it is hoped that the recent elucidation and the ongoing functionalization of the human genome may provide new insights into the etiology, course and thence treatment strategies for psychiatric illnesses such as major depression.[7] One of the most important advances in understanding psychiatric disorders has been the development of mice with genetically altered expression of a specific protein, be it a receptor, transporter, enzyme or signal transduction molecule (for recent reviews on various technologies see Bucan and Abel[8] and Tecott and Wehner[9]). These new tools have the potential to examine novel targets for antidepressant activity for which few established pharmacological tools exist. Additionally, these mice will enable better testing of the validity of current molecular theories of depression. To date there are almost 40 different strains of mice that have been generated with a phenotype that has been interpreted as being related to depression or antidepressant action (see Table 1 for details of mice thus far characterized). In the case of many of these mice strains, predictable phenotypes in depression models have been generated, largely because there is a known association between the specific targeted gene and either depression pathology and/or antidepressant action. A clear example is the noradrenaline transporter, which is the primary site of action of antidepressant agents such as desipramine and reboxetine. These drugs inhibit the reuptake of noradrenaline by the transporter, and when the gene is deleted the mouse behaves as if it already has been given antidepressants.[10] However, there are also many new targets emerging from such genetic approaches, which have thus far not been implicated in the underlying pathophysiology of depression, largely due to the fact that no pharmacological tools have been available. A recent case in point is the group III metabotropic glutamate receptor mGluR7 whose ablation in mice results in an antidepressant-like phenotype.[11]

In addition to selective target gene approaches, large-scale random mutagenesis programs are underway. This will enable researchers to demonstrate altered behavior without any assumptions of the nature of the mutated genes.[61,62] Although many species from *Caenorhabditis elegans* to primates have been used to examine aspects of depression-related pathophysiology,[63,64] the advent of genetically manipulated mice has resulted in an upsurge in the use of mice for behavioral analyses.[65]

Many batteries of mouse-specific behavioral tests have been proposed to encompass the wide possibilities of behavioral disruptions in behaviors that may occur as a result of the altered expression of the specific protein.[66–68] Interestingly, while many behavioral testing programs include models for anxiety-related behaviors, they often do not include paradigms for investigating behaviors associated with depression. Reasons for this may represent the fact that depression research is not the focus of the laboratories that propose such test batteries,[69] or perhaps it may be a reflection on how difficult it is to model depression in mice.

*Modeling depression in rodents*

Depression is a heterogeneous disorder with symptoms manifested at the psychological, behavioral and physiological level (see Tables 2 and 3), which leads to additional difficulty in attempting to mimic the disorder in the laboratory. In clinical practice, many tools have been developed and validated to better diagnose depression and the efficacy of treatment strategies in humans. These range from the diagnostic and statistical manual (DSM IV) of the American Psychiatric Association[70] to the various rating scales such as the Hamilton depression scale.[71] Further, clinicians also rely on self-reporting from patients for diagnosis in depression, and it goes without saying that no such tools are available when we shift to animal models in mice. Indeed, many of the human symptoms of depression as described in the DSM IV (such as recurring thoughts of death or suicide or having excessive thoughts of guilt) are impossible to be modeled in mice. The question therefore remains impenetrable as to whether we can ever know whether a mouse is 'depressed' (see Table 2 for a comparative analysis of symptoms of depression and analogous behaviors in mice). Evolutionary theories have been proposed for psychiatric disorders[72,73] which would plausibly predict that lower animal species can have some behaviors that are useful in modeling the human pathological state. However, such hypotheses are heavily debated and are difficult to address empirically (see discussions by Dubrovsky[74] and McLoughlin[75]). Further, the crucial advice of Crawley[65] (p 179) not to anthropomorphize human emotional behaviors to that in mice should not be understated. It is clear that evolutionary progression has enabled humans with a much more elaborated cerebral cortex that facilitates integration of complex psychological concepts also relevant to human

**Table 1** Genetically altered mice that exhibit depression or antidepressant-related behavior

| *Genetically altered mouse* | *Depression or antidepressant-related phenotype* | *Test used* | *Ref.* |
|---|---|---|---|
| *Serotonergic* | | | |
| 5-$HT_{1A}$ receptor knockout | Antidepressant-like effects | FST, TST | 12–16 |
| | Lack of effects of SSRIs | TST | |
| | | Microdialysis | |
| | REM sleep alterations | Sleep EEG | 17 |
| 5-$HT_{1B}$ receptor knockout | Increased sensitivity to the effects of SSRIs | TST | 15, 16, 18–20 |
| | | FST | 20 |
| | | Microdialysis | |
| Serotonin transporter knockout | Antidepressant-like effect on 129S6 but not C57Bl/6 background | TST | 21 |
| | No effect of fluoxetine in mice on C57Bl/6 background | TST | |
| | Desensitization of presynaptic 5-HT1A receptors | 8-OH-DPAT-induced hypothermia | 22 |
| | | 8-OH-DPAT-induced neuroendocrine effects | 22 |
| | | Electrophysiology | 23, 24 |
| 5-HT7 receptor knockout | Antidepressant-like effect | FST | 25 |
| *Noradrenergic* | | | |
| Dopamine-$\beta$-hydroxylase knockout | Blockade of antidepressant-like effects of antidepressants from a variety of classes | FST | 26 |
| $\alpha_{2A}$-Adrenoceptor knockout | Depressive-like effects and blockade of the antidepressant-like effect of imipramine | FST | 27 |
| $\alpha_{2C}$-Adrenoceptor knockout | Antidepressant-like effects | FST | 28 |
| $\alpha_{2C}$-Adrenoceptor overexpressing | Depressive-like effects | FST | 28 |
| Noradrenaline transporter knockout | Antidepressant-like effects | FST, TST | 29 |
| | Resistance to the effects of chronic social stress | FST | |
| *Monoamine oxidase* | | | |
| Monoamine oxidase A knockout | Antidepressant-like effects | FST | 30 |
| | Increased 5-HT levels | Microdialysis | 31 |
| | Increased acute neurochemical effects of SSRI | Microdialysis | |
| | Decreased baseline and SSRI-induced alterations in 5-HT dorsal raphe neurons | Electrophysiology | 31 |
| Monoamine oxidase B knockout | Antidepressant-like effects | FST | 32 |
| *Opioid* | | | |
| Mu opioid receptor knockout | Antidepressant-like effects | FST | 33 |
| Delta opioid receptor knockout | Depressive-like effects | FST | 33 |
| *GABAergic* | | | |
| Glutamic acid decarboxylase (65-kDa isoform) knockout | Antidepressant-like effects | FST | 34 |
| GABAB(1) receptor knockout | Antidepressant-like effects | FST | 35 |

| | | | |
|---|---|---|---|
| *Glutamate* | | | |
| NMDA receptor $\varepsilon 4$ subunit | Antidepressant-like effects | FST | 36 |
| mGluR7 knockout | Antidepressant-like effects | FST, TST | 11 |
| *Substance P related* | | | |
| Tachykinin $NK_1$ receptor knockout | Antidepressant-like effects | FST, TST | 37 |
| | 5-HT1A receptor desensitization | 8-OH-DPAT-induced hypothermia | 38 |
| | | Electrophysiology | 39 |
| Tac1 gene | Antidepressant-like effects | FST, TST, olfactory bulbectomy | 40 |
| *Other receptors* | | | |
| Adenosine $A_{2A}$ receptor knockout | Antidepressant-like effects | FST, TST | 41 |
| Nicotinic$\beta 2$ knockout | Insensitive to effects of chronic amitriptyline | FST, TST, LH | 42 |
| CB1 | Increased susceptibility to effects of stress | CMS | 43 |
| Nociceptin/orphanin FQ receptor | Antidepressant-like effects | FST | 44 |
| NPY-2 | Antidepressant-like effects | FST | 45 |
| Dopamine D5 receptor knockout | Antidepressant-like effects | FST | 46 |
| *HPA axis* | | | |
| Glucocorticoid-receptor-impaired transgenic | Antidepressant-like effects | FST | 47 |
| CRF-overexpressing mice | Depression-like effects | Hypercortolisimia | 48 |
| | Antidepressant-like effects | FST | 49 |
| CRF2 receptor knockout | Depression-like effects | FST | 50 |
| *Immunological* | | | |
| Tumor necrosis factor $\alpha$ knockout | Antidepressant-like effects | FST | 51 |
| Interleukin-6 knockout | Blockade of the antidepressant-like effects of *Hypericum perforatum* | FST | 52 |
| *Intracellular signaling molecules and transcription factors* | | | |
| DARPP-32 knockout | Reduced sensitivity to fluoxetine | TST | 53 |
| PDE4D | Antidepressant-like effect, lack of effect of rolipram | FST, TST | 54 |
| Emx1 | Antidepressant-like effect | FST | 55 |
| TrkB.T1 transgenic mice | Blockade of the effects of antidepressants | FST | 56 |
| $BDNF^{+/-}$ | Blockade of the effects of antidepressants | FST | 56 |
| RGS9-2 | Depression-like effect | FST | 57 |
| *Miscellaneous* | | | |
| Angiotensinogen knockout | Antidepressant-like effects | FST | 58 |
| Neural cell adhesion molecule knockout | Antidepressant-like effects | FST | 59 |
| ES(#21)-10 chimeric mice | Depression-like effect | FST | 60 |

FST, forced swim test; TST, tail suspension test; LH, learned helplessness; CMS, chronic mild stress; SSRI, selective 5-HT reuptake inhibitor.

**Table 2** Modeling DSM IV criteria

| *Depression symptom in humans* | *How it is modeled in rodents?* | *Ref.* |
|---|---|---|
| Depressed mood most of the day | Not applicable | |
| Markedly diminished interest or pleasure in all or most activities most of the day | Intracranial self-stimulation (ICSS) and progressive ratio responding in response to rewards such as sucrose can assess anhedonia | 76–78 |
| Large changes in appetite or weight gain | Easily measured | 79, 80 |
| Insomnia or excessive sleeping | Sleep architecture can be measured using EEG | 17, 81, 82 |
| Psychomotor agitation or slowness of movement | Can be assessed in terms of ease of handling | 83 |
| | Activity can be measured in novel environment and motor coordination assessed using rotarod | 84, 85 |
| Fatigue or loss of energy | Social withdrawal | 86 |
| | Energy expenditure | 87 |
| | Treadmill/running wheel | 88 |
| | Swimming | 89 |
| | Nesting behavior | 90 |
| | Active waking in EEG | 82, 91 |
| Indecisiveness or diminished ability to think or concentrate | Animal models of cognition | 65 |
| | Working memory | 92 |
| | Spatial memory | 93 |
| | Attention | 94 |
| Recurrent thoughts of death or of suicide | Not applicable | |
| Feelings of worthlessness or excessive or inappropriate guilt | Not applicable | |

depression, such as self-esteem and the ability to perceive the future, that are absent in mice. Burns summarizes this discrepancy in the concluding stanza of 'To a Mouse':

> *Still thou art blest, compar'd wi' me*
> *The present only toucheth thee:*
> *But, Och! I backward cast my e'e.*
> *On prospects drear!*
> *An' forward, tho' I canna see,*
> *I guess an' fear!*

Nonetheless, there are also many fundamental physiological and behavioral responses that have been evolutionary conserved between species, in order to regulate homeostasis. Therefore, largely through inference, we can exploit these latter responses to elucidate phenotypes relevant to emotional behaviors (see Tables 2 and 3 for a detailed description of how physiological and behavioral responses in animals compare with that seen in major depression).

Unlike other medical disorders where a pathology is well characterized (although perhaps not understood) such as diabetes or Parkinson's disease, the underlying pathophysiology of depression is still unresolved. This further enhances the difficulties faced in modeling depression in mice. Although the DSM IV is used extensively in the characterization and diagnosis of depression, it has many limitations, and clearly there are many characteristics of depression that extend beyond the DSM IV criteria. Table 2 summarizes both those instances where DSM IV criteria can and cannot be translated into behavioral traits in mice.

*Depression psychopathology: beyond DSM IV*

Depression, as well as many psychiatric disorders, can be viewed as a manifestation of an inability to cope with various lifetime stressors. This coping deficit may be due to prior exposure to acute or chronic stressors, and is strongly influenced by the individual's genetics.[95–97] Consequently, there are a large number of physiological and neurochemical alterations seen in major depressed patients, which may give clues to the causation or are at least are coincident with some of the behavioral manifestations that are delineated in DSM IV criteria of depression.[1,98] Unfortunately, it is extremely difficult to distinguish whether such hallmarks are the root cause of the disease or rather consequences of suffering from depression. Table 3 summarizes some of the most widely used strategies currently employed to characterize depression or antidepressant-like behaviors in mice based on data from the clinical literature. The vast amount of studies focusing on alterations in depressed patients or alterations in their post-mortem brains (see Connor and Leonard[99] for extensive analysis of biological markers of major depression) offers ample opportunity for researchers to investigate neurochemical and physiological changes in genetically modified animals in addition to behavioral changes. Unfortunately, too often, researchers investigate behavioral alterations in mice in total isolation from the neurochemical and physiological changes that may parallel and in many instances are responsible for the manifestation of the observed behavior. Further, one must also take a critical look at the clinical studies: many of these are

**Table 3** Modeling other hallmarks of depression

| *Dysfunction in depression in humans* | *Application in mice* | *Example* | *Comments* |
|---|---|---|---|
| ***HPA axis dysfunction*** | | | |
| Hypercorticolism[100] | Plasma corticosterone levels are easily measured by radioimmunoassay (RIA) | Increase in serum corticosterone levels in genetic mouse model of depression[82] | Worthwhile to measure both under basal conditions and following acute and chronic stress |
| Increase in ACTH secretion[101] | Measure of plasma ACTH level by RIA | Increase in plasma ACTH concentration in CRH-overexpressing mice[48] | Worthwhile to measure both under basal conditions and following acute and chronic stress |
| Adrenal hypertrophy[102,103] | Measure of weight and volume of adrenal gland post mortem | Social defeat stress induces adrenal hypertrophy in mice.[104] Increase of adrenal gland weight in CRH-overexpressing mice[48] | |
| | MRI imaging also used to assess noninvasively *in vivo* function | *In vivo* characterization of adrenal size in CRF transgenic mice[105] | Not widely used to date |
| Impairment of negative feedback[106] | Dexamethasone suppression test | Impaired dexamethasone suppression in CRH-overexpressing mice and in mice expressing RNA antisense against the glucocorticoid receptor[48,107,108] | Few studies in non-HPA axis genetically modified animals |
| Elevated CRF levels in the locus coeruleus of MD patients[109] | Immunoreactivity studies | Not tested | |
| Reduced CRF binding sites in the frontal cortex of suicides victims[110] | Binding studies | Not tested | |
| Increased number of vasopressin-expressing neurons in the PVN of depressed subjects[111] | Immunohistochemical studies | Chronic stress increases AVP mRNA levels in rat PVN[112] | |
| ***Serotonin dysfunction*** | | | |
| Supersensitivity of 5-HT1A autorecepors[113] | 8-OH-DPAT (a 5-HT1A receptor agonist)-induced hypothermia | *Depressive-like effect*: Enhanced hypothermic response to 8-OH-DPAT in helpless mice[82] | Chronic antidepressants induce blunted response to 8-OH-DPAT indicating a desensitization of 5-HT1A autoreceptors |
| | | *Antidepressant-like effect*: Blunted hypothermic response in NK1 receptor knockout mice[39] | |
| | Electrophysiological studies in dorsal raphe nucleus (DRN) | *Depressive-like effect*: Increase of 5-HT1A autoreceptor inhibition of serotonergic neurons induced by ICV injection of 8-OH-DPAT[82] | |
| | | *Antidepressant-like effect*: Alteration in the potency of the 5-HT1A receptor agonist ipsapirone to inhibit the discharge of serotoninergic neurons in DRN slices of NK1 knockout mice[39] | |

**Table 3** Continued

| *Dysfunction in depression in humans* | *Application in mice* | *Example* | *Comments* |
|---|---|---|---|
| | Microdialysis studies | *Antidepressant-like effect*: Increased sensitivity of SSRIs on extracellular levels of 5-HT in forebrain areas[18] | |
| | Binding, autoradiography and GTP$\gamma$S studies in DRN | *Antidepressant-like effect*: Reduction of 8-OH-DPAT-stimulated $^{35}$[S]-GTP-$\gamma$-S binding in 5-HTT knockout mice[114] | |
| Increase of 5-HT2A receptors in frontal cortex[115] | Binding and autoradiography studies in frontal cortex | *Antidepressant-like effect*: Administration of desipramine for 21 days induces a decrease of 5-HT2A receptors in frontal cortex of OB rats[116] | |
| | ($\pm$)DOI-induced head twitches | *Antidepressant-like effect*: Chronic SNRIs treatment decreases head twitch induced by ($\pm$)DOI[117] Depressive-like effect: Supersensitive 5-HT2A-mediated head twitching in OB mice[118] | |
| Decrease of serotonin transporter binding in the midbrain of depressed patients[119,120] | Binding studies | *Antidepressant-like effect*: Decrease of serotonin transporter binding in the hypothalamus of mice treated with antidepressants (fluoxetine, paroxetine)[121] | |
| | Voltametry studies | *Antidepressant-like effect*: Decrease in the clearance of 5-HT by chronic antidepressant treatment[122] | Not characterized in mouse as yet |
| ***Noradrenergic dysfunction*** | | | |
| Alteration in $\beta$-adrenoceptors binding in suicide victims[123] | Possibility to do binding and autoradiography studies | *Antidepressant-like effect*: Chronic citalopram and fluoxetine treatments induced downregulation of $\beta$1-adrenoceptors in rat forebrain[124] | |
| Increase in density and affinity of $\alpha$2-adrenoreceptors in the brain of depressed patients[125] | Receptor binding and autoradiography studies | *Antidepressant-like effect*: Chronic, but not acute, treatment with desipramine decreased sensitivity of $\alpha$2-adrenoreceptors in rat brain[126] | |
| Reduced concentration of tyrosine hydroxylase in the locus coeruleus of suicide victims[127] | Immunohistochemical studies | *Antidepressant-like effect*: Decreased level of tyrosine hydroxylase in locus coeruleus following chronic desipramine[128] | |
| Alteration of norepinephrine transporters in the locus coeruleus of major depressed patients[129] | Binding studies | Not tested | |
| | Voltametry studies | | |

| | | |
|---|---|---|
| Elevated binding to α2-adrenoreceptors in the locus coeruleus of depressed patients[127,130] | Binding studies | *Antidepressant-like effect*: Chronic administration of antidepressant reduced α2-adrenoreceptor binding in rat locus coeruleus[131] |
| ***Dopaminergic systems dysfunctions*** | | |
| SPECT studies showed elevated D-2 receptors binding in the striata of patients with depression[132,133] | Binding studies | Not tested |
| Elevated D2/D3 receptor binding in the amygdala of major depressed patients[134] | Binding studies | Not tested |
| Lower dopamine transporter binding in the amygdala of major depressed patients[134] | Binding studies | Not tested |
| Lower dopamine transporter binding in the stiatum of depressed patients[135] | Binding studies | Single social defeat reduces striatal dopamine transporter binding in rats[136] |
| ***Alterations of glutamatergic system*** | | |
| Decrease of glutamate level in the anterior cingulate cortex of depressed patients[137] | Microdialysis studies | Not tested |
| Alteration of high-affinity glycine-displaceable of [3H]CGP-39653 binding to Glu receptors in the frontal cortex of suicide victims[138] | Binding studies | Chronic citalopram-modified high-affinity glycine-displaceable of [3H]CGP-39653 binding to Glu receptors in frontal cortex in mice[139] |
| ***Alteration of gabaergic system*** | | |
| Decrease of GABA level in the cortex of depressed patients[140] | Microdialysis studies | Not tested |
| Alteration of GABA levels in the occipital cortex of depressed patients using SPECT studies[141] | Microdialysis studies | Not tested |
| ***Substance P systems alteration*** | | |
| Decrease of neurokinin-1 receptors in the cortex of depressed patients[142] | Binding studies | Not tested |
| ***Imidazoline receptor*** | | |
| Decrease of type 2 imidazoline receptor in the frontal cortex of depressed patients[143] | Binding and immunohistochemical studies | *Antidepressant-like effect*s: Chronic imipramine treatment upregulates IR2-imidazoline in bulbectomized rats[144] |
| ***Alteration in cell-mediated immunity and in inflammatory response system*** | | |
| Major depressed patients have reduced natural killer cell cytotoxicity[145] | Natural killer activity assessed by the $^{51}Cr$ assay | *Depressive-like effects*: 8 weeks of chronic mild stress (CMS) decreases cytotoxicity of natural killer cells in mice[146] |

**Table 3** Continued

| *Dysfunction in depression in humans* | *Application in mice* | *Example* | *Comments* |
|---|---|---|---|
| Depressed patients exhibit an impairment in concanavalin A (Con A)-induced lymphocyte proliferation[147] | T lymphocytes proliferative response to Con A assessed using [3H]-thymidine uptake assay | *Depressive-like effects*: Mice that underwent CMS exhibit a decrease in proliferative responses of splenic cells to Con A[146]<br>*Antidepressant-like effect*: Chronic treatment with fluoxetine and citalopram induces an increase of lymphocyte stimulation induced by Con A[148] | |
| Increased levels of circulating proinflammatory cytokines in depressed patients[145,149–151] | Measurement of plasma cytokine detection and quantification in mice by ELISA | *Depressive-like effects*: Increase of basal level of IL-1 (proinflammatory cytokine) in chronically stressed mice[146] | |
| Increase levels of *in vitro*-stimulated production of proinflammatory cytokines in depressed patients[152] | Measurement of proinflammatory cytokines by ELISA from mitogen-stimulated mouse splenocytes *in vitro*, or in serum/spleen following an *in vivo* LPS challenge | *Depressive-like effects*: Chronically stressed mice have increased *in vitro*-stimulated production of IL-1 and IL-2.[146] In rats, CMS induces an increased production of IL-1 and IL-2 by stimulated splenocytes[153]<br>Antidepressant-like effects: 5 weeks of imipramine treatment reverses the effects of CMS on stimulated production of IL-1 and IL-2 in rats[153] | Tricyclics appear to be more effective than SSRIs in attenuating the stimulated production of cytokines induced by LPS *in vivo* |
| Antidepressant treatment induces an increase of *in vitro*-stimulated production of anti-inflammatory cytokines in healthy humans[154] | *In vitro* stimulation of proinflammatory cytokines production induced by LPS and PHA | *Antidepressant-like effect*s: Chronic treatment with citalopram or fluoxetine induces an enhanced stimulated production of IL-10 in mice.[148] Chronic treatment with desipramine induces an increase of stimulated production of IL-10 in chronically stressed and nonstressed animals[155] | |
| ***Cell survival pathway dysfunction***<br>Alteration of CREB levels in temporal cortex in major depressed patients.[156]<br>Decrease of CREB phosphorylation in the orbitofrontal cortex of depressed patients[157] | Immunohistochemical studies | *Antidepressant-like effect*s: Chronic antidepressant treatment increases CRE-mediated gene expression and CREB phosphorylation in limbic and cerebral cortex in mice[158]<br>Chronic administration of antidepressants induces an increase of CREB expression in rat hippocampus[159] | |

| | | | |
|---|---|---|---|
| Increase of hippocampal BDNF immunoreactivity in subjects treated with antidepressants[160] | RNase protection analysis. ELISA studies | *Antidepressant-like effects*: Increase of BDNF mRNA in frontal cortex and hippocampus in mice after 21 days of antidepressant medication[161] | |
| Decrease of plasma levels of BDNF in depressed patients[162] | ELISA studies | Not tested | |
| Increase of mRNA levels of tyrosine receptor kinase B in the cerebellum of major depressed patients treated with antidepressants[163] | Immunohistochemical studies | *Antidepressant-like effect*: Chronic administration of antidepressants induces an increase of tyrosine receptor kinase B expression in rat hippocampus[159] | |
| ***Abnormal cardiovascular function*** | | | |
| High heart rate in major depressed subjects[164,165] | ECG | Increase of heart rate after exposure to 4 weeks of CMS followed 4 weeks of recovery period[88] | Heart rate measurements can be combined with temperature measurements in mice[166] |
| Reduction of heart rate variability in depressed patients[167] | ECG | Decrease of heart rate variability in chronically stressed rats[88] | |
| ***Sexual dysfunction*** | | | |
| 75% of depressed patients exhibit a decrease of libido[166,168,169] and 25–30% of major depressed patients show a decrease in duration and intensity of nocturnal penile tumescence[170] | Behavioral testing[171] | CMS induces an alteration of copulatory behavior in male rats[172] | |
| ***Depression-induced brain morphological changes*** | | | |
| Depressed patients exhibit a 19% reduction of hippocampus volume[173–175] | Neuroimaging and autopsy studies | Antidepressant-like effect: Antidepressants reverse decreases in hippocampal volume in tree shrews subsequent to chronic social stress[176] | Hippocampal volume reductions recently shown in multiple episode but not in first episode depressed patients[177] |
| Decrease of volume and neuronal densities in depressed patients[178–180] | Neuroimaging studies and autopsy studies | *Depressive-like effect*: Chronic glucocorticoids treatment induces loss of dendrites in rat prefrontal cortex[181] | |
| Enlargement of amygdala during the first episode of depression[182] | Neuroimaging studies and autopsy studies | *Depressive-like effect*: Chronic stress induces increase of dendritic branching in rat amygdala[183] | |
| Ventricular enlargement in depressed patients[184,185] | Neuroimaging studies | *Depressive-like effect*: Increased ventricle size in OB model of depression in rats[186] | |
| ***Depression-induced functional changes in brain*** | | | |
| Depressed patients exhibit abnormalities in the activity of several structures of brain such as prefrontal cortex, anterior cingulate cortex, amygdala, hippocampus[187–190] | Neuroimaging studies on awake animals[191] | Not tested | Functional neuroimaging is not yet widely established in awake mice |

**Table 3** Continued

| *Dysfunction in depression in humans* | *Application in mice* | *Example* | *Comments* |
|---|---|---|---|
| | | | Neuroimaging studies include MRI studies,[182] fMRI studies,[192] EEG[193] and PET[194] |
| ***Altered sensitivity to drugs of abuse*** | | | |
| Increased rewarding effect of smoking[195,196] | Behavioral studies using nicotine in mice | Not tested | Increased occurrence of substance abuse among depressed patients is largely thought to be to counteract the anhedonic aspects of depression[197] |
| Rewarding effects of amphetamine correlate with severity of depression[198] | Behavioral studies in mice | Increased sensitivity to amphetamine following chronic stress and olfactory bulbectomy in rats[199,200] | |

just single studies from selected small populations of patients and therefore caution should be taken for not making overinterpretations on such data sets. However, the list in Table 3 serves as a primer of some reasonable avenues of research for investigators in order to further evaluate whether their mouse of interest has phenotypic aspects of the depressed syndrome.

## Some key issues associated with using depression models

Despite the difficulties in translating human affective disorders into relevant tests in mice, numerous attempts have been made to create animal models of depression, or at least of models of some core symptoms of depression. Various paradigms have been developed to investigate whether manipulations, be they pharmacological, lesion-based, environmental or genetic, can selectively modify the behavior of mice in a manner that can be interpreted as altering depression or antidepressant-like behavior. Before we describe some of the models individually, there are a number of key points that are worth keeping in mind when using such animal models of depression.

*Does the paradigm meet validity criteria?*
Many authors have proposed criteria for animal models of depression; however, the criterion proposed by McKinney and Bunny[201] over 30 years ago is still the most cited. These authors propose that the validity of an animal model can be determined by the extent that (a) it is 'reasonably analogous' to the human disorder in its manifestations or symptomatology, (b) there is a behavioral change that can be objectively monitored, (c) the behavioral changes observed should be reversed by the same treatment modalities that are effective in humans, and (d) the system should be reproducible between investigators. Subsequently, the relative importance of the individual criterion of animal models of depression has been viewed differently among various investigators. Much commentary has focused on various definitions with regard to which type of criteria is or is not the most useful for using animal models, and the reader is referred to these reviews for extensive analysis.[64,201–206] One pragmatic proposal by Geyer and Markou[205] is that the only criteria that are necessary and sufficient for *initial* use of an animal model are that the paradigm has strong predictive validity, and that the behavioral readout be reliable and robust in the same laboratory and between laboratories. According to these authors, the satisfaction of other criteria such as construct or discriminant validity may have heuristic value and are considered as desired but not essential for the model to provide important *initial* uses in both basic neurobiological research and drug discovery. Further, it is becoming clear that a more useful strategy may be to model single endophenotypic differences (ie one clearcut

behavioral output) relevant to the disease state rather than an entire syndrome.[204,205] In parallel, similar urgings have been made for dissecting the DSM IV criteria of various psychiatric disorders into specific endophenotypes and use this modified classification as a basis for investigating novel treatments and underlying genes for specific endophenotypes.[207–209]

*Predictivity* In terms of predictive nature of animal models of depression, it is hoped that a given paradigm will respond to clinically effective antidepressant agents belonging to various pharmacological classes and interfering with different mechanisms (although it should be noted that to date all approved antidepressants are inhibitors of monoamine transporters or monoamine oxidase). Further, a valid model should also be sensitive to nonpharmacological treatments for depression such as electroconvulsive shock therapy, sleep deprivation and transcranial magnetic stimulation. The extent of predictivity of a given model would also take into account that non-antidepressant psychotropic agents such as antipsychotics, antiepileptic agents and anxiolytic medications should be inactive in the model. However, it is becoming clear that in psychiatric practice in certain situations, such agents may offer some therapeutic benefits in patients suffering from depression, although in many cases as adjunct treatments in combination with standard antidepressant treatments.[210,211] Hence, care should be taken not to exclude the validity of a model based on an as yet untested clinical principle.

The utility of many models that are sensitive to the effects of acute monoamine reuptake transporter blockade is often criticized because they fail to detect antidepressants with novel mechanisms of action. Although antidepressant-like activity has been demonstrated in such paradigms for compounds whose mechanism of action are primarily through corticotropin-releasing factor (CRF), neuropeptide Y, glucocorticoid, glutamate and substance P mechanisms,[212] this overall contention is largely true. That said, until an antidepressant with such a novel mechanism of action is successfully launched on the market, can we make clear analysis on the predictivity of the given model?

*Reliability* The issue of reproducibility of behavioral effects between laboratories is an important consideration in all aspects of behavioral analyses. This has recently been further expounded upon following the highly profiled publication of the *Multi-Center Trial of a Standardized Battery of Tests of Mouse Behavior*[213] (also see Wahlsten *et al*[214] for more comprehensive analysis). In these studies, Crabbe and colleagues demonstrate that even when every possible attempt is made to standardize the experimental conditions at various behavioral laboratories, interlaboratory differences in the outcome of such studies still emerge. Nonetheless, while many have seen these studies as an indictment of the validity of behavioral neuroscience as a whole, it should be noted that there were many behaviors which translated appropriately, at least qualitatively, between the laboratories and that within laboratories the inter-experimental variability was low (see Picciotto and Self[215] and Sapolsky[216] for excellent commentary on these studies). Further, the data largely confirm many strain-dependent differences in behavior reported in the literature. Nonetheless, the studies substantiate the fact that indefinable environmental factors affect mouse behavior. Further, they caution overinterpretation of phenotypic changes until a replication has been performed in another lab environment. One succinct example of where such analyses has prevailed has been the generation of mice that lack the 5-$HT_{1A}$ receptor, a key autoreceptor regulating 5-HT function in the brain. This mouse was generated independently on three different genetic backgrounds, in three different laboratories, and essentially the same anxious and antidepressant-like phenotype was observed.[12–14] Nevertheless, it should be noted that quantitatively the effects in all 5-$HT_{1A}$ receptor knockout mice are relatively robust, with large baseline genotypic differences in behavior observed. Further, an antidepressant-related phenotype was to be anticipated given that the receptor has long been linked with the pathophysiology of depression and the mechanism of action of antidepressants.[217] However, there is always the possibility of having the significance of more subtle behavioral effects induced by deletion of a protein, diluted through environmental factors. This is especially problematic if the targeted protein is not yet associated with antidepressant-like behaviors or in the case of large-scale mutagenesis programs where no *a priori* effect is predicted.

In recent years, there have been very reasonable arguments both for[218] and against[69,219] complete standardization of behavioral analysis. Standardization of apparatus size and testing protocol is definitely desirable to optimize the chances of reproducing similar effects between labs but as the Crabbe *et al*[213] study exemplifies it by no means ensures it. However, the converse can also be argued. By using one testing apparatus and one strain of mouse, under one condition, there is always the risk that suboptimal conditions may be generated that may be insensitive for the detection of novel effects induced by genetic deletion. An example of the latter comes from the rat literature where the forced swim test (FST; see below for details) is the most widely used animal model for assessing antidepressant activity.[212,220] However, under the standardized conditions proposed in the initial descriptions of the test selective serotonin reuptake inhibitors (SSRIs), the most widely prescribed class of antidepressant drugs today are not detected, whereas under a modified design, using an increased water depth and investigating both active and passive behaviors, also SSRIs

show antidepressant-like behaviors.[221–223] Therefore, if standardization equals rigidity, then caution needs to be exercised, since experience has told us that many traditional paradigms are receptive to further modification. Such improvements will enhance the models' utility for both the detection of novel targets for antidepressant activity and contributing to a better understanding of the underlying pathophysiology of depression.[212] As a consequence of this and in line with the assertion of van der Staay and Steckler,[218] all reports on behavioral data should include as much detailed information as possible with regard to laboratory and testing conditions. Only with this information supplied can the critical aspects of reproducibility of behavioral observations be challenged appropriately. Implied in this, every deviation from standardized behavioral methodology needs to be explicitly documented and will necessitate the revalidation of the test in its modified form for both its behavioral outcome and sensitivity of antidepressants. One cannot stress enough how minor changes in apparatus size and testing protocol can have major consequences on the behavioral outcome and sensitivity of pharmacological agents in the test.

*Is the model simple and straightforward to carry out?*
There is somewhat of a vicious circle in establishing behavioral analysis of complex traits such as depression, as in order for the test to be amenable to high-throughput methodology, which is especially needed in ENU-mutagenesis screens and quantitative trait linkage (QTL) analyses, the tests have to be quick yet meaningful. Obviously, it is debatable whether this can be achieved without sacrificing the validity of the paradigm and still truly encapsulate the endophenotypes of the disease. Another factor that is particularly relevant for such analysis in a high-throughput mode and also relevant to the pharmaceutical industry is whether the behavioral readout can be automated. Automation has two somewhat obvious advantages, firstly it speeds up both behavioral testing and data analysis and in addition removes inter-rater subjective bias. Some tests such as the FST and tail suspension test (TST) (see below) are potentially amenable to such high-throughput testing and automation.

*Animal model of depression or of antidepressant action?*
As many of the models described below were essentially validated retrospectively based on the effects seen with clinically effective antidepressant agents, it is perhaps more appropriate to consider them as models of antidepressant action rather than models of depression. Self-evidently, it is most desirable that paradigms can detect depressive-like behavior in addition to antidepressant-like behaviors. However, unlike anxiety-related behaviors, where anxiety can be provoked acutely by a variety of pharmacological (eg *m*-chlorophenylpiperazine (*m*-CPP); $\beta$-carboline; flumazinal; lactate; cholecystokinin (CCK) type B receptor agonists (tetragastrin (CCK-4) and pentagastrin (CCK-5); doxapram) or stressful situations (eg brightly lit, elevated environments; placing near scent of predator)),[224–226] it is difficult to acutely provoke depression in animals and humans. This dissociation of the two disorders is also reflected in currently available early proof-of-concept clinical trials in humans, where panic (via CCK agonist, $CO_2$ or lactate) or fear (via fear potentiated startle) provocation tests are available and carried out in healthy volunteers in addition to patients,[227] whereas for depression still 6- to 8-week trials in patients are needed before an initial efficacy signal is obtained. Interestingly, it is becoming clear that a number of interventions known to be involved in the susceptibility or induction of major depression in humans induce a depression-like effect in models such as the FST and TST. These manipulations include a genetic predisposition;[82,228] exposure to early life stressors,[229] chronic stress,[230–232] prenatal stress;[233] being in the postpartum state;[234] immunological activation;[235,236] or deprivation of dietary tryptophan.[237] In addition, withdrawal from morphine, amphetamine and phencyclidine, which in humans has been associated with depressive-like behavior, has been shown to increase immobility in the FST in rats and mice[77,238–240] and to affect intercranial self-stimulation (ICSS),[240] which further supports the use of this parameter to detect depression-like behavior and indicating the etiological validity of these paradigms.[205]

*Honey! I shrunk the rat…*
The adage that a mouse is not a small rat is as relevant in depression tests as in other behavioral paradigms. As it is obvious to anyone who has worked with both species, rats and mice are ethologically different species with individual behavioral idiosyncrasies. Historically however, most of the behavioral tests across all of psychopharmacological research today were initially established, characterized and validated in the rat. While there is an upsurge in the use of mice, fundamental changes are required to translate tests from one species to the other, beyond simply reducing the arena size. Tests have to be refined to encapsulate the particularities and peculiarities of mouse behavior. Further, one has to bear in mind that there is also species differences in the distribution and functional aspects of certain neurotransmitters and neuropeptides and their receptors between rats and mice, which may influence the behaviors. A recently observed example of this is for the expression of the galanin R1 receptor, which is largely absent from the mouse dorsal raphe nucleus whereas it is abundant in the rat nucleus.[241] This may significantly affect the interpretation of certain phenotypes in genetically modified animals.

*Are there strain differences?*
There is a line in George Orwell's Animal Farm that states that *'All animals are equal, but some animals*

*are more equal than others.'* Clearly, mice do not fit into the later assertion. The burgeoning use of genetically altered mice in behavioral pharmacology has resulted in much emphasis being placed on studying individual strain differences in both baseline behavior and in the responsiveness to psychotropic medications in mice.[242] In almost all of the behavioral models outlined below, substantial strain differences have been observed. Further analyses of both inbred and outbred strains may reveal phenotypic behavioral differences that potentially have an underlying genetic basis relevant to depression or antidepressant action (see below). Recent studies in various animal models of antidepressant action suggest that multistrain comparisons may be needed to prevent false negative screening of compounds in a given paradigm. Thereby, the detailed elaboration of strain differences in the response to antidepressant drugs could well provide new models for the detection of genes that influence the clinical effects of antidepressants. Where appropriate, strain differences in mice in various tests are discussed individually in the section on each test below.

*Are there gender differences?*

It has been long known that there is greater incidence of major depression among women than men[243] and such gender differences extend to the presentation, the course of the illness and the treatment response.[244] Yet it is somewhat paradoxical that the vast majority of animal studies (both behavioral and neurochemical) related to depression models are carried out in male animals only. Reasons for this apparent reductionism largely stem from the pragmatic approach taken by investigators that male animals are easier to use, as one does not have to take into account and control for stages within the estrus cycle and hence there is the perception of less variability.[245,246] Sexually dimorphic behavioral effects that emerge in rodents include aggression, exploration, activity levels, infant play, food intake, food preference and cognitive ability.[245,247,248] However, even between rodent species there is a differential evolutionary bias on gender differences in behavior. As exemplified here for locomotor activity, male meadow voles are more active than female voles;[249] no difference in locomotor activity was reported between male and female mice;[250] but generally male rats have less activity than their female counterpart.[247] Therefore, when one extrapolates to the human situation, it is difficult to know at what point in evolutionary history that the predisposing effects of gender on depression-related behaviors have emerged. To date there are only few studies investigating gender effects in animal models of depression; there has been somewhat conflicting evidence with some models showing increased depressive-like behavior in female animals[251,252] or no effect.[253] However, the majority of studies in various models found that females are actually *less* susceptible to the effects of the stress or behave as if they had been administered antidepressants compared to their male counterparts in the model.[254–260] It is clear that many ethologically relevant behaviors in mice have gender-dependent effects, which may result in an interference with the behavioral readout and subsequent sensitivity to antidepressant drugs.[256,257] It is therefore important to investigate gender differences between various strains of genetically modified mice and to also study how behaviors are influenced by ovarian steroids over the estrous cycle. In a recent study with CRF2 receptor knockout mice, females show a more pronounced depressive-like effect in the FST paradigm compared to male mice.[50] On the other hand, Jones and Lucki[261] have shown that female 5-$HT_{1B}$ receptor knockout mice behave as if they were administered antidepressants in both the FST and tail suspension paradigms whereas male mice show no such phenotype. In summary, although the gender differences in rodents may not be representative of those in humans, investigations of sex-related effects in rodents should not be ignored as they may unmask interesting interactions between the altered gene- and steroid-mediated behaviors.

*Is the paradigm applicable for genetic analyses?*

Quantitative trait loci (QTLs) technology is perhaps the most extensively used technique to map genes for defined behaviors in rodents.[262,263] A quantitative trait is defined as a phenotype that can vary in a measurable manner in the population. The variation can be due to combinations of genes and can be under the influence of environmental factors. A QTL is a single gene in a multiple gene system underlying a specific trait. QTLs can act in concert and can have a cumulative influence on the expression of the trait; further, epistatic effects can be detected. Such techniques have been used to further understand the genetic basis for complex disorders such as anxiety[263] and addiction.[264] This technique requires that the behavioral output within strains must be relatively easily assessed and be quantitatively stable. In terms of depression, a recent elegant QTL analysis of behavioral despair (immobility) behavior in both the FST and the TSTs revealed that there were independent and overlapping QTLs for this behavioral trait in these tests.[265] The overlapping QTL was found to encode GABAA receptor subunits.

As technologies for assessing changes in gene expression become more robust, sensitive, sophisticated and widely used,[266] it will be important to assess genetic changes in the brains of various animal models of depression and assess whether any alterations in gene expression are countered by antidepressant pretreatments.[267] To date, no such studies have been published, although a recent study by Kinnunen *et al*[268] demonstrates that prenatal stress (which has been proposed as a model of depression[269]) significantly alters genes associated with glutamatergic and gabaergic neurotransmission in the rat frontal pole;

this certainly demonstrates the promise of such approaches.

## Widely used murine models of depression

The following section focuses on some of those paradigms for assessing antidepressant and/or depression-like behaviors in mice. It is by no means an exhaustive survey but highlights some of the more widely used models. As the paradigms differ in terms of their widespread use and applicability to mice, Table 4 provides comparative analysis of models described below in addition to some lesser established ones.

*Forced swim test*

'A major problem in the search for new antidepressant drugs is the lack of animal models which both resemble depressive illness and are selectively sensitive to clinically effective antidepressant treatments' thus begins the seminal Nature paper from Porsolt *et al*,[220] which introduced the FST in 1977. Although the same statement could still preface the introduction of any new model for depression today, the FST changed the way that drug screening for antidepressants was carried out. Presently, the FST (also known as Porsolt's test; behavioral despair test) is probably the most widely and most frequently used experimental paradigm for detecting antidepressant activity, largely due to its relative reliability across laboratories and its ability to detect activity in a broad spectrum of clinically effective antidepressants.[212] Furthermore, this test is the most widely used paradigm to assess depression- and antidepressant-related phenotypes in genetically altered mice.[212,270,271] The test is based on the observation that rodents, following initial escape-oriented movements, develop an immobile posture in an inescapable cylinder filled with water. If antidepressant treatments are given prior to the test, the subjects will actively persist engaging in escape-directed behaviors for longer periods of time than after vehicle treatment. For reasons not yet elucidated, in mice, one exposure is sufficient to generate a stable immobility readout that can be countered by acute pretreatment with antidepressant agents, whereas generally two trials are required in the rat version.[272,273]

In a recent survey of 11 different strains of mice in the FST, Lucki *et al*[274] demonstrated that there was a maximal 10-fold difference in baseline immobility scores in untreated animals in the 4-min test between strains. Furthermore, the baseline level did not correlate with sensitivity to diverse antidepressants across the strains. The norepinephrine reuptake inhibitor desipramine reduced immobility in seven of the strains, with DBA/2J and C57Bl/6 being the most responsive strains. In contrast, only three strains (DBA/2J, BALB/cJ and NIH-Swiss) were sensitive to the effects of the selective serotonin reuptake inhibitor fluoxetine. Qualitatively similar baseline strain effects in baseline behavior have been subsequently replicated by two independent groups.[231,265] Interestingly, at least in the rat version of the test, chronic treatment with doses of antidepressant agents that are acutely ineffective at altering immobility is active, which adds further validity to the test in relation to the temporal effects of antidepressants in clinic[275,276] (Cryan and Lucki, unpublished observations). More data are required to determine if such an enhanced sensitivity emerges in the mouse version of the test.

Given the clinical comorbidity between depression and anxiety,[277,278] it is clear that increased anxiety states may predispose individuals to depression or be coincident with the course of the disease. Recent studies in the rat FST suggest that individual differences in immobility rely on the animal's response to novelty;[279] however, using rats that were selectively bred for their high or low anxiety, Ho and colleagues[280] failed to show any correlative effect between immobility levels and anxiety *per se*. Therefore it is unclear whether any direct correlation can be made. Interestingly, genetically modified mice that are both *more* and *less* anxious in behavioral tests can behave similarly in the FST,[11,15,34,35,45,49] showing a further dissociation between anxiety and depression-like behavior in the mouse.

The rat FST has been further modified[231] and demonstrated that the test reveals specific behavioral components of active behaviors, namely swimming, which is sensitive to serotonergic compounds such as the selective serotonin (5-HT) reuptake inhibitors and 5-HT receptor agonists, and climbing (also known as struggling), which is sensitive to tricyclic antidepressants and drugs with selective effects on catecholaminergic transmission.[212–223,281] In accordance with these studies, Alcaro *et al*[231] have recently shown that chronic treatment with the serotonergic antidepressant clomipramine increased swimming behavior, whereas the catecholaminergic drugs desipramine and amphetamine primarily increased struggling behavior in a mouse version of the test. This dissection of active behaviors in the mouse FST has also recently been extended to unveil depressive-like behaviors in mice lacking the CRF receptor 2.[50] Further studies are required to confirm if the assignment of specific behaviors in the mouse version of the FST is as powerful of a tool as its rat counterpart to assess the role of monoamines in antidepressant action.

*At swim two mice: What does immobility in the FST mean?*

> *Having placed in my mouth sufficient bread for three minutes' chewing, I withdrew my powers of sensual perception and retired into the privacy of my mind, my eyes and face assuming a vacant and preoccupied expression.*

Thus opens the classic novel '*At Swim-Two-Birds*' by the Irish writer Flann O'Brien. The behavioral state described in many ways depicts a mouse undergoing immobility in the FST. However, just as this novel

**Table 4** Comparative analysis of murine depression models

| *Behavioral model* | *Readout* | *Is it studied in mice?* | *Predictability in mice* | *Reliability in mice* | *Comments* | *Ref.* |
|---|---|---|---|---|---|---|
| FST | Immobility | Highly | High | High | Quick, easy, robust, sensitive to both acute and chronic treatments | 274, 378 |
| Modified FST | Immobility, swimming, climbing | Not well | Untested | Untested | Sensitive to acute and chronic antidepressant treatments; differentiates antidepressants from different classes including SSRIs in rat, needs to be further characterized in mouse | 50, 231 |
| TST | Immobility | Highly | High | High | Quick, easy, robust. Sensitive to acute treatments. Certain strains climb their tail | 298, 300, 302 |
| Olfactory bulbectomy | Hyperactivity in a novel environment, passive avoidance deficits | Some studies | Not clear | Needs further studies | Behavioral effects evident only following chronic treatment; mechanism of action poorly understood | 311, 326 |
| Learned helplessness | Number of escape failures, latency to escape | Some studies | Medium | Medium | Sensitive to short-term antidepressant treatments; ethical restrictions in some countries | 345, 353 |
| Chronic mild stress | Sucrose preference, ICSS, fur state | Some studies | Needs further studies | Poor reliability | Behavioral effects reversed in temporal fashion to that seen in depressed patients | 374–377 |
| Drug-withdrawal-induced anhedonia | ICSS, sucrose preference, FST, TST, learned helplessness progressive ratio (rat) | Some studies | Needs further studies | Needs further studies | Requires further validation; cannot easily assess baseline strain differences using ICSS; is probably dependent on regimen of administration of drug | 77, 238, 240, 358, 361, 379 |
| Novelty, suppressed feeding | Approach time to novel food | Some studies | Needs further studies | Needs further studies | May reflect anxiety as opposed to depression-like behavior; responds to chronic but not acute antidepressants | 380, 381 |
| Prenatal stress | Immobility (FST), endocrine parameters | Few studies | Needs further studies | Needs further studies | May not be specific to depression, also proposed as model of schizophrenia | 38, 382 |
| Neonatal clomipramine | Immobility (FST), circadian disturbances, endocrine parameters | Only characterized in rat | Only characterized in rat | Only characterized in rat | Only limited testings of antidepressants have been conducted in rat and none in mouse | 383–386 |
| Maternal deprivation | HPA axis, ICSS (rat) | Some studies | Needs further studies | Needs further studies | Only limited testings of antidepressants have been conducted in rat and none in mouse | 387, 388 |
| DRL | Response rate, reinforcement rate | Few studies | Needs further studies. Medium level of predictivity in rat | Needs further studies | Sensitive to short-term antidepressant treatments in rat | 389, 390 |
| Resident intruder | Agonistic behavior | Not well characterized as a depression model | Needs further studies | Needs further studies | Distinguishable behavioral effects only following chronic treatment; requires further validation in other laboratories | 391 |
| LPS-induced immunological activation | Temperature responses, cytokines production, endocrine parameters, sickness | Some studies | Unclear; mainly sensitive to tricyclic antidepressant | Needs further studies | Requires further validation for mouse | 392–394 |

continues to give us three separate openings to its meandering story, there are many explanations and theories for the physical adaptation of the immobility response in the FST. The posture of immobility was originally coined *'behavioral despair'* by Porsolt, largely based on the assumption that the animals have 'given up hope of escaping', that is a failure of persistence in escape-directed behavior. Other investigators have contended that the behavioral responses comprise an evolutionary preserved coping strategy[282] in which immobility behaviors represent the psychological concept of 'entrapment' described in clinical depression.[69,283,284] Thus, the development of passive behavior (immobility) disengages the animal from active forms of coping with stressful stimuli.[69] Further, as Willner[202] correctly points out, the immobility in the test is due to inability or reluctance to maintain effort rather than a generalized hypoactivity. This is correlative to the clinical findings that it is in tests that require the sustained expenditure of effort that depressed patients show their most pronounced psychomotor impairments.[285] In further support of the notion that immobility is actually a beneficial behavioral posture to adopt, Nishimura *et al*[286] demonstrated that animals with high immobility in the initial minutes of the swim test are protected against sinking following prolonged exposure to the inescapable cylinder.

Immobility is seen in other contexts in the behavioral repertoire of animals. One such response that has been well studied is the freezing behavior in response to an aversive stimulus such as shock, context of previous shock or predator.[287] This is a markedly different response than the immobility seen in the FST, specifically in terms of it being largely defensive in nature and being much more rapidly revealed upon re-exposure to the stimulus. Another defensive response in animals is tonic immobility, which is the unlearned reaction to brief manual restraint that is characterized by a catatonic-like state of reduced responsiveness, and its duration is thought to be positively related to the antecedent fear state.[288] Tonic immobility has largely been characterized in avian species but has been demonstrated in mammalian species such as the guinea pig.[289] However, FST-induced immobility is qualitatively different from these predator responses in that anxiolytic agents have little effect in FST whereas they can attenuate some of the fear responses in conditioned fear and tonic immobility paradigms.

Another immobility-like behavior is seen following repeated shocks in the learned helplessness paradigm (see below). However, FST-induced immobility especially in the mouse is not a learned response *per se,* as only one exposure is required to detect antidepressant-like behaviors reliably. Nevertheless, it is probable that the FST requires within session instrumental learning to engage in immobility. It is generally believed that in the rat version of the test, a pretest exposure is required to predict antidepressant action reliably,[272] whereas only one exposure is required in the mouse. Reasons for this remain unclear. It should be noted that robust antidepressant-like effects have been reported following just one exposure in the rat also[290] (Cryan and Lucki, unpublished observations). Some authors have argued that immobility is largely dependent on learning and memory.[291,292] However, most of the bases for such hypotheses are centered on the two-test rat model and hence are largely redundant in the mouse version of the test. Nonetheless, there has been very little research focused on the role of cognitive process in the mouse FST; however, it should be noted that the muscarinic antagonist and amnesiac scopolamine prevents the induction of immobility in the test.[293] An interesting suggestion proposed by West[291] is that immobility may be a result of an enhanced habituation and subsequent development of a preference for a familiar place due to aversive stress.

Although we caution overextrapolation of the behavioral readout in the FST, it is noteworthy that FST-induced immobility is influenced differentially by many factors including a genetic predisposition,[294] effects of stress,[230,232,233] changes in food intake,[231] alterations in sleep architecture[230] and anhedonia[77,240] in addition to antidepressant treatments both pharmacological and nonpharmacological.[273,295] As many of these factors also influence or are altered by the course of major depression in humans, swim test immobility presents itself as an attractive model for assessing depression-related factors in mice. In addition, many of the key questions posed earlier are also fulfilled by the mouse FST. However, we also warn that there are also many nondepression-related factors that can influence FST-induced immobility. For example, $M_1$ muscarinic acetylcholine receptor knockout animals are hyperactive and correspondingly have an artifactual antidepressant-like phenotype in the FST.[296] Therefore, we strongly urge that a broad analysis of a given phenotype behaviorally, physiologically and neurochemically is embarked upon in order to ensure that any change in immobility is related to antidepressant-like behavioral effects (see Table 3 for examples of such analyses).

*Tail suspension test*

This test is theoretically similar to the FST. Briefly, mice are suspended by their tails for 6 min, and the amount of time they spend immobile is recorded.[297] Acute antidepressant treatments will decrease these immobility scores. Advantages of this test include its ability to detect a broad spectrum of antidepressants, it is inexpensive, methodologically unsophisticated and easily open to automation. This automation enables the assessment of additional parameters such as power and energy of movement.[298,299] Similar to the FST, its validity is questioned by the fact that acute antidepressant treatments reverse the behavioral 'depression'. Although both the FST and TST are similar in the constructs that they purport to assess, they are probably different in terms of the biological substrates that underlie the observed behavior and

often offer converging data on a potential antidepressant.[270,300,301] Further, the TST avoids any possible confounds induced by hypothermic exposure that may be problematic in the FST, especially if a targeted gene is involved in thermoregulatory processes. Furthermore, the TST also circumvents the need of the mouse to swim, which may be relevant for examining the effects of certain genetically modified animals where motor coordination may be compromised. A relevant example of this is mice of the 129 strain, which have problems keeping afloat when tested in the FST following treatment with SSRIs.[69] However, the TST itself is also dependent on a motor readout, and animals with severe motoric phenotypes may give misleading information in the test. Moreover, some commonly used inbred strains, such as C57Bl/6, are not the ideal strain to use in the TST as they have a tendency to climb their tail.[11,302] Consequentially, we suggest that much larger groups of animals are needed if the TST is to be included in a behavioral testing battery using mice of the C57Bl/6 strain. Further, it will be helpful if investigators should delineate what percentage of animals climbed their tail in studies, in order to determine the extent of tail climbing in mice strains from various suppliers. It goes without saying that animals that do climb their tail must be removed from further analysis as they have learned that escape is possible and will quickly resume such a stature. Obviously, caution must be exercised by those investigators who use automated apparatus that do not allow for visual observation of the mice, as the potential climbing may influence the behavior. Yoshikawa *et al*[265] have claimed that they have successfully overcome the influence of this climbing behavior on TST behavior. Instead of attaching the mouse tail to a fixed support, they attach the tail to a hook that is connected to a perpendicular wire and a strain gauge, which prevents the animal climbing.

Strain differences have also been noted in the TST.[300,303] Liu and Gershenfeld[304] examined the baseline and imipramine-induced behaviors of 11 strains of mice in the TST. Clear interstrain differences were observed for baseline scores in the TST, and only three strains (DBA/2J, NMRI and FVB/NJ) responded to the antidepressant imipramine. In follow-up studies, this group performed a factor analysis of anxiety- and stress-related behaviors in 12 strains and in an F2 cross of two strains that differed in both baseline immobility and in response to imipramine in the TST (NMRI and 129S6).[305] Their analysis predicts that neither immobility nor imipramine-induced changes in immobility in the TST is related to anxiety or stress responsivity. Further, as is the case with the FST,[69] no correlation between the effects of antidepressants and the baseline immobility scores was observed in the TST.

*Olfactory bulbectomy*

The bilateral removal of the olfactory bulbs of rodents results in a complex constellation of behavioral, neurochemical, neuroendocrine and neuroimmune alterations many of which are comparable with changes seen in patients with major depression.[306–309] This model has been best characterized in the rat, with only a handful of studies available in mice.[40,118,310–315] Therefore most of the discussion must focus on the rat version of the test, with caveats where appropriate, that the effects may not be identical between both species. Superficially, the olfactory bulbectomy model may seem to be an obscure test, and indeed the rationale for its use as an animal model of depression has often been questioned based on construct and etiological validity arguments.[202] However, this model has one of the best portfolios for the prediction of known antidepressant compounds following repeated administration in the rat and is reliable between laboratories at least in the rat.[203,212] Indeed, when using a novel scoring system, Willner and Mitchell[203] have evaluated various animal models of predisposition to depression, including genetically selected lines (congenitally learned helplessness, Flinders sensitive line, Roman high-avoidance line and the Fawn hooded rat), genetically modified mice (glucocorticoid transgenic, serotonin transporter knockout; neurokinin 1 receptor knockout) and developmental models (neonatal antidepressant treatments, prenatal or neonatal stress); the olfactory bulbectomy model received the highest score in terms of validity as a model of depression.

The most consistent behavioral change of bulbectomy in both rats and mice is a hyperactive response in a novel brightly lit open field apparatus, which is reversed almost exclusively by chronic, but not acute, antidepressant treatment and at doses that do not compromise the performance of sham-lesioned control animals.[307,316,317] Recent studies have shown that the hyperactivity might be related to increases in defensive behavior[252] or alterations in aversively motivated behavior in the rat.[318] Furthermore, it has been shown that antidepressant compounds predominantly enhance habituation to novelty in the bulbectomized rat.[319] Concurrent with these studies, other groups have focused on neurochemical alterations that might account for the antidepressant-sensitive behavioral alterations. Studies in the rat have shown that following olfactory bulbectomy there are marked changes in the serotonergic,[320–322] noradrenergic,[309] glutamatergic,[323,324] cholinergic,[325,326] dopaminergic[327,328] and GABAergic[329] systems. Imaging studies demonstrated alterations in signal intensities in cortical, hippocampal, caudate and amygdaloid regions in olfactory bulbectomized rats compared to sham-operated controls.[186] In addition, ventricular enlargement was evident in bulbectomized rats. It has been suggested that these structural changes correlate somewhat with those seen in depressed patients.[186] Comparing the behavioral and biochemical effects of bulbectomy in young *vs* aged rats, Slotkin *et al*[330] suggest that this test might provide a useful animal model with which to test therapeutic interventions for geriatric depression. In

addition to an increased locomotor response to stress, bulbectomized rats have a heightened acoustic startle responsivity to stress,[331] marked deficits in circadian rhythms (also mice),[313] cognitive deficits in Morris Maze and passive avoidance learning,[326,332] elevated corticosterone[333–335] and anhedonia-like behaviors such as decreased sucrose preference and sexual behavior.[251,308] In addition, it has been shown that bulbectomized rats also exhibit increased levels of amphetamine self-administration compared to sham animals,[200] and bulbectomized mice have elevated alcohol consumption[336] compared to sham animals. Most recently, Uzunova *et al*[337] described regionally selective decreases in the neurosteroid allopregnanolone in bulbectomized rat brains. Several lines of evidence suggest that the behavioral sequelae induced by bulbectomy in the rat is not just a consequence of loss of smell, as peripheral anosmia fails to produce the syndrome.[319,338,339] The olfactory bulbectomy behavioral syndrome is largely thought to be brought about by compensatory neuronal reorganization, changes in synaptic strength and/or loss of spine density in various subcortical limbic regions such as the amygdala and hippocampus.[307,340,341]

There have been few studies examining strain-dependent effect of bulbectomy in mice. However, it has been shown that whereas bulbectomy-induced hyperactivity (locomotor activity and rearing) was observed in both C57Bl/6J mice and DBA/2J mice, only the increased rearing seen in the former strain was sensitive to attenuation by the antidepressants (amitriptyline, trazadone and imipramine).[311] Similarly, C57Bl/6J mice had deficits in both passive and active avoidance subsequent to bulbectomy, which were reversed by antidepressants, whereas the DBA/2J strain failed to show such alterations.[311] Recently, the first studies investigating the effects of bulbectomy in genetically modified animals were published. In these studies, animals with the gene for prepropeptide for substance P (Tac-1) disrupted failed to have the same level of hyperactivity as wild-type animals,[40] and these mice in addition showed antidepressant-like behavior in the FST. Obviously, future studies will have to investigate if other aspects of the bulbectomy syndrome are affected by genetic manipulations.

*Learned helplessness*

This paradigm was originally developed based on the observations that dogs subjected to repeated inescapable uncontrollable (but not those subjected to controllable) shocks demonstrate escape deficits.[342,343] The model was later translated to the rat[344] and subsequently to the mouse.[345,346] The rodent studies revealed that the behavioral deficits are sensitive to a broad spectrum of antidepressants usually after short-term treatment.[347–349] The major drawbacks of the learned helplessness model are twofold: (i) most of the depression-like symptomatology does not persist beyond 2–3 days following cessation of the uncontrollable shock.[350] Further, only a certain percentage (estimates vary somewhere between 10 and 80%) of animals develop helplessness behavior.[256,346,351] Vollmayr and Henn[352] have recently proposed key factors that can be manipulated to enhance both the usability and reliability of the learned helplessness paradigm in the rat, many of which are in principle translatable to the mouse. These include the use of a larger testing apparatus, a mild shock presentation, a relatively difficult shock avoidance task and taking into account animals that are artifactually avoiding shock due to their position in the apparatus.

As in many other paradigms, there are mouse-specific aspects and vast strain differences observable in the learned helpless test.[256,353–355] In addition to baseline differences, Shanks and Anisman[354] showed differential responsivity to three different antidepressants across the four strains of mouse tested. Chronic desipramine prevented the escape deficits in A/J, but did not affect the performance in BALB/cByJ, C57Bl/6J or CD-1 mice; repeated treatment with bupropion, in contrast, had only a modest effect in CD-1 mice. Unlike these compounds, amitriptyline was found to influence escape performance irrespective of whether the drug was acutely or chronically applied. Caldarone *et al*[256] suggest that C57Bl/6 mice are suitable for use in learned helplessness studies but caution that strains such as 129 and the hybrid B6129F1 strain may be inappropriate because nonshocked control mice of these strains show poor escape performance *per se*. Marked sexual dimorphic effects have been observed in the mouse learned helplessness paradigm, with female mice being less disrupted by the effects of inescapable shock than male animals.[256] One important caveat that must be considered with the learned helplessness paradigm is that alterations in pain sensitivity caused by pharmacological or genetic manipulation will influence the behavior of the animals. This is relevant when discussing strain effects, as prominent differences in pain sensitivities have been described between inbred mouse strains.[356] One example where such caution is pertinent is the recent analysis of mice with heterozygote expression of brain-derived neurotrophic factor.[357] These mice display antidepressant-like behavior in the learned helplessness paradigm, but the authors point out that the mice also show a reduced sensitivity to pain, which makes it impossible to dissociate the helplessness from hypoalgesia. As with many behavioral models, motor performance can also affect mouse behavior in this test.

*Drug withdrawal as a model of depression*

Reward deficits associated with withdrawal from drugs of abuse can be an animal model of the symptom of 'diminished interest or pleasure' with construct, convergent and predictive validities.[77,205,212,240,358] Therefore, amphetamine withdrawal has been proposed as a suitable substrate for inducing depression in rodents.[240,358,359] Recent studies showed that withdrawal from chronic

amphetamine regimens are characterized by decreased breaking points under a progressive ratio schedule for a sucrose solution reinforcer, and results in decrements in anticipatory and motivational measures for sexual reinforcement in rats.[358,360] Also amphetamine withdrawal increases immobility in the rat and mouse FST[77,240] and mouse TST,[240] and also promotes helpless behavior in the mouse learned helpless paradigm.[77]

The use of the intracranial self-stimulation (ICSS) paradigm has provided investigators with a reliable and quantifiable behavioral readout that enables the assessment of reduced brain reward function following withdrawal from a variety of drugs of abuse.[77,361] Many investigators have adopted ICSS procedures to mice.[76,77,362] It will be of interest to assess the effects of drug-withdrawal-induced anhedonia across various strains using ICSS, including those that have been genetically modified.

*Chronic mild stress*

The chronic mild stress (CMS) model has long been championed by Willner co-workers[363–365] as a realistic model of depression. As the name suggests this paradigm consists of exposing rodents to a series of mild unpredictable stressors during a prolonged period (usually $>2$ weeks). This stress regimen induces many long-term behavioral, neurochemical, neuroimmune and neuroendocrine alterations resembling those dysfunctions observed in depressed patients.[365] Primarily, there have been two major antidepressant-sensitive readouts characterized in the CMS model: (i) CMS depresses the consumption and preference for sucrose solution and (ii) CMS decreases brain reward function as assessed using ICSS. Both measures are correlated with anhedonia, one of the core symptoms of depression as defined in the DSM IV. These anhedonia-like behaviors have generally been shown to be reversed by chronic, but not acute, treatment with several classes of antidepressants.[365,366] Although the paradigm has been described as a model with a high predictive, construct and etiological validity,[202,363–365] two major facts limit its widespread use in assessing depression-related behavior in genetically modified animals. Firstly, this model, at least in rats where it has been for the most part characterized, has very poor reliability and could not be reproduced in many laboratories.[199,360,361,367–373] (Cryan and Norman, unpublished observations). Indeed, Willner[365] himself admits to having difficulties in re-establishing the model in his own laboratory following a move from London to Swansea.

Secondly, there is only a sporadic number of studies to date that have employed mice.[374] A recent interesting modification of the CMS model in mice has been proposed by Griebel, Belzung and co-workers,[375–377] which uses a 1–3 point scale for the assessment of the physical state of the animal's fur. Animals subjected to chronic stress do not groom themselves or take interest in the state of their fur. There is certainly some analogy between this stress-induced state and the observations that depressed patients have a reduced efficiency with which even the smallest tasks are accomplished, leading to the inability to maintain minimal personal hygiene.[376] Further, it has been shown that chronic treatment with the antidepressant fluoxetine and novel antidepressant candidates (the CRF1 receptor antagonist antalarmin and the vasopressin 1B receptor antagonist SSR149415) improved the physical state index of the mice. While this readout of model does look promising, further studies are required also because this is a very subjective readout and thus needs to be conduced in a blinded fashion. As of today, it still remains to be determined whether the CMS model can be used successfully and more reliably in mice than has been the experience in rats.

*Selective breeding in animal models of depression*

In order to discriminate potential genetic influences on 'depressive-like' behavior, several attempts have been undertaken using selective breeding programs of animals based on the individual responsiveness in animal models of depression. To date, these breeding efforts have largely focused on rat models. To this end, rats were selectively bred for susceptible to learned helplessness[352,395,396] and for high and low level of immobility in the FST.[397] Other genetic models have been developed based on an underlying alteration in the function of both cholinergic[398,399] and serotonergic[400,401] neurotransmitter systems. One mouse model that has been derived from animals bred for spontaneous high or low immobility scores in the TST[228] will be discussed in detail later. Two other lines of mice were selectively bred for their differential sensitivity to the convulsant effects of benzodiazepine receptor inverse agonist, methyl $\beta$-carboline-3-carboxylate ($\beta$-CCM). These mice also have diverging effects in anxiety tests, which is not all that surprising since $\beta$-CCM is also an anxiety-proving agent.[402] Interestingly, these mice show marked differences in FST and TST behavior.[403] However, it should be noted that as the differences in depression-related behavior in these strains are correlated responses to selection, rather than the traits on which genetic selection pressure was exerted, the relationship of those line differences to the genetic underpinnings of depression is not clear, and is at best indirect. However, it at least points to a role for GABAergic mechanism in the maintenance of immobility behavior.

It should be noted that any two genotypes that differ markedly in a trait can serve as the basis for a genetic intercross that is informative for gene mapping purposes. There is always the possibility that in undergoing such selected breeding programs the effects seen may be influenced by genetic drift. This would result in independently selected lines having qualitatively different behavioral responses.[404] Hence, it is beneficial to have multiple lines established and to perform appropriate comparisons to allow for investigation of the potential effects of such drift.[404]

In addition to selected breeding programs, which are based on the heritance of a natural trait, forward genetic approaches are underway whereby a mutant line of animals is induced randomly by mutagens such as ethylnitrosourea, and subsequently these animals' behavior is analyzed.[61,62,405] Positional cloning would enable the identification of gene targets relevant for the behavioral response.

Many genetic models inevitably use simple screening paradigms such as the FST procedures to determine that the mice indeed have relevant phenotypes. As stated previously, the use of single endophenotypic differences such as reaction to stress or pronounced elevation in ICSS thresholds in response to manipulations, as opposed to a syndrome, is a useful strategy in identifying genetic factors. Therefore, both genetic and behavioral strategies should be viewed as complementary and serving overlapping purposes. In concert, they may yield further information about the idiopathic disease state. Such models might eventually match individual patients to the most effective types of therapy for their genetic constitution or type of depression.

*The Rouen 'depressed' mice*

One of the most promising murine selective breeding programs relevant to depression are the Rouen 'depressed' or helpless mice. From a research program that was initiated in 1995 by Jean-Marie Vaugeois and colleagues at the Université de Rouen in northern France,[82,228] mice with high and low immobility in the TST have been selected. The initial studies began with the testing of 92 male and 58 female adult CD1 mice. The chosen selection criteria, which were the same at each generation, were a high immobility score ($>115$ s) and a low immobility score ($<35$ s) in the TST. From the original CD1 mice, two pairs of high and low immobility mice were bred to produce the first generation (S1). To minimize inbreeding, animals that were least related to one another were mated to produce next generations, but brother–sister mating was systematically carried out for each generation after S5. In the meantime, at least 14 generations of breeding have been generated.[82] Immobility scores and the percentage of animals reaching criteria increased moderately with each consecutive generation in the helpless animals until almost all animals reach criteria.

In addition to increased immobility in the TST, helpless animals also had an increased immobility in the FST but this did not become evident until selection had reached advanced generations, thus further suggesting the concept that both tests have overlapping yet not identical mechanisms underlying the behavioral strategy of immobility. The mice do have some alterations in locomotor activity, and it is not clear at this point of their genetic selection how much this hypoactivity contributes to the helpless behavior and vice versa. However, there was no direct correlation between activity in the open field and immobility recorded in both the FST and TST.[82] The helpless mice also have differences in sleep architecture such as a decreased wakefulness and decreased REM sleep latency. The animals have an elevated basal corticosterone, which is suggestive of a disturbance in HPA-axis function, similar to that seen in many depressed patients. Further, serotonergic dysfunction has long been associated with depression,[217] and electrophysiological, binding and 5-$HT_{1A}$ receptor agonist-induced hypothermia studies showed a supersensitive responsivity of 5-HT neurons in the dorsal raphe nucleus.[82,406] The fact that chronic treatment with antidepressant medications reverses the deficits in 5-HT firing[82] gives further credence to the utility of this genetically selected strain as a suitable model for depression. Collectively, the studies with the Rouen mice illustrate an excellent example of how complimentary behavioral, neurochemical, physiological and genetic analysis can assist in the search for mice with phenotypes relevant to depression.

## Further considerations in murine models

Behavioral, neurochemical and genetic analysis of the effects of antidepressant agents will be most relevant in 'depressed' animals in addition to that in 'normal' healthy animals. Therefore, there is some urgency in the search for appropriate mice that have a depression-related phenotype. However, the much-discussed caveats associated with interpretation of the behavioral effects in genetically altered animals cannot be understated (the major two being that of background strain differences (see earlier section) and compensatory adaptive changes[65,212]).

The ability to see the same phenotype across different strains, as in the case of 5-$HT_{1A}$ receptor knockout mice, gives further credence to the reliability of the phenotype.[12–14] The full potential of regionally selective and inducible knockouts and transgenic mice is emerging,[407] and such strategies offer many advantages over currently used techniques since such mice will certainly become valuable tools in dissecting out regionally specific circuits that may influence depression-like behaviors in addition to being involved in the actions of antidepressants. The ability to restore, albeit transiently, the phenotype in norepinephrine-deficient mice by administering the synthetic precursor L-deoxyphenylserine is a novel way to confirm that the phenotype is related to norepinephrine function as opposed to those related to adaptive changes resulting from being reared without this monoamine.[26,408] Specific behavioral changes can be confirmed by conducting multiple types of behavioral tests such as FST, TST and learned helplessness. Other behavioral analyses such as tests for locomotor activity, pain sensitivity or cognition may be necessary to specifically allocate behavioral changes to stress-induced depression as opposed to nonspecific effects. Such caveats cannot be underestimated, and overinterpretation of antidepressant-like phenotypes must be avoided. Therefore,

appropriate caution utilizing other convergent tests that draw on different antidepressant-related endophenotypes, and complimentary physiological analyses provide a program of information concerning whether a given phenotype is functionally relevant to depression-related pathology.

*Epigenetics and early-life events*

> *a devil, a born devil, on whose nature nurture can never stick*
>
> William Shakespeare, The Tempest (4:1:188–189)

In the Tempest, Prospero regards the villain Caliban as being genetically inferior and inherently incapable of being influenced by environmental factors. However, in contrast to this assertion, it should be noted that in mice there are many events that may occur during development and in the early life period that will influence the manifestation of behavior (for further extensive details on this topic see review by Meaney[409]). Indeed many models of depression have been proposed that involve prenatal stress,[233,269,410] maternal deprivation[388,411,412] or neonatal exposure to the serotonergic antidepressant clomipramine[383] (see Table 4 for further analyses of the utility of these models). It was recently shown that maternal behavior was inheritable in a nongenomic, epigenetic fashion in the rat.[413,414] The implication of early life to the behavior of mice was recently tested[415] (for detailed analysis see also commentary by Crabbe and Phillips[416]). In a series of elegant studies Francis and colleagues transferred C57Bl/6J embryos to either C57Bl/6 or BALB/c female mice—two strains that have very different behaviors across a variety of behavioral analyses. Following birth, animals were again cross-fostered to either C57Bl/6J or BALB/c mothers, resulting in genetically identical C57Bl/6J mice that have had four different well-defined intrauterine or postnatal influences. When tested in a battery of behavioral tasks all mice behaved as C57Bl/6J with the exception of those mice that had *both* intrauterine and postnatal fostering with BALB/c mother. These mice behaved similar to purchased BALB/c animals in anxiety and learning tests. This epigenetic influence on mouse behavior further demonstrates the complexity of gene–environment interactions and their influence on complex behavior. Further studies will be required to determine whether there are specific mouse strains that, like Shakespeare's Caliban, are resistant to environmental and epigenetic influences in animal models of depression.

*Knockout = antagonist: caution!*

Given the possibilities for developmental compensation and the influence of epigenetic and environmental factors on subsequent behavior in adult mice, it is clear that the lack of a specific protein during the course of early development may result in alterations in molecular pathways and neural circuits relevant to depression behavior. These alterations may result in ectopic expression of other proteins that may markedly influence behavior. It is also possible that the direction of a depression-like effect in a conventional mutant animal may not be in agreement with acute pharmacological manipulation of the very same protein. The common occurrence of pleiotropy among individual genes and overlapping functions between genes adds to the complexity faced by researchers. Further, the mechanisms underlying penetrance of dominant alleles make it difficult to construct meaningful gene dose/response relationships.[417]

Behavioral analysis of gene knockout data can uncover unexpected compensations, effects on other gene products, altered endocrine and neuronal feedback loops, and a lack of control over the temporal and spatial impact of the genetic manipulation. At this point all that can be deduced is that the given protein is involved in a molecular pathway that influences depression-related behavior. Hence validation of various findings in genetically modified animals requires additional confirmation with acute and more importantly chronic pharmacological agents as they become available.

## Conclusions

It is clear that whereas depression may not be as amenable for modeling in the laboratory as other disorders, there exist many very useful paradigms that enable researchers to investigate various aspects of the depression syndrome in genetically modified animals. Recently, Zambrowicz and Sands[418] surveyed the 100 best-selling pharmaceutical agents and demonstrated that the effects of all of these agents could have been predicted from analyzes in relevant knockouts of the given target gene. Although one must remain somewhat cautious with the authors' interpretation of the word prediction, it is clear that analysis of genetically modified mice represents an important and still growing strategy in elucidating new mechanistic approaches for developing novel treatments for various medical disorders including depression. Nonetheless, it will take perspicacious analysis at the behavioral, genetic, physiological and neurochemical levels to realize fully and confirm whether one has indeed found that mouse with alterations relevant to depression endophenotypes. Obviously, all of these studies are also guided by the fact that there is still a tremendous unmet medical need related to the treatment of patients suffering from major depression.

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