

UPDATE ARTICLE

Animal models as tools to study the pathophysiology of depression

Helena M. Abelaira, Gislaïne Z. Réus, João Quevedo

Laboratory of Clinical Neurosciences, National Science and Technology Institute for Translational Medicine (INCT-TM), Center of Excellence in Applied Neurosciences of Santa Catarina (NENASC), Graduate Program in Health Sciences, Health Sciences Unit, Universidade do Extremo Sul Catarinense (UNESC), Criciúma, SC, Brazil.

The incidence of depressive illness is high worldwide, and the inadequacy of currently available drug treatments contributes to the significant health burden associated with depression. A basic understanding of the underlying disease processes in depression is lacking; therefore, recreating the disease in animal models is not possible. Popular current models of depression creatively merge ethologically valid behavioral assays with the latest technological advances in molecular biology. Within this context, this study aims to evaluate animal models of depression and determine which has the best face, construct, and predictive validity. These models differ in the degree to which they produce features that resemble a depressive-like state, and models that include stress exposure are widely used. Paradigms that employ acute or sub-chronic stress exposure include learned helplessness, the forced swimming test, the tail suspension test, maternal deprivation, chronic mild stress, and sleep deprivation, to name but a few, all of which employ relatively short-term exposure to inescapable or uncontrollable stress and can reliably detect antidepressant drug response.

Keywords: Face validity; construct validity; predictive validity; animals models; antidepressants; depression

Introduction

Depression is a psychiatric disorder that has a poorly understood neurobiology. Many patients do not respond to existing treatments. Much research has been undertaken, and its progress is coupled to the development of animal models of human disease. As in all clinical conditions, the rapprochement between the disease and the corrective actions of drugs in laboratory animals is essential for developing effective therapies. Criteria have been established to define depressive mood disorders, such as depressed mood and/or anhedonia, changes in appetite, sleep disturbances, fatigue, and so on. These are presented in Table 1.¹

Animal models are important tools for investigating the etiology of depression, as well as progress in the development of effective therapeutic targets for its treatment. Although animal models greatly help our understanding of psychiatric disorders, they do have some limitations. For example, animals cannot observe feelings of sadness, guilt, or suicidal thoughts, symptoms mainly limited to humans.² The major obstacle is the restricted availability of validated animal models. Firstly, the ideal animal model should offer an opportunity to understand molecular, genetic, and epigenetic factors

that may lead to depression.³ By using animal models, the underlying molecular alterations and the causal relationship between genetic or environmental alterations and depression can be examined, which would afford a better insight into the pathology of depression.³ Some criteria have been established for the validation of an animal model. These criteria are currently accepted and are presented in Table 2.

Based on these criteria for validity, many animal models of depression have been and are being developed, including those based on genetic engineering, brain damage, and environmental manipulations. Table 3

Table 1 Areas of impairment in depression and corresponding diagnostic criteria

Area of impairment	DSM-IV diagnostic criteria
Mood	Depressed mood
Motivation	Markedly diminished interest or pleasure
Food	Weight loss or gain or decrease or increase in appetite
Sleep	Insomnia or hypersomnia
Motor	Psychomotor retardation or agitation
Energy	Fatigue or loss of energy
Self-esteem	Feelings of worthlessness or excessive or inappropriate guilt
Cognition	Diminished ability to think or concentrate, or indecisiveness
Hope	Recurrent thoughts of death, recurrent suicidal ideation

Correspondence: Gislaïne Z. Réus, Laboratório de Neurociências, Programa de Pós-Graduação em Ciências da Saúde, Unidade Acadêmica de Ciências da Saúde, Universidade do Extremo Sul Catarinense, CEP 88806-000, Criciúma, SC, Brazil.
E-mail: gislainezilli@hotmail.com

Table 2 Criteria for the validity of an animal model of depression

Criteria	Manifestations
1. Face validity	Behavioral manifestations should be similar to symptoms observed in patients with depression.
2. Construct validity	The pathophysiological changes that occur in patients with depression, such as changes in the HPA axis, hippocampal atrophy, and neurotransmitters must also occur in animals.
3. Predictive validity	Behavioral changes should be reversed by effective treatment (antidepressants and/or electroconvulsive therapy).

HPA = hypothalamic-pituitary-adrenal.

Table 3 Animal models of depression and its criteria of validity

Model	Criteria		
	Face	Construct	Predictive
Forced swim and tail suspension tests	-	-	+
Chronic stressors (chronic mild stress, social isolation and learned helplessness)	+	+	+
Maternal deprivation	+	+	+
Injuries (olfactory bulbectomy)	+	+	+
Stimulation of the immune system (endotoxin and inflammatory cytokines)	+	+	+

shows some existing models of depression and whether they meet each of the criteria for validity presented in Table 2.

The question therefore remains whether we can know if a mouse is “depressed”. In reality, few models of depression fully fit these validating criteria, and most models currently used rely on either the actions of known antidepressants or responses to stress.⁴ Notably, it is not necessary for an “ideal” animal model of depression to exhibit all of the abnormalities of depression-relevant behaviors, just as the patients do not manifest every possible symptom of depression. In fact, anhedonia is the core symptom of depression⁴ and most of the current models only mimic anhedonia.

It should also be noted that there is a difference between a model and a test. A model can be defined as an organism (non-human) or a particular state of an organism that reproduces aspects of human pathology, providing a certain degree of predictive validity. A test, on the other hand, provides only an endpoint – a behavioral or physiological measure (read-out) designed to assess the effect of a genetic, pharmacological, or environmental manipulation.⁵ Thus, this work aims to study the animal models of depression and determine which has the best face, construct and predictive validity.

Animal models of depression: environmental manipulations

Chronic mild stress

One of the major symptoms of depression in humans is anhedonia, a reduction in interest or pleasure in daily activities. Moreover, chronic mild stress (CMS) causes many other symptoms of depression, such as decreases in sexual, aggressive, and investigative behaviors, as well as a decrease in locomotor activity.³ Furthermore, repeated presentation of the same stressor usually leads to adaptation, which can, however, be excluded by presenting a variety of stressors in an unpredictable

sequence. According to these characteristics, many researchers use an animal model of CMS to study the neurobiology of depression, as well as to elucidate new therapeutic targets for treatment.

The first CMS paradigm was introduced by Katz et al. This model provides the basis for most of the currently used paradigms. Initial protocols included 3 weeks of exposure to electric shocks, immersion in cold water, immobilization, reversal of the light/dark cycle, and a variety of other stressors.⁶ This series of stressors could cause an increase in plasma corticosteroid levels and a reduction in sucrose preference,⁶ which suggests that chronic stress may cause anhedonia.

In fact, rats subjected to different stressors (e.g., water deprivation, food deprivation, exposure to cold temperatures, isolation, etc.) over long periods (3 weeks–3 months) exhibited behavioral deficits, such as changes in sleep and anhedonic behavior. With respect to molecular parameters, which are related to the construct validity of this animal model, changes were observed in the hypothalamic-pituitary-adrenal (HPA) system. Usually, animals subjected to a stress protocol exhibit increased levels of corticosterone and an increase in adrenal gland weight.⁷ Changes in lipids and proteins, as well as decreases in the activity of antioxidant enzymes and increases in pro-inflammatory cytokines, were observed in animals subjected to these experimental protocols.^{8,9}

In recent years, many researchers have focused on the importance of neurotrophins, which are involved in cellular plasticity. One of the most important neurotrophins is brain-derived neurotrophic factor (BDNF), which is a target of prime importance in the neurobiology and treatment of depression. In fact, it has been reported that levels of this neurotrophin are decreased in serum and in postmortem brain samples from patients with depression. Moreover, antidepressants have shown a positive effect on BDNF, increasing its expression.¹⁰ Reduced BDNF levels, as well as attenuated neurogenesis, have been found in the brains of animals exposed to a stress

protocol.¹¹ However, the opposite results – i.e., an increase in BDNF levels in stressed animals – have been reported by other authors, suggesting the existence of a compensatory mechanism in response to stress.⁷

It is important to note that many of the changes found in animals exposed to stress procedures and that serve as criteria for face validation (such as anhedonia) and for construct validation (such as changes in the HPA axis and in neurotrophin levels) are reversed by various classes of clinically effective antidepressants (e.g., fluoxetine and imipramine),¹² demonstrating the predictive validity of this animal model. However, the CMS model has two major drawbacks. One is the practical difficulty in carrying out CMS experiments, which are labor-intensive, space demanding, and have long duration. The other is that the procedure itself can be difficult to establish in a new laboratory setting, making replication across laboratories challenging.¹³

Learned helplessness

Certain types of human depression are precipitated by stressful life events, and vulnerable individuals experiencing these stressors may develop clinical depression. In this aspect, stress can be used to induce depression-like symptoms in rodent animals. One of the well-validated animal models is learned helplessness, in which a depressive-like state is induced by uncontrollable and unpredictable electrical foot-shock stress.¹⁴

Following an uncontrollable and inescapable stress, such as exposure to unavoidable electric shocks, the animals develop a state of “helplessness” such that when re-exposed to the same shocks, now with an easy escape route, the animals will either display an increased escape latency or completely fail to escape.¹⁴ Following one or more sessions of inescapable shock, rats have been shown to develop persistent changes including weight loss, alterations in sleep patterns and HPA axis activity, and a loss of spine synapses in the hippocampal regions.^{2,15}

Reduced weight, increased motor activity, reduced libido, cognitive deficits, and changes in sleep have been observed in helpless animals. In most cases, use of the behavioral model of learned helplessness causes animals to present depressive-like behavior, as is observed clinically in human patients.¹⁶ Therefore, this model presents good face validity. In fact, animals subjected to this model respond to tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors, and electroconvulsive therapy.¹⁷ Response to these antidepressant drugs was observed between 2-3 days after initiation of treatment.

Neurobiological changes have also been observed after induction of learned helplessness. Depletion of norepinephrine and serotonin, as well as changes in the NE β norepinephrine and 5-HT_{1B} serotonin receptors in the hippocampus, were reported. However, chronic administration of antidepressants reversed the changes in the NE β and 5-HT_{1B} receptors. In addition, the HPA axis appears to play an important role in this animal

model. Indeed, an increase in the vulnerability to learned helplessness has been observed in animals after antagonism of the glucocorticoid receptors. Furthermore, high levels of glucocorticoids and homocysteine – which are found in human patients with depression – have been reported in rats in an animal model of learned helplessness.¹⁸

One advantage of learned helplessness as a model is that its symptoms are parallel to those of major depression, and most can be reversed by multiple acute (subchronic) treatment with antidepressants (typically for 3-5 days).¹⁶ In addition, the cognitive (e.g., learning) and other behavioral outcomes (e.g., neurovegetative abnormalities) seem to be correlated, thus enhancing our understanding of depressive symptoms in humans. The excellent face and predictive validities of learned helplessness make it an interesting model for exploration of the pathophysiology of depression.¹⁹ Furthermore, the model can also be generally used to measure the escape performance of mice with different mutations, showing which target genes for depression may affect vulnerability to development of a depressive-like state. However, the major drawback of this model is that most of its depression-like symptoms do not persist long enough following cessation of the uncontrollable shock stimulus.¹⁵ In addition, different protocols are used in different laboratories.

Maternal deprivation

Early adverse life experiences represent one of the major risk factors for the development of mental disorders such as major depression. The early postnatal period is characterized by considerable plasticity of the developing nervous system. As such, the early postnatal environment is critical in its capacity to influence adult behavior. Preclinical studies have provided direct evidence that early life stress leads to heightened responsiveness to stress and alterations in the HPA system throughout the lifespan.²⁰ Among the paradigms used to study early adverse life events, long maternal separation in rodents mimics early life neglect/loss of parents in humans, and has been presented as one of the most potent natural stressors during development. Maternal separation was developed to examine the consequences of early adverse experiences on behavior and neurobiology, and this model has been described as a model of vulnerability to drug dependence, anxiety, stress-induced illness, and depression.²¹

In particular, maternal separation was proposed to represent an important animal model for investigation of the pathophysiology and treatment of major depression. For example, treatment with antidepressants was able to normalize anxiety-like behavior, endocrine stress response, and preference for ethanol in adult male rats subjected to maternal separation.

Corroborating these data, rats deprived of maternal care showed depressive-like behavior in the forced swimming test (FST), had increased levels of glucocorticoids, and a decrease in neurotrophins (e.g., BDNF and

neurotrophin-3 [NT-3]).^{22,23} In contrast, treatment with antidepressants in animals deprived of maternal care was able to reverse depressive-type behaviors, promoting an increase in neurotrophin levels and neurogenesis.²

Sleep deprivation

Sleep has important homeostatic functions, and sleep deprivation is a stressor that has consequences for the brain as well as for many body systems. Although sleep deprivation is not yet a well-established model of depression, many studies show that it alters important pathways related to stress.

Increased levels of messenger RNA for interleukin-1 β (a pro-inflammatory cytokine) and for cortisol have been shown in rodents after sleep deprivation. The procedure of this study consisted of handling the animals gently to prevent them from sleeping. Furthermore, 72 hours of sleep deprivation in mice was induced using the platform method, which is accomplished by placing the animal on a platform submerged in water so that, when the animal falls asleep, it falls into the water and must then climb back onto the platform, thus forcing it to stay awake. This study showed that after 72 hours of sleep deprivation, there was an increase of oxidative stress in the hippocampus. A decrease in cell proliferation has also been observed after 96 hours of sleep deprivation.⁹ Some classical tests used to assess cognitive parameters in animals, such as the inhibitory avoidance and water maze tests, show deficits in learning and memory of rodents subjected to sleep deprivation, as well as aggressive behavior and hyperactivity.²⁴

Even though many studies using mice have shown depressive-like behavior after sleep deprivation, it is important to note that studies in patients with depression have shown effects of antidepressants on selective slow-wave sleep deprivation.^{25,26} The underlying mechanisms are still unknown, however,²⁷ and may be related to the fact that the beneficial effects of sleep deprivation on depressive-like behaviors require an astrocyte-dependent signaling pathway.

Some neurotransmitters, such as dopamine and serotonin, are altered following sleep deprivation, and these alterations are associated with behavioral changes. Sleep deprivation for short or long periods also altered gene expression of several transcription factors and genes that encode neurotransmitters and proteins involved in metabolic processes and cellular plasticity.²⁸ Anhedonic behavior has been shown in rats subjected to paradoxical sleep deprivation.²⁹ Although the sleep deprivation protocol still has its limitations as an animal model of depression, it meets some of the criteria for a valid animal model, such as good face and construct validity.

Changing photoperiod

More recently, it was proposed that manipulation of the light/dark cycle could characterize a new animal model of depression.³⁰ In this model, nocturnally active mice are

exposed to long periods of artificial light (22 hours per day) for a period of 2 weeks. Exploring the interactions between these mechanisms and mood changes in diurnal animals may provide new insight into depression. Recent studies demonstrate that diurnal Fat sand rats and Nile grass rats show depression-like behavior when maintained under short-photoperiod (SP) conditions compared with animals maintained under neutral photoperiod (NP) conditions. Moreover, these behaviors were ameliorated by treatment with bright light.³¹

These animals also developed anhedonic behavior and increased motor activity. Consistent with these behavioral changes, increased levels of corticosterone and a decrease in BDNF levels in the hippocampus were also found, thus demonstrating face and construct validity. However, the predictive validity of this model should be examined, as treatment with the antidepressant imipramine was only able to prevent some of these behavioral and physiological changes.³² Diurnal mice develop depressive-type behavior when subjected to experimental conditions with a decreasing photoperiod. Treatment with the antidepressants bupropion and imipramine reversed depressive behavior in these animals as shown by the FST, but not anhedonic behavior.

Other studies using the dark phase of a 12:12 light/dark cycle showed that rats exhibited depressive-like behavior.^{33,34} On the other hand, brief or long exposures to light treatment have an antidepressant effect on the FST.^{33,34} Taken together, these studies reveal a relationship between light control and depression.

Animal models of depression: injuries

Olfactory bulbectomy

Bilateral olfactory bulbectomy (OB) results in endocrine, behavioral, immune system, and neurotransmitter changes that mimic many of the symptoms seen in human patients with major depression.³⁵ The rat olfactory system forms a part of the limbic region, which includes the amygdala and hippocampus. These are responsible for functions such as memory and emotion, and are known to have altered morphology and activity in patients with depression. After OB, there is a marked degeneration of neurons in the olfactory bulb, but also in other areas such as the hippocampus, cortex, amygdala, locus ceruleus, and raphe nuclei. In all likelihood, these focal brain changes lead to the dysfunction in serotonergic and noradrenergic systems that is observed after bulbectomy.³⁶

With regards to behavioral parameters in animals with OB, there is an increase in cannibalism and exploratory and locomotor behavior, as well as a decrease in sexual activity and behavioral and cognitive anhedonic deficits. Cellular level studies have also observed a reduction in the number of synapses and dendritic arborization in the hippocampal and cortical neurons. Furthermore, reductions were found in the concentrations of serotonin and norepinephrine in the brains of rodents subjected to OB.³⁶ Changes in the immune system are quite evident in these animals. Studies have also reported that many of these

changes, both behavioral and cellular, are reversed by several classes of antidepressants used in the clinic.¹ With both behavioral changes and changes in neurotransmitters and in the immune system occurring after OB, we can conclude that this may be a good animal model of depression, since it has both face and construct validity. Moreover, different classes of therapeutically effective antidepressants reverse the behavioral and molecular changes caused by OB, thus showing that this model presents predictive validity.

Chronic, but not acute, administration of antidepressants largely corrects most of the behavioral, endocrine, immune, and neurotransmitter changes that occur following bulbectomy. Thus, the olfactory bulbectomized rat is not only a model for detecting antidepressant activity but also one for exploring the inter-relationships between these systems, which are also dysfunctional in patients with major depression.³⁶

Animal models of depression: chemical manipulations

Stimulation of the immune system

Recently, several studies have drawn special attention to the role of the immune system in depression. Immunologic abnormalities in depression have been described for over two decades,^{37,38} but it is still unclear whether these abnormalities play a role in the pathogenesis of depression.

Cytokines are inflammatory mediators that interact with pathways related with depression, including neurotransmitter metabolism, neural plasticity, and neuroendocrine functions. Depressed patients exhibit high levels of proinflammatory mediators, such as interleukins (IL-1, IL-2 and IL-6) and tumor necrosis factor (TNF). Moreover, treatment with antidepressants has been shown to decrease levels of IL-4 in patients with depression. Table 4 lists the major cytokines and their respective functions.¹

Animal models of depression involving the immune system have been the target of criticism, but the fact remains that there is still great difficulty in understanding the complexity of the communication between the immune system and the brain. Nevertheless, a few studies have addressed this issue.¹ In rodents, the administration of endotoxin, a cell wall component of Gram-negative cells, led to behavioral changes similar to

Table 4 Cytokines implicated in depression and their main functions

Cytokine	Main functions
Interleukin-1 β (IL-1)	Fever HPA axis activation Lymphocyte activation Prostanoid synthesis Endothelial activation IL-6 synthesis
Interleukin-4 (IL-4)	Inhibits production of TNF and IL-1 Stimulates B cells
Interleukin-6 (IL-6)	Fever Acute phase protein synthesis T and B cell differentiation and activation
Interleukin-8 (IL-8)	Inflammation Neutrophil chemotaxis
Interleukin-10 (IL-10)	Inhibits inflammation Inhibits production of IL-1, IL-6, TNF and IFN- γ
Tumor necrosis factor (TNF)	Fever Endothelial activation Neutrophil activation Migration of dendritic cells to lymph nodes
Interferon- α (INF- α)	Induction of viral resistance Natural killer cell activation Macrophage activation
Interferon- γ (INF- γ)	Macrophage activation T cell differentiation

those seen in humans, such as anhedonia and sleep disorders, which are parameters of face validity. Neuroendocrine changes, which help determine construct validity, were also found. Treatment with the antidepressants fluoxetine and desipramine³⁷ reduced anhedonia in these animals (Table 5).

Animal models of depression: genetically modified animal models

Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in 5-HT biosynthesis. The discovery of a neuronal isoform, TPH2, by Walther & Bader³⁸ opened up a new way to reliably map 5-HT neurons in the brain by means of immunohistochemistry and in situ hybridization. Results confirmed wide expression, including cell bodies in DRN and MRN.^{39,40} In fact, it is now established that TPH2 is the predominant isoform in the rodent brain.^{41,42} However, early studies reported that TPH expression,

Table 5 Different animal models of depression and their effects on parameters of face and construct validity

Treatment	Face validity			Construct validity			
	Anhedonia	Motor activity	Weight	HPA axis	Neurotrophins	Proinflammatory cytokines	Hippocampal volume
Chronic stress	↑	-	↓	↑	↓ ↑	↑	↓
Maternal deprivation	↑	-	-	↑	↓ ↑	↑	↓
Learned helplessness	↑	↑	↓	↑	↓	↑	-
Olfactory bulbectomy	↑	↑	↓	-	↑	↑	-
Sleep deprivation	↑	↑	↓	↑	↓ ↑	↑	↓

HPA = hypothalamic-pituitary-adrenal.

turnover, and distribution in DRN are complex, in line with the existence of different subpopulations of 5-HT neurons in this area.⁴³ Changes in *TPH2* gene and/or protein expression in the brain have been reported in various mood disorders and have been validated in animal models.⁴⁴⁻⁴⁶ *TPH2* variants have also been extensively reported to be associated with major depression.⁴⁷⁻⁴⁹ However, whether these changes might reflect alterations in possible modulatory effects of other neurotransmitter systems on 5-HT systems is still largely unknown. To address this question directly, *TPH2* mRNA expression was measured in three different transgenic mouse models, all related to pathological states of depression and anxiety, but caused by mutations affecting different neurotransmitter systems. One of the models is the 5-HT transporter-deficient mouse (5-HTT^{-/-}).^{50,51} By mediating 5-HT reuptake in the nerve terminal (and other parts of the 5-HT neuron), 5-HTT fine-tunes the magnitude and duration of serotonergic signaling,^{52,53} which makes it the target for many antidepressant drugs, including the SSRIs.⁵⁴ These animals exhibit major adaptive changes in 5-HT neurotransmission when compared with their wild-type controls. It has been shown that lack of 5-HTT depletes 5-HT and its metabolite 5-hydroxyindoleacetic acid by 60–80% in several brain areas, such as the brainstem, striatum, hippocampus, and frontal cortex.^{50,55} Functional desensitization of 5-HT1A, 5-HT1B autoreceptors has been reported in the DRN of 5-HTT^{-/-} mutants as a consequence of high extracellular 5-HT levels in the vicinity of the serotonergic cells in the DRN. However, in terms of their target areas such as hippocampus and forebrain, increased or unaltered expression of these receptors has been reported.^{55,56} Autoradiographic labeling of 5-HT2A receptors has also revealed a 30–40% reduction in the density of these receptors in the cerebral cortex and lateral striatum of 5-HTT^{-/-} animals in comparison with wild-type mice.⁵⁷ In addition, anxiolytic- and antidepressant-like responses have been observed in 5-HTT^{-/-} mice in behavioral test paradigms such as the elevated plus maze, tail suspension test (TST), and FST.⁵⁸⁻⁶⁰

The second model consists of mice heterozygous for the vesicular glutamate transporter 1 gene (VGLUT1^{+/-}).⁶¹ As the first of three vesicular glutamate transporters (VGLUT1, VGLUT2 and VGLUT3),⁶² VGLUT1 has been shown to have a high level of expression in glutamatergic neurons in the cerebral cortex.⁶³ By concentrating glutamate in synaptic vesicles, VGLUT1 mediates glutamate release from synaptic terminals and facilitates efficient glutamatergic transmission.^{64,65} Recent studies have demonstrated that VGLUT1^{+/-} mice exhibit deficient glutamate transmission,⁶⁶ depressive-like behavior and neurochemical changes, which are related to depression and anxiety.^{67,68} Aberrations in glutamate synthesis and its dysregulation also appear to play a relevant role in major depression.⁶⁹ In keeping with this, recent postmortem studies showing decreased cortical VGLUT1 in depressed subjects⁷⁰ together with clinical findings of an excitatory inhibitory imbalance in the cortex of depressed patients^{71,72} suggest that decreased VGLUT1 levels may have clinical implications.

Finally, mice with targeted disruption of the gene encoding the cannabinoid 1 receptor (CB1R^{-/-})⁷³ were used as an additional model of mood disorders. The endocannabinoid system is a major neuromodulatory system that contributes to the control of emotional behavior.⁷⁴ Pharmacological and genetic blockade of the CB1R induces a behavioral state analogous to depression in experimental animals.⁷⁵ Thus, CB1R^{-/-} mice exhibit depressive-like symptoms, such as reduced responsiveness to reward stimuli^{74,76} and enhanced anxiety levels and sensitivity to stress.^{77,78} Moreover, the chronic absence of CB1R activity induces alterations in 5-HT-dependent negative feedback. In particular, enhanced extracellular 5-HT levels in the prefrontal cortex decreased 5-HTT binding site density and caused functional desensitization of the 5-HT1A autoreceptors. Reduced 5-HT2C receptor expression in different brain regions has also been described in these mutants.⁷⁹ In addition, according to Mato et al.,⁸⁰ mice lacking *CB1R* exert impaired post-synaptic serotonergic signaling, suggesting that CB1R^{-/-} mice are useful models to reveal more regarding the nature of cannabinoid/5-HT interactions in mood disorders.

Although genetically modified animals can provide good models of human diseases, mood disorders such as depression are more difficult to represent, as these disorders are associated with changes in several genes, which are specific for each patient.

Predictive models of antidepressant activity

Forced swimming test

One of the tests most commonly used by researchers to investigate new antidepressant drugs is the FST, first described by Porsolt et al.⁸¹ This test was developed as an animal model of depression that aimed to measure the effects of antidepressant compounds in mice. In this test, the animal is placed in a water-filled cylinder which it is unable to exit. Initially, the animal will try to escape, but eventually it adopts a posture of immobility, a passive behavior characterized by the absence of movements except for those necessary for the animal's snout to remain above the water level (Figure 1A). The test for rats consists of two swimming exposures. The first exposure is for 15 minutes and the second is performed 24 hours after the first, with an exposure period of 5 minutes. The test for mice consists of a single 6-minute exposure, with the first 2 minutes serving as a habituation period and the last 4 minutes consisting of the test itself, which yields the duration of immobility.

FST is easy to use, has very good reproducibility and is used for the selection of new antidepressant drugs. Various classes of antidepressants reduce immobility time during the FST by increasing the swimming and/or climbing time. With respect to anti-immobility, it is known that drugs affecting noradrenergic neurotransmission (e.g., imipramine) increase climbing behavior, whereas drugs affecting serotonergic neurotransmission (e.g., fluoxetine, sertraline, paroxetine, citalopram) increase swimming time.⁸² The swimming time is measured by the

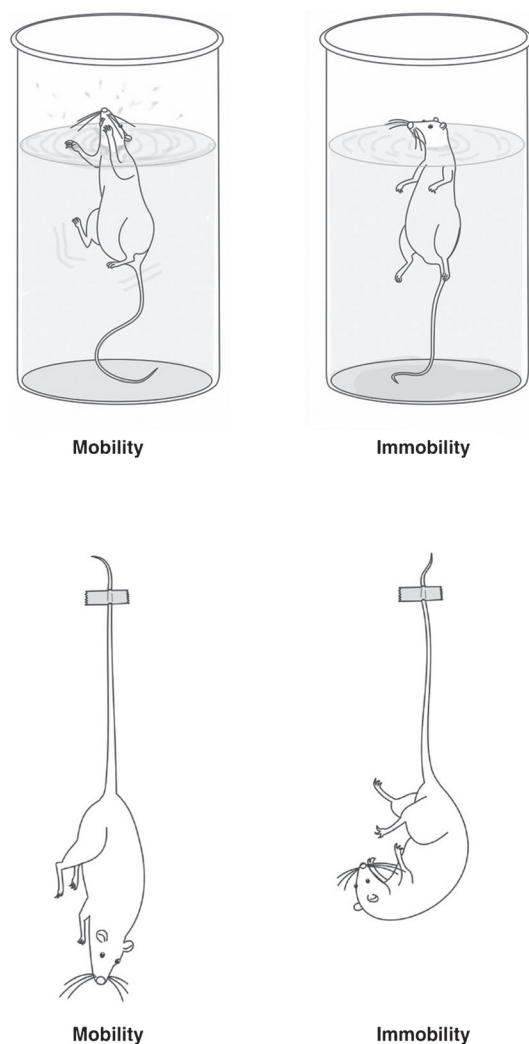


Figure 1 Schematic representation of mobility and immobility in A) the forced swimming test and B) the tail suspension test.

horizontal and vertical movements of the animals as they try to scale the cylinder walls with their paws. The effects of antidepressants on FST behavior are relatively specific, since they do not increase spontaneous motor activity, unlike psychostimulants such as amphetamine and cocaine.⁸³ Besides the effects of antidepressant drugs, the FST can also be used to evaluate the type of depressive behavior; for example, it has been demonstrated that animals subjected to a protocol of maternal deprivation exhibit increased immobility time in the FST.²³

Tail suspension test

Since its introduction almost 20 years ago, the TST has become one of the most widely used models for assessing antidepressant-like activity in mice. The test is based on the fact that animals subjected to the short-term, inescapable stress of being suspended by their tail will develop an immobile posture.¹⁵ The FST is similar to the TST, but differs in that it can be used on both rats and

in mice, whereas the TST can only be undertaken on mice. In the TST, the tails of the mice are attached and suspended by an adhesive tape (Figure 1B). The time spent immobile by the animal during a period of 6 minutes is interpreted as a measure of depressive-like behavior. Various antidepressant medications reverse this immobility and promote the occurrence of escape-related behavior.

Importantly, both the TST and FST are considered predictive models of antidepressant activity, not animal models of depression. Accordingly, they lack face and construct validity.

Conclusion

Although there are many animal models of depression, including some that have predictive, face, and construct validity within the same model, many limitations constrain their utility. It is remarkable that all animal models of depression have contributed to a better understanding of the neurobiology of this disorder, and offer new pharmacological targets for treatment. However, the development of a model that represents most symptoms of depression and meets all criteria for animal model validity would be ideal.

Acknowledgements

This study was supported in part by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq-Brazil – JQ and GZR), Instituto Cérebro e Mente (JQ), and UNESCO (JQ). JQ is a recipient of productivity grants from CNPq-Brazil. GZR and HMA receive student-ship grants from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Disclosure

The authors report no conflicts of interest.

References

- 1 DellaGioia N, Hannestad J. A critical review of human endotoxin administration as an experimental paradigm of depression. *Neurosci Biobehav Rev.* 2010;34:130-43.
- 2 Nestler EJ, Hyman SE. Animal models of neuropsychiatric disorders. *Nat Neurosci.* 2010;13:1161-9.
- 3 Chen SK, Tvrdik P, Peden E, Cho S, Wu S, Spangrude G, et al. Hematopoietic origin of pathological grooming in Hoxb8 mutant mice. *Cell.* 2010;141:775-85.
- 4 Krishnan V, Nestler EJ. The molecular neurobiology of depression. *Nature.* 2008;455:894-902.
- 5 Urani A, Chourbaji S, Gass P. Mutant mouse models of depression: candidate genes and current mouse lines. *Neurosci Biobehav Rev.* 2005;29:805-28.
- 6 Katz RJ, Roth KA, Carroll BJ. Acute and chronic stress effects on open field activity in the rat: implications for a model of depression. *Neurosci Biobehav Rev.* 1981;5:247-5
- 7 Fortunato JJ, Reus GZ, Kirsch TR, Stringari RB, Fries GR, Kapczinski F, et al. Effects of beta-carboline harmine on behavioral and physiological parameters observed in the chronic mild stress model: further evidence of antidepressant properties. *Brain Res Bull.* 2010;81:491-6.

- 8 Lucca G, Comim CM, Valvassori SS, Reus GZ, Vuolo F, Petronilho F, et al. Effects of chronic mild stress on the oxidative parameters in the rat brain. *Neurochem Int*. 2009;54:358-62.
- 9 You Z, Luo C, Zhang W, Chen Y, He J, Zhao Q, et al. Pro- and anti-inflammatory cytokines expression in rat's brain and spleen exposed to chronic mild stress: involvement in depression. *Behav Brain Res*. 2011;225:135-41.
- 10 Mao QQ, Xian YF, Ip SP, Tsai SH, Che CT. Long-term treatment with peony glycosides reverses chronic unpredictable mild stress-induced depressive-like behavior via increasing expression of neurotrophins in rat brain. *Behav Brain Res*. 2010;210:171-7.
- 11 Ibarguen-Vargas Y, Surget A, Vourc'h P, Leman S, Andres CR, Gardier AM, et al. Deficit in BDNF does not increase vulnerability to stress but dampens antidepressant-like effects in the unpredictable chronic mild stress. *Behav Brain Res*. 2009;202:245-51.
- 12 Szymanska M, Budziszewska B, Jaworska-Feil L, Basta-Kaim A, Kubera M, Leskiewicz M, et al. The effect of antidepressant drugs on the HPA axis activity, glucocorticoid receptor level and FKBP51 concentration in prenatally stressed rats. *Psychoneuroendocrinology*. 2009;34:822-32.
- 13 Willner P. Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. *Psychopharmacology (Berl)*. 1997;134:319-29.
- 14 Seligman ME, Beagley G. Learned helplessness in the rat. *J Comp Physiol Psychol*. 1975;88:534-41.
- 15 Cryan JF, Mombereau C, Vassout A. The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. *Neurosci Biobehav Rev*. 2005;29:571-625.
- 16 Takamori K, Yoshida S, Okuyama S. Availability of learned helplessness test as a model of depression compared to a forced swimming test in rats. *Pharmacology*. 2001;63:147-53.
- 17 Malberg JE, Duman RS. Cell proliferation in adult hippocampus is decreased by inescapable stress: reversal by fluoxetine treatment. *Neuropsychopharmacology*. 2003;28:1562-71.
- 18 Setnik B, de Souza FG, d'Almeida V, Nobrega JN. Increased homocysteine levels associated with sex and stress in the learned helplessness model of depression. *Pharmacol Biochem Behav*. 2004;77:155-61.
- 19 Vollmayr B, Simonis C, Weber S, Gass P, Henn F. Reduced cell proliferation in the dentate gyrus is not correlated with the development of learned helplessness. *Biol Psychiatry*. 2003;54:1035-40.
- 20 Heim C, Nemeroff CB. The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry*. 2001;49:1023-39.
- 21 Anisman H, Zaharia MD, Meaney MJ, Merali Z. Do early-life events permanently alter behavioral and hormonal responses to stressors? *Int J Dev Neurosci*. 1998;16:149-64.
- 22 Ruedi-Bettschen D, Zhang W, Russig H, Ferger B, Weston A, Pedersen EM, et al. Early deprivation leads to altered behavioural, autonomic and endocrine responses to environmental challenge in adult Fischer rats. *Eur J Neurosci*. 2006;24:2879-93.
- 23 Reus GZ, Stringari RB, Ribeiro KF, Cipriano AL, Panizzutti BS, Stertz L, et al. Maternal deprivation induces depressive-like behaviour and alters neurotrophin levels in the rat brain. *Neurochem Res*. 2011;36:460-6.
- 24 McEwen BS. Sleep deprivation as a neurobiologic and physiologic stressor: allostasis and allostatic load. *Metabolism*. 2006;55:S20-3.
- 25 Goldstein MR, Plante DT, Hulse BK, Sarasso S, Landsness EC, Tononi G, et al. Overnight changes in waking auditory evoked potential amplitude reflect altered sleep homeostasis in major depression. *Acta Psychiatr Scand*. 2012;125:468-77.
- 26 Landsness EC, Ferrarelli F, Sarasso S, Goldstein MR, Riedner BA, Cirelli C, et al. Electrophysiological traces of visuomotor learning and their renormalization after sleep. *Clin Neurophysiol*. 2011;122:2418-25.
- 27 Hines DJ, Schmitt LI, Hines RM, Moss SJ, Haydon PG. Antidepressant effects of sleep deprivation require astrocyte-dependent adenosine mediated signaling. *Transl Psychiatry*. 2013;3:e212.
- 28 Sei H, Saitoh D, Yamamoto K, Morita K, Morita Y. Differential effect of short-term REM sleep deprivation on NGF and BDNF protein levels in the rat brain. *Brain Res*. 2000;877:387-90.
- 29 Andersen ML, Hoshino K, Tufik S. Increased susceptibility to development of anhedonia in rats with chronic peripheral nerve injury: involvement of sleep deprivation? *Prog Neuropsychopharmacol Biol Psychiatry*. 2009;33:960-6.
- 30 Becker A, Bilkei-Gorzo A, Michel K, Zimmer A. Exposure of mice to long-light: a new animal model to study depression. *Eur Neuropsychopharmacol*. 2010;20:802-12.
- 31 Krivisky K, Ashkenazy T, Kronfeld-Schor N, Einat H. Antidepressants reverse short-photoperiod-induced, forced swim test depression-like behavior in the diurnal fat sand rat: further support for the utilization of diurnal rodents for modeling affective disorders. *Neuropsychobiology*. 2011;63:191-6.
- 32 Song C, Leonard BE. The olfactory bulbectomized rat as a model of depression. *Neurosci Biobehav Rev*. 2005;29:627-47.
- 33 Molina-Hernandez M, Tellez-Alcantara P. Long photoperiod regimen may produce antidepressant actions in the male rat. *Prog Neuropsychopharmacol Biol Psychiatry*. 2000;24:105-16.
- 34 Schulz D, Aksoy A, Canbeyli R. Behavioral despair is differentially affected by the length and timing of photic stimulation in the dark phase of an L/D cycle. *Prog Neuropsychopharmacol Biol Psychiatry*. 2008;32:1257-62.
- 35 Hellweg R, Zueger M, Fink K, Hortnagl H, Gass P. Olfactory bulbectomy in mice leads to increased BDNF levels and decreased serotonin turnover in depression-related brain areas. *Neurobiol Dis*. 2007;25:1-7.
- 36 Irwin MR, Miller AH. Depressive disorders and immunity: 20 years of progress and discovery. *Brain Behav Immun*. 2007;21:374-83.
- 37 Miller AH, Ancoli-Israel S, Bower JE, Capuron L, Irwin MR. Neuroendocrine-immune mechanisms of behavioral comorbidities in patients with cancer. *J Clin Oncol*. 2008;26:971-82.
- 38 Walther DJ, Bader M. A unique central tryptophan hydroxylase isoform. *Biochem Pharmacol*. 2003;66:1673-80.
- 39 Zhang X, Beaulieu JM, Sotnikova TD, Gainetdinov RR, Caron MG. Tryptophan hydroxylase-2 controls brain serotonin synthesis. *Science*. 2004;305:217.
- 40 Clark JA, Flick RB, Pai LY, Szalayova I, Key S, Conley RK, et al. Glucocorticoid modulation of tryptophan hydroxylase-2 protein in raphe nuclei and 5-hydroxytryptophan concentrations in frontal cortex of C57/Bl6 mice. *Mol Psychiatry*. 2008;13:498-506.
- 41 Gutknecht L, Waider J, Kraft S, Kriegebaum C, Holtmann B, Reif A, et al. Deficiency of brain 5-HT synthesis but serotonergic neuron formation in Tph2 knockout mice. *J Neural Transm*. 2008;115:1127-32.
- 42 Gutknecht L, Kriegebaum C, Waider J, Schmitt A, Lesch KP. Spatio-temporal expression of tryptophan hydroxylase isoforms in murine and human brain: convergent data from Tph2 knockout mice. *Eur Neuropsychopharmacol*. 2009;19:266-82.
- 43 Weissmann D, Chamba G, Debure L, Rousset C, Richard F, Maitre M, et al. Variation of tryptophan-5-hydroxylase concentration in the rat raphe dorsalis nucleus after p-chlorophenylalanine administration. II. Anatomical distribution of the tryptophan-5-hydroxylase protein and regional variation of its turnover rate. *Brain Res*. 1990;536:46-55.
- 44 Hiroi R, McDevitt RA, Neumaier JF. Estrogen selectively increases tryptophan hydroxylase-2 mRNA expression in distinct subregions of rat midbrain raphe nucleus: association between gene expression and anxiety behavior in the open field. *Biol Psychiatry*. 2006;60:288-95.
- 45 Bach-Mizrachi H, Underwood MD, Tin A, Ellis SP, Mann JJ, Arango V. Elevated expression of tryptophan hydroxylase-2 mRNA at the neuronal level in the dorsal and median raphe nuclei of depressed suicides. *Mol Psychiatry*. 2008;13:507-465.
- 46 Bonkale WL, Austin MC. 3,4-Methylenedioxymethamphetamine induces differential regulation of tryptophan hydroxylase 2 protein and mRNA levels in the rat dorsal raphe nucleus. *Neuroscience*. 2008;155:270-6.
- 47 Zill P, Baghai TC, Zwanzger P, Schule C, Eser D, Rupprecht R, et al. SNP and haplotype analysis of a novel tryptophan hydroxylase isoform (TPH2) gene provide evidence for association with major depression. *Mol Psychiatry*. 2004;9:1030-6.
- 48 Van Den Bogaert A, Slegers K, De Zutter S, Heyman L, Norrback KF, Adolfsen R, et al. Association of brain-specific tryptophan hydroxylase, TPH2, with unipolar and bipolar disorder in a Northern

- Swedish, isolated population. *Arch Gen Psychiatry*. 2006;63:1103-10.
- 49 Haghghi F, Bach-Mizrachi H, Huang YY, Arango V, Shi S, Dwork AJ, et al. Genetic architecture of the human tryptophan hydroxylase 2 Gene: existence of neural isoforms and relevance for major depression. *Mol Psychiatry*. 2008;13:813-20.
 - 50 Bengel D, Murphy DL, Andrews AM, Wichems CH, Feltner D, Heils A, et al. Altered brain serotonin homeostasis and locomotor insensitivity to 3, 4-methylenedioxymethamphetamine ("Ecstasy") in serotonin transporter-deficient mice. *Mol Pharmacol*. 1998;53:649-55.
 - 51 Murphy DL, Lesch KP. Targeting the murine serotonin transporter: insights into human neurobiology. *Nat Rev Neurosci*. 2008;9:85-96.
 - 52 Lesch KP, Mossner R. Inactivation of 5HT transport in mice: modeling altered 5HT homeostasis implicated in emotional dysfunction, affective disorders, and somatic syndromes. *Handb Exp Pharmacol*. 2006;417-56.
 - 53 Canli T, Lesch KP. Long story short: the serotonin transporter in emotion regulation and social cognition. *Nat Neurosci*. 2007;10:1103-9.
 - 54 Owens MJ, Nemeroff CB. The serotonin transporter and depression. *Depress Anxiety*. 1998;8:5-12.
 - 55 Fabre V, Beaufour C, Evrard A, Rioux A, Hanoun N, Lesch KP, et al. Altered expression and functions of serotonin 5-HT1A and 5-HT1B receptors in knock-out mice lacking the 5-HT transporter. *Eur J Neurosci*. 2000;12:2299-310.
 - 56 Mannoury la Cour C, Boni C, Hanoun N, Lesch KP, Hamon M, Lanfumey L. Functional consequences of 5-HT transporter gene disruption on 5-HT(1a) receptor-mediated regulation of dorsal raphe and hippocampal cell activity. *J Neurosci*. 2001;21:2178-85.
 - 57 Rioux A, Fabre V, Lesch KP, Moessner R, Murphy DL, Lanfumey L, et al. Adaptive changes of serotonin 5-HT2A receptors in mice lacking the serotonin transporter. *Neurosci Lett*. 1999;262:113-6.
 - 58 Holmes A, Yang RJ, Lesch KP, Crawley JN, Murphy DL. Mice lacking the serotonin transporter exhibit 5-HT(1A) receptor-mediated abnormalities in tests for anxiety-like behavior. *Neuropsychopharmacology*. 2003;28:2077-88.
 - 59 Holmes A, Murphy DL, Crawley JN. Abnormal behavioral phenotypes of serotonin transporter knockout mice: parallels with human anxiety and depression. *Biol Psychiatry*. 2003;54:953-9.
 - 60 Renoir T, Paizanis E, El Yacoubi M, Saurini F, Hanoun N, Melfort M, et al. Differential long-term effects of MDMA on the serotonergic system and hippocampal cell proliferation in 5-HTT knock-out vs. wild-type mice. *Int J Neuropsychopharmacol*. 2008;11:1149-62.
 - 61 Wojcik SM, Rhee JS, Herzog E, Sigler A, Jahn R, Takamori S, et al. An essential role for vesicular glutamate transporter 1 (VGLUT1) in postnatal development and control of quantal size. *Proc Natl Acad Sci U S A*. 2004;101:7158-63.
 - 62 Takamori S, Rhee JS, Rosenmund C, Jahn R. Identification of a vesicular glutamate transporter that defines a glutamatergic phenotype in neurons. *Nature*. 2000;407:189-94.
 - 63 Hisano S. Vesicular glutamate transporters in the brain. *Anat Sci Int*. 2003;78:191-204.
 - 64 Freneau RT, Jr, Kam K, Qureshi T, Johnson J, Copenhagen DR, Storm-Mathisen J, et al. Vesicular glutamate transporters 1 and 2 target to functionally distinct synaptic release sites. *Science*. 2004;304:1815-9.
 - 65 Wilson NR, Kang J, Hueske EV, Leung T, Varoqui H, Murnick JG, et al. Presynaptic regulation of quantal size by the vesicular glutamate transporter VGLUT1. *J Neurosci*. 2005;25:6221-34.
 - 66 Balschun D, Moechars D, Callaerts-Vegh Z, Vermaercke B, Van Acker N, Andries L, et al. Vesicular glutamate transporter VGLUT1 has a role in hippocampal long-term potentiation and spatial reversal learning. *Cereb Cortex*. 2010;20:684-93.
 - 67 Tordera RM, Totterdell S, Wojcik SM, Brose N, Elizalde N, Lasheras B, et al. Enhanced anxiety, depressive-like behaviour and impaired recognition memory in mice with reduced expression of the vesicular glutamate transporter 1 (VGLUT1). *Eur J Neurosci*. 2007;25:281-90.
 - 68 Garcia-Garcia AL, Elizalde N, Matrov D, Harro J, Wojcik SM, Venzala E, et al. Increased vulnerability to depressive-like behavior of mice with decreased expression of VGLUT1. *Biol Psychiatry*. 2009;66:275-82.
 - 69 Krystal JH, Sanacora G, Blumberg H, Anand A, Charney DS, Marek G, et al. Glutamate and GABA systems as targets for novel antidepressant and mood-stabilizing treatments. *Mol Psychiatry*. 2002;7:S71-80.
 - 70 Uezato A, Meador-Woodruff JH, McCullumsmith RE. Vesicular glutamate transporter mRNA expression in the medial temporal lobe in major depressive disorder, bipolar disorder, and schizophrenia. *Bipolar Disord*. 2009;11:711-25.
 - 71 Sanacora G, Gueorguieva R, Epperson CN, Wu YT, Appel M, Rothman DL, et al. Subtype-specific alterations of gamma-aminobutyric acid and glutamate in patients with major depression. *Arch Gen Psychiatry*. 2004;61:705-13.
 - 72 Bhagwagar Z, Wylezinska M, Jezzard P, Evans J, Ashworth F, Sule A, et al. Reduction in occipital cortex gamma-aminobutyric acid concentrations in medication-free recovered unipolar depressed and bipolar subjects. *Biol Psychiatry*. 2007;61:806-12.
 - 73 Ledent C, Valverde O, Cossu G, Petitot F, Aubert JF, Beslot F, et al. Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. *Science*. 1999;283:401-4.
 - 74 Maldonado R, Valverde O, Berrendero F. Involvement of the endocannabinoid system in drug addiction. *Trends Neurosci*. 2006;29:225-32.
 - 75 Hill MN, Gorzalka BB. Is there a role for the endocannabinoid system in the etiology and treatment of melancholic depression? *Behav Pharmacol*. 2005;16:333-52.
 - 76 Sanchis-Segura C, Cline BH, Marsicano G, Lutz B, Spanagel R. Reduced sensitivity to reward in CB1 knockout mice. *Psychopharmacology (Berl)*. 2004;176:223-32.
 - 77 Martin M, Ledent C, Parmentier M, Maldonado R, Valverde O. Involvement of CB1 cannabinoid receptors in emotional behaviour. *Psychopharmacology (Berl)*. 2002;159:379-87.
 - 78 Aso E, Ozaita A, Valdizan EM, Ledent C, Pazos A, Maldonado R, et al. BDNF impairment in the hippocampus is related to enhanced despair behavior in CB1 knockout mice. *J Neurochem*. 2008;105:565-72.
 - 79 Aso E, Renoir T, Mengod G, Ledent C, Hamon M, Maldonado R, et al. Lack of CB1 receptor activity impairs serotonergic negative feedback. *J Neurochem*. 2009;109:935-44.
 - 80 Mato S, Aso E, Castro E, Martin M, Valverde O, Maldonado R, et al. CB1 knockout mice display impaired functionality of 5-HT1A and 5-HT2A/C receptors. *J Neurochem*. 2007;103:2111-20.
 - 81 Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. *Nature*. 1977;266:730-2.
 - 82 Castagne V, Moser P, Roux S, Porsolt RD. Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. *Curr Protoc Neurosci*. 2011;Chapter 8:Unit 8.10A.
 - 83 Kang S, Kim HJ, Kim HJ, Shin SK, Choi SH, Lee MS, et al. Effects of reboxetine and citalopram pretreatment on changes in cocaine and amphetamine regulated transcript (CART) expression in rat brain induced by the forced swimming test. *Eur J Pharmacol*. 2010;647:110-6.