# Genetically Modified Mice as Tools to Understand the Neurobiological Substrates of Depression

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**Abstract**

The pathophysiological mechanisms underlying depression are still poorly understood. An initial hypothesis postulated to explain the substrates of depression was based on the involvement of monoaminergic systems. This early theory was proposed from different findings obtained using pharmacological tools and can explain the mechanism of action of the drugs currently used to treat depression. However, more recent studies have revealed that other neurobiological processes different from monoamines also participate in the substrates of depression. These mechanisms include the participation of several neuromodulatory systems, stress-related circuits and neuroplastic changes that could represent a direct substrate for these pathophysiological processes. The lack of selective pharmacological tools for several of these potential targets of depression represents an important limitation to study their potential involvement. In the last two decades, different lines of genetically modified mice have been generated with selective deletions in specific genes related to the control of emotional responses. This review summarizes the main findings that have been obtained with these novel genetic tools to clarify the neurobiological substrates of depression. A particular focus has been devoted to the advances obtained with mice deficient in specific components of the monoaminergic, opioid and cannabinoid system and those with mutations in elements of the hypothalamic-pituitary-adrenal axis.

**Key Words:** knockout mice, mutants, behavioural models, mood disorders, dopamine, serotonin, noradrenaline, endocannabinoids, opioids, HPA, GABA, glutamate, CRF.

## 1. Introduction

Depression is a chronic brain illness affecting over 350 million people worldwide [1], with women being twice as likely to suffer this disorder as men [2]. It comprises symptoms such as negative mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy, and poor concentration. Some important issues related to the treatment of depression are the large percentage of patients that do not respond to existing antidepressants (up to 30-40 %), the common appearance of residual symptoms and relapse [3]. Even though an enormous amount of research effort has been dedicated to the study of depression, finding new treatment strategies has been problematic. The elucidation of the pathophysiological mechanisms underlying depression would provide important advances to identify novel therapeutic targets for this mental disease. Indeed, although it is known that the two major precipitating factors of depression are stress and genetic predisposition [4], the aetiology of depression is still unknown. The fact that the most effective pharmacotherapy for depression at present is still based on monoamine reuptake inhibitors has reinforced the old "monoamine deficiency" hypothesis of depression. However, multiple studies have revealed the key role of other neurochemical systems in the neurobiological substrate underlying this disorder [5].

Early studies provided evidence for the involvement of monoamines (dopamine, noradrenaline and serotonin), and other neurotransmitters such as acetylcholine, gamma-aminobutyric acid, glutamate in mood and affective processing [3, 6]. More recent studies have also revealed the participation of some neuropeptides, such as the endogenous opioid system and lipid mediators, like the endocannabinoids in the etiopathogenesis of depression [7]. In addition, hypothalamic-pituitary-adrenal (HPA) axis dysregulation is considered to

play an important role in the pathophysiology of depression [8, 9]. Thus, stress-mediated activation of the HPA axis leads to functional and morphological damage to the hippocampus that results in altered forebrain processing of information, and specific symptoms of depression in vulnerable individuals [3]. Accordingly, certain subpopulations of depressed patients show small reductions in hippocampal volume with ventricular enlargement [10]. Functional neuroimaging studies have also involved other brain areas in the emotional alterations found in depressed patients, such as the amygdala and frontal cortex [11]. In fact, deep brain stimulation of the subgenual cingulate cortex or the nucleus accumbens produces antidepressant effects in treatment-resistant individuals [11, 12]. While these approaches are promising for understanding the neural circuits involved in depression, there is still a need for further studies in order to identify the precise mechanisms governing the numerous and complex symptoms observed in depression.

One of the most relevant recent research strategies developed to investigate the neurobiological mechanisms leading to this complex mental disorder, as well as the genetic factors conferring susceptibility for depression has been the use of genetically modified mice [13]. The use of these mutant mice combined with various animal models of depressive-like behaviour and pharmacological approaches have provided a great amount of information for understanding depression. In this review, we have summarized the main findings obtained by using genetically modified mice for understanding the neurobiological substrates involved in depression. Specifically, we will describe some of the genetic mouse models that have been used to evaluate the substrates of depression related to monoamine, cannabinoid, glutamate, gamma-aminobutyric acid (GABA) and opioid neurotransmission systems, as well as other models linked to neurogenesis and neuroplasticity.

## 2. Animal models in depression research

Modelling the symptoms of human depression in laboratory animals represents a great challenge in neuropsychiatric research due to the complex emotional features, and the difficulties to mimic the multidimensional aspects of the disease. Some symptoms of depression such as feelings of low self-worth and rumination are practically impossible to model in experimental animals, while others like learned helplessness/despair, failure to cope with stress, and anhedonia have been successfully modelled in several animal species [14]. Depression models should fulfil three main criteria: *face validity*, which refers to the need for the animal model to reproduce the symptoms observed in humans; *construct validity* is the requirement for similar causative factors or similar neurobiological mechanisms; and *predictive validity*, which is the necessity for the depressive symptoms to be reversed by available antidepressants [6, 15].

Acute paradigms of depressive-like behaviour are founded on the observation that animals exposed to inescapable stress fail to attempt to escape when given the option [16]. The two most widely used acute depression models based on this principle are the forced swim test [17], and the tail suspension test [18]. The forced swim test consists of placing the mice inside a cylinder full of water from which it cannot escape. The mouse will first swim energetically attempting to escape, and then it will adopt immobile postures along with short periods of swimming. In the tail suspension test, mice suspended by the tail causing a dynamic stress will also show alternate periods of trying to escape and immobility. The degree of immobility in both of these tests is thought to be a measure of behavioural despair, and it is sensitive to antidepressant treatment, although the sensitivity varies between tests [14]. A more sophisticated behavioural paradigm to evaluate this despair-like

behaviour is the learned helplessness model, where animals subjected to uncontrollable and inescapable exposure to electric shocks will either display increased escape latency or completely fail to escape [16].

Chronic models of depressive-like behaviour include repeated olfactory bulbectomy, restraint stress, unpredictable chronic mild stress, the resident–intruder model, and maternal separation. Bilateral olfactory bulbectomy induces many behavioural and neurochemical alterations similar to the ones displayed by depressed patients [19]. Although the construct and face validities of this model have been questioned, its reliability for predicting antidepressant-like effects after repeated administration has been shown in rats [20, 21]. The chronic mild stress model involves exposing animals to a variety of intermittent physical stresses for long periods of time [22]. This model induces brain and behavioural adaptations resembling those in depressed patients and has good predictive validity, although it suffers from poor reproducibility among different laboratories [23]. The resident intruder paradigm involves subjecting an intruder mouse to repeated bouts of social defeat by a resident aggressive mouse, which leads to the development of a submissive behaviour [24]. Following repeated defeat encounters, rodents exhibit reduced social interactions, decreased locomotion, anhedonia, increased stress-induced immobility, and alterations of the HPA axis function [6, 25]. The maternal deprivation model entails separating pups from their dam for varying lengths of time during the postnatal period [26, 27]. In rats, it has been shown that maternal separation leads to anhedonia, decreased social motivation, and anxiety-like behaviours and long-lasting physiological changes that can persist into adulthood [28]. However, this paradigm still needs to be characterized in mice as a valid model of depressive-like behaviour. Finally, several studies have demonstrated that chronic

corticosterone treatment produces both dose and time dependent depressive-like symptoms [29].

The behavioural outcomes most frequently used to evaluate the anhedonic manifestations induced by these chronic experimental procedures are obtained by using the sucrose preference test [30], the novelty-suppressed feeding test [31], or the intracranial self-stimulation procedure [32]. Some of these behavioural measures of anhedonia were found to be sensitive to reversal upon chronic antidepressant treatment [14, 23]. Nevertheless, further studies are needed to characterize the predictability and reproducibility of most of these models [23, 33].

**3. Genetic animal models to study the role of the monoaminergic system in depression**

The monoamine hypothesis of depression postulates that the underlying cause of depression is a deficiency in the levels of serotonin, noradrenaline and dopamine in the CNS (central nervous system). This theory is supported by the mechanism of action of antidepressants, which elevate monoamine levels and relieve the symptoms of depression. However, direct evidence for a primary monoaminergic dysfunction in depression has not been clearly established [34]. Thus, antidepressants increase brain monoamine levels quite rapidly, while the beneficial effects on the symptomatology occur after several weeks of treatment. Current data indicate that increased levels of monoamines levels following antidepressant treatment induce slow-onset neuroplastic changes in the hippocampus, including an increase in brain derived neurotrophic factor (BDNF)-mediated signalling [35] and upregulation of the cAMP response element-binding (CREB) in the hippocampus [36],

which may participate in the antidepressant action [5]. **Table 1 shows a summary of the genetic models used in depression research based on the monoaminergic system.**

### 3.1. The serotonergic system

In humans, deficits in serotonergic neurotransmission have been observed in subjects suffering from depression [37], and selective serotonin reuptake inhibitors (SSRIs) are an effective treatment for this illness [38]. Polymorphisms in the human serotonin transporter gene (*SLC6A4*) gene have been shown to influence mood states [39, 40]. Thus, clinical studies show that carriers of the *s* allele are at greater risk for developing affective disorders [41-43], and respond less to SSRI treatment [44]. Similarly, a C(-1019)G polymorphism in the promoter region of the serotonin 5-$HT_{1A}$ receptor gene has been identified in humans [45]. The G(-1019) allele leads to the over-expression of 5-$HT_{1A}$ receptor in the dorsal raphe nucleus, and the G/G genotype has been associated with depression, suicide, and poor response to SSRIs [45, 46].

#### *3.1.1. Serotonin transporter*

Knockout mice lacking the serotonin transporter (5-HTT) [47] display a robust anxiogenic-like phenotype [48], in the open field test [49], elevated plus maze [49, 50], light-dark box test [50], and novelty supressed feeding paradigm [51, 52]. In addition, exposure to mild foot-shock or predator odours during the postnatal period increases anxiety-like behaviour in 5-HTT knockout mice but not in wild-type controls [53, 54], and chronic mild stress lasting for 28 days increased the latency to start feeding in the novelty supressed feeding test [55]. In parallel, these knockout mice display a less aggressive phenotype than wild-

type mice in the resident intruder paradigm, with reduced social interaction and slower attack latency [56, 57], suggesting that deletion of the 5-HTT gene also leads to reduced emotional learning and increased social anxiety.

The depressive-like phenotype observed in 5-HTT knockout depends on the genetic background. Thus, knockout mice on the C57BL/6J background do not show differences in immobility time in the forced swim test nor the tail suspension test compared to wild-type mice, although repeated exposure to stress increases immobility time in these mice [58, 59]. Mice from the CD1 background are more prone to show behavioural despair (increased immobility in the forced swim test and tail suspension test), and anhedonia in the sucrose preference test [50]. Finally, mice from the 129S6 background display either increased or decreased immobility depending on the test used (forced swim test or tail suspension test, respectively) [51]. In addition, physiological alterations appear with time in 5-HTT knockout mice, which show obesity at about 3 months of age with an associated increase of insulin, leptin and hyperglycaemia [60].

Biochemical studies have provided evidence pointing to changes in the activity of the hypothalamic-pituitary-adrenal (HPA) axis in 5-HTT knockout mice. Thus at baseline, corticotropin releasing factor mRNA and plasma corticosterone levels are lower in knockout compared to wild-type mice [61, 62]. In agreement, corticotropin releasing factor-1 receptor density was increased and glucocorticoid receptor (GR) expression was reduced in the pituitary of knockout mice [62]. Consistent with the impaired regulation of the HPA axis, knockout mice show a greater increase in adenocorticotropic hormone and plasma adrenaline than wild-type controls under stressful conditions such as after exposure to the elevated plus maze or to restrain stress [61, 63, 64]. In contrast, no changes in brain protein

levels of BDNF have been observed in 5-HTT knockout mice compared to wild-type animals [65].

In vivo microdialysis studies show that extracellular serotonin levels are prominently increased in the substantia nigra, prefrontal cortex and striatum of 5-HTT knockout mice [66-68]. In contrast, these mice are characterized by a decrease in serotonin tissue content in the brainstem, frontal cortex, striatum and hippocampus [47], by an increase in the synthesis and turnover of serotonin [68], and by a decrease in serotonin clearance [69]. Moreover, knockout mice also show lower serotonin neuronal firing rate in the dorsal raphe nucleus [70]. As a result of this dysregulation in serotonin neurotransmission, several neuroadaptations can be found in the expression and function of serotonin receptors, including 5-$HT_{1A}$, 5-$HT_{1B}$, 5-$HT_{2A}$, 5-$HT_{2C}$ and 5-$HT_3$ receptors [66, 71-75]. Consequently, these compensatory changes in receptor function along with the morphological abnormalities [59] observed in constitutive 5-HTT knockout mice are conceivably the basis for their pro-anxiety phenotype, and for the fact that, contrary to what was expected from pharmacological studies with SSRIs in humans, 5-HTT knockout mice do not show antidepressant-like behaviour.

### *3.1.2. 5-$HT_{1A}$ receptor mutant mice*

5-$HT_{1A}$ receptors have been implicated in the mechanism of action of antidepressants. Hence, it has been hypothesized that the delayed therapeutic action of these compounds is due to slow-onset 5-$HT_{1A}$ receptor desensitization [76]. In agreement, the selective pharmacological blockade of 5-$HT_{1A}$ autoreceptors has been shown to increase the antidepressant-like effects of SSRIs by elevating synaptic serotonin levels [77]. The genetic

deletion of 5-$HT_{1A}$ receptors leads to increased anxiety-like responses such as avoidance of novel and fearful environments and to an antidepressive-like phenotype in the forced swim and the tail suspension test [78] [79, 80]. Moreover, acute fluoxetine administration decreased immobility in the tail suspension test in wild-type mice, but not in 5-$HT_{1A}$ receptor knockout mice [81], further supporting the involvement of these receptors in the antidepressant effects of SSRIs. Interestingly, the targeted rescue of 5-$HT_{1A}$ receptor in the hippocampus and cortex, but not in dorsal raphe nucleus reversed the anxiogenic-like phenotype in constitutive 5-$HT_{1A}$ knockout mice [82]. In addition, through time-locked conditional mutagenesis, it was demonstrated that the absence of 5-$HT_{1A}$ receptor in the hippocampus and cortex during the post-natal period, but not during adulthood leads to anxiety-like behaviour [82]. These data highlighted the relevance of forebrain of 5-$HT_{1A}$ heteroreceptors in selectively influencing anxiety-like behaviour during early developmental periods. However, more recent studies have also implicated 5-$HT_{1A}$ autoreceptors in the control of anxiety-like processes. Thus, mice with normal 5-$HT_{1A}$ autoreceptor expression in the dorsal raphe nucleus and conditional deletion of 5-$HT_{1A}$ heteroreceptors in the forebrain during the postnatal period exhibit an enhanced depressive-like, but not anxiety-like phenotype [83]. This phenotype was not present if heteroreceptors were deleted during adulthood, underscoring the role of forebrain serotonergic signalling during development in depressive-like behaviour. Likewise, conditional over-expression of 5-$HT_{1A}$ heteroreceptors in the forebrain leads to a depression-resistant phenotype in males but not in females [84]. Conversely, mice with selective suppression of 5-$HT_{1A}$ autoreceptors throughout life displayed increased anxiety-like behaviours as adults [83], whereas the over-expression of autoreceptors in the dorsal raphe nucleus during adulthood

gives rise to a depressive-like phenotype, and a lack of response to SSRI treatment, a phenotype similar to the one observed in patients with the 5-$HT_{1A}$ risk allele [85]. These studies suggest that the integrity of both 5-$HT_{1A}$ receptor auto- and hetero-receptors during development is necessary for normal processing of anxiety and depressive-like behaviours.

Tissue levels of serotonin and its major metabolite, 5-hydroxyindoleacetic acid in the cortex, hippocampus, striatum, amygdala and in the dorsal raphe nucleus were found to be normal in 5-$HT_{1A}$ receptor knockout mice [80], [86], [87]. Similarly, in vivo microdialysis studies have shown that basal extracellular levels of serotonin do not differ between 5-$HT_{1A}$ receptor knockout and wild-type controls in the dorsal raphe nucleus or the frontal cortex [88], [89], and pharmacological or electrical stimulation of serotonin release in slices of raphe, cortex and hippocampus were not modified in these mice [80] [90]. However, electrophysiological experiments report that 35% of raphe nucleus neurons have increased firing rate in 5-$HT_{1A}$ receptor knockout mice under basal conditions [90], and conditional mice with low expression of 5-$HT_{1A}$ autoreceptors showed an increase spontaneous neuronal firing in the dorsal raphe nucleus compared with mice with high expression of these receptors [85], suggesting a lack of normal auto-regulatory activity by 5-$HT_{1A}$ receptor in these genetically modified mice.

*3.1.3. 5-$HT_{1B}$ receptor mutant mice*

Constitutive 5-$HT_{1B}$ receptor knockout mice [91] show a sex-linked antidepressant-like phenotype. Thus, female 5-$HT_{1B}$ receptor knockout mice have reduced basal immobility in the tail suspension test and the forced swim test compared to male knockout and to female wild-type mice [92]. Ex vivo neurochemical studies revealed that under basal conditions,

serotonin release was increased in midbrain and hippocampus, but not in frontal cortex slices of these knockout mice [93]. However, in vivo microdialysis experiments showed no differences in basal extracellular levels of serotonin in the frontal cortex and ventral hippocampus in knockout mice [94]. Several compensatory changes have been observed in 5-$HT_{1B}$ receptor knockout mice that could explain these discrepancies between *ex vivo* and *in vivo* neurochemical results, including alterations in the response to psychostimulants [95], hypersensitivity of 5-$HT_{1A}$ somatodendritic receptors [96], and decreased functionality of 5-$HT_{2C}$ receptors [97].

*3.1.4. 5-HT4 receptor mutant mice*

Both anxiogenic and anxiolytic-like responses have been reported in $5HT_4$ receptor knockout mice depending on the experimental paradigm. Thus, 5-$HT_4$ receptor knockout mice generated from a 129/Sv background exhibit a decrease in crossings in the open-field test, suggesting anxiogenic-like behaviour; although corticosterone levels were not modified following the elevated plus maze test [98]. In addition, these mice show an attenuated response to stress-induced hypophagia, indicating an anxiolytic-like phenotype [98]. 5-$HT_4$ receptor knockout mice display a selective reduction in serotonin brain tissue concentration in the dorsal raphe nucleus, but not in the hypothalamus, nucleus accumbens, amygdala, hippocampus and striatum, and show decreased basal serotonin neuronal activity in the dorsal raphe nucleus [99]. Other changes observed in the dorsal raphe nucleus of 5-$HT_4$ receptor knockout mice include an increase in the levels of 5-HT transporters and in the sensitivity of 5-$HT_{1A}$ receptors [99].

*3.1.5. 5-HT7 receptor mutant mice*

The hypothesis that the 5-$HT_7$ receptor might be a good therapeutic target for depression comes from indirect neuroanatomical data showing the localization of these receptors in corticolimbic areas associated with affective processes [100]. In addition, these receptors were downregulated in the hypothalamus after chronic antidepressant treatments [100]. Subsequent studies in 5-$HT_7$ receptor knockout mice reported an antidepressant-like phenotype in these mutants, which exhibited lower immobility time in the forced swim and tail suspension tests as compared to wild-type controls [101, 102]. These results suggest that 5-$HT_7$ receptor antagonists may represent a new class of antidepressants with faster therapeutic action [103].

*3.1.6. Tryptophan-hydroxylase-2 mutant mice*

Constitutive knockout mice lacking tryptophan-hydroxylase-2 display a complete lack of serotonin synthesis, a marked reduction in serotonin concentrations in the brain, and developmental alterations such as reduced body weight and size have been observed [104-106]. These mice exhibit less time in the centre of the open field and increased marble-burying, pointing to an anxiogenic-like phenotype [105]. Maternal care in these mutant mice is also altered along with an increase in aggressive behaviour [106]. Time and tissue specific conditional tryptophan-hydroxylase-2 knockdown mice have been recently generated [107], and will provide an excellent tool to evaluate in future studies the precise implication of this enzyme in depression.

A knockin mouse has been recently generated carrying a single nucleotide polymorphism (SNP) with a substitution of an $Arg^{441}$ to $His^{441}$ at the mouse tryptophan-hydroxylase-2

gene, which is analogous to a SNP found in a cohort of Eastern US geriatric depressed patients [108]. These knockin mice display a marked depressive-like phenotype as measured in the tail suspension test, increased anxiety in the light-dark test and increased aggressive behaviour. A striking deficiency in serotonin tissue levels was observed in these mice, with decreases of up to 80%. In terms of compensatory changes, an increase in 5-$HT_{2A}$ receptor functionality, but no changes in presynaptic receptor function and 5-HTT expression were observed. These studies using tryptohan-hydroxylase-2 knockout and knockin mice support the data implicating a serotonin deficiency in anxiety and depression [109].

### 3.2. The Dopaminergic System

The role of dopamine in depression is underscored by converging lines of evidence showing that dopamine agonists such as pramipexole [110], and dopamine re-uptake inhibitors have antidepressant effects [111], while dopamine depletion and dopamine antagonists reduce motivation and induce a depressive state [112, 113]. In addition, depression is often associated with neuropsychiatric disorders characterized by dopaminergic depletion such as Parkinson's disease [114] and drug addiction [115]. Moreover, genetic studies have provided evidence for a link between polymorphisms in genes related to the dopaminergic system and the vulnerability for depression [116]. One SNP is the 9-repeat/10-repeat in the dopamine transporter (DAT) gene that may convey a risk for depression inducing higher DAT activity and hence, lower synaptic levels of dopamine [117]. The 48-bp variable number tandem repeat polymorphism in the dopamine D4 receptor gene is the only variant to show some association with depression, with the 2-

repeat allele increasing the risk for depression [118]. Interestingly, the highly prevalent 4-repeat allele of the D4 receptor gene [119] has been shown to protect against depression in the Japanese population [120]. Finally, two polymorphisms in the dopamine D2 receptor gene, the Taq1A and the C957T have been shown to increase the vulnerability for depression [116].

*3.2.1. Dopamine transporter*

The DAT is essential for the re-uptake of dopamine into the synapse, and thus for regulating dopaminergic neurotransmission. Psychostimulants like cocaine and amphetamine, and antidepressants such as nomifensin and bupropion block DAT and increase dopamine levels in corticolimbic structures. Consequently, DAT function has been implicated in a variety of neuropsychiatric disorders including drug addiction, schizophrenia, attention deficit disorder, and depression [121]. Hence, the use of mice lacking DAT [122] has provided important insights into the mechanisms involved in many of these disabling brain disorders.

DAT knockout mice show hyperlocomotion and stereotypic behaviour in a novel environment [122], and neophobia in the zero-maze test [123]. DAT knockout mice are also impaired in several types of cognitive function in the eight-arm radial maze [124], and the Morris water maze [125], and show deficits in sensorimotor gating in the prepulse inhibition test [126]. Interestingly, DAT knockout mice show an antidepressant-like phenotype in the forced swim test and the tail suspension test [127]. Moreover, DAT knockout mice show increased consumption of sucrose in the sucrose preference test indicating reduced anhedonia [127]. Neurochemical studies using *in vivo* microdialysis

with the no-net-flux technique revealed that basal extracellular levels of dopamine in the striatum of DAT knockout mice were markedly increased [128]. This hyperactivity of the dopaminergic system was confirmed in cyclic voltammetry experiments performed in striatal slices that show a prolongation in dopamine clearance rate [122, 128]. In contrast, the quantity of dopamine released after a single stimulation is lower than in wild-type mice, consistent with lower intracellular levels of striatal dopamine, and a decrease in tyrosine hydroxylase activity, the rate-limiting enzyme of dopamine biosynthesis [122, 129]. Paradoxically however, striatal dopamine synthesis rate is increased, possibly due to over activity of the few tyrosine hydroxylase molecules observed in knockout mice compared to wild-type controls [128, 129]. Several compensatory alterations have been observed in DAT knockout mice including a downregulation of postsynaptic D1 and D2 receptors [122], and reduced mRNA and functionality of presynaptic D2 receptors [130], while the density of D3 receptors is increased [131].

*3.2.2. Dopamine receptor mutant mice*

Several studies have investigated the role of dopamine receptors in depression and anxiety using genetically modified mice. Thus, $D_3$ receptors, which are autoreceptors regulating neuronal activity and dopamine release [132], mostly in limbic areas [133], are downregulated in depressed patients, and these alterations are reversed by antidepressant treatment [134]. Constitutive knockout mice lacking $D_3$ receptors show increased levels of extracellular dopamine in the nucleus accumbens [135, 136], and locomotor hyperactivity [137]. However, inconsistent results in terms of anxiety-like behaviour have been reported, with studies showing either an anxiolytic-like phenotype in knockout [138],

or no differences with respect to wild-type in the elevated plus maze [139]. Moreover, investigating the antidepressant-like phenotype in these mutants, studies have reported no differences between knockout and wild-type in terms of immobility time in the forced swim test, although knockout mice display a higher sensitivity to antidepressants in this paradigm [140]. In contrast, $D_4$ receptor knockout mice exhibit a clear anxiety-like phenotype as shown by less centre exploration in a novel open-field, reduced novel object exploration, and greater preference for the home-base in the emergence test than wild-type controls [141], but no data is yet available as to the depressive-like phenotype of these genetically modified mice.

### 3.3. The Noradrenergic System

Noradrenaline also plays an important role in depression. Thus, selected inhibitors of noradrenaline uptake such as reboxetine, induce antidepressant effects in humans [142]. In addition, it has been shown that patients with treatment-resistant depression or those suffering from “decreased positive affect” respond better to antidepressants that enhance noradrenergic neurotransmission such as selective noradrenaline re-uptake inhibitors than to SSRIs [142]. Genetic studies examining the role of human polymorphisms in different components of the noradrenergic system with depression have mostly evaluated their relationship with antidepressant response rather than to the basis of depression. Nonetheless, some evidence points out a linkage between variants at the noradrenaline transporter (NAT) and depression [143], and polymorphisms in $\alpha_{2A}$-adrenoreceptors with suicidal behaviour [144].

*3.3.1. Noradrenaline transporters*

NAT is involved in the re-uptake of excess noradrenaline released from noradrenergic terminals, and represents a substrate for the mechanism of action of different drug classes including antidepressants like reboxetine and desipramine, and atomoxetine used in the treatment of attention deficit disorder [145]. NAT was the first monoamine transporter to be cloned [146], although mice lacking NAT were not developed until the year 2000 [147]. NAT knockout mice display physiological alterations such as reduced body weight and lower basal body temperature, and show an antidepressive-like phenotype in both the forced swim and the tail suspension tests [147, 148] [149], and an increase in sucrose consumption [149]. In addition, these knockout mice are unaffected by chronic stress since immobility time is increased in the forced swim test, and sucrose preference is decreased in wild-type mice following restraint stress and social defeat, but not in NAT knockout mice [149]. Resilience to depressive-like behaviours in knockout mice is also observed in the resident intruder test, where they do not display social avoidance as wild-type control mice do [149]. In line with these findings, knockout mice show lower blood pressure and heart rate in response to chronic exposure to fearful environments [150].

Neurochemical studies indicated that noradrenaline concentrations in the cerebellum, prefrontal cortex and hippocampus homogenates of NAT knockout mice were decreased, while noradrenaline synthesis was increased with respect to wild-type littermates [147]. Interestingly, noradrenaline content was not changed in the striatum of knockout mice, suggesting that noradrenaline is taken-up by DAT in dopaminergic neurons [147]. Microdialysis experiments showed enhanced noradrenaline extracellular levels in the hippocampus and cerebellum of knockout mice [147, 151]. Accordingly, α1-

adrenoreceptors were downregulated in the spinal cord, hippocampus and cerebral cortex [147, 151, 152], while the density of α2-adrenoreceptors that are predominantly located at presynaptic neurons was increased in the spinal cord of knockout compared to wild-type mice [152]. Other autoradiographic studies have reported decreased binding of β-adrenoreceptors in the cortex of knockout mice [15].

### *3.3.2. Dopamine-ß-hydroxylase mutant mice*

Constitutive knockout mice lacking the dopamine beta-hydroxylase enzyme, involved in the synthesis of noradrenaline, have been generated [153]. Although these mice lack endogenous noradrenaline and adrenaline, they show no differences in immobility time with respect to heterozygous or wild-type controls when tested in the forced swim and the tail suspension tests under baseline conditions [154]. Interestingly, despite the fact that no changes in the expression of the NAT were observed in knockout mice, they were insensitive to the selective noradrenaline reuptake inhibitors, desipramine and reboxetine. These results indicated that the behavioural effects of these antidepressants are mostly mediated by endogenous noradrenaline [154].

### *3.3.3. Adrenergic $\alpha_{2A}$ and $\alpha_{2C}$ receptor mutant mice*

The constitutive deletion of $\alpha_{2A}$-adrenergic receptors induced a depressant-like phenotype in the forced swim test, and lack of sensitivity to the tricyclic antidepressant, imipramine [155]. In addition, knockout mice were also more sensitive to stress since they exhibited more anxiety-like behaviours in the open field and the light-dark box after saline injection

[155]. In contrast, the genetic deletion of $\alpha_{2C}$-adrenergic receptors produced antidepressant-like effects in the forced swim test, whereas the overexpression of this receptor induced the opposite phenotype [156]. In addition, plasma corticosterone levels assessed following different stressors were attenuated in $\alpha_{2C}$-knockout mice, while an increase was observed in $\alpha_{2C}$-overexpressing mice [156]. Brain tissue levels of L-DOPA, dopamine and noradrenaline were higher in $\alpha_{2A}$ -adrenergic and $\alpha_{2C}$-adrenergic knockout mice compared with wild-type, an indication of increased catecholamine biosynthesis [157]. Together, these data suggest that the development of subtype-selective $\alpha_{2A}$-adrenergic receptors agonists, and $\alpha_{2C}$-adrenergic receptors antagonists could have therapeutic relevance in the treatment of depression.

### 3.4. Other components of monoaminergic systems

*3.4.1. Monoamine oxydase A and B mutant mice*

Monoamine oxydase-B (MAO-B) knockout mice showed similar levels of dopamine, serotonin and noradrenaline in the brain with respect to wild-type mice, and no differences in the synthesis, storage, uptake, and release of dopamine between genotypes were observed [158, 159]. These mutant mice do exhibit an up-regulation of $D_2$-like receptors in the striatum, and a functional supersensitivity or $D_1$ receptors, but no differences in DAT binding [159]. Mice lacking the MAO-B gene do not show alterations in anxiety-like responses, but exhibit a depressive-like phenotype in the forced swim test [158]. On the contrary, MAO-A knockout mice show alterations in serotonin metabolism, and increased levels of serotonin and noradrenaline [160]. These mutant mice display a reduced anxiety- and antidepressant-like phenotype, showing more time in the centre of an open field and

less immobility time in the forced swim test [160]. However, it is still unknown if these behavioural traits are due to alterations in monoamine metabolism or to structural changes observed in the cerebral cortex of these mice [161].

*3.4.2. Vesicular monoamine transporter (VMAT) mutant mice*

The VMAT-2, mostly localized within the CNS [162], is a protein that transports monoamines from the cytoplasm into synaptic vesicles [163]. Since treatment with reserpine, an irreversible inhibitor of the VMAT, leads to depletion of vesicular monoamine stores and induces depressive-like symptoms in humans [164], it has been investigated whether VMAT-2 mutant mice would show symptoms of depression. However, the constitutive deletion of the VMAT-2 gene in homozygous mice is lethal [165]. Thus, heterozygous mice have been generated, which show a depressive-like phenotype in the forced swim and tail suspension tests with respect to wild-type controls, and these changes were reversed by antidepressants. They also exhibit less preference for low concentrations of sucrose, suggesting an anhedonic effect of this gene deletion [166]. These heterozygous mice did not show anxiety-like behaviours or modifications in basal concentrations of plasma corticosterone, indicating that VMAT-2 selectively modulates depressive-like responses [166].

## 4. Genetic animal models to study the role of the endogenous opioid system in depression

Multiple studies have suggested that the endogenous opioid system plays a key role in the control of emotional behaviour and the etiopathogenesis of anxiety and depressive disorders [7]. This opioid system consists of different families of opioid peptides acting on three types of opioid receptors, μ- (MOP), δ- (DOP) and κ-opioid receptor (KOP). MOP, DOP and KOP are $G_{i/o}$-coupled receptors that have been cloned and characterized at the biochemical, pharmacological and molecular level [167]. Three families of endogenous opioid peptides, the derivatives from proopiomelanocortin, proenkephalin and prodynorphin, have been also identified [168]. Proopiomelanocortin is the precursor of the endogenous opioid peptide beta-endorphin, which shows high affinity for MOP and DOP; proenkephalin is the main precursor of the endogenous enkephalins, which presents higher affinity for DOP than MOP, and the main opioid peptides derived from prodynorphin are the dynorphins that have preferential affinity for KOP [167]. The neurons containing endogenous opioid peptides and receptors are largely distributed within the CNS, but these peptides and receptors are also present in several peripheral tissues. This wide distribution is related to the important role that the opioid system plays in the control of several physiological responses including emotional behaviour [168]. The generation of several lines of knockout mice deficient in the different components of the endogenous opioid system [169] has provided novel tools to clarify the exact involvement of the opioid system in emotional disorders. **Table 2 shows a summary of the genetic models used in depression research based on the opioid system.**

Constitutive deletion of the MOP gene leads to an anxiolytic- and antidepressive-like phenotype in several behavioural models, which suggests that the endogenous MOP tone would exert a negative effect on anxiety and depression [170]. In agreement, corticosterone responses to stress were reduced in MOP knockouts revealing the facilitatory role of MOP in emotional responses to stress [171]. In spite of these emotional alterations, the anxiolytic-like effects of benzodiazepines were unaltered [172] or even enhanced [173] in MOP knockout mice, which could be related to an upregulation of the benzodiazepine receptor system in these mutants [174]. MOP knockouts also present an enhancement in 5-$HT_{1A}$ receptor levels and reduction of M1 muscarinic receptors in several brain areas related to emotional behaviour control. Both neurochemical changes could participate in the emotional phenotype revealed in these mutants [175]. These MOR knockouts also show a decreased response to social defeat stress and a decreased expression of BDNF, suggesting that MOR could be involved in the behavioural consequences of social stress and consequent dysregulation of BDNF expression [176].

Studies in knockout mice have revealed that DOP plays an opposite role to MOP in the control of emotional responses. Thus, DOP knockout mice present the opposite emotional phenotype to MOP knockouts, i.e., anxiogenic- and depressive-like responses were revealed in different behavioural paradigms in mice lacking DOP [170]. In agreement, the anxiolytic [177]- and antidepressant-like responses induced by the inhibition of the enzymes involved in endogenous enkephalins degradation were not modified in MOP knockout mice, suggesting an involvement of DOP in these emotional responses of endogenous enkephalins [178]. Therefore, an endogenous enkephalin tone acting on DOP would promote anxiolytic and antidepressant effects.

The endogenous opioid tone on KOP does not seem to play a major role in emotional control. Indeed, constitutive KOP knockout mice show similar locomotor responses and anxiety-like behaviour in the elevated plus maze and zero maze than wild-type mice [179].

Constitutive knockout mice lacking the pre-proenkephalin gene displayed increased anxiety-like responses in different behavioural paradigms including the zero-maze, light-dark box and startle-reactivity test, but not in the social interaction test. These results were obtained in knockout male mice in a C57BL/6J background [177, 180], and are in agreement with the proposed anxiolytic effects promoted by the endogenous enkephalin tone [170]. Similar results were obtained in the open-field, light dark box and fear conditioning test in female pre-proenkephalin knockout mice in a mixed C57BL/6J and 129 strains [181]. However, this phenotype was dependent on the genetic background since pre-proenkephalin knockout on a DBA/2J background only present the anxiogenic-like phenotype in the zero-maze and social interaction test. In contrast to these results, the selective down-regulation of the pre-proenkephalin gene in the central nucleus of the amygdala by local microinjection of a lentiviral vector decreased anxiety in the elevated plus-maze and fear conditioning paradigm in rats [182]. The use of different experimental approaches and animal species could influence this recent contradictory result. Pre-proenkephalin knockout mice did not exhibit any behavioural change in the forced swim [183, 184] and tail suspension tests [183], which is in contrast with the anxiolytic- and antidepressant-like responses induced by endogenous enkephalins [178].

Several studies have reported increased anxiety-like responses in knockout mice with a constitutive deletion of the prodynorphin gene in different behavioural models including the elevated plus maze, zero maze, light dark box, social interaction test [177, 185], and the

fear conditioning and delayed extinction in contextual conditioning paradigms [186]. These knockout mice showed reduced neuronal activity in key limbic structures during extinction, suggesting that dynorphins play an important role in fear extinction. In agreement, humans carrying a functionally relevant polymorphism in the dynorphin gene also showed reduced fear extinction [186]. However, the opposite phenotype has also been reported by other researchers. Thus, an anxiolytic-like phenotype was revealed in the open-field, elevated plus maze and light-dark test in prodynorphin knockout mice that was fully reversed by the systemic administration of the selective KOP agonist U-50488H [187]. A more recent result also reported an anxiolytic-like phenotype in the open-field, elevated plus maze and light-dark test in these prodynorphin knockouts [188]. In agreement, the anxiogenic-like responses induced in the elevated plus maze by central administration of corticotrophin releasing factor were suppressed in prodynorphin knockout mice [189]. A depressive-like phenotype was reported in these prodynorphin knockout mice in the tail suspension test, whereas no changes were revealed in the forced swim model [188]. Further studies would be required to understand these apparent contradictory results that have been found in prodynorphin knockout mice, mainly with regards to the anxiety phenotype, which are also in contrast with the lack of modification anxiety-related responses in KOP knockout mice [179].

Constitutive knockout mice lacking beta-endorphin show decreased anxiety-like responses in the zero maze [177]. In agreement, the anxiogenic-like effects induced by nicotine administration were attenuated [190], and the anxiolytic responses induced by ethanol were enhanced in these knockout mice [191]. However, contradictory results have also been found in the emotional responses of these knockout mice. Indeed, enhanced anxiety-like

responses have been reported in these beta-endorphin knockout mice in the elevated plus maze and light-dark model [191].

## 5. Genetic animal models to study the role of the endocannabinoid system in depression

The endocannabinoid system has emerged as a major neuromodulatory system involved in the control of mood and emotional behaviour [192]. The components of the endocannabinoid system include at least two well-characterized cannabinoid $G_{i/o}$-coupled receptors; $CB_1$ and $CB_2$ cannabinoid receptors, their endogenous ligands and the enzymes related to the synthesis and degradation of these endocannabinoids [193]. The $CB_1$ receptor, is highly abundant in the brain, and is expressed in all of the main brain structures involved in emotion-related behaviours, such as the hypothalamus, amygdala, limbic system, habenula, cortex and hippocampus [194]. $CB_2$ receptors are predominantly located in peripheral and brain immune cells, and a low expression has been reported in neurons in specific brain areas [195-197]. Findings obtained in mice genetically modified for different endocannabinoid system components demonstrate the involvement in the control of the HPA axis, neuroplasticity, reward pathways and the modulation of the monoaminergic system, suggesting a possible role of the endocannabinoid system in the aetiology of depression [198, 199]. Most of these results were obtained in $CB_1$ knockout mice, whose phenotype exhibit several similarities with clinical features of depressive disorders and has been proposed as an animal model of depressive-like behaviour [200]. Thus, mice lacking $CB_1$ receptor show an anxiogenic-like phenotype and increased sensitivity to exhibit anhedonia after chronic unpredictable mild stress exposure [201], increased passive stress-

coping behaviours [202, 203] and an enhanced vulnerability to behavioural inhibition after repeated or acute severe stress [204]. Moreover, mice lacking $CB_1$ receptors do not respond to benzodiazepines [205, 206], suggesting that these receptors play a pivotal role in the mechanism of action of anxiolytic drugs probably as a consequence of their involvement in the control of GABAergic responses [207]. $CB_1$ knockout mice also exhibit other manifestations of an anhedonic state, as revealed by the reduced responsiveness to natural rewarding stimuli [208] and to all prototypical drugs of abuse [209]. These observations are in agreement with the well-known activation of mesolimbic dopaminergic pathway by the stimulation of $CB_1$ receptor, which produces rewarding effects and facilitates the motivation to seek for rewarding stimuli [209].

The enhanced sensitivity of $CB_1$ knockout mice to stress-induced behavioural responses is associated to increased corticosterone serum levels, indicating a hyperactivity of the HPA axis in response to stress [203, 205, 210]. Endocannabinoids inhibit the HPA axis through $CB_1$ receptor activation [211, 212], which suggests an impairment in the negative feedback regulation of the HPA axis in the absence of $CB_1$ receptor [213]. Sustained elevations of glucocorticoids promoted by stress produce detrimental effects in synaptic plasticity, dendritic morphology [214] and neurogenesis [215], even leading to neuronal death in the hippocampus [216]. These hippocampal changes in synaptic connectivity induced by stress might reduce the inhibitory control that this structure exerts on the HPA axis, leading to a positive feedback process with pathological consequences that could induce hippocampal volume reduction and cognitive impairments, as described for depressive patients [217, 218]. Therefore, the hyperactivity of the HPA axis in $CB_1$ knockout mice could be related to the plasticity impairment that has been also described in these mutants, which mimics

that observed in depressive patients [35]. In agreement, mice lacking $CB_1$ receptors exhibit defective neurogenesis in the hippocampus [219], increased susceptibility to neurotoxic insults [220] and impaired release of trophic factors [202, 203, 221]. BDNF seems to be a key mediator in the $CB_1$ receptor-dependent mechanisms of neuroprotection [221] and to be a specific target for the impairment in plasticity induced by stress in $CB_1$ knockout mice. Indeed, an impairment in hippocampal BDNF has been reported in $CB_1$ mutants related to the altered response to stress, as revealed by the complete abolition of their depressive-like behaviour by the intra-hippocampal administration of BDNF [203].

The role of the endocannabinoid system in regulating emotional behaviour could also be explained by the modulation of serotonin release exerted by $CB_1$ receptors [222-224]. Thus, mice lacking $CB_1$ receptor exhibited increased serotonin extracellular levels in the prefrontal cortex, which could be due to the alteration of several components involved in serotonin negative feedback [224]. Thus, $CB_1$ knockout mice showed decreased 5-HTT binding site density in the frontal cortex and the hippocampus, indicating impairment in the serotonin reuptake from the synaptic terminal [225]. Moreover, animals lacking the $CB_1$ receptor exhibited a functional desensitization of 5-$HT_{1A}$ autoreceptors and a reduction in the expression of the 5-$HT_{2C}$ receptors in dorsal raphe nuclei, leading to a reduction in the inhibitory effect on serotonin neurons [224]. These findings are apparently in contrast to the classical monoaminergic hypothesis of depression considering the depressive-like phenotype of $CB_1$ mutants. However, the alterations on serotonin neurotransmission previously mentioned could be a compensatory mechanism for counteracting the stress-induced emotional impairments in mice lacking $CB_1$ receptor, since similar alterations have been described after antidepressant treatments [226, 227]. As a result, the deregulation of

glucocorticoid release and the deficiency of neurotrophic mechanisms could prevail upon the alterations on serotonin neurotransmission, leading to the depressive-like phenotype exhibited by $CB_1$ mutant mice [228]. However, the possibility that reduced 5-HTT levels and 5-$HT_{1A}$ desensitization could be the primary mechanism responsible for the depressive-like phenotype exhibited by $CB_1$ knockout mice cannot be rule out. Thus, an impaired 5-HTT function [229, 230] and a 5-$HT_{1A}$ desensitization [231-233] have been associated to an increased vulnerability to depressive symptoms in animal models as well as in humans.

Additional molecular pathways contributing to the depressive-like phenotype and the increased vulnerability to deleterious effects of stress in mice lacking $CB_1$ receptor have been identified by studying gene expression profile of mutants at basal conditions and after repeated stress exposure. Several genes coding for neurotransmitter receptors, neurotrophic factors, neuropeptides and hormones receptors were differentially expressed in $CB_1$ knockout mice [234]. These microarray results suggested that the lack of $CB_1$ receptor induced a gene expression pattern mainly in the raphe nuclei at basal conditions that mimics the consequences of stress exposure, which could confer to mutants the increased sensitivity to stress.

In contrast to $CB_1$ receptor, little is still known about the role of $CB_2$ receptors in the control of emotional behaviour. The genetic overexpression of $CB_2$ receptors in mice produced an endophenotype resistant to stress-induced depressive-like behaviours as well as to anxiogenic stimuli [196, 235]. These reports suggest that the involvement of $CB_2$ receptor in the emotional responses is related to its role in the regulation of BDNF expression, revealed by the increased hippocampal levels of this neurotrophic factor in mice overexpressing $CB_2$ receptor [196]. Moreover, mice overexpressing $CB_2$ receptor

showed an alteration of corticotropin releasing factor and proopiomelanocortin levels, which reveals the role of CB2 receptors in the controls of the HPA axis. These responses, together with the regulation exerted by $CB_2$ receptors on the GABAergic system, as revealed by the impaired action of anxiolytic drugs and the differential expression of GABA receptor subunits in those mutant mice [235], could also be relevant for the control of emotional behaviour.

The fatty acid amide hydrolase (FAAH) enzyme is another component of the endocannabinoid system involved in the control of emotional responses. This enzyme participates in the enzymatic degradation of anandamide [236], one of the two main endocannabinoids involved in the modulation of emotional behaviour through the activation of $CB_1$ receptor. Thus, knockout mice lacking FAAH exhibited an antidepressant-like phenotype when exposed to the forced swim and tail suspension tests, as well as anxiolytic-like responses in the open field and elevated plus maze [237, 238]. Moreover, FAAH mutants exhibited enhanced brain serotonergic transmission in dorsal raphe, prefrontal cortex and hippocampus [237, 238]. This antidepressant and anxiolytic-like phenotype of FAAH knockout mice was reversed by the administration of the $CB_1$ receptor antagonist rimonabant, demonstrating that this emotional phenotype was due to increased anandamide levels acting on $CB_1$ receptors [237, 238]. These findings are in agreement with the depressive- and anxiogenic-like phenotype of $CB_1$ knockout mice, and confirm the crucial role of $CB_1$ receptors in the modulation of emotional behaviour. **Table 2 shows a summary of the genetic models used in depression research based on the endocannabinoid system.**

## 6. Genetic animal models targeting the hypothalamic–pituitary–adrenal (HPA) axis

Stressful life events play a crucial role in the aetiology of depression [239], and the HPA axis controls the influence of stress on this disorder. The repeated exposure to stressful situations is counteracted by neurobiological systems that maintain behavioural homeostasis in most individuals, and this equilibrium can be disrupted leading to emotional disorders [240, 241]. Corticotropin releasing hormone (CRH) and arginine–vasopressin (AVP) in the hypothalamus control the activity of the HPA axis on stress-related responses. Both hormones activate the secretion of the adrenocorticotropic hormone from the pituitary. Adenocorticotropic hormone stimulates corticoid release (cortisol in humans and corticosterone in rodents) from the adrenal cortex, which interact with GR with low-affinity and mineralocorticoid receptors with high-affinity in multiple central and peripheral targets [242]. Glucocorticoids exert a negative feedback control in the hypothalamus on CRH and AVP secretion through the activation of GR expressed, among others, by hippocampal and paraventricular nucleus neurons. Glucocorticoids also lead to a decrease in the synthesis of serotonin by enhancing the conversion of its precursor tryptophan into a different molecule, kynurenine [243].

The corticosteroid hypothesis of depression is based on dysfunctions of the HPA axis consisting on the persistent hyperactivity of HPA through a deficit in the negative feedback regulation [244, 245]. This dysfunction appears when the intensity or frequency of stressors exceeds an individual-specific threshold [246, 247]. Usually, individuals show habituation to chronic stress due to decreased HPA axis responses, although the mechanism underlying this habituation has not been fully elucidated. This habituation is considered a mechanism of plasticity to protect the organism from the potentially damaging effects of

hypercorticosteroidism [248]. The over activity of HPA axis in depressed patients is not a simple consequence of depression, but constitutes an important risk factor predisposing vulnerable individuals [240, 241, 244].

Epigenetic changes programme the genome without altering the nucleotide sequence mostly through covalent modifications of DNA such as methylation [242]. Certain environmental events, mainly those early in life may cause variations in epigenetic programming serving as an adaptive response of the genome to future life events [249] However, when the later environment events were not predicted, the previous epigenetic reprogramming would result in maladaptations that contribute to the risk of developing emotional disorders [242, 250]. Evidence is growing to support the hypothesis that adverse early environments underlie vulnerability to emotional disorders through epigenetic influence [251]. Thus, maternal factors [252], prenatal stress and early-life stress [253, 254] can affect long-term behavioural and neuroendocrine consequences in rodents as revealed by multiple cross-fostering studies in different strains of mice and rats [255].

The generation of genetically engineered mice with selective mutations in particular components of the HPA axis has improved the knowledge of their role in the control of emotional responses.

A mouse model with impaired GR function was first generated with the insertion of a DNA stretch of the GR into the mouse genome in reverse direction under control of a neuron specific promoter [256]. These GR antisense transgenic mice showed several behavioural and neuroendocrine manifestations similar to those revealed by depressive states, which include reduced negative feedback sensitivity to corticoids and enhanced stress hormone response following pharmacological or emotional stimuli [257-259]. The constitutive GR

null mutant mice were not viable due to severely impaired lung development and respiratory failure [260]. GR-heterozygous knockout mice with a 50% GR gene dose reduction were successfully generated and revealed enhanced learned helplessness after stress exposure and reduced feedback regulation of HPA system [261]. Conditional mutant mice were also generated with specific deletion of GR in the hippocampus, hypothalamus and paraventricular nucleus using the Cre/loxP-recombination system [262]. These mice showed impaired HPA axis regulation, resulting in increased glucocorticoid levels that lead to a Cushing-like syndrome and impaired behavioural response to stress [262]. A different line of conditional GR knockout mice with time-dependent, forebrain-specific disruption of these receptors showed the relevance of GR expression in the onset of depression. These mice developed impaired negative feedback regulation, hyperactivity of HPA axis and increased depression-like behaviour [263, 264]. Another line of conditional GR knockout mice has been generated exhibiting impaired dimerization and glucocorticoid response-element transactivation ($GR^{dim}$), but intact cross-talk with other transcription factors, such as transrepression of AP-1-driven genes. The homozygous $GR^{dim/dim}$ mice are viable and show impairments in several physiological functions of the GR, suggesting that activities of these receptors dependent on DNA binding are not essential for survival [265]. Transgenic mice with a conditional GR overexpression specifically in forebrain have been proposed to be a model for increased anxiety-related behaviour not secondary to altered levels of stress hormones [266]. These mice displayed increased anxiety and depressive-like behaviours, and a consistently wider range of reactivity in both positive and negative emotionality tests that may reflect a bipolar-like disorder [266].

Clinical evidence reveals that CRH is elevated in depressed humans. Different mutants with alterations in the CRH system have been generated. CRH knockout mice present glucocorticoid deficiency and HPA hyporeactivity to stress [267]. In contrast, mice overexpressing CRH show increased corticosterone levels, development of a Cushing-like syndrome and no alteration of anxiety-related behaviour [268-270]. Two different CRH receptors have been identified; CRH-1 and CRH-2. CRH has a higher affinity for CRHR1 than for CRHR2, while urocortin, a CRH-related peptide, is thought to be the endogenous ligand for CRHR2. CRHR1 null mutant mice showed a severe impairment in the stress response of the HPA system. Their basal plasma levels of adenocorticotropic hormone were normal, suggesting the presence of compensatory mechanisms, but social defeat increased plasma adenocorticotropic hormone in these mutants, whereas forced swim stress did not modify these levels [271]. Conditional mutant mice lacking CRHR1 in anterior forebrain and limbic brain structures, but not in the pituitary are also available. These mutants were hypersensitive to stress, and corticotropin and corticosterone levels remained elevated after stress showing that limbic CRHR1 modulates anxiety-related behaviour independently of HPA axis function [272]. On the other hand, constitutive CRHR2 knockout mice are hypersensitive to stress and display increased anxiety-like behaviour. These mutant mice have normal basal feeding, but decreased food intake following food deprivation [273, 274]. To further elucidate the roles of both CRH receptors and determine their interaction in behaviours, mice deficient in both CRHR1 and CRHR2 were generated [275, 276]. The female double-mutant mice displayed anxiolytic-like behaviour, but the male double-mutant showed enhanced anxiety-like behaviour compared with the females. Double-mutant mice also displayed an even greater impairment of their HPA axis response to stress

than that of the CRHR1-mutant mice. CRH mRNA levels were elevated in CRHR1- and double-mutant mice, and urocortin and vasopressin mRNA levels were increased in CRHR2- and double-mutant mice. These results indicate that both CRHR1 and CRHR2 have critical roles in gene regulation and the maintenance of homeostasis in response to stress [276]. **Table 2 shows a summary of the genetic models used in depression research based on the HPA axis.**

## 7. Other genetic animal models to evaluate the substrates of depression

CREB protein is a critical integrator of neural plasticity sensitive to a large variety of environmental and pharmacological stimuli, including antidepressant compounds. The functional significance of this transcription factor in the learned helplessness paradigm was determined in inducible transgenic lines of mice that express either CREB or a dominant-negative mutant of CREB (mCREB) in forebrain regions. Blockade of CREB by overexpression of mCREB in transgenic mice or by viral expression of mCREB in the nucleus accumbens produces an antidepressant-like effect, whereas overexpression of CREB by itself results in the opposite phenotype [277]. In addition, mCREB expression decreased the levels of prodynorphin in the nucleus accumbens, and dynorphin antagonism in this brain structure produced an antidepressant-like effect similar to that observed after blockade of CREB. These results demonstrate the role of nucleus accumbens CREB-dynorphin in learned helplessness responses, and suggest that this signaling cascade contributes to depressive symptoms [277]. Constitutive CREB deletion in mice increases stress resilience and enhances antidepressant effects in the forced swimming and tail suspension test [278] supporting that CREB inactivation contributes to an antidepressant-

like behaviour, in agreement with the biochemical and molecular studies indicating that antidepressant drugs upregulate CREB [279]. Therefore, the genetic blockade of CREB activity produced similar antidepressant-like effects in both mice and rats **(see Table 3 for summary).**

GABA-A receptors characterized by the presence of the α3 subunit are the major GABA-A receptor subtype expressed in brain monoaminergic nuclei, and are in a unique position to regulate monoaminergic functions. Knockout mice with a targeted deletion of this α3 subunit did not exhibit any gross change of anxiety-like behaviour, but show an antidepressant-like phenotype in the forced swim test [280]. The metabotropic GABA-B receptors predominantly function as heterodimers of GABA-B1 and GABA-B2 subunits, although GABA-B1 can also form functional receptors in the absence of GABA-B2. Mice lacking the GABA-B1 subunit have important changes in anxiety- and depressive-like responses [281]. These mice did not show the effects of benzodiazepines in anxiety-like responses suggesting that this deletion alters GABA-A receptor function *in vivo* [281]. Both GABA-B1 and GABA-B2-deficient mice were more anxious than wild type in the light-dark box paradigm. In contrast, these mice exhibited an antidepressant-like phenotype in the forced swim test. Therefore, heterodimeric GABA-B1,2 receptors are required for the normal regulation of emotional behaviour [282].

The GABA transporter subtype 1 (GAT1), which transports extracellular GABA into presynaptic neurons, plays an important regulatory role in the function of GABAergic systems. GAT1 knockout mice showed lower levels of basal corticosterone, depressive- and anxiety-like behaviours in comparison to wild-type mice in different behavioural models, and are insensitive to the effect of selected antidepressants and anxiolytics.

Therefore, the absence of GAT1 affects emotional behaviour in mice by enhancing the GABAergic activity, and modifying serotonergic and HPA axis activity [283]. The 65-kDa isoform of glutamic acid decarboxylase (GAD65) plays an essential role for GABA synthesis in the CNS. GAD65 knockout mice showed an increase of GABA levels in the amygdala, hypothalamus and parietal cortex. These mutants displayed increased anxiety-like behaviour, reduced inter-male aggression, and an antidepressant-like phenotype in the forced-swim test. Therefore, GAD65-mediated GABA synthesis may be crucially involved in the control of emotional behaviour. Aggressiveness and possibly other social behaviours may be especially prone to regulation through GAD65-mediated GABA synthesis [284]. **Table 2 shows a summary of the genetic models used in depression research based on the GABAergic system.**

Glutamatergic neurotransmission has been strongly implicated in the pathophysiology of affective disorders, such as major depression and anxiety. This neurotransmission is mediated by several metabotropic and ionotropic receptor subfamilies including multiple NMDA receptor subunits; NR1 and NR2 (NR2A-2D) [285]. Little is currently known about the specific role of NMDA subunits in emotional behaviour due to a lack of subunit-specific ligands. A mouse gene-targeting approach has examined the role of the NR2A subunit in anxiety- and depressive-related behaviours. NR2A knockout mice showed antidepressant-like profile in the forced swim and tail suspension tests and decreased anxiety-like behaviour across multiple tests. These results support a role for the NR2A subunit in the modulation of emotional behaviours in rodents and provide insight into the role of glutamate in the pathophysiology and treatment of mood and anxiety disorders [285]. The role of group III metabotropic glutamate receptors (mGluR4, mGluR6, mGluR7,

mGluR8) in emotional disorders has not been investigated in depth due to the lack of specific pharmacological tools. Mice with a targeted deletion of the gene for mGluR7 showed antidepressant-like phenotype in both the forced swim and the tail suspension tests, and anxiolytic activity in different behavioural tests suggesting that mGluR7 may play a pivotal role in emotional behaviour. Therefore, drugs acting at mGluR7 may provide novel treatments for depression and anxiety [286]. Antidepressant-like effects of metabotropic glutamate mGluR5 antagonists have been reported [287]. In agreement, mGluR5 knockout mice showed an antidepressant-like phenotype in the forced swim test, and the antidepressant effects of the mGluR5 antagonist, 2-methyl-6-(phenylethynyl)-pyridine (MPEP) were not observed in these knockout mice, whereas the tricyclic antidepressant imipramine remained active in these mutants. These findings substantiate the hypothesis that mGluR5 antagonism is associated with antidepressant-like effects [287]. **Table 2 shows a summary of the genetic models used in depression research based on the glutamatergic system.**

Members of the muscarinic acetylcholine receptor family are thought to participate in emotional behaviour control. Thus M1 muscarinic receptor deficient mice showed antidepressant-like effects in the forced swim test an anxiolytic-like phenotype suggesting that M1 receptor knockout mice are hyperactive under stressful conditions [288]. Neuropeptide Y (NPY) has also been involved in the pathophysiology of depression and anxiety, although it is unknown which of the five cloned NPY receptors mediate these functions. Y2 receptor knockout mice showed anxiolytic-like phenotype in several behavioural paradigms and antidepressant-like effects in the forced swim test. Considering the presynaptic location of these receptors, their deletion may result in enhanced release of

NPY, GABA and/or glutamate in brain areas linked to anxiety control and stress-related behaviour such as the amygdala. These results suggest that Y2 receptors play an important role in the generation of anxiety- and stress-related behaviours in mice [289]. Y4 receptor knockout mice also exhibit an anxiolytic phenotype in several behavioural models, and reduced depression-like behaviour in the forced swim and tail suspension tests similar to Y2 receptor knockout mice. This anxiolytic and antidepressant-like phenotype was enhanced in Y2/Y4 double knockout mice compared with single Y2 and Y4 knockout mice [290] **(see Table 3 for summary).**

The potential role of different adenosine receptors has also been investigated using genetically modified mice (**see Table 2 for summary**). Adenosine A3 receptor knockout mice showed a depressive-like phenotype in the forced swim and tail suspension tests [291]. In contrast, the genetic disruption of the adenosine A2 receptor produced antidepressant-like effects [292].

Neurotrophic systems play a key role in the pathogenesis of depression [293]. This disorder has been frequently linked to impairments in neural resilience, with altered expression of BDNF, its high affinity receptor, tyrosine kinase-B, and the transcription factor, CREB [294]. The neurotrophic hypothesis of depression postulates that reduced activity of the CREB-BDNF-tyrosine kinase-B pathway causes a depressive state. In agreement, the activation of this pathway has been involved in the molecular mechanisms of antidepressant therapy [295-298]. To clarify the implication of this system in depression several mutant mouse lines have been developed consisting in heterozygous, conditional or forebrain-specific knockouts of BDNF since the complete deletion of BDNF is lethal [299]. Heterozygous mutation resembles the situation in humans because only 50% of BDNF is

deleted, although these mutant mice do not exhibit a clear depressive-like phenotype [300-302] and only aggressive and anxiety-like behaviour has been revealed [303]. Conditional mutant mice lacking BDNF expression in forebrain are prone to develop anhedonia [304]. Finally the involvement of BDNF on depression has been demonstrated by the antidepressive phenotype shown by mutant mice overexpressing BDNF [305] (**see Table 3 for summary)**.

## 8. Concluding remarks

The use of genetically modified mice has provided important advances in the understanding of the neurobiological mechanisms underlying emotional disorders. Most of the studies modelling depression in mice have used the behavioural despair paradigms, while other more sophisticated models such us the chronic mild stress and social defeat paradigm are less commonly used. Nonetheless, these genetically engineering mice have been particularly useful to elucidate the participation on these disorders of several targets that are devoid of pharmacological tools with selective affinity. These genetic tools have provided new insights to understand the role of the monoaminergic systems in the substrates of depression. Indeed, the lack of an antidepressant-like phenotype in mice with a mutation that inactivates 5-HTT, and that therefore mimics the mechanism of action of the antidepressants acting on this target, suggests that other neurobiological mechanisms should be involved in the therapeutic effects of these compounds. Interestingly, a clear antidepressant phenotype was revealed in mice with a deletion of specific serotonergic receptors, such as 5-HT1A, 5-HT1B and 5-HT7, which underlines the interest of targeting a more specific component of the serotonergic system than 5-HTT to generate novel

antidepressant compounds. In contrast to the results obtained in 5-HTT knockout mice, the genetic deletion of the other main monoamine transporter systems, DAT and NAT, resulted in a clear antidepressant phenotype. These two monoamine transporter systems could therefore represent interesting targets to explain the mechanism of action of the antidepressants.

The results obtained in genetically modified mice have clarified the important role of the endogenous opioid and endocannabinoid system in the pathophysiological mechanisms underlying emotional disorders. The different components of the endogenous opioid system seem to play an opposite role in the control of emotional responses. Thus, endogenous enkephalinergic tone on DOP produces antidepressant- and anxiolytic-like effects, whereas the endogenous tone on MOP results in the opposite emotional responses, i.e., depressive- and anxiogenic-like effects. These findings underline the potential interest of DOP for the development of novel therapeutic strategies for emotional diseases. The role of KOP and the endogenous beta-endorphin and dynorphin tone on emotional behaviour have not been yet clearly elucidated, and further studies would be required to clarify the contradictory results that have been obtained so far. The endocannabinoid system also plays a major role in the control of emotional responses. Indeed, an appropriate endocannabinoid tone acting on $CB_1$ receptors could confer protection against the deleterious effects of stressful stimuli that account for depressive- and anxiogenic-like responses. In agreement with this role of the endogenous cannabinoid tone, the genetic deletion of the enzyme involved in the enzymatic degradation of anandamide produced an antidepressant- and anxiolytic-like phenotype. $CB_2$ cannabinoids receptors also seem involved in the emotional control since the overexpression of these receptors also produced an antidepressant- and anxiolytic-like

phenotype. These novel findings reveal the potential interest of $CB_1$ and $CB_2$ cannabinoids receptors, as well as FAAH, as potential targets for the development of therapeutic strategies against depression and anxiety.

Finally, findings obtained in genetically modified mice deficient in different components of the HPA axis have confirmed the crucial role of this system in the pathogenesis of depression. Therefore, all these genetic tools have allowed the identification of several neurobiological targets that are involved in the substrates of depression, different from the monoamine transporters that are the current target of the antidepressant compounds now available. The identification of these novel targets provides important advances for the development of new antidepressants acting on different neurobiological targets than the monoamine transporters.

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