Interest of using genetically manipulated mice as models of depression to evaluate antidepressant drugs activity: a review

Alain M. Gardier*, Bruno P. Guiard, Jean-Philippe Guilloux, Christelle Repérant, François Coudoré, Denis J. David
Fac. Pharmacie, Univ. Paris Sud, EA 3544, Chatenay-Malabry cedex F-92296, France

INTRODUCTION

Depression and anxiety disorders are common public health problems with a 17% lifetime prevalence [1]. Major depressive disorder is defined by episodes of depressed mood lasting for more than 2 weeks accompanied by additional symptoms including disturbed sleep and appetite, reduced concentration, excessive guilt, and suicidal thoughts [2]. However, the molecular mechanisms underlying these diseases are still poorly understood. Much of our understanding about them comes from pharmacological experiments performed following administration of antidepressant drugs in healthy, non-depressed animals. This is a real problem because these drugs have several limitations: antidepressant drugs such as selective serotonin [5-hydroxytryptamine (5-HT)] reuptake inhibitors (SSRIs) are effective in treating mood as well as anxiety disorders [3]. However, while these drugs produce a relatively fast blockade of the serotonin transporter (SERT) in vitro, the onset of an appreciable clinical effect on mood is slow, taking several weeks to occur [4]. This delayed onset of action is likely...
related to the requirement of adaptations of presynaptic receptors (see below), i.e. a functional desensitization of inhibitory 5-HT1A autoreceptors [5]. This latency represents an important problem because major depressive disorders are often associated with a high risk of suicide. Thus, we need to improve our experimental conditions and to develop new animal models of psychiatric disorders to understand better the mechanisms limiting the effects of currently available treatments. The search for a rapid-acting antidepressant drug has been a subject of intense research for several decades.

In addition, around 30% of depressed patients treated with antidepressant drugs already on the market are resistant to these treatments. It has been relatively easy to show a depression-resistant phenotype in some mutant animals [mainly knockout (KO) mice] with a single deletion of a particular gene. However, showing an altered onset of action of SSRI in these mice, which can be reversed by a chronic antidepressant treatment has been more complicated.

Animal models are indispensable tools to screen for new antidepressant drugs or new combination of therapies that may have a more rapid efficacy to correct depressive symptoms. These models may also provide insights into the neuropathology that underlies the idiopathic disease state of depression [6]. Ideally, an animal model developed in psychopharmacology should reflect the human psychiatric disease. It is thus necessary to evaluate its validity, reliability, and utility [7].

- The animal model should reproduce behavioral states of the disease, i.e., the symptoms of depression observed in human (face validity).
- These symptoms are underpinned by the same neurochemical mechanisms as that in humans (construct validity).
- The behavioral changes observed in the new animal model should be reversed by chronic treatment with a wide variety of antidepressant drugs (predictable validity).

Major depressive disorders (MDD) result from a combination of interacting environmental, genetic, and epigenetic factors [2].

Environmental factors such as stressors are postulated to play a role in the etiology of the disease and to increase the susceptibility to MDD. Thus, effects of chronic antidepressant treatment have been tested, for example, in the chronic mild stress paradigm that causes behavioral changes in rodents that parallel symptoms of depression (see [8] for a review). Some studies were designed to evaluate the antidepressant-like activity of drugs in this model. Chronic exposure to mild unpredictable stress can decrease the consumption of palatable sweet solutions. Stressed animals were also sensitive to food reward in the place conditioning procedure; these effects are reversed by chronic treatment with tricyclic or atypical antidepressant drugs.

Genetic factors play a key role in the etiology of depression, a multifactorial illness [9]. It is thus necessary to identify those involved in this etiology. In the quest for better animal models of MDD, genetically manipulated rodents (mainly mice: see below) have been generated by homologous recombination [10], and their phenotype has been evaluated by using various neurochemical and behavioral tests.

Generation of KO mice with targeted mutations of specific genes is one of the most important tools available for determining the functions of gene products in the brain. This technique involves the direct introduction by homologous recombination of a mutation in a gene of interest. This approach is able to target any gene/any type of mutation; however, it is necessary to produce a new construction for each gene/mutation studied.

Animal models such as KO or transgenic mice have been developed to better understand the underlying neurochemical mechanisms leading to diseases (for example, anxiety, depression) in humans. The invalidation of a gene by mutagenesis in animals allows the identification of functions impaired by the mutations.

The mouse is a model organism of choice for this purpose because a lot of its genes have an equivalent in humans. In addition, the genome of the mouse is easy to modify by homologous recombination. This strategy allowed the creation of relevant mouse models of human disease. Numerous biological and biochemical functions of the mouse are similar to those of humans.

Genetic background is a fundamental parameter for the analysis of the phenotype of KO mice. Historically, mutant mice were established using embryonic stem cells of the 129/Sv line. However, the establishment of mutant lines preferably on a genetic C57BL/6 background is now largely recognized even though some limitations of the use of this strain exist in some behavioral tests [11].

The characterization of the phenotype of these KO mice relies on the availability of a large set of behavioral tests evaluating their basal anxiety-like and depressive-like states, associated with changes in their locomotor activity. By comparing the results obtained in wild-type (WT) controls with those in KO mice, it is then possible to screen for molecules capable of correcting these symptoms, thus having anxiolytic-like and/or
antidepressant-like pharmacological activities in these experimental models. For example, among the most useful behavioral tests, the Porsolt forced swim test (FST; [12]), and tail suspension test (TST; [13]) are stress paradigms aimed at screening potential antidepressants in controls (effects) vs. KO (no effects) mice. In these behavioral tests, the animal is placed in a situation that induces a state of helplessness or behavioral despair, such as being forced to swim or being suspended by the tail. When the animal realizes that it cannot escape, it stays in an immobile position. Antidepressant drugs such as SSRI reduce this period of immobility. Although the FST and TST have been widely used to describe the basal phenotype of genetically manipulated mice, they are not animal models of depression, but simple and rapid behavioral tests are used to screen the antidepressant-like activity of novel molecules following their acute administration. The following tests are used to screen drug activities:

- **Antidepressant-like activity:**
  - Forced Swim test (Porsolt et al., 1977) [12]
  - Tail suspension test (Steru et al., 1985) [13]
- **Anxiolytic-like activity:**
  - Elevated plus maze
  - Open field paradigm (Dulawa et al., 2004) [14]
  - Black and White box
  - Stress-induced hyperthermia
- **Mixed antidepressant/anxiolytic-like activity:**
  - Novelty suppressed feeding (David et al., 2007) [15]

In the search for a better animal model, we expected that KO mice exhibit alterations resembling those classically observed in depressed patients, notably regarding chronic stress, changes in sleep-wakefulness or body weight and food intake alterations. In addition, increases in plasma corticosterone levels, changes in serotonin metabolism index in brain tissue homogenates, firing activity of 5-HT neurons in the nucleus raphe dorsalis and consequences of serotonin (5-HT1A) autoreceptor stimulation are also expected. Thus, in the present review, we will give a few examples of different lines of KO mice exhibiting a decrease in serotonergic tone, which evokes that associated with endogenous depression in humans. All these behavioral impairments and serotonergic dysfunctions must be studied by comparing data obtained in WT controls vs. KO mice first in basal conditions, then following a chronic treatment with an antidepressant drug. Some of these KO mice provide an opportunity to approach genes influencing susceptibility to anxiety and depression.

### CONSTITUTIVE KO MOUSE ENGINEERING

A genetically manipulated organism (GMO) or genetically engineered organism is an organism whose genetic material has been altered using genetic engineering techniques. These techniques are generally known as recombinant DNA technology in which DNA molecules from different sources are modified in vitro (combination into one molecule; mutation; deletion) to create a new gene in ES cells. This DNA is then transferred in vivo into an organism: the addition of a genetic material into his genome modifies the expression of endogenous traits or generates new traits – Genetic engineering. The production of a GMO was made possible through a series of scientific advances including the discovery of DNA and the creation of the first recombinant bacteria in 1973, i.e. *Escherichia coli* expressing a salmonella gene [16]. This led to concerns in the scientific community about potential risks from genetic engineering. In 1978, the company Genentech announced the creation of an *E. coli* strain producing the human protein insulin [17].

In the field of Neuroscience – Neuropharmacology, examples of GMOs include transgenic animals (genetically manipulated by recombinant DNA methods), mainly mice, which are now widely used in research. These tools are indispensable in many areas of research including those that study the mechanisms of human and other diseases or fundamental biological processes in eukaryotic or prokaryotic cells. Transgenic animals are used as experimental models on which we can perform phenotypic tests with genes whose function is unknown. In addition, these animals were generated because they are susceptible to certain compounds or to acute or chronic stressors for testing in biomedical research.

A KO mouse is a GMO that has had one or more of its genes made inoperable through a gene KO. KO is a route to learning about a gene that has been sequenced, but has an unknown or incompletely known function; this technique allowed the generation of numerous animal models of human pathologies, then to set up new and more efficient therapies. By inactivating the gene and studying the mouse for any resulting differences compared with WT controls, we can infer the probable physiological function of that gene. Mice became a favorite subject for KO experiments because they are easy to breed laboratory animals, are less expensive to feed than to feed rats, and live in smaller cages in animal facilities.
The first KO mice were generated by homologous recombination and produced by Martin Evans in 1981–1987 [18], for which he was awarded the Nobel Prize for Medicine in 2007 with Mario R. Capecchi and Oliver Smithies. In 1981, Evans and Kaufman isolated pluripotent, undifferentiated embryonic cells from mouse blastocysts to grow them in culture. Cultures of embryo-derived pluripotent cells exhibit both a normal karyotype and a high ability of differentiation. Blastocyst injection studies using isolated XY embryo-derived cell lines have shown that they produce a high proportion of live-born animals that are overtly chimeric. Several chimeric male mice, derived from these cell lines, have proved to be functional germ-line chimeras [19]. One year later, the same group of scientists used cultured mouse ES cells, mutagenized by retroviral insertion and selected for loss of hypoxanthine-guanosine phosphoribosyl transferase (HPRT) activity, to construct chimeric mice [20]. Evans group obtained a potential animal model for Lesch-Nyhan syndrome through introduction of mutations of the gene coding for HPRT into mice. This rare neurological disorder is caused by an inherited deficiency in the level of activity of this enzyme, which alters purine metabolism. Strains of mutant mice have the same biochemical defect as Lesch-Nyhan patients. Male mice carrying the mutant alleles are viable and analysis of their cells shows a total lack of HPRT activity.

In parallel, the Capecchi group studied the integration of DNA microinjected into cultured mammalian cells and showed evidence for homologous recombination between injected plasmid and DNA molecules [21].

A report on the first mutant mice obtained by homologous recombination in ES cells was published in 1989 [22]. This methodology then spread very quickly among the community of mouse geneticists and there are so far more than 12 000 strains of mice (more than 5000 genes targeted) obtained by directed mutagenesis. Today, the level of sophistication is such that all types of genetic modification (point mutation, deletion, translocation) can be obtained and that it is possible to induce mutation in a given cell type and/or at a time point during the development.

In a little <20 years, the mutant mice obtained by gene targeting have become indispensable to the study of virtually all areas of biology and physiology of the mouse. They have helped to expand dramatically our understanding of the function of genes and the genome, and shed new light on development, immunology, neurobiology, physiology, and so on.

Thus, it is the encounter between two independent approaches, ES cells and homologous recombination that led to a revolution in the genetic approach to the biology of mammals. The ability to generate changes programmed from the mouse has been used to create many mouse models of human diseases. These models are vital to biomedical research because they allow experimental dissection of diseases, the identification of new therapeutic targets, and the development of new therapies.

**MUTATION OF SEROTONERGIC ELEMENTS**

Knockout mice are interesting tools for experimental pharmacology because they very often exhibit changes in phenotypes resembling those induced by chronic treatment with antidepressants. KO mice for specific serotonergic targets (i.e., 5-HT transporter, 5-HT1B, 5-HT1A and 5-HT4 receptors) represent an alternative to the use of pharmacological tools, which have been until recently the main approach to investigate the involvement of this monoamine in mood disorders and improve antidepressant therapy.

**SERT KO mice**

We will only briefly describe these mutants in the present review because excellent reviews are regularly released by these authors [23,24].

The sodium-dependent, high affinity SERT provides the primary mechanism for inactivation of 5-HT after its release into the synaptic cleft. To further evaluate the function of SERT, the murine gene was first disrupted by homologous recombination by the KP Lesch and DL Murphy groups [25]. Despite evidence that excess extracellular 5-HT levels during embryonic development, including that produced by drugs that inhibit the SERT, may lead to severe craniofacial and cardiac malformations, no obvious developmental phenotype was observed in these mutant mice. These SERT KO mice were generated by standard homologous recombination techniques with the use of a targeting construct in which exon 2 of the transporter-coding region was partially deleted. A DNA fragment containing SERT exon 2 was replaced by a phosphoglycerate kinase neomycin-polyA expression cassette. One hundred and twenty-nine R1 ES cells were cultured, transfected, and subjected to double selection. Clones were then microinjected in C57BL/6J blastocysts to obtain chimeric progeny. Chimeric male mice were mated to female mice on the CD-1 and C57BL/6J background strains. Pups were then genotyped to select.
heterozygous SERT +/- and SERT KO mutants [25]. Why are these mice an interesting model in neuropsychopharmacology? Starting in the mid 1990s, Lesch and Murphy groups suggested that KO SERT mice can be viewed either as an experimental model to study the mechanism of action of antidepressants because SERT is a key regulator of extracellular 5-HT levels and the main target of antidepressant drugs of the SSRIs family, or as an animal model of depression.

Indeed, in Humans, a long (l) allele and a short (s) allele of the SERT gene have been described. Lesch et al. (1996) [26] provided the first demonstration that a functional polymorphism in the promoter region of the SERT gene (SLC6A4) is associated with anxiety- and depression-related personality traits and antidepressant drug resistance [27]. Of course, these traits can be amplified by gene–environment and gene–gene interactions. A reduced SERT function associated with a greater amygdala neuronal activity (a brain region activated in response to emotion, fear, stress, and anxiety) was found in individuals with one or two copies of the short (s) allele of the SERT promoter [28]. In addition, humans with one or two copies of the short (s) allele of the SERT promoter polymorphism exhibit more depressive symptoms and suicidality in relation to stressful life events than individuals homozygous for the long (l) allele. Thus, these psychiatric disorders could be primarily attributable to altered intracellular and enhanced extracellular 5-HT concentrations during development and/or adulthood. It can be hypothesized that a functional polymorphism in the promoter region of the SERT gene limits the influence of stressful life events on depression [29]. Thus, therapeutic responses and side effects following treatment of depressed patients with SSRI were also associated with SERT gene (SLC6A4) variants (see [24] for a review).

Serotonin transporter function-modifying gene variants in humans apparently produce many phenotypes that are similar to those found in KO mice. Mutations resulting in reduced (in SERT +/- mice) or completely abrogated SERT function (in SERT KO mice) have led to the identification of more than 50 different phenotypic changes associated, for example, with an increased anxiety and stress-related behaviors in basal conditions as described in humans. Other behavioral and neurochemical effects of various pharmacological agents (mainly serotonergic drugs) were investigated early after birth or in adult SERT KO mice [30,31].

Serotonin transporter KO mice display modest changes in basal dialysate 5-HT levels compared with control mice as measured by intracerebral in vivo microdialysis in the striatum and frontal cortex [32,33]. Not surprisingly, the absence of normal responses to SSRI antidepressants in SERT KO mice demonstrated that effects on SERT are a critical principle mechanism of action of members of this class of antidepressants [25]. Specific labeling with radioligands and antibodies, and competitive RT-PCR, mainly showed that 5-HT1A receptor protein and mRNA levels were significantly decreased in the dorsal raphe nucleus (DRN; presynaptic 5-HT1A receptors) and increased in the hippocampus (postsynaptic 5-HT1A receptors) in SERT KO vs. WT control mice [34].

It is well recognized that, in mice treated chronically with an SSRI or constitutive SERT KO mice, a functional desensitization of 5-HT1A autoreceptors is observed. In line with these effects, a robust and time-dependent downregulation of the 5-HT transporter SERT occurred in rodents following chronic SSRI administration [35,36].

We have learnt more with another SERT KO model also generated by homologous recombination, the targeted deletion also involving exon 2 of the transporter-coding region. However, in this model, ES cells derived from a 129S6/SvEv background strain. Resulting chimeras were then backcrossed to 129S6/SvEv mice [37]. Interestingly, a transient inhibition of SERT during early development with fluoxetine, an SSRI, produced abnormal emotional behaviors in adult WT mice. This effect mimicked the behavioral phenotype of adult SERT KO mice showing a reduced exploratory behavior, i.e. anxiogenic-like state, in the elevated-plus-maze test [38]. Thus, when serotonin reuptake is blocked, an excess of extracellular 5-HT in synaptic cleft can lead to overstimulation of pre- and/or post-synaptic 5-HT receptors, and to abnormal behaviors. These data highlighted the fact that 5-HT neurotransmission exerts a critical role in the maturation of brain systems that modulate emotional function in the adult. Thus, a developmental mechanism may explain how low-expressing SERT promoter alleles increase vulnerability to psychiatric disorders. These data were recently confirmed by using a pharmacological approach; an early exposure to SERT inhibitors from postnatal day 4 (P4) to P21 produced abnormal emotional behaviors in adult mice [39]. Thus, SERT KO mice show an increased anxiety and stress-related behaviors suggesting the occurrence of an anxiogenic-like state. To our knowledge, no studies have tried to reverse this effect. However, sleep impairment occurring at adulthood in SERT KO mice can be totally
or partially reversed by a 5-HT synthesis inhibitor, para-chlorophenylalanine, or a 5-HT1A receptor antagonist, WAY 100635, initiated at postnatal day 5 [30]. A developmental loss of a protein in KO mice generally produces altered behaviors in screening tests of antidepressant-like activity that in line with those produced by chronic antidepressant treatment [37]. Here, SERT KO mice display an anxiogenic-like phenotype, while a long-term treatment (e.g., 4 weeks) with an antidepressant drug is often anxiolytic in animals models such as the open field paradigm [14]. These discrepancies could be a compensatory response to constitutive deletion of the 5-HT transporter and may contribute to the anxiogenic-like state observed in KO mice. In line with these findings, it was found that healthy individuals carrying a gene polymorphism of the short (s) allele of the SERT promoter have increased anxiety-related traits, increased amygdala reactivity, and elevated risk of depression. The neural mechanism underlying this complex genetic association seems to involve a reduced gray matter volume in short-allele carriers in limbic regions critical for processing of negative emotion, particularly cingulate cortex and amygdala [40]. Thus, not surprisingly, the absence of normal responses to SSRI antidepressants in SERT KO mice demonstrates that actions on SERT are a critical principle mechanism of action of members of this class of antidepressants [31].

5-HT receptor KO mice
The monoaminergic hypothesis of depression suggests that it results from a deficiency of brain 5-HT and/or norepinephrine functions. This hypothesis dominated the field for decades and was mainly supported by the effectiveness of antidepressant drugs such as imipramine derivatives and SSRI that increase 5-HT neurotransmission by preventing the reuptake of these neurotransmitters. It was thus not surprising that researchers working in the field of anxiety and depression first focused their attention on various KO mice lacking a particular 5-HT receptor subtype exerting an inhibitory feedback control on serotonergic neurons. The first interesting KO model to study brain serotonin-related disorders was 5-HT1B receptor KO mice [41].

Brain 5-HT has been implicated in a number of physiological processes and pathological conditions. These effects are mediated by at least 14 different 5-HT receptors. Advanced knowledge in the physiopathology of mood disorders was made when the inactivation of the genes encoding for the 5-HT1A, 5-HT1B or 5-HT4 receptor sub-types was possible in mice. Serotonin is involved in the regulation of mood disorders, a fact that has been established with the discovery that drugs that deplete 5-HT precipitate depression, whereas increasing brain 5-HT levels has antidepressant effects [42]. In addition, the idea that 5-HT may also affect anxiety is now obvious because some SSRI can also be prescribed in the treatment of generalized anxiety.

One possible explanation for the long delay of action of antidepressant drugs could be a negative feedback control exerted by somatodendritic 5-HT1A and/or nerve terminal 5-HT1B autoreceptors on 5-HT release [43]. It was thus logical that molecular biologists focused their attention on these presynaptic receptors to generate mutant mice lacking 5-HT autoreceptors as new tools to study stress-related behaviors.

In an attempt to dissect the contribution of individual 5-HT receptor subtypes to behavior, various KO mice have been generated by homologous recombination. At the cellular level, SSRI-induced blockade of the selective transporter SERT results in a rapid suppression of the firing activity of 5-HT neurons in the brainstem [44]. Consequently, despite the 5-HT reuptake inhibition also taking place at nerve terminals, there is a decrease in 5-HT cell firing via activation of 5-HT1A (somatodendritic) or 5-HT1B (nerve terminal) autoreceptors [45] leading to a moderate increase in extracellular 5-HT levels at serotonergic terminal levels. To alleviate this problem occurring following acute SSRI treatment, 5-HT1A autoreceptor antagonists have been co-administered with antidepressants [46]. It is thus logical to believe that 5-HT1A or 5-HT1B receptor KO mice may be interesting animal models, which should display a higher serotonergic tone. Very recently, the 5-HT4 receptor also became a new center of interest because agonists seem to display an antidepressant-like activity with a rapid onset of action.

5-HT1B receptor KO mice
5-HT1B receptors are expressed throughout the brain of rodents. These receptors are located in the axon terminals of both 5-HTergic and non-5-HTergic neurons where they act as inhibitory autoreceptors or hetero-receptors, respectively. These receptors have been difficult to study because of the diversity of their localization and the absence of highly selective receptor antagonists. In these conditions, 5-HT1B KO mice are important tools to model mood disorders because these receptors play a major role in the regulation of 5HT release in various brain regions including the hippocampus.

It was shown that mice lacking the 5-HT1B receptor sub-type did not exhibit any obvious developmental or
behavioral defects [41]. However, the hyperlocomotor effect of the 5-HT1A/1B receptor agonist RU24969 was absent in KO mice indicating that this effect is mediated by 5-HT1B receptor activation. Moreover, when confronted with an intruder, KO mice attacked the intruder faster and more intensely than did WT control mice, suggesting the participation of 5-HT1B receptors in aggressive behavior. These latter results suggest that terminal 5-HT1B receptors play a role in mood disorders. These data might be related to the fact that a class of 5-HT1 receptor agonists, termed serenics, has anti-aggressive properties, and with the findings that certain impulsive aggressive behaviors are associated with deficits in central serotonin [47].

Then, some attempts have been made to study the consequences of the constitutive lack of 5-HT1B receptor on the regulation of basal and evoked-release 5-HT at nerve terminals either in vitro [48] or in vivo [49,50]. Thus, from midbrain, frontal cortex and hippocampus preloaded slices obtained from WT and 5-HT1B KO mice, it was shown that, in the absence of any drug, [3H]5-HT release was increased in midbrain and hippocampus, but not in frontocortical slices of 5-HT1B KO mice. The selective 5-HT1B receptor agonist CP 93129 and the 5-HT1B/D agonist sumatriptan, inhibited [3H]5-HT release in hippocampus and cortical slices obtained from control mice, but had no effect in mutants. In slices containing midbrain raphe nuclei, CP 93129 had no effect in either group. In contrast, sumatriptan inhibited [3H]5-HT release in both genotypes. This latter effect was blocked by the 5-HT1D antagonist GR 127935, but not by the 5-HT1A antagonist (+)WAY 100135, thus suggesting that a 5-HT1D-like receptor, negatively regulates 5-HT release in mouse midbrain raphe nuclei [48].

Interestingly, we compared basal extracellular 5-HT levels as measured by in vivo microdialysis in conscious WT vs. 5-HT1B receptor KO mice. In the frontal cortex and ventral hippocampus, basal 5-HT release did not differ between the two strains of mice studied. The infusion via reverse microdialysis of the selective 5-HT1B receptor agonist CP-93,129 significantly decreased basal 5-HT release in the WT, but had no effect in KO mice. The mixed 5-HT1B/5-HT1D receptor agonist sumatriptan gave similar results. These results confirmed that in mice, 5-HT1B autoreceptors inhibit 5-HT release at nerve terminals located in the frontal cortex and ventral hippocampus [49].

In addition, the effects of some antidepressant drugs were evaluated in 5-HT1B KO mice following their acute [49,50] or chronic administration [46]. A single dose of paroxetine increased extracellular 5-HT levels in both genotypes, but these effects were potentiated in the ventral hippocampus, but not in the frontal cortex, in 5-HT1B KO mice compared with WT mice [50]. Furthermore, using the FST, we found that SSRIs decreased immobility of WT mice, and this effect was absent in 5-HT1B KO mice showing therefore that activation of 5-HT1B receptors mediated the antidepressant-like effects of SSRIs. However, some compensatory changes resulting from the KO may actually mediate this effect (see below). Our results suggest that 5-HT1B autoreceptors limit the effects of SSRIs particularly in the hippocampus, while postsynaptic 5-HT1B heteroreceptors are likely required for the antidepressant activity of SSRIs [50–52].

To date, only one intracerebral in vivo microdialysis study described neurochemical responses following chronic SSRI administration in 5-HT1B KO mice [46]. Results were disappointing as a chronic administration of paroxetine via osmotic minipumps (1 mg/kg per day for 14 days) did not alter basal extracellular 5-HT levels in the frontal cortex and ventral hippocampus in these KO mice compared with WT controls. No differences were found between genotypes following chronic treatment. Paroxetine’s pharmacokinetic properties in KOs were similar to those found in the control group. These data suggest that presynaptic 5-HT1B receptors retain their capacity to limit 5-HT release mainly in the ventral hippocampus following chronic paroxetine administration, i.e. these autoreceptors were not desensitized [46]. However, the absence of 5-HT1B autoreceptor desensitization remains somewhat equivocal because in vitro evidence in guinea-pigs (the animal of choice to study the 5-HT1B receptor) indicated that the electrically-evoked release of [3H]-5-HT was enhanced in the hippocampal and cortical slices after sustained administration of SSRIs [53].

Interestingly, female 5-HT1B receptor KO mice displayed a significantly reduced immobility than either male 5-HT1B KO mice or male and female WT mice in the TST and FST [54]. Those authors concluded that these KO mice demonstrate a sex-linked disinhibition of 5-HT release that sustained higher baseline levels of hippocampal 5-HT and behavioral vulnerability to 5-HT depletion.

What are the main drawbacks occurring in KO mice? Constitutive KO mice are powerful tools to study the role of a protein. However, they are generated by homologous recombination in which a gene is KO during embryonic life, generally affecting the whole organism
throughout its lifetime [42]. Thus, compensatory changes are likely to occur in these KOs. Table 1 summarizes the main findings. In 5-HT1B KO mice, alterations in presynaptic neuronal activity suggest that one compensatory mechanism may involve the dopaminergic system. Indeed, constitutive deletion of the 5-HT1B receptor enhanced the effects of psycho- stimulants in the nucleus accumbens and basal or cocaine-evoked dopamine release in projection areas of mesostriatial or mesoaccumbens dopamine neurons [55]. An alternative compensatory mechanism would be that a decrease in the efficiency of G-protein coupling to 5-HT1A receptors has developed in 5-HT1B KO mice [56]. In our laboratory, we tested for adaptive compensatory changes that may have occurred in the functional activity of somatodendritic 5-HT1A receptors during the development of constitutive 5-HT1B KO mice. Thus, we studied the decrease in body temperature induced by an acute subcutaneous injection of the 5-HT1A receptor agonist, 8-hydroxy 2(di-n-propyl(amino)tetralin (8-OH-DPAT). We found a higher efficacy of 8-OH-DPAT-induced hypothermia in 5-HT1B KO than in WT mice suggesting that an adaptive thermoregulatory process involving a hyperfunctional activity of somatodendritic 5-HT1A receptors in KO mice lacking 5-HT1B receptors [57]. Heart rate and temperature in 5-HT1B KO mice also increased markedly in response to transportation and handling procedures suggesting a physiological hyper-reactivity of these KO mice [58]. Furthermore, 5-HT1B KO mice show a compensatory reduction in 5-HT2C receptor-mediated functions such as smaller reductions in food intake and locomotor activity in response to administration of 5-HT2C receptor agonists [59]. These effects result from a long-term adaptation to the loss of 5-HT1B receptor function in these KO mice. Decreased basal heart rate and increased basal body temperature (i.e. exaggerated autonomic responses to novel cage stress) were also described in 5-HT1B KO mice [60].

### 5-HT1A receptor KO mice

5-HT1A receptors are pre- and post-synaptic receptors expressed in a number of brain regions to which serotonergic neurons project, including the frontal cortex, hippocampus, and amygdala. As with presynaptic autoreceptors, activation of postsynaptic 5-HT1A receptors leads to hyperpolarization of the neuron and the consequent inhibition of neurotransmitter release. This effect appears to be mediated through a biochemical signaling pathway in which 5-HT1A receptors activate a G protein (Gi)-coupled inwardly rectifying potassium channel.

The 5-HT1A receptor sub-type represents a potentially more important regulatory site for modulating the

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8-OH-DPAT, 8-hydroxy 2(di-n-propyl(amino)tetralin; HPA, hypothalamus pituitary adrenal; KO, knockout; HT, hydroxytryptamine.

*Parameters related to the symptoms of anxiety and/or depression.
actions of serotonin in the brain compared with the nerve terminal 5-HT1B receptor sub-type. The role of this serotonin receptor sub-type in the mechanism of action of antidepressant drugs such as SSRIs has been extensively studied. Indeed, a ligand that preferentially antagonizes somatodendritic 5-HT1A autoreceptors can enhance the antidepressant-like activity of SSRIs by increasing 5-HT levels in the synaptic cleft following the blockade of its selective transporter located on the presynaptic membrane [61]. In other words, the selective blockade of inhibitory autoreceptors may augment the ability of SSRIs to increase synaptic serotonin levels [62]. This hypothesis is related to the functional desensitization of 5-HT1A autoreceptors, which occur following a chronic SSRI administration.

The role of 5-HT1A receptors in anxiety states is also recognized, thanks to pharmacology and later to 5-HT1A KO mice. Anxiety in mice is defined as a high level of avoidance of novel and unfamiliar environment and increased fear reaction. Other aspects of anxiety such as autonomic activation, increased stress responsiveness, and neuroendocrine abnormalities have also been described in 5-HT receptor KO mice [63]. In 1998, three different groups demonstrated that the 5-HT1A KO mouse is not only an interesting animal model of anxiety-related disorders but also helps to predict the anxiolytic-like potential of novel agents. They examined the consequences of 5-HT1A receptor deletion on anxiety states by comparing the behaviors of WT vs. KO mice in various paradigms (elevated-plus-maze, open field, etc…) that measure an animal’s willingness to explore open spaces. Mice that prefer to stay longer near protective walls of the box are considered to be more anxious than animals with greater exploratory activity. Such assays serve as reasonable predictors of anxiolytic-like drug activity. These groups also studied WT and 5-HT1A KO mice in models that predict the efficacy of antidepressant drugs such as the FST and TST.

The results were obtained following a target inactivation of this gene by homologous recombination on different genetic backgrounds in three different laboratories and tested under similar, but not identical, experimental conditions (Miklos Toth group [64]; René Hen group [65]; LH Tecott group [66]), i.e. BALB/c, 129/Sv and Swiss, respectively (Table II). They all came to the same conclusion that 5-HT1A KO mice have an increased tendency to avoid a novel and fearful environment and to escape a stressful situation, and behaviors were consistent with an increased anxiety and stress response. In the FST [64,65] as well as in the TST [66], 5-HT1A KO mice displayed a shorter immobility time

<table>
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<td>129/Sv mice</td>
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<td>DRN: effects of an acute fluoxetine dose on dialysate 5-HT levels are potentiated in mutants; DRN and FCx: effects of an acute paroxetine dose on dialysate 5-HT levels are potentiated in mutants; Decreased basal immobility; no effect of a single dose of SSRI</td>
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DRN, dorsal raphe nucleus; SSRI, selective serotonin reuptake inhibitors; HT, hydroxytryptamine; EPM, elevated plus maze.
suggesting that lack of functional 5-HT1A receptors favors a less depressed, at least under these experimental conditions [62]. The phenotype of this 5-HT1A KO mouse seems paradoxical as heightened anxiety is most often associated with depression [65]. It must be underlined that these behavioral tests have been performed without any injection of antidepressants to these KO mice; these experimental conditions are not the most appropriate ones because these tests have been designed and validated in order to screen the antidepressant-like activity of novel compounds. 5-HT1A KO mice also exhibited a spontaneous decreased immobility in the FST, without any antidepressant drug injection. This effect is commonly associated with an acute antidepressant treatment in WT mice [12,65].

Although the core phenotype of anxiety can be reproduced in KO mice in various inbred and outbred backgrounds, abnormalities in 5-HT dynamics and resistance to the anxiolytic drug diazepam have been observed in one, but not on other genetic backgrounds of 5-HT1A KO mice, i.e. in mice generated by M. Toth on the Swiss Webster genetic background. This indicates that while the development of anxiety is an invariable consequence of receptor deficit, other features induced by receptor loss are strongly modulated by other genes [63].

Inducible KO strategies that allow eliminating protein expression acutely brought new important information. The 5-HT1A receptor is currently the only 5-HT receptor sub-type for which this strategy was applied. The use of constitutive KO mice for these 5-HT receptor sub-types does not allow the discrimination between the roles of these 5-HT1A receptors according to their presynaptic (autoreceptors) and postsynaptic (heteroreceptors) locations. For this purpose, a conditional rescue strategy has been recently applied; these mice express the 5-HT1A receptor primarily in the hippocampus and cortex, but not in the raphe nuclei [67]. The authors found that mice lacking 5-HT1A receptors throughout the brain showed pronounced anxiety-like behavior, while those having a selective restoration of the 5-HT1A receptors in the forebrain had a normal behavior. Behavioral and neurochemical experiments performed in these mice also suggest that postnatal developmental processes help to establish adult anxiety-like behaviour. Indeed, by using mice in which the 5-HT1A receptor can be knocked out temporarily, the authors show that the absence of postsynaptic 5-HT1A receptors in the hippocampus and cortex of newborn mice does indeed lead to anxiety-like behavior, whereas its KO during adult life has no effect. Thus, postsynaptic 5-HT1A receptors located in the forebrain regulate anxiety, while those in the hindbrain are less involved. Anxiety seems to be linked to the presence of 5-HT1A receptors in specific brain regions, at a particular period of development: these data add a new layer of understanding of the involvement of 5-HT in the physiopathology of anxiety.

Neurochemical experiments (especially intracerebral in vivo microdialysis) performed in 5-HT1A KO mice nicely complemented these behavioral informations. Based on the role of the somatodendritic 5-HT1A autoreceptors in the feedback regulation of the 5-HT system, an increased serotonergic neurotransmission was expected to explain the anxiety-like behavior of receptor-deficient animals. This view is consistent with earlier studies showing that pharmacological activation of the 5-HT system (e.g. either by a 5-HT receptor agonist, or by an acute SSRI treatment) is anxiogenic in animal models as well as in humans [63,64]. However, it was surprising to observe that 5-HT1A KO mice had normal brain tissue levels of 5-HT and 5-hydroxyindole-acetic acid (the major 5-HT metabolite). In addition, by using intracerebral in vivo microdialysis, it was also shown that basal extracellular 5-HT levels did not differ between WT and 5-HT1A KO mice, neither in raphe nuclei, nor in the frontal cortex at serotonergic tonic nerve terminals [68,69]. These data are consistent with a lack of control of 5-HT1A autoreceptors on 5-HT release in these brain regions of these KO mice. It suggests that decreases in presynaptic 5-HT1A receptor density because of genetic defects or environmental stressors might result in heightened anxiety, without changes in 5-HT neurotransmission [65]. Further investigations are necessary to explain this behavioral change and to try to link them to specific alterations of other neurotransmitter systems. As benzodiazepines are indirect agonists of gamma-aminobutyric acid (GABA) A receptors and anxiolytics of reference, a blunted inhibitory GABAergic neurotransmission may occur in the brain of 5-HT1A KO mice. Indeed, binding of benzodiazepines are reduced and GABAergic inhibition is impaired in these KO mice [70]. These changes were observed in the amygdala and hippocampus. These data suggest a close relationship between 5-HT1A receptors and GABA A receptors in limbic regions involved in the control of fear and anxiety.

Pharmacological studies performed in KO mice also provided interesting data. Indeed, it was shown that the 5-HT1A receptor agonist of reference 8-OH-DPAT reduces extracellular 5-HT levels to 30% of basal values.
in raphe nuclei in WT mice, but not in 5-HT1A KO mice. Fluoxetine or paroxetine (SSRI) increase dialysate 5-HT levels in raphe nuclei and frontal cortex in a dose-dependent manner in both genotypes, but this effect was markedly more pronounced in 5-HT1A KO mice [68,69]. The data reflect a lack of the inhibitory feedback control exerted by 5-HT1A autoreceptors in conditions of enhanced 5-HT neurotransmission. In addition, these KOs helped to study the pharmacological properties of pindolol, a β1,2 adrenoceptor antagonist, in the brain. Indeed, this compound shortens the delay of action of SSRI in depressed patients (see a recent meta-analysis [71]), but it was not sure that this effect was mediated by somatodendritic 5-HT1A autoreceptor blockade. We thus studied the effects of (+)-pindolol-paroxetine co-administration in genetic and pharmacological approaches in 5-HT1A KO mice [69]. Paroxetine dose-dependent increased cortical dialysate 5-HT levels in both genotypes, but the effects were greater in KOs (+)-pindolol potentiated the effects of paroxetine on cortical dialysate 5-HT levels in controls, but not in 5-HT1A KO mice. Similar responses were obtained following local intra-raphe perfusion by reverse microdialysis of (+)-pindolol. In the FST, an acute paroxetine administration dose-dependently decreased the immobility time in both strains of mice, but the response was much greater in 5HT1A KO mice compared with WT controls. In contrast, (+)-pindolol blocked paroxetine-induced decreases in the immobility time. These findings confirm that, when combined with a SSRI, (+)-pindolol behaves as an antagonist of presynaptic 5-HT1A auto-receptor in mice, but its blockade of paroxetine-induced antidepressant-like effects in the FST may be because of its binding to other neurotransmitter receptors, likely located postsynaptically [69].

It was also showed, by using 5-HT1A KO mice, that radiological methods disrupting antidepressant-induced neurogenesis also block behavioral responses to antidepressants. Neurogenesis in adult mammalian brain can be divided into several steps including proliferation of neural stem cells, their maturation, migration and differentiation into neurons in adult hippocampus [72,73]. The survival, i.e. the balance between life and death of new neurons occurs in few specialized brain regions such as the olfactory bulb and the granular cell layer of the dentate gyrus of the hippocampus. 5-HT1A KO mice made on 129/Sv background were insensitive to the neurogenic and behavioral effects of the SSRI fluoxetine [74]. Suppression of hippocampal neurogenesis by X-irradiation of a restricted region of mouse brain containing the hippocampus abolished the behavioral effects of antidepressant drugs [74]. At that time, these data suggest that: (i) the behavioral effects of chronic antidepressants may be mediated by the stimulation of proliferative step of neurogenesis in the hippocampus; (ii) 5HT1A postsynaptic receptor is necessary to the effects of SSRIs on adult neurogenesis in mice on 129Sv background, but not on Balb/cJ background because behavioral effects of chronic fluoxetine in BALB/cJ mice do not require adult hippocampal neurogenesis or the 5-HT1A receptor function [75]; and (iii) the etiology of depression could involve neurodegeneration and impairments of growth of new neurons. Although being attractive, this neurogenesis hypothesis of depression is still a matter of debate [76–79]. Can depressive-like behavior occur without impairments in neurogenesis? If neurogenesis is necessary to observe antidepressant-like activity of imipramine and SSRI, is it sufficient?

5-HT1A KO mice also improved our knowledge of the regulation of vigilance states. It has been shown that this receptor sub-type plays a key role in the control of spontaneous sleep–wakfulness cycles, as well as in homeostatic regulation and stress-induced adaptive changes of paradoxical sleep [80]. Indeed, 5-HT1A KO mice expressed higher amounts of paradoxical sleep than WT control mice during both the light and the dark phases. In WT mice, a 5-HT1A receptor antagonist promoted paradoxical sleep, while a 5-HT1A receptor agonist had an opposite effect. By contrast, 5-HT1A receptor ligands did not affect sleep in KO mice. Finally, in contrast to WT mice, 5-HT1A KO mice did not exhibit any rebound of paradoxical sleep after either a 9-h instrumental paradoxical sleep deprivation or a 90-min immobilization stress. Thus, in the mouse, pre- and postsynaptic 5-HT1A receptors seem to participate to basal regulation of paradoxical sleep and to its adaptive changes occurring following stress [80].

5-HT1A KO mice also exhibit abnormalities reminiscent of symptoms of stress-related psychiatric disorders, i.e. a hippocampal deficit in 5-HT1A receptors, contributes to the cognitive abnormalities often seen in these disorders [81]. Indeed, these KO mice showed a deficit in hippocampal-dependent learning and memory tests such as the Morris water maze. Furthermore, synaptic plasticity in the hippocampus, and limbic neuronal excitability were also impaired in 5-HT1A KO mice as compared with WT control mice. These data demonstrate that 5-HT1A receptors are required for maintaining normal hippocampal functions. It also plays a role in
hippocampal-related symptoms such as cognitive disturbances observed in stress-related disorders.

Taken together, these data underline the main results obtained by using 5-HT1A KO mice in the field of neuropsychopharmacology. The importance of the role of this 5-HT receptor sub-type was discovered or confirmed in mood and stress-related disorders (anxiety, depression), in various aspects of the mechanism of action of SSRIs (its impact on 5-HT neurotransmission and on neurogenesis), in the regulation of sleep as well as in learning and memory. By using these KO mice as an experimental tool, it has been clearly demonstrated that 5-HT1A KO mice represent a genetic animal model of anxiety with both construct and face validities (see [63] for a review). Apart from these advantages, there are, however, some drawbacks of using autoreceptor KO mice: In both cases (5-HT1B and 5-HT1A autoreceptors), the loss of presynaptic autoreceptor function did not result in an increased basal serotonergic activity suggesting that these autoreceptors likely do not exert a tonic control on 5-HT release. However, the interpretation of a standard gene KO experiment is often complicated by possibilities of long-term developmental compensatory changes ([62] and see above for 5-HT1B KO mice).

5-HT4 receptor KO mice

Interestingly, recent findings pointed out another serotonergic receptor sub-type as being a novel putative target for antidepressant-like activity: 5-HT4 KO mice have therefore been recently generated to study their mechanism of action. These KOs display normal feeding and motor behaviors in basal conditions, but attenuated response to stress-induced hypophagia and novelty-induced exploratory activity [82]. Furthermore, they exhibit a reduced spontaneous electrical activity of 5-HT neurons in raphe nuclei associated with diminished brain tissue levels of 5-HT and 5-HIAA suggesting a tonic excitatory influence of 5-HT4 receptor type. Cumulative, systemic administration of the SSRI citalopram, reduces 5-HT cell firing by 30% in WT animals, and completely inhibits 5-HT neuron firing in the 5-HT4 KO mice. Other changes in the DRN of the KO mice include increases in the levels of the selective 5-HT transporter SERT and its mRNA [83]. However, the mechanisms by which 5-HT4 receptors mediate a tonic positive influence on the firing activity of DRN 5-HT neurons and 5-HT content remain to be determined. 5-HT4 KO mice also exhibit an increase in neuronal network excitability, which is unusual in the context of a genetic deficit that disrupts the ability of stress to reduce feeding and body weight.

Recent data suggest that 5-HT4 KO mice are a novel interesting animal model of mood disorders. In line with this hypothesis, it was shown that 5-HT4 receptor agonists could be a putative class of antidepressants with a rapid onset of action [84]. Indeed, in rats, 5-HT4 receptor agonists reduce the immobility time in the FST, thus displaying an antidepressant potential. Moreover, such compounds modify rat brain parameters considered to be the key markers of antidepressant action (desensitization of 5-HT1A autoreceptors, increased tonic on hippocampal postsynaptic 5-HT1A receptors, and enhanced of the active phosphorylated form of a transcription factor, the cyclic adenosine monophosphate responsive element binding protein cyclic AMP responsive element binding protein (CREB) protein and neurogenesis in the adult hippocampus); these effects were maximal after only 3 days of agonists treatment, while they are observed only after 2–3 weeks of treatment with SSRIs [84]. Then, a 3-day regimen with the 5-HT4 receptor agonist was sufficient to reduce the diminution of sucrose intake (which reflects anhedonic-like behavior) consequent to chronic mild stress in rats. This widely-accepted stress model of depression postulates that genetic factors contribute to biological vulnerability. The use of this latter animal model is important because it allowed analysis of behaviors with a good face and predictive validities and determining a time course of the response, which paralleled rapid and sustained electrophysiological responses in rats [84]. Here, the 5-HT4 receptor agonist RS 67333 indeed displays a more rapid onset of action (sucrose consumption) than classical antidepressant drugs.

Thus, the 5-HT4 receptor is a novel promising target in the field of anxiety and depression, which must be further explored. Indeed, if 5-HT4 receptor agonists display a rapid onset of antidepressant action, the opposite, i.e. a longer onset of antidepressant action could be observed in 5-HT4 KO mice. The use of other preclinical tests will be required to further evaluate this hypothesis before conducting clinical trials in depressed patients. For example, in the chronic mild stress model, the decrease in sucrose consumption is often associated with body weight loss, and physical state degradation (i.e. a degradation of coat state) and a decrease in grooming behavior in the splash test. The predictive validity of this model can be analyzed as these altered responses can be reversed by chronic SSRI administration [85]. In addition, it is well known that 5-HT4
receptor agonists can have side effects, especially on the gastrointestinal system and heart (atrial arrhythmia), which could limit their prescription in depressed patients.

To conclude, one of the main interests of these serotonin receptor KO mice for various sub-types (5-HT1A, 5-HT1B or 5-HT4) is to help better delineate relevant brain regions and serotoninergic circuits/paths involved in the regulation of mood disorders, as well as to learn more about the mechanism of action of antidepressant and anxiolytic drugs.

**Mutation of a Neurotrophic Factor**

**Brain-derived neurotrophic factor and neurogenesis in adult hippocampus**

As mentioned above, understanding the etiology and physiopathology of depression is a major challenge facing psychiatry. One recent hypothesis of depression stipulates that an impairment of neurogenesis in the adult hippocampus could precipitate depressive states. Isolation, characterization, and use of stem cells from the brain were first described by Fred H. Gage (see [86] for a review). Then, the regulation of neurogenesis phenomenon in the adult dentate gyrus of mammals was detailed by Elizabeth Gould [87,88]. It has been recently shown that chronic fluoxetine treatment accelerates the maturation and functional integration of newborn, immature neurons in the dentate gyrus in WT 129SvEv adult male mice [89]. The role of adult neurogenesis in both the pathophysiology and treatment of depression was progressively described in the mid 1990s (see [90] for a review), and lately [74]. Indeed, animal studies have shown that neurogenesis can be decreased by a variety of stimuli (aging; various stressors such as chronic mild stress; glucocorticoids) and antidepressant drugs are able to reverse the effects [73].

Brain-derived neurotrophic factor, belongs to the family of neurotrophins (together with Nerve Growth Factor NGF, NT-3, NT-4 and NT-5), is active as a homodimer and its biological effects appear following the activation of its high-affinity protein kinase receptor family TrkB (Tropomyosine-related kinase B). In humans, a clinical study reported reduced BDNF in the brains of unmedicated depressed patients [91]. We can thus infer that a decreased levels of specific neurotrophic factors (BDNF, NT-3, but not NGF: [92]), could participate to the hippocampal atrophy observed in depressed patients[93]. Chronic, but not acute, SSRI treatment by increasing 5-HT neurotransmission causes an increase in BDNF protein levels and expression (mRNA) most notably in the dentate gyrus granular cell layer of the hippocampus in adult rats [94,95]. However, it was recently demonstrated that acute treatment with antidepressants could also promote TrkB receptor phosphorylation within 30 min, indicating that antidepressants could induce BDNF release as well [96]. This cascade of events may contribute to the therapeutic effects of antidepressant drugs: BDNF in the adult hippocampus might be involved in this delay of onset of SSRIs. BDNF requires activation of the high-affinity protein kinase receptor family TrkB to exert its biological effects. Furthermore, reciprocal interactions between BDNF and 5-HT in the central nervous system have been proposed [97]. BDNF has trophic effects on 5-HT neurons in the central nervous system [98]. In these conditions, in our laboratory, we tried to understand the connection between BDNF and 5-HT systems by using a combined genetic and pharmacological approach.

The actual knowledge regarding the relationship between BDNF and 5-HT neurotransmission in the hippocampus is limited. For example, is there any reciprocal effect of BDNF on 5-HT neurotransmission? We reasoned that, if BDNF reduction plays a pivotal role in depression, an increase in hippocampal BDNF through its local delivery would improve the efficacy of SSRI treatment. Thus, to answer this question, we used adult WT or mutant mice and developed a dual experimental strategy by inducing either a decrease (data obtained in constitutive heterozygous BDNF +/- mice: 103) or an increase (data obtained following intra-hippocampal injection of BDNF in rats as well as in WT mice: 105, 111) in brain BDNF protein levels. First, we will examine below whether heterozygous BDNF +/- mice can develop behavioral abnormalities associated with specific dysfunctions of the brain serotonergic system.

**Constitutive heterozygous BDNF +/- mice**

The first BDNF mutant mice were generated by Ernfors et al., in 1994 [99] because this member of the neurotrophin family can prevent the death of particular peripheral sensory neurons and of central motor neurons as well as dopaminergic and cholinergic neurons of the basal forebrain during development. These authors showed that KO mice lacking BDNF (KO BDNF) have severe deficiencies in coordination and balance, associated with excessive degeneration in several sensory ganglia including the vestibular ganglion. Survival of sympathetic, midbrain dopaminergic and motor neurons was not affected.
Because of the early postnatal lethality of BDNF null mice, most studies using genetically manipulated mice referred to constitutive heterozygous BDNF +/- mice [100] or mice lacking its main receptor TrkB [101], or mice overexpressing the truncated isoform of the TrkB receptor [96,102].

Heterozygous BDNF +/- mice generated on a 129Sv genetic background, in which brain BDNF protein levels are decreased by half [100], develop enhanced intramale aggressiveness and hyperphagia accompanied by significant weight gain in early adulthood; these behavioral abnormalities are known to correlate with 5-HT dysfunction [103]. Young adult BDNF +/− mice show alterations in the expression of several 5-HT receptors in the cortex, hippocampus, and hypothalamus. The heightened aggressiveness can be normalized by chronic fluoxetine treatment [103]. Thus, from this pioneer study, we have learned that endogenous BDNF is critical for the normal development and function of central 5-HT neurons and for the elaboration of behaviors that depend on these nerve cells. Proliferation of adult progenitors and survival of immature neurons are significantly decreased in BDNF +/- mice [104]. Therefore, BDNF +/- mice provide a useful model to study human psychiatric disorders related to dysfunction of serotonergic neurons.

We have recently shown that constitutive BDNF +/- mice have increased basal extracellular 5-HT levels in the hippocampus associated with a decreased 5-HT reuptake capacity [105]. In keeping with these results, the SSRI paroxetine failed to increase hippocampal dialysate 5-HT levels in BDNF +/- mice compared with WT littermates. Using in vitro synaptosome techniques, we found a significant reduction in [3H]5-HT uptake in hippocampal synaptosomes, which reveals a decrease in SERT function. These results provide evidence that constitutive reductions in BDNF modulate SERT reuptake capacity in adult hippocampus. Results obtained by using chronoamperometry confirmed that 5-HT clearance rate increased markedly with age, and suggests that the profoundly reduced ability of 5- and 10-month-old BDNF+/− mice to clear 5-HT is not because of a decrease in the total number of SERT, but may be a result of functional deficits, e.g. in the machinery/signaling required for insertion of SERT into the plasma membrane and/or activation of the SERT once it is positioned to take up 5-HT from extracellular fluid [106].

By contrast, when BDNF protein levels was increased following its local infusion into adult hippocampus in WT mice, we found that BDNF decreased basal extracellular levels of 5-HT in the hippocampus, as measured by intracerebral microdialysis. In addition, perfusion with BDNF decreased KCl-evoked elevations of 5-HT, this effect being blocked by the non-selective antagonist of TrkB receptors, K252a. Thus, in adult hippocampus, a single injection of BDNF through TrkB activation, enhances SERT function. We hypothesized that such acute effects of BDNF would counteract early effects of SSRIs, which might, in part, account for some delay in therapeutic effect [107].

Age-related loss of serotonin axons in the hippocampus was potentiated in BDNF +/- mice compared with WT mice, particularly the CA1 sub-region [108]. By contrast, aging BDNF +/- mice showed increased serotonin innervation of the basomedial nucleus of the amygdala. The noradrenergic system was also altered in the BDNF +/- mice. Indeed, these mice showed reduced numbers of cell bodies and fibers in the locus coeruleus compared with age-matched WT mice, whereas no changes were observed in dopaminergic innervation with respect to genotype. Thus, reduced BDNF protein levels in the whole brain was associated with altered serotonergic and noradrenergic innervation in aging mice and, in particular, with accelerated loss of serotonergic innervation to the hippocampus.

Despite these results, the use of constitutive KO mice did not definitively allow determining: (i) the brain regions where BDNF mediates its excitatory effects on 5-HT system; and (ii) whether this neurotrophic factor plays a major role in the regulation of 5-HT during development and/or in adulthood. Conventional KO technology has limitations, such as lethal phenotype, or when gene function at a certain developmental stage must be elucidated [109].

**Inducible BDNF KO mice**

An inducible KO mouse is not by definition ‘tissue specific’, as the promoter is not necessarily restricted to particular tissue(s). A conditional deletion of a gene can be obtained by using a tetracycline-controlled gene expression system in the brain [109].

Inducible/conditional KO mice technology has the advantage of allowing the KO to take place after development/embryogenesis. For example, the strategy of conditional KO, is based on a tissue-specific inactivation of the gene of interest using a recombinase deleting the DNA fragment located between the two Lox-P recombinase-specific sites. A mouse bearing the recombinase-specific sites (introduced by homologous
recombination in ES cells) is bred with a mouse expressing the recombinase (generated by homologous recombination or transgenesis). The tissue-specific expression of the recombinase allows the inactivation of the gene of interest only in the tissue where the recombinase is expressed. The final performance of the model is strongly dependent on the quality of the recombinase expressing mouse.

Results obtained with conditional KO mice with floxed BDNF alleles allowed spatial and temporal regulation of BDNF deletion [110]. The use of conditional deletion and tissue specific BDNF KO mice was made possible by the development of more precise temporal and spatial regulation of gene expression [111]. Conditional deletion of BDNF in the postnatal brain leads to obesity and hyperactivity [110]. In addition, these mice allow dissection of the role of BDNF in depression-related behaviors and the responses to antidepressant drugs in two sub-regions of the hippocampus, the dentate gyrus and CA1, using the viral-mediated localized BDNF knockdown strategy [112].

Monteggia et al. (2004) [113] used an inducible KO system to show that deleting BDNF in broad forebrain regions of adult mice attenuates the effects of desipramine, an antidepressant, in the FST, connecting the BDNF role in adult brain with the antidepressant-like activity of these drugs. More recently, the same group injected different systems [i.e., adeno-associated virus (AAV-Cre to obtain KO adult mice) or AAV-GFP to obtain control mice] bilaterally into the dentate gyrus or CA1 of the hippocampus to selectively knockdown BDNF expression [112]. Then, a series of behavioral tests measuring locomotor activity, fear learning, depression, and anxiety-related behaviors was performed. Similar to what was found in total forebrain (including the hippocampus) in constitutive BDNF +/- mice, mice lacking BDNF in the CA1 or dentate gyrus did not show differences in baseline locomotion, anxiety, or depression-like behavior compared with control mice. However, dentate gyrus KO adult mice showed attenuated response to desipramine and citalopram, two common antidepressants, in the FST, whereas CA1 KO mice showed normal response to desipramine. This is the first study showing regional specificity of BDNF deletion within the hippocampus and how it affects antidepressant action [114]. These results are in good agreement with studies showing that infusions of BDNF into the hippocampus produce antidepressant-like effects in neurochemical (in mice: [107]) and behavioral tests (in rats: [92]; in WT mice: [115]).

Brain-derived neurotrophic factor conditional KO show gender differences in depression-related behaviors leading to an individual’s vulnerability to depression. By generating two independent lines of conditional BDNF KO mice in which the BDNF gene is deleted selectively in forebrain. Monteggia et al., (2007) [116] showed that male conditional BDNF KO mice exhibit hyperactivity, but normal depression-related behaviors. In contrast, female conditional BDNF KO mice display normal locomotor activity, but a striking increase in depression-like behavior. A conditional loss of BDNF gene in both male and female mice attenuates the actions of the antidepressant desipramine in the FST. These gender differences in depression-related behaviors in conditional BDNF KO mice provide direct evidence for a role of BDNF in depression. The results reinforce the hypothesis that forebrain BDNF may be essential in mediating antidepressant efficacy.

In agreement with all these data obtained in rodents, in humans, a common single-nucleotide polymorphism in the BDNF gene [a methionine (Met) substitution for valine (Val) at codon 66, Val66Met: 117], was found to be associated with alterations in brain anatomy and memory [118]. A ‘knock in’ BDNF mouse [BDNF(Met/Met)] that reproduces the phenotypic hallmarks described in humans was generated [119]. The variant allele BDNF(Met) was expressed in brain at normal levels in these mice, but its secretion from neurons was defective. When placed in stressful environments, BDNF(Met/Met) mice exhibited increased anxiety-related behaviors. Surprisingly, these behavioral changes were not normalized by the antidepressant, fluoxetine. Thus, a variant BDNF seems to play a key role in genetic predispositions to anxiety, and maybe depressive disorders.

These studies performed in genetically manipulated mice suggest that BDNF/TrkB receptor signaling plays a pivotal role in the action of antidepressants, rather than in the development and expression of depression per se [114]. It would be interesting to look at the postsynaptic sub-types of monoamine receptors activated by indirect receptor agonists (SSRI), in adult neurogenesis in hippocampal subregions of BDNF mutant mice compared with WT littermates. We need more time to obtain similar knowledges from a large set of different subclasses of non-monoaminergic antidepressant drugs. As these BDNF +/- mice display blunted neurochemical and behavioral responses to serotoninergic antidepressants, this strain of mice can be viewed as an animal model of resistance to these drugs rather than as a model of depression.
CONCLUSION

Discerning the neurobiology underlying antidepressant- and/or anxiolytic-like activities of SSRI may facilitate the development of new drugs to treat major depressive episodes or generalized anxiety.

Apart from the brain monoaminergic systems (5-HT, NE), other targets are of potential importance in the identification of new therapeutics by using pre-clinical tools. Indeed, molecular alterations that underlie the pathology or treatment of depression and brain regions and pathways involved are still poorly understood. The TWIK-1 related K+ channel (TREK-1) protein is a background K+ channel regulated by various neurotransmitters including 5-HT. In mice, the deletion of its gene (Kcnk2, also called TREK-1) led to animals with an increased efficacy of 5-HT neurotransmission and changes in different behavioral tests related to antidepressant-like activity [120]. TREK-1-deficient (Kcnk2) mice showed behavior similar to that of naïve animals treated with classical antidepressants such as fluoxetine. These results indicate that alterations in the functioning, regulation or both of the TREK-1 channel may alter mood, and that this particular K+ channel may be a potential target for new antidepressants.

Progress in the field of DNA sequencing have resulted in the complete genome sequences of man and different model organisms (Drosophila, mice, rat, etc.). KO animal models are experimental tools for understanding genetic vulnerability to anxiety, depression and their respective pharmacological treatments. One of the main interests of animal models of depression is to discover susceptibility genes with strong link to psychiatric disorders, thus allowing the identification of people at risk. However, the genetic association of known polymorphism with depression and anxiety (e.g. cingulated cortex, amygdala and emotion [40])

The recent literature clearly demonstrates how each genetically modified mouse can help to understand the action of antidepressant.

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