Mutant mouse models and antidepressant drug research: focus on serotonin and brain-derived neurotrophic factor

Alain M. Gardier

Several lines of knockout (KO) mice have been evaluated as models of depression-related behavioral and neurobiological changes, and used to investigate molecular and cellular mechanisms underlying the activity of antidepressant drugs. Adult neurogenesis and brain 5-hydroxytryptamine (5-HT)/ neurotrophic factor interactions have recently attracted great interest in relation to the mechanism of action of antidepressant drugs. The present review focuses primarily on genetic manipulation of the serotoninergic (5-HT) system. Basal neurochemical and behavioral changes occurring in mice lacking the 5-HT transporter (SERT), which is the main target of antidepressant drugs, as well as in those lacking G protein-coupled serotonin receptors (e.g. 5-HT1B, 5-HT1A, and 5-HT4 receptors) are described and evaluated. The importance of KO mice for neurotrophic factors, particularly for brain-derived neurotrophic factor and its high-affinity receptor (R-TrkB), is also addressed. Constitutive KO, tissue specific, or inducible KO mice targeting both 5-HT and brain-derived neurotrophic factor systems may potentially make an important contribution to knowledge of the pathophysiology and treatment of depression. ***Behavioural Pharmacology*** **20:18–32 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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University Paris-Sud, EA 3544 Faculty of Pharmacy, Chatenay-Malabry Cedex, France

Correspondence to Alain M. Gardier, University Paris-Sud, EA 3544, Faculty of Pharmacy, 5, rue J-B Clement, Tour D1, 2e etage, Serotonine et Neuropharmacologie, Chatenay-Malabry Cedex F-92296, France
E-mail: alain.gardier@u-psud.fr

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Introduction

Depression and anxiety disorders are common public health problems with a 17% lifetime prevalence (Levinson *et al.*, 2006). However, the molecular and cellular mechanisms underlying these disorders are still poorly understood. Antidepressant drugs such as selective serotonin [5-hydroxytryptamine (5-HT)] reuptake inhibitors (SSRIs) are effective in treating mood as well as anxiety disorders (Morilak and Frazer, 2004). However, these drugs have several limitations. In particular, while they produce a relatively fast blockade of the 5-HT transporter (SERT) *in vitro*, the onset of an appreciable antidepressant-like effect *in vivo* is slow, taking several weeks to occur in humans (Katz *et al.*, 2004) as well as in animals (Dulawa *et al.*, 2004). Experiments carried out in rodents showed that this delayed onset of action is likely related to the requirement of adaptations of presynaptic receptors (see below), that is, a functional desensitization of inhibitory 5-HT1A autoreceptors (Blier and de Montigny, 1994). This latency represents an important problem because major depressive disorders (MDD) are often associated with a high risk of suicide. Thus, the search for the origin of these diseases and for rapidly acting antidepressant drugs has been a subject of intense research for several decades.

The use of genetically manipulated rodents (mainly mice: see below) has contributed to answers, at least in part, to these questions. Many preclinical studies have already been carried out in the field of anxiety and depression, but mostly in healthy, 'nondepressed' animals. New animal models are needed to understand better the underlying mechanisms limiting the effects of currently available treatments of MDD, and factors leading to disorders such as anxiety and depression. It is now widely recognized that MDD results from a combination of interacting environmental and genetic factors (Mill and Petronis, 2007). In humans, environmental factors such as stressors are postulated to play a role in the etiology of the disorder and to increase the susceptibility to MDD. It has also been shown that, in mice, genetic factors play a key role in the etiology of depression-like behaviors (El Yacoubi *et al.*, 2003). Since the early 1990s, animal models such as knockout (KO) or transgenic rodents have been developed to identify basal functions and behaviors impaired by the mutation of a gene of interest. These genetically manipulated mice can also provide information on the mechanism of action of antidepressants. This is the case when a selective mutation of a gene alters responses in neurochemical and/or behavioral tests carried out after acute or chronic drug administration.

The first KO mice were generated by homologous recombination (Silva *et al.*, 1992). The mouse is a model organism of choice for this purpose because: (i) many of its genes have an equivalent in humans, (ii) numerous biological and biochemical functions of the mouse are similar to those of humans, and (iii) the genome of the mouse is easily manipulable by homologous recombination.

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This strategy allowed the creation of relevant animal models of human brain disorders. Genetic background is a fundamental parameter for the analysis of the phenotype of KO mice. Historically, mutant mice were established using embryonic stem (ES) cells of the 129/Sv line. However, the establishment of new mutant lines on a genetic C57BL/6 background is now generally preferred, even though there are some limitations to the use of this strain in some behavioral tests (Mayorga and Lucki, 2001).

The characterization of the phenotype of these KO mice relies on the availability of a large set of behavioral tests evaluating basal anxiogenic-like and depressive-like states, taking into account any alterations in locomotor activity. For example, among the most useful behavioral tests, the Porsolt forced swim test (FST; Porsolt *et al.*, 1977) and tail suspension test (TST; Steru *et al.*, 1985) are stress paradigms aimed at screening potential antidepressants in controls (effects) versus KO (no effects) mice. In these behavioral tests, the animal is placed in a situation that induces immobility, which is counteracted by antidepressant drugs such as SSRIs. 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 used to screen the antidepressant-like and/or anxiolytic-like activities of novel molecules after their acute administration.

A better animal model might be represented by KO mice exhibiting alterations resembling those classically observed in depressed patients, notably regarding chronic stress, changes in sleep–wakefulness, or alterations in body weight and food intake. In addition, increases in plasma corticosterone levels, changes in serotonin metabolism index in brain tissue homogenates, in firing activity of 5-HT neurons in the nucleus raphe dorsalis and consequences of serotonin (5-HT1A) autoreceptor stimulation are also studied. Thus, this study presents examples of different lines of KO mice exhibiting a decrease in serotonergic tone, comparable with that associated with endogenous depression in humans. The behavioral impairments and serotonergic dysfunctions measured in KO mice are studied by comparing them with wild-type (WT) control mice, first in basal conditions, then after an acute or chronic treatment with an antidepressant drug. Some of these KO mice provide an opportunity to approach genes influencing susceptibility to anxiety and depression.

Recently, compelling evidence suggested that antidepressant drugs exert their behavioral activity in adult rodents through cellular and molecular changes in the hippocampus as well as in other brain structures. Chronic antidepressant treatment can regulate the expression of brain-derived neurotrophic factor (BDNF; Nibuya *et al.*, 1995) and stimulate adult neurogenesis (proliferation, differentiation, and survival of neural progenitor) in the dentate gyrus of rats (Malberg *et al.*, 2000) and mice (Santarelli *et al.*, 2003; David *et al.*, 2007). Studies carried out in heterozygous BDNF $^{+/-}$ mice as well as 5-HT1A KO mice have contributed to this research.

Thus, this study focuses first on mutations of specific serotonergic targets (i.e. 5-HT transporter, 5-HT1B, 5-HT1A, and 5-HT4 receptors), because such KO mice very often exhibit changes in phenotypes resembling that induced by chronic treatment with antidepressants. KO mice for these targets 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 antidepressant therapy. In addition, BDNF KO mice are described because BDNF–5HT interactions are now attracting considerable interest.

Mutation of serotonergic elements

5-hydroxytryptamine transporter knockout mice

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 (Bengel *et al.*, 1998; see reviews by Canli and Lesch, 2007; Murphy and Lesch, 2008). 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 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 (Bengel *et al.*, 1998). It has been suggested that these 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.

In humans, a long allele and a short allele of the SERT gene have been described. Lesch *et al.* (1996) provided the first demonstration that a functional polymorphism in the promoter region of the SERT gene (SLC6A4) is associated with anxiety-related and depression-related personality traits and antidepressant drug resistance (Serretti *et al.*, 2005). Of course, these traits can be amplified by gene–environment and gene–gene interactions. A reduced SERT function associated with greater neuronal activity in the amygdala (a brain region activated in response to emotion, fear, stress, and anxiety) was found in individuals with one or two copies of the short allele of the SERT promoter (Hariri *et al.*, 2002). In addition, humans with one

or two copies of the short allele of the SERT promoter polymorphism exhibit more depressive symptoms and suicidality in relation to stressful life events than individuals homozygous for the long 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 (Caspi *et al.*, 2003). Thus, therapeutic responses and side effects after treatment with SSRIs in depressed patients were also associated with SERT gene (SLC6A4) variants (for review, see Murphy and Lesch, 2008).

SERT function-modifying gene variants in humans apparently produce many phenotypes that are similar to those found in KO mice. Indeed, SERT KO mice are interesting in relation to both depression and anxiety. 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 increased anxiety, stress and depression-related behaviors in basal conditions (e.g. changes in body weight: Holmes *et al.*, 2002; sleep alterations: Alexandre *et al.*, 2006) 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 (Alexandre *et al.*, 2006; for review, see also Fox *et al.*, 2007). Thus, these models are very relevant for studying hereditary influences on depression.

SERT 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 (Mathews *et al.*, 2004; Szapacs *et al.*, 2004). Not surprisingly, the absence of normal responses to SSRI antidepressants in SERT KO mice showed that effects on SERT are a critical mechanism of action of members of this class of antidepressant (Bengel *et al.*, 1998). Specific labeling with radioligands and antibodies, and competitive real-time-PCR, 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 versus WT control mice (Fabre *et al.*, 2000). In line with these effects, a robust and time-dependent downregulation of the SERT occurred in rodents after chronic SSRI administration (Piñeyro *et al.*, 1994; Benmansour *et al.*, 2002).

More has been learned with another SERT KO model, also generated by homologous recombination, that the targeted deletion also involves exon 2 of the transporter-coding region. However, in this model, ES cells were derived from a 129S6/SvEv background strain, and resulting chimeras were then backcrossed to 129S6/SvEv mice (Lira *et al.*, 2003). Interestingly, a transient inhibition of SERT with fluoxetine, a SSRI, administered during early development (between postnatal days 4 and 21, P4 and P21) produced abnormal emotional behaviors in adult WT mice. This effect mimicked the behavioral phenotype of adult SERT KO mice, showing a reduced exploratory behavior, that is, an anxiogenic-like state, in the elevated-plus-maze test (Ansorge *et al.*, 2004). Thus, when 5-HT reuptake is blocked, an excess of extracellular 5-HT in synaptic cleft can lead to overstimulation of presynaptic and/or postsynpatic 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. Therefore, 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 WT mice (Ansorge *et al.*, 2008).

Thus, SERT KO mice show increased anxiety-related and stress-related behaviors, suggesting the occurrence of an anxiogenic-like state. To our knowledge, no studies have tried to reverse these behavioral effects. However, sleep impairment occurring at adulthood in SERT KO mice (in which rapid eye movement sleep is enhanced compared with WT mice; Alexandre *et al.*, 2006) 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 (Alexandre *et al.*, 2006). Here, SERT KO mice displayed an anxiogenic-like phenotype, whereas a long-term treatment (e.g. 4 weeks) with an antidepressant drug is often anxiolytic in procedures such as the open field (Dulawa *et al.*, 2004). In line with these findings, it was found that healthy individuals carrying a gene polymorphism of the short 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 (Pezawas *et al.*, 2005). Thus, not surprisingly, the absence of normal responses to SSRI antidepressants in SERT KO mice (Holmes *et al.*, 2002; Fox *et al.*, 2008) shows again that actions on SERT are a critical mechanism of action of members of this class of antidepressants (Fox *et al.*, 2007).

5-hydroxytryptamine receptor knockout mice

The monoaminergic hypothesis of depression suggests that depression results from a deficiency of brain 5-HT and/or noradrenaline functions. This hypothesis

dominated the field for decades and was mainly supported by the effectiveness of antidepressant drugs such as imipramine derivatives and SSRIs that increase 5-HT neurotransmission by preventing the reuptake of these neurotransmitters.

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 cell body 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 (Blier, 2001). Consequently, despite the 5-HT reuptake inhibition also taking place at nerve terminals, there is a decrease in 5-HT cell firing through activation of 5-HT1A (somatodendritic) or 5-HT1B (nerve terminal) autoreceptors (Rutter *et al.*, 1995; Artigas *et al.*, 1996), leading to a moderate increase in extracellular 5-HT levels at serotonergic terminals. To alleviate this problem occurring after acute SSRI treatment, 5-HT1 autoreceptor antagonists have been coadministered with antidepressants (Gardier *et al.*, 2003). It is, thus, logical to believe that 5-HT1A or 5-HT1B receptor KO mice may be interesting animal models displaying a higher serotonergic tone. Thus, these models are very relevant for studying the role of 5-HT autoreceoptors in the mechanism of antidepressant action. In addition, this review will also focus on mice lacking the 5-HT4 receptor because, very recently, this KO mouse was used to show that agonists of this receptor subtype elicit an antidepressant-like activiy with a rapid onset of action.

5-HT1B receptor knockout mice

5-HT1B receptors are expressed throughout the brain of rodents. These receptors are located in the axon terminals of both serotonergic and nonserotonergic neurons, where they act as inhibitory autoreceptors or heteroreceptors, 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, as these receptors play a major role in the regulation of 5HT release in various brain regions, including the hippocampus.

Mice lacking the 5-HT1B receptor subtype do not exhibit any obvious developmental or behavioral defects (Saudou *et al.*, 1994). 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. These data might be related to the fact that a class of 5-HT1 receptor agonists, termed serenics, have antiaggressive properties, and to the findings that certain impulsive aggressive behaviors are associated with deficits in central 5-HT (Ramboz *et al.*, 1996).

Subsequent studies examined the consequences of the constitutive lack of 5-HT1B receptor on the regulation of basal and evoked-release of 5-HT at nerve terminals, which were investigated either *in vitro* (Piñeyro *et al.*, 1995) or *in vivo* (Trillat *et al.*, 1997, 1998). Using slices obtained from the brains of 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 frontal cortex slices of 5-HT1B KO mice. The selective 5-HT1B receptor agonist CP 93129 and the 5-HT1B/1D agonist sumatriptan, inhibited [3H]5-HT release in hippocampus and cortical slices obtained from control mice, but had no effect in KOs. 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 (Piñeyro *et al.*, 1995).

Basal extracellular 5-HT levels as measured by in-vivo microdialysis have also been compared in conscious WT versus 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 through reverse microdialysis of the selective 5-HT1B receptor agonist CP-93129 significantly decreased basal 5-HT release in the WT mice, 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 (Trillat *et al.*, 1997).

In addition, the effects of some antidepressant drugs were evaluated in 5-HT1B KO mice after their acute (Trillat *et al.*, 1997, 1998) or chronic (Gardier *et al.*, 2003) administration. A single dose of paroxetine increased extracellular 5-HT levels in both genotypes, and these effects were potentiated in the ventral hippocampus, but not in the frontal cortex, in 5-HT1B KO mice compared with WT mice (Trillat *et al.*, 1998). Furthermore, SSRIs decreased the immobility of WT mice in the FST, and this effect was absent in 5-HT1B KO mice, showing that activation of 5-HT1B receptors mediates the antidepressant-like effects of SSRIs in the FST. Our results suggest that activation of terminal 5-HT1B autoreceptors limits the effects of SSRIs, particularly in the hippocampus, whereas postsynaptic 5-HT1B heteroreceptors are likely required for the antidepressant activity of SSRIs (Trillat *et al.*, 1998; Malagié *et al.*, 2001; De Groote *et al.*, 2002).

Conversely, Mayorga *et al.* (2001) found that the immobility time in the TST was increased by fluoxetine in 5-HT1B KO mice compared with WT mice. These data suggest that

activation 5-HT1B heteroreceptors also limits the antidepressant-like activity of fluoxetine. More behavioral studies in KO mice are needed, to establish whether antidepressant-like effects of SSRIs are mediated by presynaptic or postsynaptic 5-HT1B receptors.

To date, only one intracerebral in-vivo microdialysis study has described neurochemical responses after chronic SSRI administration in 5-HT1B KO mice (Gardier *et al.*, 2003). The results were disappointing, because chronic administration of paroxetine through 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. The pharmacokinetic properties of paroxetine were similar in KOs to those found in the control group. These data suggest that terminal 5-HT1B receptors retain their capacity to limit 5-HT release, mainly in the ventral hippocampus, after chronic paroxetine administration, that is, these autoreceptors were not desensitized (Gardier *et al.*, 2003). However, the absence of 5-HT1B autoreceptor desensitization remains somewhat equivocal, as in-vitro evidence in guinea pigs indicated that the electrically evoked-release of [3H]5-HT was enhanced in hippocampal and cortical slices after sustained administration of SSRIs (El Mansari and Blier, 1996). The guinea pig is the animal of choice to discriminate between 5-HT1B and 5-H1D receptors (Wilkinson and Middlemiss, 1992). Indeed, several studies suggested that the guinea pig 5-HT terminal autoreceptor is a 5-HT1D receptor. These findings reinforce the species homology between the 5-HT1B receptors in humans and 5-HT1D receptors in guinea pig.

Interestingly, female 5-HT1B receptor KO mice displayed a significantly reduced basal immobility, relative to either male 5-HT1B KO mice or male and female WT mice in the TST and FST (Jones and Lucki, 2005). It was concluded that these KO mice show a sex-linked disinhibition of 5-HT release that sustained higher baseline levels of hippocampal 5-HT and behavioral vulnerability to 5-HT depletion.

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 knocked out during embryonic life, generally affecting the whole organism throughout its lifetime (Snyder, 2002). 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 psychostimulants in the nucleus accumbens and basal or cocaine-evoked dopamine release in projection areas of mesostriatal or mesoaccumbens dopamine neurons (Shippenberg *et al.*, 2000). An alternative compensatory mechanism would be a decrease in the efficiency of G-protein coupling to 5-HT1A receptors in 5-HT1B KO mice (Ase *et al.*, 2002). In our laboratory, we tested for adaptive compensatory changes that may have occurred during development in the functional activity of somatodendritic 5-HT1A receptors in 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-propylamino)tetralin (8-OH-DPAT). We found a higher efficacy of 8-OH-DPAT-induced hypothermia in 5-HT1B KO than in WT mice, suggesting an adaptive thermoregulatory process involving a hyperfunctional activity of somatodendritic 5-HT1A receptors in KO mice lacking 5-HT1B receptors (Gardier *et al.*, 2001). Heart rate and temperature in 5-HT1B KO mice also increased markedly in response to transportation and handling procedures, suggesting a physiological hyperreactivity of these KO mice (Bouwknecht *et al.*, 2000). 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 (Clifton *et al.*, 2003). These effects result from a long-term adaptation to the loss of 5-HT1B receptor function in these KOs. Decreased basal heart rate and increased basal body temperature (i.e. exaggerated autonomic responses to novel cage stress) have also been described in 5-HT1B KO mice (Groenink *et al.*, 2003).

5-HT1A receptor knockout mice

5-HT1A receptors are presynaptic and postsynaptic 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 seems 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 subtype represents a potentially more important regulatory site for modulating the actions of 5-HT in the brain, compared with the nerve terminal 5-HT1B receptor subtype. The role of this 5-HT receptor subtype 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 is able to 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 (Artigas, 1993). In other words, the selective blockade of inhibitory autoreceptors may augment the ability of SSRIs to elevate synaptic 5-HT levels (Julius, 1998). This hypothesis is related to the functional

Table 1 Main phenotypic changes found in 5-HT1B KO mice

Tests	Effects	References
Intracerebral in-vivo microdialysis	Increased dopamine release in the nucleus accumbens, but not in the striatum	Shippenberg *et al.* (2000)
Stimulation of [(35)S]GTP(γ)S incorporation by a 5-HT1B receptor agonist 5-CT	Decreases in efficiency of G-protein coupling to 5-HT1A receptors	Ase *et al.* (2002)
Hypothermia induced by 8-OH-DPAT	Higher efficacy of a 5-HT1A receptor agonist to induce hypothermia	Gardier *et al.* (2001)
Body temperature responses to various stimuli	Increased heart rate and increased temperature in response to transportation and handling	Bouwknecht *et al.* (2000)
Food intake[a], locomotor activity	Reduction in 5-HT2C receptor-mediated functions	Clifton *et al.* (2003)
Body weight[a]	Increased (+17% in males, +9% in females) compared with wild-type mice	Bouwknecht *et al.* (2001)
Effects of stress and 5-HT1A receptor agonists on corticosterone release through HPA axis activation	Larger brains[a] relative to body weight Normal basal corticosterone levels in plasma More aggressive[a], but normal plasma testosterone levels	
Basal heart rate and body temperature of 5-HT1B KO mice, and their autonomic responses to novel cage exposure and to reversal of the light-dark rhythm	Increased autonomic responses to stress[a]	Groenink *et al.* (2003)

5-CT, 5-carboxyamidotryptamine; 5-HT, 5-hydroxytryptamine; 8-OH-DPAT, 8-hydroxy 2(di-n-propylamino)tetralin; [(35)S]GTP(γ)S, guanosine 5′-O-(γ-[(35)S]thio)triphosphate; HPA, hypothalamus-pituitary-adrenal; KO, knockout.
[a]Parameters related to the symptoms of anxiety and/or depression.

desensitization of 5-HT1A autoreceptors that occurs after chronic SSRI administration.

In 1998, three different groups showed that the 5-HT1A KO mouse is an interesting animal model of anxiety-related disorders, and can also be used to predict the anxiolytic-like potential of novel agents. The results were obtained following targeted inactivation of this gene by homologous recombination on different genetic backgrounds in three different laboratories, with testing under similar, but not identical, experimental conditions (Heisler *et al.*, 1998; Parks *et al.*, 1998; Ramboz *et al.*, 1998; using BALB/cJ, 129S6/SvEv, and Swiss mice, respectively: Table 2). All the three studies 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, behaviors consistent with increased anxiety and stress responses. In the FST (Parks *et al.*, 1998; Ramboz *et al.*, 1998) as well as in the TST (Heisler *et al.*, 1998), 5-HT1A KO mice displayed a shorter immobility time, suggesting that lack of functional 5-HT1A receptors favors an antidepressant-like effect, at least under these experimental conditions (Julius, 1998). The phenotype of this 5-HT1A KO mouse seems paradoxical, as heightened anxiety is most often associated with depression (Ramboz *et al.*, 1998). However, these experiments did not involve any administration of antidepressants to these KO mice; these are not the most appropriate experimental conditions, as these tests were designed and validated to screen for antidepressant-like activity.

Although the core phenotype of anxiety can be reproduced in KO mice from various inbred and outbred backgrounds, abnormalities in 5-HT dynamics and resistance to the anxiolytic drug diazepam have been seen in one [i.e. in mice generated by Toth (2003) on the Swiss Webster genetic background], but not on other genetic backgrounds of 5-HT1A KO mice; this indicates that, although the development of anxiety is an invariable consequence of receptor deficit, other features induced by receptor loss are strongly modulated by other genes.

Inducible KO strategies enable the acute elimination of protein expression in adult animals. The 5-HT1A receptor is currently the only 5-HT receptor subtype to which this strategy has been applied. The use of constitutive KO mice for these 5-HT receptor subtypes does not discriminate 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 (Gross *et al.*, 2002). The authors found that mice lacking 5-HT1A receptors throughout the brain showed pronounced anxiety-like behavior, whereas those having a selective restoration of 5-HT1A receptors in the forebrain had normal behavior. Behavioral and neurochemical experiments carried out in these mice also suggested that postnatal developmental processes help to establish adult anxiety-like behavior. Indeed, by using mice in which the 5-HT1A receptor could be knocked out temporarily, it was shown 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, whereas those in the hindbrain

Table 2 Main phenotypic changes found in 5-HT1A KO mice

Genetic background	Tests	Effects	References
BALB/cJ mice	Open field, FST	Increased tendency to avoid a novel and fearful environment	Parks *et al.* (1998)
	Hippocampal-dependent learning and memory test; synaptic plasticity and neuronal excitability	Impaired	Sarnyai *et al.* (2000)
129S6/SvEv mice	Anxiety-related disorder (EPM, open field) Electrophysiology in brain slices	Decreased immobility in the FST But no significant difference from wild-type controls in the electrically evoked-5-HT release	Ramboz *et al.* (1998)
C57BL/6J mice	Home-cage activity, rotarod, open field, elevated-plus-maze, novel object, tail suspension test	Elevated anxiety levels in various assays Antidepressant-like responses in a tail-suspension assay	Heisler *et al.* (1998)
C57BL/6J from Toth (2003); Parks *et al.* (1998)	Intracerebral in-vivo microdialysis	DRN effects of an acute fluoxetine dose on dialysate 5-HT levels were potentiated in KOs	Bortolozzi *et al.* (2004)
		DRN and FCx effects of an acute paroxetine dose on dialysate 5-HT levels were potentiated in KOs	Guilloux *et al.* (2006)
129S6/SvEv from Ramboz *et al.* (1998)	Intracerebral in-vivo microdialysis (striatum, hippocampus)	Effects of low fluoxetine dose were potentiated in the striatum, but not in the hippocampus	Knobelman *et al.* (2001)
	Tail suspension test	Decreased basal immobility; no effect of a single dose of SSRI	Mayorga *et al.* (2001)

5-HT, 5-hydroxytryptamine; DRN, dorsal raphe nucleus; EPM, elevated-plus-maze; FCx, frontal cortex; FST, forced swim test; KO, knockout; SSRI, serotonin-selective reuptake inhibitors.

are less involved. Anxiety seems to be linked to the presence of 5-HT1A receptors in a specific brain region, at a particular period of development: these data add a new layer to the understanding of the involvement of 5-HT in the pathophysiology of anxiety.

Neurochemical experiments (especially intracerebral in-vivo microdialysis) carried out in 5-HT1A KO mice complement well with these behavioral studies. On the basis of the role of somatodendritic 5-HT1A autoreceptors in the feedback regulation of the 5-HT system, an increase in 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 and also in humans (Parks *et al.*, 1998; Toth, 2003). However, it was surprising to observe that 5-HT1A KO mice had normal brain tissue levels of 5-HT and 5-hydroxyindoleacetic 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 at serotonergic tonic nerve terminals in the frontal cortex (Bortolozzi *et al.*, 2004; Guilloux *et al.*, 2006). 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. This suggests that decreases in presynaptic 5-HT1A receptor density, caused by genetic defects or environmental stressors, might result in certain conditions in heightened anxiety, without changes in 5-HT neurotransmission (Ramboz *et al.*, 1998). Further investigations are necessary to explain these behavioral changes and try to link them to specific alterations of other neurotransmitter systems. As benzodiazepines are indirect agonists of γ-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 is reduced and GABAergic inhibition is impaired in the amygdala and hippocampus of these KO mice (Sibille *et al.*, 2000). 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 carried out in KO mice have also provided interesting data. The 5-HT1A receptor agonist of reference, 8-OH-DPAT, reduced extracellular 5-HT levels in the raphe nuclei to 30% of basal values in WT mice, but not in 5-HT1A KO mice. Fluoxetine or paroxetine (SSRI) increased 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 (Bortolozzi *et al.*, 2004; Guilloux *et al.*, 2006). 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 have also been used to study the mechanism of action of the β1,2 adrenoceptor antagonist, pindolol. This compound is known to shorten the delay of action of SSRIs in depressed patients (see the recent meta-analysis of Ballesteros and Callado, 2004), but it was uncertain whether this effect was mediated by somatodendritic 5-HT1A autoreceptor blockade. We thus studied the effects of coadministration of (+/−)-pindolol and paroxetine in 5-HT1A KO mice (Guilloux *et al.*, 2006).

Paroxetine dose-dependently 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 after local intraraphe 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 5-HT1A 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 autoreceptor in mice, but its blockade of paroxetine-induced antidepressant-like effects in the FST may be because of its binding to other neurotransmitter receptors, which are likely located postsynaptically (Guilloux *et al.*, 2006).

5-HT1A KO mice have also been used to study antidepressant-induced neurogenesis. Neurogenesis in the adult mammalian brain can be divided into several steps including proliferation of neural stem cells, and their maturation, migration, and differentiation into neurons in adult hippocampus (Malberg *et al.*, 2000; Duman *et al.*, 2001). The survival, that is, the balance between life and death of new neurons, occurs in a 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 the 129S6/SvEv background, were insensitive to the neurogenic and behavioral effects of chronic treatment with the SSRI fluoxetine (Santarelli *et al.*, 2003). Suppression of hippocampal neurogenesis by X-ray irradiation of a restricted region of mouse brain containing the hippocampus also abolished the behavioral effects of antidepressant drugs (Santarelli *et al.*, 2003). At the time, these data suggest that: (i) the behavioral effects of chronic antidepressants may be mediated by the stimulation of the proliferative step of neurogenesis in the hippocampus; (ii) the 5-HT1A postsynaptic receptor is necessary to the effects of SSRIs on adult neurogenesis in mice on 129S6/SvEv background, but not on BALB/cJ background, as the behavioral effects of chronic fluoxetine in BALB/cJ mice do not require adult hippocampal neurogenesis or 5-HT1A receptor function (Holick *et al.*, 2008); and (iii) the etiology of depression could involve neurodegeneration and impairments of growth of new neurons. Although attractive, this neurogenesis hypothesis of depression is still a matter of debate (Vollmayr *et al.*, 2003; Sahay and Hen, 2007; Schmidt and Duman, 2007; Cunningham and Watson, 2008).

5-HT1A KO mice also exhibit cognitive abnormalities reminiscent of symptoms of stress-related psychiatric disorders, namely, a deficit in hippocampal-dependent learning and memory tests, such as the Morris water maze. Synaptic plasticity in the hippocampus and limbic neuronal excitability were also impaired in 5-HT1A KO mice as compared with WT control mice (Sarnyai *et al.*, 2000). These data show that 5-HT1A receptors are required for the maintenance of normal hippocampal functions, and play a role in hippocampal-related symptoms, such as the cognitive disturbances observed in stress-related disorders.

Taken together, these data obtained using 5-HT1A KO mice show or confirm a role of this 5-HT receptor subtype in mood and stress-related disorders (anxiety, depression), in various aspects of the mechanism of action of SSRIs (their impact on 5-HT neurotransmission and on neurogenesis), in the regulation of sleep as well as in learning and memory. It can be argued that 5-HT1A KO mice represent a genetic animal model of anxiety with both construct and face validities (for review, see Toth, 2003). Apart from these advantages, however, receptor KO mice also have some drawbacks: 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 (Julius, 1998 and see above for 5-HT1B KO mice).

5-HT4 receptor knockout mice

Interestingly, recent findings have identified another 5-HT receptor subtype as potentially relevant to the mechanism of action of antidepressant drugs. 5-HT4 KO mice display normal feeding and motor behaviors in basal conditions, but attenuated response to stress-induced hypophagia and novelty-induced exploratory activity (Compan *et al.*, 2004). These results provide the first example of a genetic deficit that disrupts the ability of stress to reduce feeding and body weight and suggest that 5-HT4 receptors may be involved in stress-induced anorexia. Furthermore, these mice exhibit a reduced spontaneous electrical activity of 5-HT neurons in raphe nuclei associated with diminished brain tissue levels of 5-HT and 5-hydroxyindoleacetic acid, suggesting a tonic excitatory influence of the 5-HT4 receptor. Cumulative, systemic administration of the SSRI citalopram, reduced 5-HT cell firing by 30% in WT animals, but completely inhibited 5-HT neuron firing in 5-HT4 KO mice. Other changes in the DRN of the KO mice include increases in levels of the selective SERT and its mRNA (Conductier *et al.*, 2006). However, the mechanisms by which 5-HT4 receptors mediate a tonic positive influence on the firing activity of DRN 5-HT neurons and on 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 the 5-HT4 KO mouse is a novel interesting animal model of mood disorders. In line with this hypothesis, it was shown in WT rats that 5-HT4 receptor agonists could be a putative class of antidepressants with a rapid onset of action (Lucas *et al.*, 2007). 5-HT4 receptor agonists reduced immobility time in the FST, thus displaying an antidepressant potential. Moreover, a 3-day regimen with the 5-HT4 receptor agonist RS 67333 was sufficient to reduce the decrease in sucrose intake (which reflects anhedonic-like behavior) consequent to chronic mild stress in rats, a model of depression that has good face, predictive, and construct validities (Willner, 2005). The use of this latter animal model is important because it was possible to determine a time course of the response, which paralleled rapid and sustained electrophysiological responses in rats (Lucas *et al.*, 2007). In 5-HT4 KO mice, RS 67333 displayed a more rapid onset of action (restoration of sucrose consumption) than classical antidepressant drugs. 5-HT4 receptor agonists have also been shown, in WT rats, to modify brain parameters considered to be the key markers of antidepressant action (desensitization of 5-HT1A autoreceptors, increased tonus on hippocampal postsynaptic 5-HT1A receptors, enhanced phosphorylation of a transcription factor, the cyclic AMP responsive element-binding protein, and neurogenesis in the adult hippocampus). Again, these effects were maximal only after 3 days of agonists treatment, whereas they are observed only after 2–3 weeks of treatment with SSRIs (Lucas *et al.*, 2007).

Thus, the 5-HT4 receptor is a novel promising target in the field of anxiety and depression, which must be further explored. However, it is well known that 5-HT4 receptor agonists can have side effects, especially on the gastrointestinal system and heart (atrial arrhythmia; Duman, 2007), which could limit their prescription in depressed patients.

Mutation of a neurotrophic factor

Brain-derived neurotrophic factor and neurogenesis in adult hippocampus

One recent hypothesis of depression stipulates that an impairment of neurogenesis in the adult hippocampus could precipitate depressive states. Beginning with the description of the isolation, characterization, and use of stem cells from the brain (for review, see Gage *et al.*, 1995), and the regulation of neurogenesis in the dentate gyrus of adult mammals (Cameron and Gould, 1994; Gould, 2007), the role of adult neurogenesis in both the pathophysiology and treatment of depression was progressively revealed in the mid 1990s (for review, see Duman *et al.*, 1999), and more recently (Santarelli *et al.*, 2003). Chronic fluoxetine treatment accelerates the maturation and functional integration of newborn, immature neurons in the dentate gyrus in WT 129S6/SvEv adult male mice (Wang *et al.*, 2008b). Conversely, 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 these effects (Duman *et al.*, 2001).

BDNF belongs to the family of neurotrophins (together with nerve growth factor, NT-3, NT-4, and NT-5). BDNF is active as a homodimer, and its biological effects seem after the activation of its high-affinity protein kinase receptor family tropomyosine-related kinase B (TrkB). In humans, a clinical study reported reduced BDNF protein levels in the brains of unmedicated depressed patients (Dwivedi *et al.*, 2006). We can thus infer that decreased levels of specific neurotrophic factors (BDNF, NT-3, but not nerve growth factor: Shirayama *et al.*, 2002), could contribute to the hippocampal atrophy observed in depressed patients (Sheline *et al.*, 1996). Chronic, but not acute, SSRI treatment, by increasing 5-HT neurotransmission, causes an increase in BDNF protein levels and expression (mRNA) in adult rats, most notably in the dentate gyrus granular cell layer of the hippocampus (Nibuya *et al.*, 1995, 1996). However, it was recently shown that acute treatment with various antidepressants could also promote TrkB receptor phosphorylation within 30 min after treatment, thus indicating that antidepressants could also induce BDNF release (Rantamäki *et al.*, 2007). This cascade of events may contribute to the therapeutic effects of antidepressant drugs. BDNF in the adult hippocampus might be involved in the delay of onset of SSRIs. Furthermore, reciprocal interactions between BDNF and 5-HT in the central nervous system have been proposed (Mattson *et al.*, 2004). BDNF has trophic effects on 5-HT neurons in the central nervous system (Mamounas *et al.*, 2000). In this context, we tried, in our laboratory, to use a combined genetic and pharmacological approach to understand the connection between BDNF and 5-HT systems.

Little is known about the relationship between BDNF and 5-HT neurotransmission in the hippocampus. 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 should 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, Guiard *et al.*, 2008) or an increase (data obtained after intrahippocampal injection of BDNF in rats as well as in WT mice, Benmansour *et al.*, 2008; Deltheil *et al.*, 2008a) in brain BDNF protein levels.

In 1994, the first BDNF mutant mice were generated by Ernfors *et al.* (1994). BDNF 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, and it was found that KO mice lacking 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. Most studies have used either constitutive heterozygous BDNF$^{+/-}$ mice, which have a decrease rather than an absence of BDNF expression (heterozygous BDNF$^{+/-}$, Korte *et al.*, 1995), or mice overexpressing the dominant-negative truncated splice variant of BDNF receptor TrkB (TrkB.T1) in postnatal cortical and hippocampal neurons (Saarelainen *et al.*, 2003; Sairanen *et al.*, 2005) because of the early postnatal lethality of BDNF null mice.

Constitutive heterozygous BDNF$^{+/-}$ mice

Heterozygous BDNF$^{+/-}$ mice generated on a 129S6/SvEv genetic background, in which BDNF protein levels are decreased by half (Korte *et al.*, 1995), develop enhanced intermale aggressiveness and hyperphagia accompanied by significant weight gain in early adulthood; these behavioral abnormalities are known to correlate with 5-HT hypofunction (Lyons *et al.*, 1999). 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 (Lyons *et al.*, 1999). This pioneering study showed 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 (Lee *et al.*, 2002). Therefore, BDNF$^{+/-}$ mice provide a useful model to study human psychiatric disorders related to hypofunction 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 (Guiard *et al.*, 2008). 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 the hippocampus, indicating 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, which suggests that the profoundly reduced ability of 5-month 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 because of functional deficits, for example, 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 (Daws *et al.*, 2007).

In contrast, when BDNF protein levels were increased following its local infusion into adult hippocampus in WT mice, this caused a decrease in 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 nonselective 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 of the delay in therapeutic effect (Benmansour *et al.*, 2008).

Age-related loss of 5-HT axons in the hippocampus was potentiated in heterozygous BDNF$^{+/-}$ mice compared with WT mice, particularly in the CA1 subregion (Luellen *et al.*, 2007). In contrast, aging BDNF$^{+/-}$ mice showed increased 5-HT innervation of the basomedial nucleus of the amygdala. The noradrenergic system was also altered in the BDNF$^{+/-}$ mice; these mice showed reduced number 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 were associated with altered serotonergic and noradrenergic innervation in aging mice and, in particular, with accelerated loss of serotonergic innervation to the hippocampus.

Other brain-derived neurotrophic factor knockouts

Saarelainen *et al.* (2003) recently showed that the behavioral effects of two antidepressants, imipramine and fluoxetine were abolished in transgenic TrkB.T1 mice with inhibited TrkB signaling in brain. Thus, these mice were resistant to the effects of antidepressants in the FST, suggesting that normal TrkB receptor signaling is required for the behavioral effects typically produced by antidepressants. Sairanen *et al.* (2005) used the same mouse strain to investigate the role of BDNF signaling in antidepressant-induced neurogenesis. They found that the antidepressant-induced increase in the surviving neurons seen in the hippocampus in WT mice 3 weeks after treatment was essentially lost in TrkB.T1 mice. These data suggest that antidepressants increase turnover of hippocampal neurons rather than neurogenesis *per se* and that BDNF signaling is required for the long-term survival of newborn neurons in mouse hippocampus. These experiments performed after a chronic 21-day imipramine treatment need to be extended to additional antidepressants.

Apart from these results, a double-KO mouse model was developed by breeding SERT KO mice with BDNF$^{+/-}$,

producing SERT $^{-/-}$ × BDNF $^{+/-}$ mice (Ren-Patterson *et al.*, 2005). The authors tested the hypothesis that reduced BDNF availability during development might exaggerate the consequences of absent SERT function. These mice had significantly increased anxiety-like behaviors compared with WT or single KO mice as measured using the elevated-plus-maze test. In addition, hypothalamic and hippocampal neurons exhibited 25–30% reductions in dendrites in double-KO mice compared with control mice. These findings support the hypothesis that genetic changes in BDNF expression interact with brain 5-HT neurotransmission and modulate anxiety and stress-related behaviors. This double-KO mouse provides a valuable animal model to evaluate epistatic interactions of BDNF and SERT gene polymorphisms in neuropsychiatric disorders.

It remains to be determined: (i) in which brain region(s) BDNF exerts 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.

Inducible brain-derived neurotrophic factor knockout mice

Conventional KO technology has limitations, such as lethal phenotype or the inability to study gene function at particular developmental stages (Aiba and Nakao, 2007). In addition, when mice develop without the protein of interest, developmental compensations may have taken place, contributing to an observed phenotype. Inducible strategies allowing the timing of expression of a gene to be regulated are currently being developed (Stark *et al.*, 1998) and have been applied to the BDNF gene. Inducible/conditional KO mouse technology has the advantage of allowing the KO to take place after development/embryogenesis.

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. An inducible KO mouse is not by definition 'tissue specific', as the promoter is not necessarily restricted to particular tissue(s). However, conditional deletion of a gene can be obtained by using a tetracycline-controlled gene expression system in the brain (Aiba and Nakao, 2007).

The term 'floxed' describes the sandwiching of a DNA sequence between two lox-P sites. This is used for Cre-lox recombination, for example, which has been used to investigate the role of BDNF in postnatal neuronal brain. The development of conditional KO mice with floxed BDNF alleles allowed spatial and temporal regulation of BDNF deletion (Rios *et al.*, 2001). The subsequent extension of this technique to produce both conditional deletion and tissue-specific BDNF KO mice was made possible by the development of more precise temporal and spatial regulation of gene expression (Berton *et al.*, 2006).

Conditional deletion of BDNF in the postnatal brain leads to obesity and hyperactivity (Rios *et al.*, 2001). Monteggia *et al.* (2004) used an inducible KO system to show that deleting BDNF in broad forebrain regions of adult mice also attenuates the effects of the antidepressant desipramine in the FST, indicating a role for BDNF in the adult brain in the antidepressant-like activity of this drug. It has also been possible to dissect the role of BDNF in depression-related behaviors and responses to antidepressant drugs in two subregions of the hippocampus, the dentate gyrus and CA1, using a viral-mediated localized BDNF knockdown strategy (Adachi *et al.*, 2008). Different systems [i.e. adeno-associated virus (AAV-Cre to obtain KO adult mice) or AAV-GFP to obtain control mice] were injected bilaterally into the dentate gyrus or CA1 of the hippocampus to selectively knockdown BDNF expression (Adachi *et al.*, 2008). Then, a series of behavioral tests measuring locomotor activity, fear learning, depression-related behaviors, and anxiety-related behaviors was carried out. Similar to what was found in total forebrain (including the hippocampus) constitutive BDNF $^{+/-}$ mice, mice lacking BDNF in the CA1 or dentate gyrus were similar to control mice in baseline locomotion, anxiety-like, or depression-like behavior. However, dentate gyrus KO adult mice showed attenuated responses in the FST to desipramine and citalopram, two commonly used antidepressants, whereas CA1 KO mice showed a normal response to desipramine. This is the first study showing regional specificity of BDNF deletion within the hippocampus and how this affects antidepressant action. These results are in good agreement with the opposite strategy, which showed that infusions of BDNF into the hippocampus produce antidepressant-like effects in neurochemical (in mice: Benmansour *et al.*, 2008) and behavioral tests (in rats: Shirayama *et al.*, 2002; in WT mice: Deltheil *et al.*, 2008b).

Conditional BDNF KOs show sex differences in depression-related behavior in the FST. By generating two independent lines of conditional BDNF KO mice in which the BDNF gene was deleted selectively in forebrain, Monteggia *et al.* (2007) showed that male conditional BDNF KO mice exhibited hyperactivity, but normal depression-related behavior. In contrast, female conditional BDNF KO mice displayed normal locomotor

activity, but a striking increase in a depression-like behavior. However, a conditional deletion of BDNF gene attenuated the actions of the antidepressant desipramine in the FST in both male and female mice. Although the results reinforce the hypothesis that loss of BDNF from forebrain regions contributes to vulnerability for depression, a larger battery of behavioral tests predicting antidepressant-like activity would need to be used to investigate this hypothesis.

Connection between rodents and humans

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, Pröschel *et al.*, 1992], was found to be associated with alterations in brain anatomy and memory (Egan *et al.*, 2003). A 'knock in' BDNF mouse [BDNF (Met/Met)] that reproduces the phenotypic hallmarks described in humans was generated (Chen *et al.*, 2006). 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 in several tests (elevated-plus-maze, open field). Surprisingly, these behavioral changes were not completely normalized by the antidepressant, fluoxetine. Thus, these data suggest that a variant BDNF may play a key role in genetic predispositions to anxiety.

These studies carried out 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* (Wang *et al.*, 2008a). It would be interesting to investigate the role of the different subtypes of postsynaptic monoamine receptors activated by indirect receptor agonists (SSRIs), in adult neurogenesis in hippocampal subregions of BDNF mutant mice compared with WT littermates. It will also be necessary to accumulate similar information from a large set of different subclasses of nonmonoaminergic antidepressant drugs. As $BDNF^{+/-}$ mice display blunted neurochemical and behavioral responses to serotonergic antidepressants, this strain of mouse could be viewed as an animal model of resistance to these drugs rather than as a model of depression.

Conclusion

During the past 15 years, serotonergic KO mice have contributed to the analysis of the role of presynaptic and postsynaptic 5-HT1A and 5-HT1B receptors in the mechanism of action of antidepressants. This genetic approach can be associated with a pharmacological approach or can replace it when selective receptor ligands, agonists or antagonists are missing. Research involving neurotrophic factors is an excellent example of this experimental benefit; in 1995, it was shown in rats that chronic antidepressant drug treatments caused an increase in the induction and prolonged expression of BDNF, which could protect neurons from the damaging effects of stress. Subsequently, studies carried out in heterozygous $BDNF^{+/-}$ mice largely supported this hypothesis. In addition, the use of $BDNF^{+/-}$ mice highlighted the close relationship between 5-HT and BNDF systems in the brain. This body of evidence suggests that the development of brain-penetrating agents that directly bind and activate TrkB receptors in the brain should be of interest in the future.

Thus, the recent literature clearly shows how some genetically manipulated mice can help to determine phenotypic changes linked to anxiety/depression-related behaviors as well as to understand the mechanism of action of antidepressants. This strategy can lead to the discovery of new targets of potential importance in the treatment of anxiety-related and depression-related disorders. We should thus favor the use of mice instead of rats in our experimental protocols because, appropriately modified, they can serve as negative controls, that is 'super-antagonists', to verify the selective action of new drugs.

The use of a KO strategy to study the role of ion channels in the mechanism of action of antidepressants could be even more interesting than studying G protein-coupled receptors. It was recently shown that the 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 behavior in different tests related to antidepressant-like activity (Heurteaux *et al.*, 2006). These results indicate that alterations in the functioning, regulation or both of the TREK-1 channels may alter mood, and that this particular $K+$ channel may be a potential target for new antidepressants. In addition, an ion channel receptor complex forms a pentameric protein whose subunits composition is characteristic of a brain region. The subunit composition of functional receptors is difficult to establish by using a classical pharmacological approach. By using KO mice for one (simple KO) or more (double or triple KO) subunits of the ion channel complex, it is possible to study, for example, the pentamerous (e.g. GABA, glutamate, nicotinic acetylcholine) receptor complexes selectively expressed on monoaminergic neurons.

The generation of receptor KO mice has offered a new approach to study processes underlying mood disorders. KO animal models are experimental tools for understanding genetic vulnerability to anxiety, depression, and their respective pharmacological treatments. One of the main interests of genetically manipulated mice as animal models of depression is to discover susceptibility genes

with strong link to psychiatric disorders, thus allowing the identification of people at risk; SERT polymorphism and genetic variant BDNF (Val66Met) polymorphism are good examples. As discussed in this review, these 5-HT and BDNF KO models have helped to determine a group of genes involved in alterations in anatomy and function of brain circuits critical for stress regulation and susceptibility for anxiety and depression (Pezawas *et al.*, 2005).

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