Opinion TRENDS in Pharmacological Sciences Vol.28 No.3

The developing use of heterozygous mutant mouse models in brain monoamine transporter research

Allan V. Kalueff, Renee F. Ren-Patterson and Dennis L. Murphy

Laboratory of Clinical Science, Building 10, Room 3D41, National Institute of Mental Health, 10 Center Drive, 1264 Bethesda, MD 20892-1264, USA

5-Hydroxytryptamine (5-HT), dopamine and norepinephrine are important monoamine neurotransmitters implicated in multiple brain mechanisms and regulated by high-affinity transmembrane monoamine transporters. Although knockout mice lacking 5-HT, dopamine or norepinephrine transporters are widely used to assess brain monoamine processes, these models have several methodological limitations. There is mounting evidence that heterozygous mutant mice with reduced (but not abolished) monoamine transporter functions could provide models with greater relevance to the genetics of human disorders, which only rarely involve complete loss-of-function mutations. Here, we discuss why heterozygous mouse models, in addition to knockout mice, might be useful for brain monoamine transporter research.

Introduction

5-Hydroxytryptamine (5-HT), dopamine and norepinephrine are key brain monoamine neurotransmitters that are implicated in multiple normal and pathological brain mechanisms [1–5]. High-affinity uptake by the plasma membrane monoamine transporters SERT, DAT and NET has an important role in the regulation of brain monoamine signaling, although less-specific uptake by other transporters might also contribute to this modulation (Table 1).

Plasma membrane monoamine transporters are established targets for many pharmacological agents, including antidepressants, psychostimulants and neurotoxins [1–5]. In addition to pharmacological manipulations, various transgenic and mutant mice are widely used to assess the role of monoamines and their transporters in brain pathogenesis (Tables 1 and 2). Most of the pharmacogenetic studies using mutant mice tend to report knockout ($^{-/-}$) versus wild type ($^{+/+}$) data (see Refs [4–6] for review) and consider heterozygous ($^{+/-}$) phenotypes as subtle or potentially intermediate [7].

Although heterozygous monoamine transporter mutant mice display intermediate responses in some tests (Table 1), recent data show that they might also differ qualitatively from both wild-type and knockout mice (Table 2). Collectively, these findings indicate that heterozygous mice merit more scrutiny and could lead to interesting novel genetic mouse models beyond knockout mice.

Because mouse phenotypes, including behaviors and drug responses, can provide relevant models for human disorders [1–4,6–9], researchers could benefit from an in-depth re-evaluation of the existing mutant mouse models and their use in brain monoamine research. In this article, we summarize the conceptual and methodological advantages of using heterozygous mutant mice in addition to monoamine-transporter-knockout mouse models.

Methodological and conceptual aspects of knockout mouse models: 'more normal' or 'less normal'?

Among monoamine transporter heterozygous mice, *Sert* (*Slc6a4*)$^{+/-}$ mutants have been studied most extensively (Table 2) and provide most of the evidence discussed here. In general terms, for each individual phenotype, heterozygous (compared with knockout) mutants can display unaltered, partial–intermediate, enhanced or principally new phenotypes. Can the situations in which heterozygous mutants display intact or less-affected phenotypes be used advantageously? Consider neuronal apoptosis, levels of which are reduced in *Sert*$^{-/-}$ mice but which remain unaltered in *Sert*$^{+/-}$ mice [10]. Because apoptosis can nonspecifically affect multiple brain mechanisms, *Sert*$^{+/-}$ mice might represent a less confounded model of transporter dysfunction for behavioral and pharmacological studies.

Similarly, an excess of 5-HT (a key regulator of brain development) from birth leads to pronounced developmental abnormalities in *Sert*$^{-/-}$ mutants, including aberrant somatosensory cortex (rescued by early postnatal 5-HT-depleting agents) [9,11]. Accompanied by deficits in somatosensory, corticothalamic and trigeminal-spinal physiology [11], these abnormalities can impede the behavioral responses of adult mice in this genetic model. By contrast, although *Sert*$^{+/-}$ mice are also affected by an excess of extracellular 5-HT, their barrel pattern is present (although reduced) [9,11], implying a better validity of heterozygous mice as a less developmentally confounded alternative to knockout mutants.

Another example is the reduced baseline corticosterone and altered body temperature reported in *Sert*$^{-/-}$ mice [12,13]. Clearly, this could nonspecifically affect the activity and metabolism of these mice, in addition to affecting the pharmacodynamics of drugs tested in this model. By

Corresponding author: Kalueff, A.V. (kalueva@mail.nih.gov).
Available online 2 February 2007.

Table 1. Brain monoamines and their plasma membrane transporters[a]

Dopamine	Norepinephrine	5-HT
General pharmacology		
Crucial for control of motor activity and control, brain reward mechanisms and cognitive functions	Regulates arousal, alertness, sleep, attention, memory, learning and cerebral plasticity	Regulates brain development, aggression, anxiety- and depression-related behaviors, motor activity, sleep, nociception and reward mechanisms
Acts at dopamine D_1–D_5 metabotropic presynaptic and postsynaptic receptors	Acts at several subtypes of α and β metabotropic presynaptic and postsynaptic receptors	Acts at 14 metabotropic and ionotropic presynaptic and postsynaptic receptors
Synthesized from tyrosine by tyrosine hydroxylase, metabolized by monoamine oxidase (MAO)-A, MAO-B and catechol-*O*-methyl transferase	Synthesized from dopamine by dopamine-β-hydroxylase, metabolized by MAO-A	Synthesized from tryptophan by tryptophan hydroxylase, metabolized by MAO-A
Plasma monoamine transporters		
DAT takes up dopamine, which is then transported by VMAT2 into synaptic vesicles	NET takes up norepinephrine, which is then transported by VMAT2 into synaptic vesicles	SERT takes up 5-HT, which is then transported by VMAT2 into synaptic vesicles
Selectively inhibited by DAT blockers, nonselectively inhibited by cocaine [2,3]	Selectively inhibited by NET blockers, inhibited less selectively by SNRIs, nonselectively inhibited by cocaine [2,3]	Selectively inhibited by SSRIs, inhibited less selectively by SNRIs or SRIs, nonselectively inhibited by cocaine [1–3]
Knockout mouse genetic models		
Altered neurochemistry and physiology (relative to wild type)[b]		
Absent DAT binding [2,3]	Absent NET binding [3]	Absent SERT binding [1,5]
↑ Extracellular dopamine (∼fivefold) [2,3]	↑ Extracellular norepinephrine (∼twofold) [3]	↑ Extracellular 5-HT (∼5–10-fold) [1,5]
↓ D_1 and D_2 receptors [2,3]		↓5-HT_{1A}, 5-HT_{1B}, ↑ 5-HT_{2A}, 5-HT_{2C} and 5-HT_3 receptors [1,5]
↓ Bodyweight (knockout mice) [2,3,19,24]	↓ Bodyweight (knockout mice) [3,24]	↑ Bodyweight (knockout mice) [20] Hyperthermia (knockout females) [12] ↓ Basal corticosterone [13]
Altered behavioral responses (relative to wild type)[b]		
↑ Stereotypic locomotion (absent from heterozygous mice) [6,21]	↑ Motor activity [24]	↓ Motor activity [15,22,23]
↑ Anxiety (absent from heterozygous mice) [21]	↓ Novelty exploration (↑ anxiety) [3]	↑ Anxiety (also in heterozygous females in some tests) [7,8,12,17,18,22,23,35,36] Spontaneous 5-HT-syndrome-like behaviors [23]
Altered drug responses (relative to wild type)[b]		
Absent cocaine-induced hyperactivity [6,31]	↑ Hyperactivity after psychostimulants [3]	Absent MDMA- and RU24969-induced hyperactivity [1,15]
Intact cocaine reward [6,31,37,38]	↑ Cocaine reward [3]	↑ Cocaine reward [1,6,31,37,38]
↑ Fluoxetine reward (absent from heterozygous mice) [38]	↑ Fluoxetine reward (absent from heterozygous mice) [38]	Absent fluoxetine reward [38]
	↑ Sensitivity to chemically induced seizures [39]	↑ Sensitivity to drugs evoking 5-HT syndrome [23]

[a]Abbreviations: DAT, dopamine transporter; MDMA, 3,4-methylenedioxymethamphetamine ('ecstasy'); NET, norepinephrine transporter; RU24969, 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole (mixed 5-HT_{1A-1B} agonist); SERT, 5-HT transporter; SNRI, 5-HT- and norepinephrine-reuptake inhibitor; SRI, 5-HT-reuptake inhibitor.
[b]Unless stated otherwise, heterozygous mice display intermediate phenotype.

contrast, $Sert^{+/-}$ mice do not have these abnormalities [12], and therefore could be more appropriate for testing stress and drug responsivity *in vivo*.

Unlike knockout mice, heterozygous mutants such as $Sert^{+/-}$ mice retain sensitivity to transporter ligands (sometimes showing hypersensitivity to these agents [14]) or to selected receptor ligands (e.g. agonists of various 5-HT receptors [12,15]). Thus, the possibility of testing a wider spectrum of psychotropic drugs and their combinations represents another example of why heterozygous monoamine transporter mouse models could be useful in brain monoamine research.

Moreover, altered motor activity and body weight have been reported in all three monoamine-transporter-knockout models (Table 1). Together with the skeletal anomalies that occur in $Sert^{-/-}$ and *Dat* (*Dat1*, *Slc6a3*)$^{-/-}$ mice [16], these factors are likely to affect performance in almost every behavioral paradigm. For example, they could compromise locomotion in the forced-swim or tail-suspension tests, and therefore affect interpretations [17,18] of animal responsivity to potential antidepressant drugs. The fact that heterozygous mice are unlikely to have such problems [19–22] minimizes possible confounding factors and enables a clearer focus on monoamine-transporter-related mechanisms and behavioral pharmacology.

Finally, the fact that heterozygous mutants sometimes have subtle or normal phenotypes implies the presence of compensatory mechanisms that ameliorate mutation-evoked deficits. Potentially important for normal brain homeostasis, these compensatory mechanisms might also be a focus of investigation, for which heterozygous mutant animals would represent ideal objects.

Altered phenotypes in plasma membrane monoamine transporter heterozygous mouse models

Although intermediate phenotypes of heterozygous monoamine transporter mutant mice are seen in some tests (Table 2), they can be used advantageously for monoamine research. For example, less-extreme heterozygous phenotypes avoid floor or ceiling effects (which are

Table 2. Selected phenotypic features of monoamine transporter heterozygous and knockout single- or double-mutant mice relative to wild type[a,b]

Genetic models and endpoints	Phenotype		Refs
	+/−	−/−	
DAT mutants			
↑ Exploratory novelty seeking	++	0	[21]
↑ Voluntary ethanol consumption	++m, 0f	++m, 0f	[19]
↑ Ethanol preference	0m, ++f	0	[19]
Polydipsia	0m, ++f	++	[26]
SERT mutants			
Sensitivity to SSRIs	++	Absent	[36]
↑ Cortical and hippocampal 5-HT_3 receptors	++	++	[24]
↓ Midbrain 5-HT_{1A} receptors	+m, 0f	++	[24]
↑ Saline-injection-induced plasma ACTH	++	++	[9,13]
8-OH-DPAT-induced hypothermia	0m, +f	Absent	[13]
↑ Corticosterone response to 8-OH-DPAT	++	++	[13]
↑ Risk assessment behaviors	++	0	[18]
↓ Open-field habituation	++m, 0f	++m, 0f	[12]
↑ Aggression after repeated testing	+m	Absent	[15]
***Vmat2*$^{-/-}$ mice**			
↓ Amphetamine conditioning reward	++	[c]	[32]
↑ Amphetamine locomotion	++	[c]	[32]
↑ MPTP toxicity	++	[c]	[32]
↓ Body weight	++m, 0f	[c]	[19]
DAT × SERT double mutants			
↑ Baseline hyperactivity, *Dat*$^{-/-}$ × *Sert* mutants	++	++	[31]
↓ Striatal 5-HT, *Dat*$^{-/-}$ × *Sert* mutants	+	++	[31,37,38]
↑ Cocaine-induced hyperactivity, *Dat*$^{-/-}$ × *Sert* mutants	++	++	[31,37,38]
Cocaine reward, *Dat*$^{-/-}$ × *Sert* mutants	Absent	Absent	[31,37,38]
Cocaine reward, *Sert*$^{-/-}$ × *Dat* mutants	++	Absent	[31,37,38]
NET × SERT double mutants			
↑ Cocaine reward, *NET*$^{-/-}$ × *Sert* mutants	0	++	[20,38]
SERT × BDNF double mutants			
↓ Brain 5-HT, *Sert*$^{-/-}$ × *Bdnf*$^{+/-}$ mutants	+	[c]	[33]
↑ Body weight, *Sert*$^{-/-}$ × *Bdnf*$^{+/-}$ mutants	+	[c]	[20]

[a]Abbreviations: ACTH, adrenocorticotrophic hormone; MPTP, *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 8-OH-DPAT, 8-hydroxy-2-(di-*N*-propylamino)tetralin (a selective agonist of 5-HT_{1A} receptors); ++, robust difference; +, intermediate phenotype; 0, no difference.
[b]Where sex difference is reported, m = males, f = females.
[c]Mice die shortly after birth.

more likely in the extreme knockout phenotypes). Such bidirectionality of an animal model based on heterozygous mice could have many practical applications, including testing drugs with unknown profiles and searching for novel psychotropic drugs.

In addition, careful comparative analyses of data from both mutant genotypes enable further investigation of different monoamine-transporter-related brain anomalies and might lead to interesting findings. For example, whereas reduced aggression in *Sert*$^{-/-}$ mice [15] might be partially explained by the hypoactivity of these mice [22,23], *Sert*$^{+/-}$ mice with normal activity levels also display reduced aggression, confirming the link between low aggression and reduced SERT function. By contrast, repeated testing caused increased aggression in *Sert*$^{+/-}$, but not *Sert*$^{-/-}$, mice [15]. This enables distinction between several behavioral domains (i.e. inactivity versus initial or stress-evoked aggression), further supporting the use of heterozygous mice in modeling different brain disorders.

As mentioned earlier, heterozygous mutant mice can also show a stronger phenotype than do their wild-type littermates. For example, a recent study reported increased sensitivity of *Sert*$^{+/-}$ mice to the selective 5-HT-reuptake inhibitor (SSRI) fluvoxamine (compared with *Sert*$^{+/+}$ mice and in contrast to the complete insensitivity in *Sert*$^{-/-}$ mice) [14], strongly supporting the use of this mouse model in monoamine research and in screening various psychotropic drugs. Finally, heterozygous monoamine transporter mutant mice can also show a phenotype that changes in a different direction from wild-type mice compared with the phenotype of homozygous mutant mice. In a recent study, for example, *Dat*$^{+/-}$ mutants showed normal activity but exhibited more exploration and less anxiety than did wild-type and knockout mice (i.e. differing markedly in their novelty seeking) [21]. Collectively, these findings demonstrate that heterozygous mice can lead to novel genetic models of monoamine-related brain mechanisms that merit further study.

In addition to constitutive knockout mice, time- and tissue-specific genetic targeting (conditional knockout) might also be an interesting direction of research. Although conditional knockout models have not yet been reported for monoamine transporters, they (and the more mildly altered heterozygous conditional knockouts) might enable the modeling of a wider spectrum of brain anomalies, thus offering new insights into monoamine neurobiology.

Clinical relevance of heterozygous mutant models

Another important issue is whether the models based on heterozygous mutant mice are relevant to human clinical situations [24]. Two lines of evidence seem to support this notion strongly. First, reduced transporter function due to genetic polymorphisms, rather than complete loss of transporters, is common in human populations (at least for

the SERT transporter) and is sometimes accompanied by distinct clinical phenotypes that differ in drug responsivity [25]. Therefore, heterozygous mouse models (e.g. *Sert*$^{+/-}$ mice), rather than mice completely lacking monoamine transporters, represent a clinically relevant model of human polymorphism-related disorders [7,14,20] (Figure 1).

Second, heterozygous mice sometimes display interesting gender differences in their phenotypes that are mostly consistent with well-known gender differences in human clinical phenotypes. In some tests, for example, heterozygous female mice are more sensitive than males (Table 2), indicating the potential use of the former as clinically relevant sex-specific genetic models. The need for such models has long been recognized in biological psychiatry and in many other medical disorders characterized by gender differences in cardiovascular, autoimmune and some neurological mechanisms [1,24]. Whereas *Sert*$^{+/-}$ female (but not *Sert*$^{+/-}$ male) mice exhibited pronounced pharmacogenic hypothermia, ethanol preference was higher in *Dat*$^{+/-}$ (versus *Dat*$^{+/+}$ or *Dat* $^{-/-}$) female mice and was unaltered in any of the male groups (Table 2). Similarly, polydipsia was limited to female *Dat*$^{+/-}$ mice and resembled that of *Dat*$^{-/-}$ mice of either sex [26]. Recent human data seem to parallel these findings, with gender differences in anxiety responses in humans with reduced SERT expression [27]. Consistent with these clinical and experimental findings, well-documented sex steroid modulation of monoamine receptors [28] and transporter functions, including DAT [19] and SERT [29], and recent data in SERT × BDNF (brain-derived neurotrophic factor) double mutants [20,30] further support this notion, indicating that new psychotropic drugs can be created based on steroids that modulate monoamine transporters and that heterozygous mice of both sexes could be particularly useful for such studies.

Genetic interactions: lessons from double-knockout mice and other mutant mouse models

In addition to their use as a 'less affected' alternative to knockout models, heterozygous mice can also be used to assess the effects of another gene disruption in double-knockout studies and to examine their effects on brain neurochemistry and pharmacology [31]. For example, whereas each single knockout produces a specific effect on brain levels of monoamines, *Dat*$^{-/-}$ × *Sert*$^{+/-}$ mice had 30% less 5-HT than did *Sert*$^{+/-}$ mice with *Dat*$^{+/+}$ alleles [20,31]. These data show that heterozygous *Dat*$^{-/-}$ × *Sert*$^{+/-}$ mice cannot maintain normal striatal tissue 5-HT dynamics, whereas the absence of such changes in *Dat*$^{-/-}$ mice with *Sert*$^{+/+}$ alleles supports the idea that the uptake of 5-HT by DAT provides a fall-back, not a normal, transporter function that can substitute for the partial loss of SERT in the striatum of heterozygous mice [20]. In line with this, cocaine-conditioned place preference was increased in *Sert*$^{-/-}$ (but not *Sert*$^{+/-}$) mice, whereas double mutants (*Sert*$^{+/-}$ × *Dat*$^{-/-}$ or *Sert* $^{-/-}$ × *Dat*$^{-/-}$ mice, but not *Sert*$^{+/+}$ × *Dat*$^{+/-}$ or *Sert* $^{-/-}$ × *Dat*$^{+/-}$ mice) demonstrated considerable reduction in this behavior [20,31]. Consistent with this notion, cocaine-induced hyperlocomotion was more pronounced in *Sert*$^{+/-}$ × *Dat*$^{-/-}$ or *Sert* $^{-/-}$ × *Dat*$^{-/-}$ compared with *Sert*$^{+/+}$ × *Dat*$^{+/-}$ or *Sert*$^{+/+}$ × *Dat* $^{-/-}$ mice [20]. Thus, double-mutant mouse models that include heterozygous mice emerge as a valuable tool with which to examine genetic and gene–environment interactions in models of neuropsychiatric disorders.

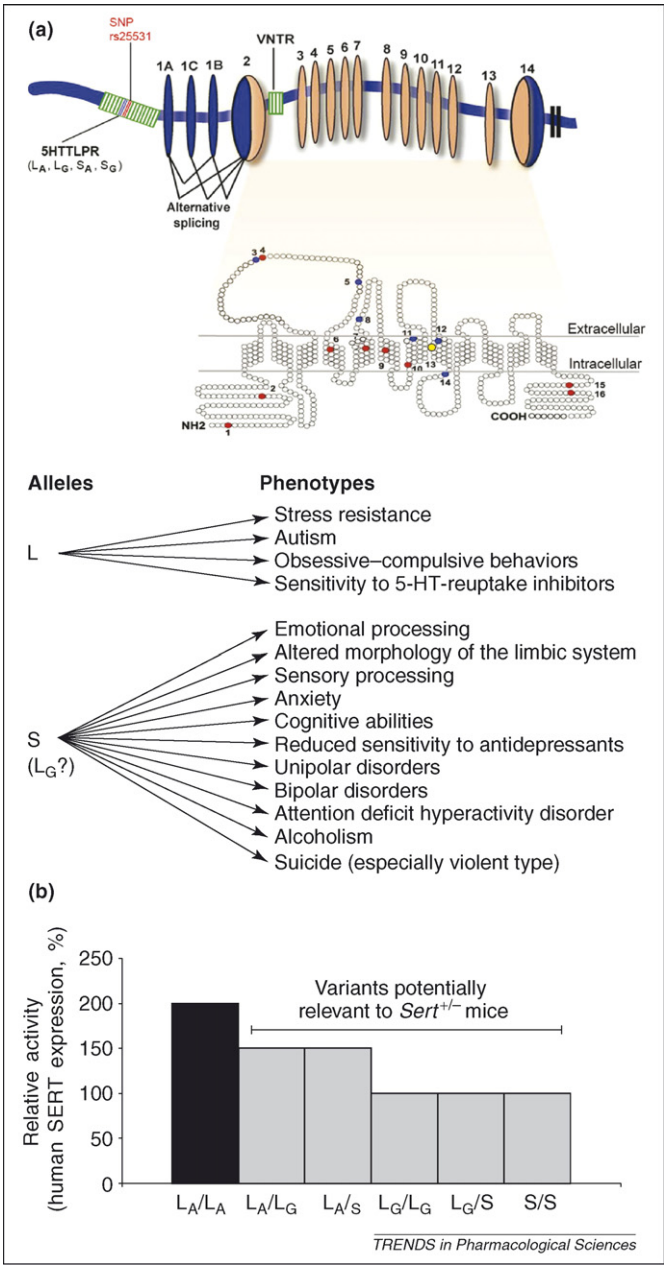

Figure 1. Genetic organization of SERT and its functions. **(a)** Human SERT genetic variants and psychiatric phenotypes. The human *SERT* gene, located on chromosome 17q11.1-q12, has a 43-bp insertion–deletion polymorphism in the promoter region (5-HTTLPR) that has long been considered as functionally biallelic and associated with multiple psychiatric phenotypes [1,40]. The long (L) allelic variant has 16 repeated elements and was thought to display the highest transcriptional activity. By contrast, the short, less active S variant has only 14 repeated elements and displays the lowest transcriptional activity [1]. Recently, the functional single nucleotide polymorphism rs2553l within 5-HTTLPR has been reported, resulting in two forms of L allele – 'active' L_A and 'less active' L_G (functionally similar to the S allele) [40]. The intronic variable number tandem repeats region (VNTR) represents another functional polymorphism of the *SERT* gene (not discussed here). **(b)** Potential parallels between human and mouse SERT genetic variance. Although some studies tend to parallel *Sert*$^{-/-}$ mice with human S/S or S/L genotypes, there is more evidence to suggest that *Sert*$^{+/-}$ mice are a closer parallel to humans with less-active *SERT* alleles, displaying a similar ~50% reduction in sites of action and function of the protein [1,9,14]. This implies that *Sert*$^{+/-}$ mice could be more appropriate for mimicking human brain disorders that are associated with reduced SERT functions.

Finally, an important area of investigation in which heterozygous models are indispensable for brain monoamine research includes testing mutant mouse strains in which the full genetic knockout of specific 'critical' genes is lethal (Table 2). For example, vesicular monoamine transporter 2 (VMAT2) or BDNF genetic knockouts lead to death shortly after birth [19,24,32,33]. By contrast, *Vmat2* (*Slc18a2*)$^{+/-}$ and *Bdnf*$^{+/-}$ mice are viable, display altered monoamine neurotransmission and some behavioral and physiological anomalies (Table 2). These mice, in addition to double-mutant models that use these heterozygotes, seem to be particularly useful for studying the role of these 'critical' genes in brain monoamine-mediated mechanisms and their interaction with genes encoding monoamine transporters and other genes. For example, using *Sert*$^{-/-}$ × *Bdnf*$^{+/-}$ mutant mice, it has been shown that reduced BDNF availability during development exaggerates the consequences of absent SERT function and leads to reduced brain 5-HT levels and increased anxiety [33]. These findings demonstrate important interactions between the two genes in the modulation of brain functions, and parallel recent clinical data [34].

Concluding remarks

The importance of heterozygous mice for neurogenetic and pharmacogenetic research applies to virtually all of the mutant mouse models. Overall, the mounting data reviewed here indicate that heterozygous mutant mice are an important tool in monoamine research, the multiple benefits of which must be considered and the use of which in experimental pharmacogenetics should be encouraged. With several physiological, pharmacological and behavioral features that create a qualitative or quantitative alternative to the well-established knockout models, some of these models (e.g. *Sert*$^{+/-}$ mice) seem to be relevant to human genetic polymorphisms (Figure 1) and modeling influences from multiple interacting genes and the environment [9,20].

More studies are needed to assess brain dysfunction and aberrant pharmacology in male and female mice that have reduced SERT, DAT or NET functions. Although further analysis is needed to examine the use of other heterozygous mouse models (especially monoamine receptor mutants or mice with ablated enzymes of monoamine metabolism), the data summarized here show how knowledge of the benefits and limitations of heterozygous and knockout monoamine transporter mutant mouse models enables a better understanding of brain monoamine systems and offers new insights into their role in various brain disorders.

Acknowledgements

This research was supported by the Intramural Research Program of the National Institute of Mental Health (NIMH/NIH). We thank Theresa DeGuzman for her valuable help with the illustrations.

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