A mouse model to address unresolved antidepressant issues

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Selective serotonin reuptake inhibitors (SSRIs) are among the most widely used drugs in psychiatry. SSRIs were initially developed as antidepressant drugs, but—in analogy with the widespread central functions of serotonin (5-hydroxytryptamine; 5-HT) (1)—they are nowadays used in the treatment of a variety of psychiatric conditions, including autism spectrum disorders, obsessive compulsive disorder, and eating disorders. Besides their wide application, their popularity is presumably related to their limited side effects and relative safety in adults. However, there are also drawbacks related to SSRI use. Patients experience clinical effects only after several weeks of treatment (2), there are substantial individual differences in the response to SSRIs (3), and early life exposure to SSRIs may lead to hazardous developmental outcomes (4). Despite extensive research, underlying mechanisms have not yet been clarified, which hampers the design of faster, more effective, and safer antidepressant drugs. In PNAS, a new mouse model is presented (5) that offers the possibility to come closer to a resolution of these major questions in the field. The unique knockin mouse bears a mutation in the serotonin transporter (SERT) gene that eliminates high-affinity recognition of a variety of SSRIs, as well as cocaine.

The new transgenic mouse of Thompson et al. (5) carries a modified copy of SERT that bears a single amino acid substitution, I172 > M172, proximal to the 5-HT binding site. The M172 substitution significantly reduces the potency of SSRIs to (*i*) reduce SERT affinity and 5-HT reuptake in vitro, (*ii*) reduce the 5-HT_{1A} receptor-dependent firing rate of serotonergic raphe neurons, (*iii*) reduce 5-HT clearance in vivo, (*iv*) increase extracellular 5-HT levels in vivo, and (*v*) affect depression-related behavioral symptoms. Importantly, the amino acid substitution does not affect the recognition of 5-HT itself. As a consequence, the animals show a normal growth, basal SERT protein and forebrain/midbrain 5-HT, dopamine, and norepinephrine levels are unchanged, and the transgenic animals do not differ from I172 control mice for each of the five parameters assessing SERT function (Table 1). This new mouse model shows that SERT is the sole target of SSRIs and, thereby, has heuristic value for dissecting the role of SERT and altered 5-HT signaling in the in vivo actions of SSRIs, and other SERT inhibitors.

Table 1. Summary of the main features of SERT rodent models

Rodent model	SERT protein levels	SERT function	SSRI sensitivity	Anxiety, depression
SERT knockout	↓↓	↓↓	↓↓	↑↑
SERT overexpressing	↑↑	?	↓	↓↓
SERT GK/EK polymorphism	?	↓	-	↑↓
SERT M172 knockin	-	-	↓↓	-

The transgenic models involve mice except for the SERT knockout, which has been generated in both mice and rats (6). The other models involve SERT-overexpressing mice (12–16), C57BL/6J mice bearing a naturally occurring polymorphism in the SERT gene (17), and M172 knockin mice characterized by elimination of SSRI and cocaine recognition by SERT (5). ↓, decrease; ↑, increase; -, no effect; ?, unknown.

What other SERT models have been generated thus far? First, we have the SERT knockout mice and rats (6). Because of the absence of SERT, SSRI recognition is lost and extracellular 5-HT levels are constitutively increased. Thereby, they could be viewed as animal models for chronic SSRI exposure. However, rather than showing low levels of anxiety- and depression-like symptoms, they show increases, which are hypothesized to be attributed to a variety of compensatory changes (Table 1). Indeed, 5-HT receptor expression, sensitivity, and/or function is altered, neuroplastic changes have been noted, and the animals show metabolic (increased fat deposition, obesity) phenotypes (6–8). Although these compensatory changes are highly relevant in the understanding of the link between the human SERT promoter polymorphism and personality traits (9, 10), they undermine the unraveling of the precise mechanisms by which SSRIs exert their clinical effects. Nonetheless, there is a striking overlap between SERT knockout phenotypes and effects of prenatal SSRI exposure, suggesting that SERT knockouts at least predict the long-term consequences of developmental SSRI exposure (11).

Developmental Changes in SERT Knockout Rodents Hamper Understanding SSRI Mechanisms

Second, SERT-overexpressing mice have been generated. In seemingly linear opposition to SERT knockout mice, these mice show enhanced 5-HT2A/C receptor function (12), up-regulation of the stress-related protein urocortin I (13), reduced anxiety (14, 15), reduced body weight (13), and a reduction in stimulus-evoked extracellular 5-HT [5-HT]o levels (16) (Table 1). However, in both SERT knockout and SERT-overexpressing mice tissue, 5-HT levels were decreased (14) and evoked [5-HT]o was frequency-insensitive. In addition, the SSRI citalopram failed to restore the magnitude of evoked [5-HT]o to wild-type levels in SERT-overexpressing mice (16). Therefore, there may be adaptations in the 5-HT release process caused by life-long changes in SERT expression, which hampers the use of the SERT knockout-overexpressing model in the understanding of SSRI effects.

Third, the group of Blakely (17) studied the impact of naturally occurring gene variation in the SERT gene among strains of mice and identified a gene variation, marked as "GK," which reduced the 5-HT reuptake capacity of SERT compared with the "EK" variant. This variant was associated with an increase in anxiety-like phenotypes and a reduction in depression-like phenotypes. However, the EK/GK variation did not alter the antidepressant effect of citalopram (17) (Table 1). A potential explanation is that the EK/GK variant was not located in the predicted binding pocket for citalopram.

What is the added value of the new mouse model of Thompson et al. (5) over these three SERT models? A common caveat of the three SERT models is that they are not suited to address the three main issues related to SSRIs: the delayed SSRI responses,

Author contributions: J.R.H. wrote the paper.

The author declares no conflict of interest.

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the individual differences in SSRI responses, and the age-dependent effects of SSRIs. The I172 knockin mouse (5) holds the promise to bring advances in the understanding of SSRI mechanisms. The authors show that the SSRIs fluoxetine and citalopram are ineffective in the tail suspension and forced swim tests for behavioral despair. Although the drugs were applied acutely, these acute tests have proven to be predictive of antidepressant efficacy (18). In view of the initial worsening of symptoms upon the start of SSRI treatment and the delayed clinical antidepressant effects, elaboration of the behavioral paradigms with tests for anxiety (e.g., elevated plus maze) and chronic SSRI effects (e.g., olfactory bulbectomy, novelty-induced feeding suppression test) are very important; they may reveal as to whether the M172 mutation reduces or offsets the initial anxiogenic responses to SSRIs and, thereby, accelerates the antidepressant response. However, it may appear that the mutation contributes to SSRI nonresponsiveness. Either way, the outcome will help in the understanding of these SSRI effects in humans, who carry the I172 variant (5).

However, to translate findings to humans, it will be critical to study SSRI effects on SERT function and behavior in M172 knockin mice bred on a genetic background characterized by high SSRI sensitivity. That is, the SSRIs were found to increase immobility in the tail suspension and forced swim tests in control I172 mice (5), which runs counter to the traditional view that SSRIs decrease immobility in these tests (18). As the authors speculate, this observation was most likely related to the 129S6/S4 background of the mice, which is associated with poor and opposite SSRI responses (19).

The I172 knockin mouse holds the promise to bring advances in the understanding of SSRI mechanisms.

SERT M172 Knockin Mouse May Serve to Unravel SSRI Function

Although SSRI treatment in adults is associated with antidepressant effects, preclinical studies suggest that prenatal and adolescent SSRI exposure leads to paradoxical anxiogenic and depressive effects and some other hazardous outcomes (4). The mechanisms underlying this intriguing phenomenon remain to be clarified, and the M172 mouse may help to reveal the neurodevelopmental pathways underlying this SSRI paradox.

Beyond SSRIs, other drugs target the SERT, like cocaine and amphetamines. Mechanisms underlying individual differences in sensitivity to these drugs and the transition from impulsive drug use to compulsive drug abuse remain to be identified, and association studies in humans and SERT knockout studies in rodents suggest a contribution of serotonergic genes to reward related processes (6, 20). Thompson et al. (5) show that cocaine-mediated SERT kinetics are significantly reduced in M172 mice, which challenges further assessment of the new mouse model in, e.g., the cocaine-induced conditioned place preference test.

The approach of Thompson et al. (5) to generate a mouse model based on hydrophobicity assessments and high-resolution crystal structures of the SERT protein, and subsequent identification of gene variation underlying important residues for ligand recognition, is not only innovative, but also points toward the possibility to generate mutants bearing a SERT that fails to recognize endogenous 5-HT. In view of the critical, yet underestimated, role of 5-HT in neurodevelopmental disorders, such a model would be of indispensable value to advance the understanding of the neurobiological mechanisms underlying these disorders.

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