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Cellular and Molecular Alterations in Mice With Deficient and Reduced Serotonin Transporters

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Abstract

The function of serotonin transporters (SERTs) is related to mood regulation. Mice with deficient or reduced SERT function (SERT knockout mice) show several behavioral changes, including increased anxiety-like behavior, increased sensitivity to stress, and decreases in aggressive behavior. Some of these behavioral alterations are similar to phenotypes found in humans with short alleles of polymorphism in the 5-hydroxytryptamine (5-HT) transporter-linked promoter region (5-HTTLPR). Therefore, SERT knockout mice can be used as a tool to study 5-HTTLPR-related variations in personality and may be the etiology of affective disorders. This article focuses on the cellular and molecular alterations in SERT knockout mice, including changes in 5-HT concentrations and its metabolism, alterations in 5-HT receptors, impaired hypothalamic–pituitary–adrenal gland axis, developmental changes in the neurons and brain, and influence on other neurotransmitter transporters and receptors. It also discusses the possible relationships between these alterations and the behavioral changes in these mice. The knowledge provides the foundation for understanding the cellular and molecular mechanisms that mediate the SERT-related mood regulation, which may have significant impact on understanding the etiology of affective disorders and developing better therapeutic approaches for affective disorders.

Index Entries: 5-HT metabolism; 5-HT_{1A} receptors; 5-HT receptors; HPA axis; stress; development; dopamine transporter; anxiety.

Introduction

Serotonin transporter (SERT or 5-hydroxytryptamine transporter [5-HTT]) is a member of the Na^+- and Cl^--coupled transporter gene family, which contains a putative 12-transmembrane domain with intracellularly oriented amino and carboxyl termini. SERT is located on the axons, dendrites perikarya, and terminals of serotonin neurons *(1)*. The function of the SERT is to remove 5-HT from the synaptic cleft and transfer it back to serotonin nerve terminals for enzymatic degradation or recycling. By doing so, SERT controls the concentration of 5-HT in the synaptic cleft and the

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actions of 5-HT at 5-HT receptors. Numerous studies have shown that SERT function is important in the regulation of emotional states. Compounds that inhibit the function of SERT (selective serotonin re-uptake inhibitors [SSRIs]) have been used both as antidepressants and for treatment of other psychiatric disorders *(2)*. Although the mechanisms mediating the therapeutic effects of SSRIs remain unclear, most evidence suggests that the effects of SSRIs on mood regulation are mediated by the adaptive changes in serotonergic systems. Blockade of SERT increases 5-HT concentrations in the synaptic cleft, which further produces changes in 5-HT receptors and their signal transduction cascades. These changes alter protein synthesis and result in the therapeutic alterations in emotional states observed in SSRI treatment *(3)*.

Several polymorphisms and mutations have been reported in SERT promoter or coding regions. Among these, the polymorphism with 43-basepair (bp) insertion/deletion in the promoter region of human SERT (5-HT tranporter-linked promoter region [5HTTLPR]) has been found to be associated with personality traits and some psychiatric disorders, including depression *(4–8)*. The long alleles (43-bp insertion) have higher efficiency in the expression of SERT *(5,9)*. A study using human lymphocytes showed that SERT expression in the cells carrying short alleles (43-bp deletion) is only 50% of that in the long allele cells. Individuals carrying the short alleles may be associated with anxiety-related personalities, such as neuroticism *(5,9,11)*. Studies have found that the short allele carriers are more sensitive to stress. For example, the amygdaloid response to fearful-face stimuli is increased in the short allele carriers, as assessed by blood oxygen level-dependent functional magnetic resonance imaging *(12)*. Caspi et al. recently showed that a major depressive episode correlates with the number of stressful life events in short allele carriers but not in homozygous long/long allele carriers *(4)*. Furthermore, the adrenocorticotropic hormone (ACTH) response to separation stress is higher in the peer-reared monkey with 5-HTTLPR long/short alleles compared to those with long/long alleles *(13)*. These data suggest that genetic variations in the expression of SERT may contribute to the development of personality traits that potentially trigger several affective disorders. Therefore, understanding the cellular and molecular mechanisms mediating the behavioral variations induced by alteration in the function of SERT will provide a foundation for understanding the etiology of affective disorders.

To study the effects of SERTs on emotional regulation, mice with constitutive deficit of SERT were created by two independent laboratories with deletions at the second exon *(14)* and the first exon *(15)*, respectively. The homozygous SERT knockout mice had no detectable SERT binding sites, whereas the heterozygous had about 50% reduction in SERT binding sites. Numerous studies have been conducted on these mice. Several behavioral studies have demonstrated that SERT knockout mice are more anxious, more sensitive to stress, and less aggressive and have increased antidepressant-like behavior (Table 1; refs. *15–18)*, suggesting that constitutive deficit or reduction of SERT function produces emotional alterations. These phenotypes in SERT knockout mice—especially in heterozygous SERT knockout mice ($SERT^{+/-}$)—are similar to those in humans with short alleles of 5-HTTLPR. Therefore, SERT knockout mice can be used as a model to study the mechanisms underlying genetic variation in SERT function that is related to emotional alterations. Several articles have discussed the behavioral changes in SERT knockout *(7,19–21)*; these articles are listed in Table 1. The present article focuses on the cellular and molecular alterations of these mice, which will help explain the mechanisms underlying the behavioral changes.

Alteration in 5-HT and Its Metabolism

The function of SERT is to uptake 5-HT from synaptic cleft back to serotonergic nerve terminals. One can expect that reducing or absent SERT will decrease 5-HT uptake, resulting in an

Table 1
Summary of Behavioral Changes in SERT Knockout Mice

Behavior	Tests	Alterations	Reference
Anxiety-like	Elevated plus maze	Increased	*18*
	Light–dark box		*15*
	Open field		*16*
Stress sensitivity	ACTH response to minor stress	Increased	*38, 50*
Locomotor activity	Open field	Decreased	*18*
	Home cage activity		*50*
Aggression	Resident/intruder	Decreased	*17*
Antidepressant-like	Tail suspension	Increased	*82*
	Force swimming	Decreased	
Body weight		Increased after age 3 mo	*10*

increase in extracellular concentration of 5-HT and a decrease in 5-HT content in the cells. Consistent with these expectations, studies using radiolabeled 5-HT found that 5-HT uptake is completely absent in homozygous SERT knockout mice ($SERT^{-/-}$). On the other hand, the 5-HT uptake in heterozygous SERT knockout mice ($SERT^{+/-}$) is almost intact *(14)*. However, a recent report using chronoamperometry showed about 60% reduction of 5-HT uptake in $SERT^{+/-}$ mice *(22)*. Consistently, the extracellular 5-HT clearance rate was significantly reduced in both $SERT^{+/-}$ and $SERT^{-/-}$ mice *(23)*.

Using microdialysis, Fabre et al. detected that the extracellular 5-HT concentration in the striatum of $SERT^{-/-}$ mice was about six times higher than their $SERT^{+/+}$ littermates *(24)*. This result was further supported by a report using the zero net flux technique *(25)*. Mathews et al. detected an eightfold increase in the extracellular 5-HT concentration in the striatum of $SERT^{-/-}$ mice relative to their SERT normal littermates ($SERT^{+/+}$; ref. *25*). Conversely, 5-HT content in the brain tissue was significantly reduced in $SERT^{-/-}$ mice, whereas only slight reduction of 5-HT content was observed in the brain in $SERT^{+/-}$ mice *(14)*. As compensatory effects, the synthesis rate of 5-HT in the brain is increased in SERT knockout mice *(26)*. However, the mechanisms mediating the increase of 5-HT synthesis remain unknown. The activity and protein levels of trytophan hydroxylase, a key enzyme for 5-HT synthesis in the brain are not altered in SERT knockout mice (ref. *26* and Li, unpublished data, 1998). Furthermore, degradation rate of 5-HT in the brain of SERT knockout mice is decreased *(26)*. The extracellular concentration of the 5-HT metabolite 5-HIAA is significantly reduced in both $SERT^{+/-}$ and $SERT^{-/-}$ mice relative to $SERT^{+/+}$ mice *(24,25)*. However, neither type A nor type B of monoamine oxidase activity is altered in the brain of SERT knockout mice *(25)*. Because these enzyme assays are measured in vitro, the activity of these enzymes may be regulated by other factors in vivo, although the activities of these enzymes are not changed.

Alteration in 5-HT Receptors

At least 15 5-HT receptors have been identified *(27,28)*. Alterations in the concentration of 5-HT in the synaptic cleft may produce adaptive changes in these receptors, including alterations in their expression, posttranslation modification, and signal transduction. Several 5-HT receptors have been characterized in SERT knockout mice, including $5\text{-}HT_{1A}$, $5\text{-}HT_{1B}$, $5\text{-}HT_{2A}$, $5\text{-}HT_{2C}$, and $5\text{-}HT_3$ receptors.

5-HT$_{1A}$ Receptors

$5\text{-}HT_{1A}$ receptors are known to play a role in the regulation of anxiety behaviors *(29–31)*.

Studies have demonstrated that deletion of 5-HT_{1A} receptors increases anxiety-like behaviors in mice *(32–34)*. Because behavioral studies have shown that SERT knockout mice are more anxious than their $SERT^{+/+}$ littermates *(15,16,18,19,35,36)*, we hypothesized that 5-HT_{1A} receptors are desensitized in SERT knockout mice. Autoradiography of ^{125}I-MPPI (a 5-HT_{1A} antagonist) binding was used to assess the density of 5-HT_{1A} receptors in the brain of SERT knockout mice *(35,37)*. The density of 5-HT_{1A} receptors is region-specifically reduced in the hypothalamus, amygdala, septum, and dorsal raphe of SERT knockout mice. These changes were observed in all three strains of the SERT knockout mice (CD1, C57/B6, and sv129), suggesting that reduction in the density results from a deficit of SERT. These data are consistent with other studies that have used both autoradiography of 5-HT_{1A} agonist and antagonist binding assays and immunohistochemistry *(24)*.

Because of the reduction in the density of 5-HT_{1A} receptors, the functions of 5-HT_{1A} receptors in SERT knockout mice are desensitized. The hormonal responses and hypothermic response to 5-HT_{1A} agonist (the markers of the function of 5-HT_{1A} receptor in the hypothalamus and dorsal raphe nucleus, respectively) were decreased in SERT knockout mice *(35,38)*. Furthermore, 5-HT_{1A} agonist-induced reduction in the firing rate of 5-HT neurons is blunted in SERT knockout mice *(39,40)*, suggesting a desensitization of 5-HT_{1A} autoreceptors. To determine the mechanisms that mediate the reduction of 5-HT_{1A} receptors, the expression of 5-HT_{1A} receptors and their G-protein coupling were examined using *in situ* hybridization, competitive reverse transcriptase polymerase chain reaction, and 8-OH-DPAT-stimulated ^{35}S-guanosine triphosphate (GTP)-γ-S binding *(24,37)*. The reduction of 5-HT_{1A} messenger RNA (mRNA) was detected only in the dorsal raphe of SERT knockout mice and not in the other brain regions. Additionally, the results from these studies suggest that the reduction in the density of 5-HT_{1A} receptors does not result from the decrease of G protein coupling *(37)*. Furthermore, G proteins (G_i and G_o proteins) coupled to 5-HT_{1A} receptors are not decreased in the hypothalamus and midbrain of SERT knockout mice. Indeed, some are even increased, suggesting a compensatory effect of the reduction of 5-HT_{1A} receptors *(37)*. Together, these data suggest that the alteration of 5-HT_{1A} receptors in SERT knockout mice may be mediated by the regulations in translational and posttranslational levels.

Interestingly, the reduction in the density of 5-HT_{1A} receptors is more extensive in female than male SERT knockout mice. This is consistent with the observation that the increased anxiety-like behaviors are more substantial in female than male SERT knockout mice. However, the reduction in 5-HT_{1A} mRNA does not appear to be gender-mediated, suggesting that the reduction of 5-HT_{1A} receptors is not mediated by the transcriptional regulation. Bouali et al. reported that gonadectomy diminishes the gender difference of desensitization of 5-HT_{1A} receptors in the dorsal raphe neurons of SERT knockout mice *(41)*. They found that testosterone prevented and estradiol produced the desensitization of 5-HT_{1A} receptors in the dorsal raphe of SERT knockout mice but not $SERT^{+/+}$ mice. These data demonstrate that sexual hormones play an important role in regulation of 5-HT_{1A} receptors in SERT knockout mice, suggesting a synergistic effect of estrogen, an opposite effect of testosterone, and a reduction of SERT function on desensitization of 5-HT_{1A} receptors. Surprisingly, gonadectomy did not alter 5-HT_{1A} receptors in the dorsal raphe of normal mice *(41)*. Several studies have found that ovariectomy desensitizes 5-HT_{1A} autoreceptors in rat and monkey *(42–46)*.

Considering that SERT knockout mice have constitutively deficient or reduced SERT function, a time-course (1 d to 8 wk postnatally) of alteration in 5-HT_{1A} receptors during postnatal development was examined in SERT knockout mice. The study found that the reduction in the density of 5-HT_{1A} receptors was more extensive in the first week after birth. The most dramatic changes during development were observed in the amygdala and dorsal raphe of SERT knock-

out mice (Li, in preparation, 2006). Together, these data suggest that the reduction in expression of 5-HT_{1A} receptors may occur during prenatal development. Because 5-HT plays an important role during development and 5-HT_{1A} receptors are the earliest developing 5-HT receptors *(47–49)*, one can speculate that increases in extracellular concentration of 5-HT resulting from a deficit of SERT in an early development stage would alter the expression of 5-HT_{1A} receptors. This early reduction in 5-HT_{1A} receptors may account for the similarity of the behavioral alterations between SERT knockout mice and 5-HT_{1A} receptor knockout mice.

To overcome the decreased 5-HT_{1A} receptors, compensatory changes are developed during later developmental and adult stages, such as increasing 5-HT_{1A} receptor-coupled G protein concentrations and maintaining 5-HT_{1A} mRNA levels. Therefore, the degree of the reduction in the density of 5-HT_{1A} receptors is attenuated in adult SERT knockout mice. Furthermore, because expression of 5-HT_{1A} receptors in the hippocampus and cortex do not peak until postnatal week 3, the density of 5-HT_{1A} receptors in these brain regions is not altered in SERT knockout mice.

The next issue is whether the reduction in 5-HT_{1A} receptors mediates increased stress response and anxiety-like behavior observed in SERT knockout mice. To address this question, recombinant adenoviruses containing 5-HT_{1A} sense or antisense sequences were used to manipulate 5-HT_{1A} receptors *(50)*. Injection of recombinant adenovirus containing 5-HT_{1A} sense sequence into the hypothalamus of SERT knockout mice restored the density of 5-HT_{1A} receptors in the medial hypothalamus. The restoration normalized increased sensitivity to stress in SERT knockout mice. These data suggest that the reduction of 5-HT_{1A} receptors in the medial hypothalamus of SERT knockout mice may have contributed to their increased sensitivity to stress. However, reduction of the density of 5-HT_{1A} receptors in the hypothalamus by injection of adenovirus containing 5-HT_{1A} antisense sequence did not significantly increase stress sensitivity in normal mice. Together with the data from others *(51–53)* and our studies that systemic injection of 5-HT_{1A} agonists inhibits ACTH responses to stress, these results suggest that 5-HT_{1A} receptors in the medial hypothalamus are involved in the inhibition of stress responses. The reduction in the density of 5-HT_{1A} receptors in the hypothalamus of SERT knockout mice attenuates their inhibitory effects on stress responses but may not be the primary cause for the increased stress sensitivity.

Restoration of hypothalamic 5-HT_{1A} receptors in SERT knockout mice was not expected to normalize their reduced locomotor activity *(50)*. These results were confirmed by injection with recombinant adenovirus containing 5-HT_{1A} antisense sequence in the hypothalamus of the normal mice. Because no evidence supports the role of hypothalamus in motor function and the locomotor activity is examined on novelty-based tests, we believe that 5-HT_{1A} receptors may be related to defensive behaviors that are known to be controlled by the hypothalamus *(50)*. In a study by Li et al., restoration of hypothalamic 5-HT_{1A} receptors did not affect the increased anxiety-like behaviors *(50)*, suggesting that 5-HT_{1A} receptors in the hypothalamus do not directly regulate anxiety-like behaviors. Because fear and anxiety behaviors are regulated by the amygdala, we hypothesize that the increase in anxiety-like behaviors is related to the reduced density of 5-HT_{1A} receptors in the amygdala *(24,37)*.

5-HT_{1B} Receptors

5-HT_{1B} receptors could be further divided as autoreceptors located in the serotonergic nerve terminals as well as postsynaptic receptors in several brain regions, such as basal ganglia. The brain regions containing the highest density of 5-HT_{1B} receptors are striatum and substantia nigra. Fabre et al. *(24)* reported that the density of 5-HT_{1B} receptors in the substantia nigra of $SERT^{+/-}$ and $SERT^{-/-}$ were reduced about 17 and 29%, respectively, relative to their $SERT^{+/+}$ littermates. The reduction in the density of 5-HT_{1B} receptors was not observed in

the globus pallidus of SERT knockout mice. 5-HT_{1B} agonist-stimulated GTP-γ-S binding was decreased in the substantia nigra of SERT knockout mice to the same degree as the density of 5-HT_{1B} receptors *(24)*, suggesting that the decreased 5-HT_{1B} agonist-stimulated GTP-γ-S binding represents a reduction in the density of 5-HT_{1B} receptors but not areduction of G protein coupling of 5-HT_{1B} receptors.

5-HT_{2A} Receptors

5-HT_{2A} receptors are distributed in most brain regions, with the highest density occurring in the cortex. The function of 5-HT_{2A} receptors may be related to psychotic disorders. Although several studies have reported that chronic SSRIs alter 5-HT_{2A} receptors, the data were not consistent. Interestingly, the density of 5-HT_{2A} receptors in SERT knockout mice is altered in opposite directions between the brain regions. Using 5-HT_{2A} antagonist (^{3}H-MDL 100907) and agonist (^{125}I-DOI) binding, Rioux et al. found, and we confirmed, that the density of 5-HT_{2A} receptors in the ventral striatum and claustrum was significantly reduced in $SERT^{+/-}$ and $SERT^{-/-}$ mice *(54,55)*. On the other hand, the density of 5-HT_{2A} receptors in the septum and hypothalamus was significantly increased in SERT knockout mice *(54)*. Although we did not observe the alteration in the density of 5-HT_{2A} receptors in the cortex with ^{125}I-DOI binding, Rioux et al. reported a decrease in 5-HT_{2A} binding sites in the cortex of SERT knockout mice using ^{3}H-MDL 100907 binding assay. The observations may be different because the total number of 5-HT_{2A} receptors (determined by antagonist binding; ^{3}H-MDL 100907) are reduced, but the activated 5-HT_{2A} receptors (determined by agonist binding; ^{125}I-DOI) are not changed in the cortex of SERT knockout mice. These differential alterations of 5-HT_{2A} receptors in SERT knockout mice suggest that the regulation of 5-HT_{2A} receptors may be different between the brain regions. Studies have demonstrated that stimulation of 5-HT_{2A} receptors in the hypothalamus potentiates stress-induced ACTH response, whereas stimulation of 5-HT_{1A} receptors inhibits stress-induced ACTH response *(52)*. The increased 5-HT_{2A} receptor and decreased 5-HT_{1A} receptors in SERT knockout mice may contribute to the increased sensitivity to stress in these mice.

5-HT_{2C} Receptors

The highest density 5-HT_{2C} receptors are located in the choroid plexus. The amygdala, lateral habenular nucleus, and thalamus contain relatively high densities of 5-HT_{2C} receptors *(54,56,57)*. Conversely to 5-HT_{1A} receptors, stimulation of 5-HT_{2C} receptors produces an anxiogenic effect *(30,58)*. The expression of 5-HT_{2C} receptors can be regulated by RNA editing and alternative splicing *(60–63)*. Studies have shown that stress and fluoxetine, a SERT inhibitor, can trigger the RNA editing and alter the ratio of 5-HT_{2C} isomers, resulting in alteration of the 5-HT_{2C} functions *(64–66)*. Therefore, in our study, we hypothesized that 5-HT_{2C} receptors may be altered in SERT knockout mice. Using autoradiography of ^{125}I-DOI binding in the presence of MDL-100907 (a 5-HT_{2A} antagonist) to block 5-HT_{2A} binding sites, we demonstrated that the density of 5-HT_{2C} receptors in the choroid plexus and amygdala was significantly increased *(34)*. This elevation was not observed in other brain regions. However, the mRNA levels of 5-HT_{2C} receptors in the amygdala were not changed, whereas 5-HT_{2C} mRNA was reduced in the choroid plexus and lateral habenular nucleus. These data suggest that the increase in the density of 5-HT_{2C} receptors did not result from an increase in their gene expression. The alteration may occur in the posttranscription and posttranslational levels.

It is particularly interesting that 5-HT_{2C} receptors are increased in the amygdala, which controls fear and anxiety behaviors. Therefore, the increase in 5-HT_{2C} receptors and decrease in 5-HT_{1A} receptors in the amygdala of SERT knockout mice may be related to the increased anxiety-like behavior of these mice. On the other hand, it is unlikely that the later onset of

obesity in SERT knockout mice resulted from alteration in 5-HT_{2C} receptors, as observed in 5-HT_{2C} knockout mice *(67–69)*, because no reduction of 5-HT_{2C} receptors was detected in SERT knockout mice.

5-HT_3 Receptors

5-HT_3 receptors are the only ligand-gated ion channel receptor in the 5-HT receptor family. They are located in both central nervous system (CNS) and peripheral tissues. Studies have shown that administration of 5-HT_3 antagonists produces an anxiolytic effect, suggesting that 5-HT_3 receptors are related to regulation of anxiety behaviors. Using autoradiography of S-methoxyl-(^{3}H) zacopride (a 5-HT_3 antagonist) binding assay, Mossner et al. reported that the density of 5-HT_3 receptors was significantly increased in the frontal cortex, parietal cortex and hippocampus CA3 region of SERT knockout mice *(70)*. However, the 5-HT_{3A} mRNA level was found to be reduced in the hippocampus CA1 region *(70)*. The frontal cortex innervates to amygdala to control the fear and anxiety behaviors. The increased density of 5-HT_3 receptors in the frontal cortex may contribute to the increased anxiety-like behaviors in SERT knockout mice, although there is no evidence that 5-HT_3 receptors in the frontal cortex are related to the regulation of anxiety-like behaviors.

5-HT_3 receptors are also located in the enteric neurons and epithelial cells of the gastrointestinal system. These receptors may play a role in control of intestinal motility. Studies have found increased colon motility and, subsequently, increased water content in the stool of SERT knockout mice *(71)*. Researchers have hypothesized that adaptive changes of 5-HT receptors in the gastrointestinal system of SERT knockout mice may be responsible for these alterations *(71,72)*. Liu et al. determined the expression and the function of 5-HT_3 receptors in the intestinal epithelial cell and enteric neurons *(72)*. They detected a reduction of 5-HT_{3B} mRNA, although the mRNA level of 5-HT_{3A} receptors was normal in SERT knockout mice. Furthermore, the 5-HT-evoked fast inward current in myenteric neurons, a 5-HT_3 receptor-mediated response, is reduced in $SERT^{-/-}$ mice, suggesting a desensitization of 5-HT_3 receptors *(72)*. The desensitization of intestinal 5-HT_3 receptors may be related to the increased intestinal motility and stool watery in SERT knockout mice.

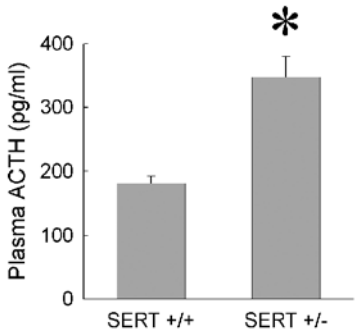

Fig. 1. Increased ACTH response to elevated plus maze stressor in $SERT^{+/-}$ mice. The data represent as mean ± SEM (n = 8–10). *, significant difference from $SERT^{+/+}$ mice.

Impaired Hypothalamic–Pituitary–Adrenal Gland Axis

Besides increased anxiety-like behaviors, SERT knockout mice also showed increased sensitivity to stress. Minor stressors, such as handling or saline injection, increase ACTH secretion in $SERT^{+/-}$ and $SERT^{-/-}$ mice *(38)*. ACTH response to elevated plus maze, a psychological stressor, is significantly higher in $SERT^{+/-}$ mice than in $SERT^{+/+}$ mice (Fig. 1). Although decreased 5-HT_{1A} receptors and increased 5-HT_{2A} receptors in the hypothalamus of SERT knockout mice may play certain roles (as discussed earlier), it is unlikely that they are primary reasons for the increased sensitivity to stress.

The activity of the hypothalamic–pituitary–adrenal (HPA) axis is known to be related to stress responses. Studies have found that although ACTH response to stress is increased

in SERT knockout mice, their corticosterone responses are not significantly altered in most of cases *(38,40)*. These data led to the hypothesis that the HPA axis is impaired in SERT knockout mice. Using the systemic corticotrophin release factor (CRF) challenge test, our recent studies demonstrated that ACTH response to CRF was elevated in $SERT^{-/-}$ mice compared to $SERT^{+/+}$ mice, suggesting a sensitized CRF receptor in the pituitary (Li, unpublished data, 2006). Similarly, Tjurmina et al. found that ACTH concentration in the pituitary in SERT knockout mice was reduced following immobilization stress, suggesting that ACTH in the pituitary is depleted by over-release *(73)*. Conversely to ACTH response to CRF, the corticosterone response to CRF was not increased in SERT knockout mice, suggesting a desensitization of ACTH receptors in adrenal cortex (Li, unpublished data, 2006).

To test the feedback regulation of the HPA axis in SERT knockout mice, a dexamethasone challenge test was performed. Systemic injection of dexamethasone significantly reduced corticosterone secretion in $SERT^{+/+}$ mice (~90% reduction). The corticosterone response to dexamethasone was attenuated in $SERT^{+/-}$ (~60% reduction) and was completely blunted in $SERT^{-/-}$ mice, suggesting that the feedback regulation of the HPA axis in SERT knockout mice is desensitized (Li, unpublished data, 2006). Together, these data suggest that the HPA axis in SERT knockout mice may be impaired. It is important to further characterize alterations in the HPA axis in SERT knockout mice. The information from SERT knockout mice will have a significant impact on understanding the mechanisms that mediate 5-HTTLPR-related increase in stress responses in humans.

Influence on Neurons and Brain Development

5-HT plays an important role in the development of the CNS. Alteration in the concentration of 5-HT induced by constitutive reduction or deficit of SERT can be expected to influence development of the CNS. Indeed, SERT knockout mice are a good model for studying the role of SERT and 5-HT in the development of the CNS. Among the limited studies that have been reported, the investigation into the impaired cortex layer four-barrel pattern formation in SERT knockout mice is the most complete. Persico et al. *(74)* found that the barrel pattern formation was impaired in SERT knockout mice. This impaired barrel pattern can be restored by administration of the tryptophan hydroxylase inhibitor p-chlorophenylalanine no later than postnatal day 1 (P1; ref. *74*), suggesting that excess of 5-HT concentration in the synaptic clefts is responsible for the impaired barrel pattern formation. Salichon et al. *(75)* further demonstrated that the impaired barrel pattern formation can be prevented by constitutively disrupting $5\text{-}HT_{1B}$ receptors, suggesting that $5\text{-}HT_{1B}$ receptors mediate the effect of increased 5-HT on barrel pattern formation.

Consistent with the impaired barrel pattern formation, the whisker-to-somatosensory cortex pathway is desensitized in SERT knockout mice *(76)*. Using glucose utilization response to whisker stimulation, Esaki et al. found that the whisker-stimulation-induced increase of glucose utilization in SERT knockout mice was reduced in the nuclei that were involved in four major whisker-to-somatosensory cortex pathways. This reduction was restored by injection of p-chlorophenylalanine on P0 and P1. Furthermore, Esaki et al. demonstrated that the basal glucose utilization was decreased in most of the brain regions of SERT knockout mice, suggesting a reduced neuronal activity. This could have resulted from the inhibitory effect of increased 5-HT concentration in synaptic cleft, because it has also been noted in chronic fluoxetine-treated animals (fluoxetine is a SERT inhibitor; ref. *77*).

Studies have shown that 5-HT is involved in cell apoptosis *(78)*. It is interesting to investigate the cell apoptosis in SERT knockout mice. Persico et al. found that the programmed cell death on P1 was reduced in several brain regions of $SERT^{-/-}$ mice *(79)*. Similarly, they also reported an increase in cell density of the

cortex in adult SERT$^{-/-}$ mice and 5-HT-staining neurons in the raphe and ventromedullar nuclei of aged SERT$^{-/-}$ mice *(79)*. Conversely to the observation in the C57/B6 strain of mice, Lira et al. reported that serotonergic neurons in the dorsal raphe were reduced about 50% in adult SERT$^{-/-}$ mice with the 129S6/SvEv strain *(15)*. These contrary results may have resulted from differences in the strain of mice, the age of mice, and the brain regions studied between the two studies.

Compensatory Effects of Other Neurotransmitter Transporters

Extracellular concentration of 5-HT in SERT$^{-/-}$ mice is five- to sixfold higher than in SERT$^{+/+}$ mice. Compensatory effects of 5-HT uptake are probably developed in these mice. The most convincing data involve the compensatory effect of dopamine transporters. Zhou et al. reported that the 5-HT-staining cells of SERT$^{-/-}$ mice were significantly increased in substantia nigra and ventral tegmental area, the brain regions that contain dopamine neurons *(80)*. This increase in 5-HT-containing cells can be blocked by treatment with a dopamine transporter inhibitor *(80)*, suggesting that the dopamine transporter uptakes 5-HT in SERT$^{-/-}$ mice. These data are further supported by a primary neuronal culture study. Pan et al. *(81)* reported that addition of 10 μ*M* of 5-HT to midbrain–hindbrain culture from SERT$^{+/+}$ mice did not increase 5-HT staining cells, whereas the 5-HT treatment increased the number of 5-HT-staining cells in the culture from SERT$^{-/-}$ mice. This increase in the 5-HT-staining cells was blocked by treatment with the dopamine transporter inhibitor nomifensine *(81)*. These data strongly support that dopamine transporters in SERT$^{-/-}$ mice can uptake 5-HT.

There is no direct evidence that norepinephrine transporters can uptake 5-HT. However, Holmes et al. found that desipramine, a norepinaphrine transporter inhibitor, induced antidepressant-like behavior is potentiated in SERT$^{-/-}$ mice *(82)*, suggesting an upregulation of the noradrenergic system.

Another compensatory effect may be mediated by organic cation transporters (OCTs). The OCTs are nonspecific transporters with a low affinity for organic cations, including serotonin and other monoamine neurotransmitters. These transporters are located mainly in the kidney and liver, with a low density in the brain. There are three subtypes of OCT: OCT1, OCT2, and OCT3. OCT1 and OCT3 are able to uptake serotonin. Schmitt et al. reported that the expression of OCT3, but not OCT1, was increased in the hippocampus of SERT$^{-/-}$ mice. This increase of OCT3 is not detected in other brain regions *(83)*. Furthermore, Chen et al. found that the OCT1 and dopamine transporters were expressed in mouse intestine *(71)*. The enteric distribution of OCT1 is similar to SERT. The expression of OCT1 in the small intestine appears to be increased in SERT$^{-/-}$ mice. Additionally, 5-HT-immunoreactive cells were observed in the intestine, although some have suggested that enteric neurons do not synthesize 5-HT. Chen et al. speculated that OCT1 and DAT uptake 5-HT together in the intestine neurons of SERT$^{-/-}$ mice *(71)*. However, the 5-HT synthesis could not be ruled out because 5-HT was detected in the intestine and was increased in SERT$^{-/-}$ mice *(71)*. These data suggest that OCT may be one of the compensatory effects for lack of SERT function.

Alteration in Other Neurotransmitter Receptors

Besides 5-HT receptors, several other neurotransmitter receptors have also been studied, including γ-aminobutyric acid $(GABA)_A$, $GABA_B$ receptor *(84)*, and adenosine receptors. Because SERT knockout mice showed the increased anxiety-like behaviors, it is reasonable to examine GABA receptors in these mice. La Cour et al. determined the function of $GABA_A$ and $GABA_B$ receptors in the hippocampus and dorsal raphe nucleus of SERT knockout mice using an electrophysiological approach and agonist-stimulated GTP-γ-S binding. They found that the $GABA_A$-agonist-

induced inhibition of firing rate was not altered in the dorsal raphe nucleus or hippocampus of SERT$^{-/-}$ mice compared to SERT$^{+/+}$ mice. On the other hand, $GABA_B$-agonist-induced inhibition of firing rate was reduced about 40% in the dorsal raphe nucleus, but not in the hippocampus, of SERT$^{-/-}$ mice. Consistently, GTP-γ-S binding stimulated by the $GABA_B$ agonist baclofen is significantly reduced in the dorsal raphe nucleus, but not in the hippocampus, of SERT$^{-/-}$ mice. These results suggest that $GABA_B$ receptors in the dorsal raphe of SERT$^{-/-}$ mice are desensitized, which could result from decreased G protein coupling or reduced density of $GABA_B$ receptors in the dorsal raphe nucleus of SERT$^{-/-}$ mice.

$GABA_B$ receptors co-express with 5-HT_{1A} receptors in serotonin neurons. Stimulation of these $GABA_B$ receptors inhibits the firing of serotonergic neurons *(85)*. The desensitization of $GABA_B$ receptors may be a negative feedback response to the reduced firing rate of serotonergic neurons *(39)*. An alterative explanation is that the desensitization of $GABA_B$ receptors results from reduced availability of G proteins and/or signal transduction pathways that are coupled to $GABA_B$ receptors in the dorsal raphe 5-HT neurons. In SERT knockout mice, increased extracellular 5-HT constitutively stimulates 5-HT_{1A} autoreceptors in 5-HT neurons, resulting in a desensitization of these 5-HT_{1A} receptors. The desensitization of 5-HT_{1A} receptors may be mediated not only by the reduction in the density of the receptors but also by the reduction in 5-HT_{1A}-receptor-coupled G proteins and the components of signal transduction pathways. Because $GABA_B$ receptors share G proteins and signal transduction pathways with 5-HT_{1A} receptors in the 5-HT neurons, the reduced G proteins and signal transduction result in the desensitization of $GABA_B$ receptors in these neurons. Further studies are needed to determine the mechanisms underlying the desensitization of $GABA_B$ receptors in the dorsal raphe of SERT$^{-/-}$ mice.

Mossner et al. examined the density of adenosine receptors in SERT knockout using autoradiography *(86)*. The study showed that the density of adenosine-1 receptors was increased in the dorsal raphe nucleus but not in the other brain regions of SERT$^{-/-}$ mice *(86)*. Because the dorsal raphe contains serotonin neurons, the increase in adenosine-1 receptors may be related to the feedback regulation of 5-HT release in SERT knockout mice. Conversely, adenosine-2 receptors are decreased in the nucleus accumbens of SERT$^{-/-}$ mice compared to SERT$^{+/+}$ mice. This downregulation may be mediated by increased extracellular 5-HT. Although adenosine receptors can be regulated by 5-HT, the mechanisms and the physiological functions of these regulations remain unknown. Therefore more studies are needed to explain the role of these changes in adenosine receptors in SERT knockout mice.

Conclusion

These studies on SERT knockout mice suggest that a deficit or reduction of SERT function produces various cellular and molecular alterations in a brain-region- and time-specific manner. These variations of adaptive changes may result from the differences in cellular regulation among individual types of cells and the developmental duration of individual organ systems. For example, 5-HT_{1A} receptors may be reduced during the embryo stage, but sensory changes occur in the first week of postnatal stage. Because most studies were conducted in adult mice, the changes that occur during prenatal development are unknown. From our preliminary data on 5-HT_{1A} receptors, the alterations during early developmental stages (prenatally and week 1 postnally) might result from increased extracellular 5-HT. During postnatal development, however, further changes may occur to compensate for the adaptive changes produced in early development. Therefore, the changes observed in adult SERT knockout mice may be less extensive than those in early developmental stages. Thus, alterations in SERT knockout mice are very complicated. They occur not only in the serotonergic system

Table 2
Comparison Between Short Alleles of 5-HTTLPR and $SERT^{+/-}$ Mice

	Short alleles of 5-HTTLPR (human)[a]	$SERT^{+/-}$ mice [b]
SERT expression (cells)	50% reduction *(6)*	50% reduction *(14)*
SERT density (adult)	Normal *(11)* or slight reduction	50% reduction *(14)*
5-HT uptake (adult)	Slightly reduced in platelets *(11)*	Slightly reduced in brain *(14)*
5-HT_{1A} receptors	Reduced *(89)*	Region-specifically reduced *(37)*
ACTH response to stress	Increased *(13)*	Increased *(38)*
Glucocorticoid response to stress	No change *(13)*	No change *(38)*
Personality	Increased neuroticism in some studies *(5)*	Increased anxiety-like behaviors in some studies *(18)*

[a]Comparing with *l* alleles of 5-HTTLPR.
[b]Comparing with SERT +/+ mice

but also in other neurotransmitter systems. The data observed may represent only a small portion of the alterations in SERT knockout mice. Therefore, it is essential to study the adaptive changes during the early development of SERT knockout mice.

The cellular and molecular alterations may account for the behavioral changes observed in these mice. Certain behavioral changes may be mediated by multiple factors. For example, the increased anxiety-like behavior could be a synergistic effect of desensitization of 5-HT_{1A} and 5-HT_3 receptors and supersensitization of 5-HT_{2C} receptors in SERT knockout mice. Additionally, the increased sensitivity to stress may be related to an impaired HPA axis, decreased 5-HT_{1A} receptors, and increased 5-HT_{2A} receptors in the hypothalamus of SERT knockout mice. Therefore, it is important to understand the integration of alterations in SERT knockout mice.

The studies on the cellular and molecular changes in SERT knockout mice will not only provide fundamental knowledge regarding the effects of SERT on the development of mice, but they will also provide information regarding the mechanisms underlying effects of polymorphism or mutations of SERT in humans. More than 25 variants (SNPs, indels, a VNTR, and a functional mutation) of *SERT* gene and its promoter region have been discovered *(21)*. Although we do not know whether most of these variants alter the function of SERTs, the two regulatory region polymorphisms *(21)* and at least one mutation *(87,88)* have been found to alter the function of SERT and may be related to personality and some psychiatric disorders. Table 2 lists the similarities between $SERT^{+/-}$ mice and short alleles of 5-HTTLPR polymorphisms (a well-studied SERT polymorphism). Therefore, studying the cellular and molecular alterations in SERT knockout mice will have an important impact on understanding the mechanisms that mediate constitutive alteration of SERT-function-induced phenotypes in humans.

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