Are serotonin transporter knockout mice 'depressed'?: hypoactivity but no anhedonia

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Although the serotonin transporter is a key target for antidepressants, its exact role in depression etiology remains unclear. While serotonin transporter knockout mice are a potential model to examine this problem, their depression profile is unclear in several 'despair' tests, and may be confounded by their hypoactivity phenotype (confirmed here by marble-burying and bedding tests). To assess depression in these mice, we evaluated wild-type, heterozygous, and serotonin transporter knockout C57BL/6 male mice on a well-validated, anhedonia-based depression paradigm, the sucrose preference test. Overall, all three genotypes showed similar sucrose preference, indicating an unaltered hedonic state. These results demonstrate that depression-like behavior (unlike hypoactivity) is not a baseline phenotypic feature of serotonin transporter knockout mice, suggesting anew that these mice do not represent a genetic model of depression. *NeuroReport* 17:1347–1351 © 2006 Lippincott Williams & Wilkins.

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Introduction

Depression is a complex human disorder characterized by depressed mood, anhedonia, loss of energy and low self-esteem [1]. Serotonin is a key brain neurotransmitter implicated in many disorders including depression, anxiety, autism and obsessive–compulsive disorder [2,3]. High-affinity uptake by a transmembrane serotonin transporter (SERT) plays a crucial role in the regulation of serotonergic neurotransmission [2,4]. SERT is widely distributed throughout the brain, and is the target of many psychotropic drugs, including selective serotonin reuptake inhibitors, the most widely prescribed antidepressants [2].

SERT knockout (SERT−/−) mice have emerged as a useful tool to examine the role of serotonin and SERT in various normal and pathological brain mechanisms [4]. SERT−/− mice, which are available on several different genetic backgrounds, demonstrate elevated extracellular serotonin, downregulation of some serotonin receptors, and numerous behavioral abnormalities [3–12]. Together with the key role of SERT in antidepressant action, this raises the possibility of using SERT−/− mice as a genetic model of anxiety and depression.

Numerous models are used in behavioral phenotyping of various mutant mice [1,6,8,13–17]. Although an anxious hypoactive phenotype is consistently seen across different tests and genetic backgrounds in SERT−/− mice [9,10], data are conflicting with respect to their depression-related behaviors. Using two popular 'behavioral despair' models of depression (the tail suspension and the forced swim tests [8]), several groups have examined depressiveness in SERT−/− mice. While SERT−/− mice on 129S6 background showed increased despair in the forced swim test but an antidepressant-like reduction of immobility in the tail suspension test, mutants on C57BL/6 background displayed unaltered tail suspension and forced swim behavior [11,12]. As background differences and limitation of a phenotype to one test only are commonly inconsistent with a strong effect of mutation, caution is needed when interpreting data on altered depression-like behavior in SERT−/− mice.

Indeed, several factors might confound these results. For example, high dependence of 'despair' tests upon locomotor activity, and hypoactivity (consistently replicated in SERT−/− mice [6,9,11,18]) would seem to limit the utility of these tests in SERT−/− mice. In addition, C57BL/6 genetic background is considered a poor choice for the tail suspension test, and 129 strains are considered a poor choice for the forced swim test [13,14]. Finally, high baseline anxiety in SERT−/− mice may nonspecifically affect performance of mice in the tail suspension and forced swim tests. Taken together, this suggests that a depression phenotype in SERT−/− mice cannot be regarded as solidly established only by 'despair' tests such as the forced swim and tail suspension tests, and would benefit from re-evaluation using other depression paradigms.

Among several well-known depression paradigms, anhedonia-based models [1,8], such as the sucrose preference test [15–17], seem to be particularly suitable for such studies.

First, the sucrose preference test is highly relevant to human pathogenesis (assessing core symptoms of depression: loss of appetitive motivation, anhedonia) [15–17]. Second, anhedonic responses in this test generally correlate well with the forced swim and tail suspension test results. Finally, anhedonia in the sucrose preference test is less dependent on baseline activity and anxiety [16], and therefore may not be confounded by hypoactive, anxious phenotype of SERT−/− mice. In the present study, we assessed depression in SERT+/+, SERT+/−, and SERT−/− mice, using a relatively active, nonanxious C57BL/6 strain as a genetic background. Given its sensitivity to sucrose [19–21] and evoked anhedonia in the sucrose preference test [16,17], this background seemed to be particularly appropriate for this study of SERT−/− mice. To assess genotype differences in activity, we also tested all three genotypes in the marble-burying and the bedding digging activity tests.

Methods

Male SERT+/+, SERT+/− and SERT−/− mice (n=10 in each group) on a C57BL/6 genetic background [4] were used. Mice (30–35 g; 3–6 months old) were littermates produced by 19–21 heterozygous backcrosses. They were experimentally naïve and housed individually for 10 days before testing in a facility approved by the American Association for Accreditation of Laboratory Animal Care, with food and water freely available under a 12 h light–dark cycle (lights on at 06:00 h). Experimental protocols complied with the National Institutes of Health Guidelines and were approved by the National Institute of Mental Health (NIMH) Animal Care and Use Committee.

Sucrose preference test

In this test, the animals were tested for 3 days [16,17], with a free choice between two bottles, one with sucrose (3% in tap water) and another with tap water. To eliminate potential side preferences, the position of bottles were switched every 24 h. The consumption of water, sucrose solution and total liquid intake (milliliter) was assessed daily for a total of 3 days. Liquid intake was also assessed relative to body weight (milliliters per gram body weight). The preference for sucrose was calculated as a percentage of the consumed sucrose solution to the total volume of liquid consumed [17]. Three percent sucrose solution was chosen for this study based on good sensitivity of background C57BL/6 mice to >1% sucrose in the sucrose intake tests, and to chronic mild stress-evoked anhedonic depression, as assessed by the sucrose preference test [16,17,19,20].

Marble-burying and bedding tests

One day later, the animals were tested in the marble-burying test [22]. The test was performed in the home cage, the standard rooted plastic cage (30 × 20 × 15 cm), with a 2-cm-thick layer of bedding (aspen shavings). Each animal was briefly removed from its cage, and six glass marbles (1.2 cm in diameter) were placed evenly on the bedding. The animal was then returned to its home cage, and the number of nonburied (<1/3), partially buried (>2/3), fully buried and total buried (fully + partially) marbles were analyzed 30 min later, according to the procedure described in [22].

The following day, the mice were subjected to the bedding (digging) test, another widely used test of activity in mice [22]. On the day of the experiment, the animals were transported to the testing room, and allowed at least 1 h to acclimate. Each mouse was then placed individually in a clean rooted plastic cage with 250 ml of bedding placed in one corner. The mice were allowed to dig in their cages for 30 min, and total area (square centimeter) left uncovered by bedding was measured using a custom-made grid (standard 1 cm^2 squares) placed beneath the transparent bottom of the cage.

One week later, the mice were tested in the marble-burying test in an unfamiliar environment (novel cage). The animals were transported to the testing room, and allowed at least 1 h to acclimate. Each mouse was then placed individually in the clean rooted plastic cage with a 2-cm-thick layer of bedding for 30 min. The mouse marble-burying activity was then assessed, as described earlier.

Statistics

All data are expressed as means ± SEM. Data were analyzed by a Kruskal–Wallis test followed by a Dunn's post-hoc test (for total 3-day consumption scores). Daily liquid consumption in the sucrose preference test was analyzed using two-way analysis of variance (factors: genotype, testing day). A probability of less than 0.05 was considered statistically significant in all tests.

Results

Figure 1 summarized data on sucrose consumption in all three genotypes used in the present study. Overall, there were no differences in total (3 day) liquid consumption among all three genotypes [H=2.1, NS (water); H=2.9, NS (sucrose solution); H=2.07, NS (total liquid)]. As body weight did not differ in all three groups (SERT+/+ mice: 33.5 ± 1.2 g; SERT+/− mice: 31.5 ± 1 g; SERT−/− mice: 35.4 ± 1.7 g, NS), liquid intake calculated per body weight was also unaltered in all three groups (Fig. 1). Likewise, there was an equally strong preference for sucrose solution in all three genotypes (H=1.02, NS). Finally, daily consumption of liquids did not vary significantly across testing days in any of the three genotypes, and sucrose preference was also essentially the same in all groups (Fig. 1).

In contrast, digging (bedding test) and marble-burying in two different contexts (home cage and unfamiliar cage) showed a striking reduction of digging/burying activity in SERT−/− mice (Table 1), indicating pronounced behavioral differences between genotypes, with a strong hypoactive phenotype in mutant mice lacking SERT.

Discussion

Basal liquid intake and sucrose preference in this study were similar to those in other sucrose preference studies using the C57BL/6 mouse strain [20,21], confirming good validity and reproducibility of this model. Similar sucrose preference in all three genotypes (Fig. 1) indicates that genetic ablation of SERT does not affect the mouse taste ability, and replicates our prior studies with these mice showing no genotype differences in the sensitivity to several other tastants, such as saccharine and quinine (C.H. Wichems, A.M. Andrews, Q. Li, K.P. Lesch, D.L. Murphy, unpublished data). This allowed us to use anhedonia-based sucrose preference paradigm to examine whether SERT−/− mice have altered baseline hedonic responses.

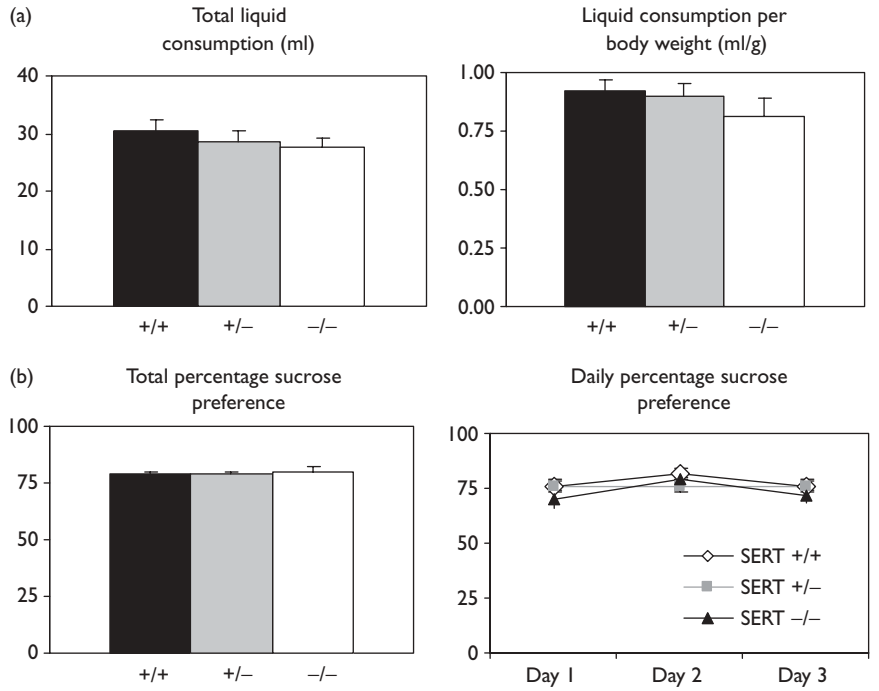

Fig. 1 Sucrose consumption in serotonin transporter (SERT) + / + , SERT + /−, and SERT−/− mice (n=10 in each group) in the 3-day sucrose preference test. (a) Total and relative (to the body weight) volume of liquid consumption. (b) Total and daily sucrose preference (calculated as a percentage of the consumed sucrose solution to the total volume of liquid consumed).

Table 1 Marble-burying and digging behaviors in serotonin transporter (SERT) + / + , SERT + /−, and SERT−/− mice (n=10 in each group)

Groups	Parameters			
	Number of nonburied marbles (< 1/3)	Number of partially buried marbles (> 1/3)	Number of fully buried marbles	Total (partial + fully) buried marbles
Marble-burying tests				
Home cage				
SERT + / +	1.5 ± 0.54**	2.0 ± 0.47	2.5 ± 0.58*	4.5 ± 0.54**
SERT + /−	2.3 ± 0.56	1.2 ± 0.29	2.5 ± 0.45*	3.7 ± 0.56*
SERT−/−	4.3 ± 0.65	1.1 ± 0.59	0.60 ± 0.43	1.7 ± 0.65
Statistics	H=8.79, P=0.012	NS	H=7.85, P=0.19	H=8.79, P=0.012
Novel cage				
SERT + / +	1.4 ± 0.27**	1.4 ± 0.37	3.2 ± 0.47**	4.6 ± 0.27**
SERT + /−	1.4 ± 0.43**	1.6 ± 0.52	3.0 ± 0.39*	4.6 ± 0.43**
SERT−/−	4.0 ± 0.39	0.9 ± 0.35	1.1 ± 0.35	2.0 ± 0.39
Statistics	H=15.50, P=0.0004	NS	H=11.20, P=0.0037	H=15.60, P=0.0004
Bedding test	Square left uncovered with bedding (cm^2)			
SERT + / +	64.5 ± 15.2*			
SERT + /−	50.4 ± 16.7**			
SERT−/−	147.9 ± 14.7			
Statistics	H=13.04, P=0.0015			

*P < 0.05, **P < 0.01 vs. SERT−/− group; Dunn's post-hoc test for significant Kruskal–Wallis data.

Using marble-burying and bedding tests, we showed a marked, approximately two-fold reduction in activity of SERT−/− mice (Table 1), despite their well-known increased anxiety (as assessed previously in numerous studies [6,10]), which normally correlates with increased digging in mice [22]. In addition to lower home-cage activity [9], these findings demonstrate the extent to which, in some but not all paradigms [4,23], multiple behaviors (including forced swim and tail suspension test performance) may be confounded by the SERT−/− mouse hypoactivity.

In contrast, our results in the sucrose preference test demonstrate unaltered performance in SERT + /− and SERT−/− mice (Fig. 1). All three genotypes showed clear ~70–80% sucrose preference, meeting the criterion for

absent anhedonia (>65%) in this model [17]. Collectively, this implies unaltered baseline hedonic behavior in SERT−/− mice. These findings are in line with unaltered tail suspension and forced swim test performance of SERT−/− mice on C57BL/6 background, which was not an artifact or strain-specific anomaly (as assessed by intact tail suspension test response to acute imipramine and desipramine but not, as expected, to the serotonin reuptake inhibitor fluoxetine) [6,11,12]. In behavioral phenotyping research, C57BL/6 strain is the reference strain, bidirectionally sensitive to manipulations affecting depression. Therefore, an intact profile of SERT−/− mice on this background in three different models [12] (Fig. 1), supports an absence of notable depression-like behaviors.

As these data do not support earlier reports on altered 'despair' depressiveness in SERT−/− mice on 129S6 background [11,12], several important aspects merit a detailed discussion here. Analyzing the validity of these tests, we note that the forced swim and tail suspension tests demonstrate good predictive validity for acute antidepressant effects, but have poor face (homology to clinical symptoms) and construct (relevance to pathogenesis) validity. In contrast, the anhedonia-based sucrose preference test is characterized by good construct, face and predictive validity [1], including responsivity to imipramine and the serotonin reuptake inhibitor citalopram, and is therefore highly relevant to depressive pathogenesis [1,15,17]. While one can argue that the forced swim, tail suspension and sucrose preference tests may reflect different subtypes of depression (i.e. despair vs. anhedonia, which might be differentially affected in different mouse models), there is generally a good correlation between these depression tests in mice with truly altered depression-related behavior (e.g. [17]). This implies that if a depression phenotype exists in our SERT−/− mice, these tests will detect it, especially using sucrose preference test and C57BL/6 genetic background.

Total liquid intake was unaltered in this and in another study analyzing drinking in SERT−/− mice [24], suggesting that liquid metabolism is not affected by SERT genetic ablation. Although the same study reported reduced ethanol (15–20%) consumption in SERT−/− mice, this phenomenon developed only after several weeks of daily ethanol drinking, more likely representing a model of alcoholism rather than anhedonia [7,24]. The lack of anhedonia in SERT−/− mice in our study coincides with their unimpaired performance in other reward-based behavioral paradigms (e.g. cocaine place preference [23]). Finally, two groups have recently assessed the hypothalamo-pituitary-adrenal system in SERT−/− mice on different backgrounds, reporting lower basal corticosterone in these mice [7,25], generally inconsistent with most frequently reported elevated cortisol levels in depressed adult humans. Collectively, this further confirms that SERT−/− mice do not represent a model of depression.

Interestingly, although heterozygous SERT+/− mice display a 50% reduction in SERT binding, altered brain morphology and disturbed serotonergic neurotransmission [2,6,7], their anhedonia-related behavior was also unaffected (Fig. 1). In line with unaltered forced swim and tail suspension responses in SERT+/− mice [12], this observation indirectly supports the notion that depression represents a separate behavioral domain insensitive to the loss of one or two SERT alleles.

Do our findings coincide with the clinical literature? In general, there are conflicting data on the SERT gene and its polymorphisms in human depression, including numerous reports negating such a link [26–29]. Notably, one recent study analyzed SERT gene–environment interaction in depression, and reported higher risk of depression with the short SERT SS genotypes and S alleles only in stressed patients [29]. From this point of view, our data reporting unaltered depression in unstressed SERT−/− mice seem to strikingly parallel these findings.

We note, however, that the main task of this study was to assess the baseline hedonic profile in SERT−/− mice, testing their potential utility as a genetic model of depression. Ruling out this hypothesis, our experiments, however, do not preclude stress-evoked alterations in hedonic behavior in these mice, and the possibility that chronically stressed SERT−/− mice may have interesting depression-related behaviors [18]. The important question as to why SERT−/− mice are not 'depressed' in the present study also remains to be investigated further. One possibility can be that SERT−/− mice (despite their lifelong anxiety and stress experiences) may be protected from 'depression' by virtue of having SERT inhibited – the situation equivalent to lifelong antidepressant treatment.

Conclusion

In summary, despite pronounced hypoactivity (assessed here by the marble-burying and bedding tests) and a well-known anxious phenotype, anhedonic responses in the sucrose preference test are not present in SERT−/− mice on C57BL/6 genetic background. Given the sensitivity of this background to the sucrose preference and anhedonic depression, our data do not support the utility of SERT−/− mice as an animal model of depression. These results further contribute to the complexity of behavioral phenotype of SERT−/− mice, and may foster a clearer focus on various interesting behavioral domains that may be affected in this genetic model.

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