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Hypolocomotion, anxiety and serotonin syndrome-like behavior contribute to the complex phenotype of serotonin transporter knockout mice

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Although mice with a targeted disruption of the serotonin transporter (SERT) have been studied extensively using various tests, their complex behavioral phenotype is not yet fully understood. Here we assess in detail the behavior of adult female SERT wild type (+/+), heterozygous (+/−) and knockout (−/−) mice on an isogenic C57BL/6J background subjected to a battery of behavioral paradigms. Overall, there were no differences in the ability to find food or a novel object, nest-building, self-grooming and its sequencing, and horizontal rod balancing, indicating unimpaired sensory functions, motor co-ordination and behavioral sequencing. In contrast, there were striking reductions in exploration and activity in novelty-based tests (novel object, sticky label and open field tests), accompanied by pronounced thigmotaxis, suggesting that combined hypolocomotion and anxiety (rather than purely anxiety) influence the SERT −/− behavioral phenotype. Social interaction behaviors were also markedly reduced. In addition, SERT −/− mice tended to move close to the ground, frequently displayed spontaneous Straub tail, tics, tremor and backward gait – a phenotype generally consistent with 'serotonin syndrome'-like behavior. In line with replicated evidence of much enhanced serotonin availability in SERT −/− mice, this serotonin syndrome-like state may represent a third factor contributing to their behavioral profile. An understanding of the emerging complexity of SERT −/− mouse behavior is crucial for a detailed dissection of their phenotype and for developing further neurobehavioral models using these mice.

Keywords: Activity, anxiety, behavioral phenotype, knockout mice, serotonin syndrome-like behavior, serotonin transporter

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Serotonin is a key neurotransmitter in the brain, whose dysfunctions are implicated in many disorders including anxiety, depression, aggression, hyperactivity, autism, attention deficit disorder, alcoholism and schizophrenia (Gingrich & Hen 2001; Lesch 2002). Through high-affinity uptake, the transmembrane serotonin transporter (SERT) is an integral regulator of serotonergic neurotransmission and homeostasis (Blakely 2001; Kim *et al.* 2005; Murphy *et al.* 2004; Schmitt *et al.* 2003; Zhou *et al.* 2002). SERT is widely distributed throughout the brain in humans and animals, and is targeted by numerous psychotropic drugs, including serotonin reuptake inhibitors (SRIs) and selective SRIs, the most widely used antidepressant medications (Murphy *et al.* 2004; Torres *et al.* 2003; Wong & Licinio 2004).

SERT knockout (−/−) mice represent a useful tool to assess the role of serotonin and SERT in various normal and pathological brain mechanisms (Bengel *et al.* 1998; Bouali *et al.* 2003; Mannoury la Cour *et al.* 2004; Mossner *et al.* 2000; Murphy *et al.* 2003; Vogel *et al.* 2003). Expressing non-functional truncated SERT, these mutants have been constructed on several different genetic backgrounds and are widely used in neuroscience research (Holmes *et al.* 2003a, 2003b; Lanfumey *et al.* 2000; Lira *et al.* 2003; Murphy *et al.* 2001, 2003, 2004; Qu *et al.* 2005; Ravary *et al.* 2001; Wisor *et al.* 2003; Zhao *et al.* 2006). SERT +/− and −/− mice have gene dose-proportionate increases in extracellular concentrations of serotonin along with decreases in intracellular concentrations of serotonin, associated with alterations in binding sites, signaling and function of several of serotonin's pre- and postsynaptic receptor subtypes (Bengel *et al.* 1998; Kim *et al.* 2005; Li *et al.* 1999, 2000, 2003; Mathews et al 2004). Several studies have noted numerous behavioral baseline alterations in SERT mutant mice. For example, SERT −/− mice show increased anxiety-like behavior and altered behavioral despair responses, in addition to decreased overall home cage activity (Holmes *et al.* 2002a, 2002b, 2003a, 2003b).

Our study aimed to perform an expanded behavioral phenotyping of SERT wild type (+/+), heterozygous (+/−) and knockout −/− mice, including an in-depth ethological analysis of their activity and emotionality, as well as an assessment of behavioral domains that have not been studied previously. Specifically, over a period of 2 months, the behavior of SERT +/+, +/− and −/− mice was assessed and compared on a battery of behavioral tests including nest building, grooming, sticky label, novel object, ethogram,

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social interaction, tics, wire hanging, balancing, rope climbing, food finding, locomotion and chewing pattern tests. All three genotypes were examined in order to more fully assess and compare their behavioral profiles. Female mice were chosen for this study, as there are fewer published reports on the behavior of female SERT −/− mice, and more pronounced behavioral and physiological alterations in female SERT −/− mice in some tests have been reported recently (Bouali *et al.* 2003; Cornelissen *et al.* 2005; Holmes *et al.* 2003a).

Methods

Animals

Subjects were female SERT +/+ ($n = 8$), +/− ($n = 7$) and −/− ($n = 7$) mice of a strain generated on a C57BL/6J genetic background (Bengel *et al.* 1998). Mice (20–25 g, 3–5 months old at the beginning of the experiments) were littermates produced by 19–21 heterozygous backcrosses. Animals were experimentally naive and were housed individually throughout the study in a facility approved by the American Association for Accreditation of Laboratory Animal Care, with food and water freely available (except where noted) and a 12-h light/dark cycle (lights on at 600 h).

Procedures

Experimental protocols complied with National Institutes of Health Guidelines and were approved by the NIMH Animal Care and Use Committee. On testing days, the mice were transported to the experimental room and left undisturbed for 1 h to acclimate to the testing room. All experiments were performed between 1300 and 1700 h, and behavioral phenotyping was always performed blind to genotype.

Nest building

Nonmaternal nest building behavior was evaluated by introducing a piece of paper towel (25 × 30 cm) to each cage for 3 days (Bulloch *et al.* 1982; Moretti *et al.* 2005). Nests were then examined by observers blind to the genotype of the mice using the following scoring system (Kalueff *et al.* 2006): 0, no nest; 1, primitive flat nest (pad-shaped, consisting of a flat tissue that slightly elevates a mouse above the bedding); 2, more complex nest (including wrapping and biting the paper towel); 3, complex cup-shaped nest (shredded paper interwoven to form the walls of the cup); 4, complex hooded nest (walls form a ceiling so the nest becomes a hollow sphere with one opening). The height of each nest (cm) was also measured.

Grooming test

Each animal was placed in a clear Plexiglas cylinder (20 cm in diameter, 30 cm high), where the latency (seconds) to groom, the number and the duration (seconds) of grooming bouts, and transitions between specific types of grooming were recorded for 5 min. The following types of grooming were recorded: paw licking, nose/face grooming, head washing, body grooming and tail/genital licking. Grooming interruptions greater than 5 seconds were considered as separate bouts. The percentages of interrupted bouts and 'correct' and 'incorrect' transitions (against cephalocaudal order) were analyzed as previously described (Kalueff & Tuohimaa 2004). The number of vertical rears and defecation boli were also recorded. In all experiments, the apparatus was cleaned between the animals with 30% ethanol to remove any olfactory cues.

Sticky label test

Four days after the grooming test, a sticky label (0.5 × 1 cm) was placed on the base of the hind paw of each mouse (Hunter *et al.* 2000). The mice were then observed in an observation cylinder, scoring the latency (seconds) to attempt removal, the number of attempts to remove and the latency (seconds) to remove the sticky label, with a cutoff time of 5 min.

Novel object test

On the day after the sticky label test, the mice were subjected to a novel object, a gray plastic cone (3 × 5 cm), which was introduced to the observation cylinder after a 2-min acclimation period. During the next 3 min, experimenters recorded the latencies (seconds) to approach or touch the object and the number of approaches (< 2 cm) and touches.

Ethograms

Four days later, each animal was placed in an observation cylinder for 5 min, during which the frequency and sequence of behavior was recorded, including horizontal activity (horizontal locomotion episodes), protected (wall leaning) and unprotected (paws in the air) vertical rears, grooming bouts, defecation, urination, freezing (animal inactive >5 seconds) and Straub tail (Fig. 2B).

Social interaction test

On the day after the ethograms experiment, the interaction between SERT +/+ and SERT −/− mice was observed for differences in sociability. One SERT +/+ mouse and one SERT −/− mouse, chosen randomly, were simultaneously placed in an observation cylinder and allowed to interact for 5 min. The following behaviors were assessed: initiation of sniffing, following, touching, heterogrooming and attacking the other animal. Individual behaviors such as self-grooming bouts, rears and Straub tail episodes were also recorded separately for each mouse.

Straub tail test

As the social interaction and ethograms experiments revealed pronounced genotype-related differences in Straub tail, we assessed this index in a separate experiment 1 day later. Specifically, the mice were examined individually in the observation cylinder for 5 min, scoring the latency (seconds), frequency and total duration (seconds) of Straub tail (Fig. 2B).

Wire hanging

On the same day, following the Straub tail test, the animals were suspended by their forepaws on a standard wire hanger 20 cm from the ground. The latency (seconds) to fall was measured with a cutoff time of 2 min. In addition, hanging was rated using the following scale: 0, no hanging; 1, 'poor' hanging (using only two paws); 2, 'good' hanging (using all four paws).

Balancing test

Four days after the wire hanging test, the mice were assessed in the horizontal rod test (Kalueff & Tuohimaa 2005). The rod was a 130-cm aluminum tube (2 cm in diameter), elevated to a height of 25 cm above a cushioned table and fixed to two Plexiglas sidewalls (1.5 × 50 × 50 cm) that prevented mice from escaping sideways. The rod was divided into 13 sectors (10 cm each) by line drawings. Each mouse was placed individually in the center of the rod and observed for 5 min, scoring total sectors visited (four paws), the number of hind leg slips (missteps) and falls. If an animal fell, the observer immediately placed it at the same location from which it fell.

Rope climbing, visual abilities and righting reflex tests

The same day, mice were allowed to climb a rope (1 cm in diameter), and the latency (seconds) to reach a 40-cm mark was recorded with a cutoff time of 3 min. The visual ability of the mice was then tested using a visual placing test (Pinto & Enroth-Cugell 2000). Briefly, each animal was lifted by the base of the tail (to a height of 15 cm) and then lowered to a table surface, assessing the animal's ability to extend its forelimbs toward the surface using a 2-point scale (0, no extension; 1, extension of forelimbs). The righting reflex was then assessed by dropping each mouse from a height of 30 cm above a cushioned floor and rated using a 2-point scale: 0, landing on flank/back; 1, normal righting (landing on all four feet).

Food finding test

Eight days later, mouse olfactory functions and food neophobia were assessed in the food finding test. To induce hunger, all mice were food deprived for 24 h prior to testing. On the test day, a small block of cheese (1 × 1 × 2 cm) was placed in the corner of each home cage, and the animals were observed for 5 min for their latency (seconds) to approach/sniff the food, the number of approaches, latency (s) to touch/lick the food, the number of touches, vertical rears and grooming bouts.

Tics and body position assessment

As previous experiments revealed frequent tics and aberrant body position in SERT −/− mice, in a separate experiment (following a 2-week rest period) we assessed these indices in SERT +/+, +/− and −/− mice by placing each mouse individually in the observation cylinder and videotaping for 3 min. Tapes were subsequently analyzed by an experienced observer (blind to the genotype), scoring mouse tics, as described previously (Nordstrom & Burton 2002). Tics were defined as any very brief isolated head and/or body jerk or shake. Tic incidence was assessed as the mean number of tics observed in individual mice during a 3-min videotape. In addition, we assessed the number (percentage) of animals displaying low/flat body posture and/or backward gait (Fig. 2 B) at some point during the testing period.

Stereotypic chewing test

One week later, a circular plastic canvas (7.1-g plastic mesh screens, 10 cm in diameter) was placed in the home cage of each animal for a period of 12 days, to assess their chewing activity (Chou-Green *et al.* 2003). At the end of the experiment, each canvas was weighed (g), and the number of spokes remaining exposed (per g) were counted for each canvas.

Activity patterns (Ethovision)

Because previous experiments did not allow us to obtain spatial, angular and velocity characteristics of mouse horizontal activity, in a separate experiment (during the last two days of the chewing test) we examined mouse behaviors using the Noldus Ethovision Video Tracking system (Version 3.0; Noldus, Wageningen, The Netherlands). On the day of experiments, mice were moved in their home cages to a dimly lit testing room (indirect red light) for 1 h to acclimate. Each animal was then placed individually in an observation cylinder for 5 min. White paper was fixed to outside of the walls and floor of the cylinder for a better contrast. Total distance traveled (cm), time spent moving (%), vertical rears, as well as relative (total distance divided by moving time) and maximal velocity (cm/seconds), angular velocity (degrees/seconds) and mean meander (degrees/cm; reflecting overall turning of the animal) were recorded and calculated for each animal. In order to assess thigmotaxis (preference of 'protected' walls vs. 'unprotected' open areas), the arena was divided into central and peripheral (< 5 cm from the walls) virtual zones. Total duration in center (seconds), the percentage

of time spent in center:periphery and the frequency of central entries was calculated for each genotype.

Statistics

All data are expressed as mean ± SEM. Data were analyzed by a Kruskal–Wallis test followed by a Dunn's *post hoc* test. Social interaction data were analyzed by the Mann–Whitney *U*-test for comparisons between the +/+ and −/− genotypes. Ethovision data were analyzed using one-way ANOVA (factor: genotype) followed by Tukey's *post hoc* tests. Per-minute distribution of horizontal and vertical activity in the same test was analyzed using a two-way ANOVA (factors: genotype, minutes). A probability of less than 0.05 was considered statistically significant in all tests.

Results

Overall, there were no significant differences between the three genotypes in nest-building activity (nest rating 2.8 ± 0.34 (SERT +/+); 3.3 ± 0.36 (SERT +/−); 2.8 ± 0.34 (SERT −/−); $H = 1.26$, NS; height 6.1 ± 0.76 cm (SERT +/+); 6.9 ± 0.69 cm (SERT +/−); 8.0 ± 0.58 cm (SERT −/−); $H = 2.6$, NS) indicating that this behavioral domain was unaltered in female SERT +/− and −/− mice.

Likewise, there were no differences in self-grooming activity between the three genotypes (latency to groom: 56.5 ± 15.1 seconds (SERT +/+); 62.0 ± 13.9 seconds (SERT +/−); 62.6 ± 19.2 seconds (SERT −/−); $H = 0.04$, NS; time spent grooming: 19.0 ± 2.9 seconds (SERT +/+); 20 ± 3.1 seconds (SERT +/−); 22.1 ± 3.1 seconds (SERT −/−); $H = 0.5$, NS; number of bouts: 8.5 ± 1.64 (SERT +/+); 9.1 ± 2.3 (SERT +/−); 10.7 ± 2.2 (SERT −/−); $H = 0.4$, NS). In addition, mice of all three genotypes demonstrated similar sequencing (patterning) of their grooming, as assessed by the percentages of incorrect transitions (51 ± 3% (SERT +/+); 50 ± 1% (SERT +/−); 51 ± 2% (SERT −/−); $H = 0.22$, NS) and interrupted bouts (8 ± 4% (SERT +/+); 17 ± 8% (SERT +/−); 18 ± 5% (SERT −/−); $H = 1.99$, NS).

In contrast, the sticky label test (Fig. 1) revealed differences between the genotypes for the number of attempts at removal ($H = 7.4$, $P = 0.025$; SERT +/− > SERT −/−, $P < 0.05$) but not for the latencies to attempt removal ($H = 5.6$, NS) or to remove ($H = 2.4$, NS) the label. As reported in Table 1, there were also significant differences between the genotypes in the novel object test, where SERT −/− mice showed increased neophobia (fewer approaches) compared to SERT +/+ mice.

Figure 2A shows ethograms reflecting generally similar patterning of behavioral activity in mice of all three genotypes, with the exception of fewer protected vertical rears and frequent Straub tail in SERT −/− mice, which was lacking in SERT +/+ and +/− mice.

Table 1 shows that SERT −/− mice differed markedly from their SERT +/+ counterparts in the social interaction test, showing less exploration, overall hypoactivity and a marked increase in Straub tail frequency.

In a separate experiment assessing Straub tail in detail (Fig. 2B, C), we again found significant genotype differences in the Straub tail scores (frequency: $H = 12.21$, $P = 0.0022$; latency: $H = 10.63$, $P = 0.0049$; duration: $H = 8.48$, $P = 0.014$). Overall, the SERT −/− group demonstrated higher Straub tail scores (compared to SERT +/+ and +/− mice: frequency 5.1 ± 0.8 (vs. 1 ± 0.5 and 1 ± 0.4; $P < 0.01$); latency 60 ± 34.5 seconds (vs. 236 ± 32.8 and 245 ± 26.1 seconds; $P < 0.05$); duration 16 ± 6.0 seconds (vs. 3.4 ± 1.7 and 2.9 ± 1.6 seconds; $P < 0.05$), respectively).

There were no differences between the three genotypes on the wire hanging test (hanging score: 1.88 ± 0.12 (SERT +/+); 1.86 ± 0.14 (SERT +/−); 1.86 ± 0.14 (SERT −/−); $H = 0.013$, NS; latency to fall: 49 ± 13 seconds (SERT +/+); 91 ± 19 seconds (SERT +/−); 47 ± 15 seconds (SERT −/−); $H = 3.35$, NS). Mice of all three genotypes also demonstrated unimpaired rope climbing (latency to climb 40 cm: 104 ± 25 seconds (SERT +/+); 77 ± 26 seconds (SERT +/−); 85 ± 18 seconds (SERT −/−); $H = 1.04$, NS), horizontal rod performance (sectors visited: 19 ± 8.4 (SERT +/+); 22 ± 4.8 (SERT +/−); 33 ± 11.5 (SERT −/−); $H = 1.06$, NS; missteps: 12.3 ± 3.4 (SERT +/+); 14.7 ± 3.9 (SERT +/−); 13.3 ± 6.3 (SERT −/−); $H = 0.82$, NS; falls: 4.6 ± 0.78 (SERT +/+); 5.6 ± 0.89 (SERT +/−); 4.1 ± 1.03 (SERT −/−); $H = 0.85$, NS), as well as equal righting reflex and visual placing test scores (1 ± 0 in each group, NS).

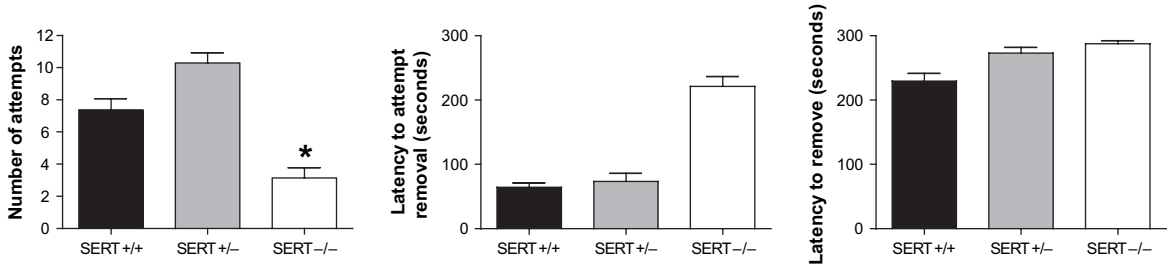

Figure 1: Performance of SERT mice on the sticky label test (mean ± SEM). *$P < 0.05$ vs. +/− mice (Dunn's test for significant Kruskal–Wallis data).

Table 1: Behavioral performance of SERT +/+, +/− and −/− mice in a battery of tests

Test/Behaviors	SERT +/+	SERT +/−	SERT −/−	Kruskal–Wallis results
Novel object test (observation cylinder, 3 min)				
Latency to approach (seconds)	17 ± 8	26 ± 19	57 ± 23	$H = 4.38$, NS
Latency to touch (seconds)	151 ± 19	132 ± 24	177 ± 3	$H = 1.72$, NS
Number of approaches	13 ± 2	10 ± 2	4.6 ± 1**	$H = 9.48$, $P = 0.009$
Number of touches	0.3 ± 0.2	0.7 ± 0.4	0.1 ± 0.1	$H = 1.68$, NS
Social interaction test (observation cylinder, 5 min)				
Initiated sniffs	16 ± 3	—	4 ± 1*	—
Follows	2.7 ± 0.6	—	0.1 ± 0.1*	—
Touches	1.4 ± 0.7	—	0.3 ± 0.3	—
Self-grooming	7 ± 1.3	—	2.6 ± 1.0*	—
Vertical rears	13 ± 2.7	—	2 ± 1.0*	—
Attacks	0.4 ± 0.4	—	0	—
Straub tail (frequency)	1 ± 0.5	—	5 ± 1.3*	—
Food neophobia (home cage, 5 min)				
Latency to approach (seconds)	10 ± 3	20 ± 9	12 ± 3	$H = 1.14$, NS
Latency to touch/lick (seconds)	30 ± 15	108 ± 45	200 ± 44**	$H = 9.30$, $P = 0.0096$
Number of approaches	5 ± 0.5	3 ± 1	4 ± 1	$H = 3.59$, NS
Number of touches	7 ± 1	4 ± 1	1 ± 0.5**	$H = 11.53$, $P = 0.0031$

*$P < 0.05$ (Mann–Whitney *U*-test for social interaction test), **$P < 0.01$ vs. SERT +/+ mice (Dunn's test for significant Kruskal–Wallis data).

Analyzing mouse performance in the food finding test, we found unimpaired olfactory and motor abilities, as assessed by approach latency and frequency, but a significant genotype difference in the latency to touch food and the number of contacts with the food (Table 1).

Analyzing mouse tics and body position, we found that all three groups displayed similar occurrences of backward gait ($H = 2.27$, NS) but showed robust genotype differences in body posture ($H = 14.40$, $P = 0.0007$), with more SERT −/− mice demonstrating low/flat body position, in addition to displaying significantly more tics ($H = 11.49$, $P = 0.0032$), than their SERT +/+ counterparts (Fig. 2C).

Figure 3 shows the behavioral performance of SERT +/+, +/− and −/− mice assessed by Ethovision. Overall, reduced horizontal activity in SERT −/− mice ($F_{2,21} = 6.006$, $P = 0.01$) was accompanied by decreased total per cent duration moving ($F_{2,21} = 7.38$, $P = 0.004$), reduced number of visits to the center ($F_{2,21} = 14.572$, $P < 0.0001$), duration in center ($F_{2,21} = 9.140$, $P = 0.002$), and per cent duration in center:-periphery ($F_{2,21} = 5.47$, $P = 0.013$), as well as increased meander ($F_{2,21} = 8.67$, $P = 0.002$) and angular velocity ($F_{2,21} = 10.04$, $P = 0.001$), but unaltered relative ($F_{2,21} = 0.76$, NS) and maximal velocity ($F_{2,21} = 0.89$, NS). In addition, analyzing the per minute distributions of activity in these mice, there were significant genotype ($F_{2,19} = 6.01$, $P = 0.01$) and time ($F_{1,19} = 23.25$, $P = 0.0001$), but not genotype × time ($F_{2,19} = 1.38$, NS), effects for total horizontal distance. Likewise, there was a significant time ($F_{1,19} = 8.54$, $P = 0.009$), but not genotype ($F_{2,19} = 3.07$, NS) or genotype × time ($F_{2,19} = 2.12$, NS), effect for vertical rears (Fig. 3A), indicating that unlike activity levels, the temporal patterning of mouse horizontal and vertical exploration was not altered by genetic ablation of SERT.

Finally, as can be seen in Fig. 4, there was a significant genotype effect for chewing activity in the chewing test (canvas weight: $H = 6.55$, $P = 0.038$) but not the number of spokes ($H = 3.98$, NS). In this test, SERT −/− mice produced less chewing than their SERT +/− littermates ($P < 0.05$).

Discussion

Overall, major sensory functions, such as olfaction, vision and vestibulation, appear to be unaffected by genetic ablation of SERT in +/− and −/− mice and are therefore unlikely to influence their performance. This was an unexpected finding, given previous reports on abnormal formation of somatosensory maps in these mice, including poorer barrel field development in SERT +/− mice and their lack in SERT −/− mice, associated with decreased glucose utilization in this somatosensory pathway (Esaki *et al.* 2005; Persico *et al.* 2001; Salichon *et al.* 2001; Xu *et al.* 2004). While these data suggest that whisking—an important part of mouse exploration—may indeed be affected in SERT +/− and −/− mice, contributing to their behavioral abnormalities reported previously (e.g., Holmes *et al.* 2003a, 2003b, 2003c), further studies are needed to assess the role of aberrant barrel fields in SERT −/− mouse behavior, as well as the extent to which compensatory brain mechanisms may counterbalance these impairments.

Another important factor to consider is that SERT −/− mice demonstrated overall hypoactivity in various tests reported here, particularly on the open field (Fig. 3) and social interaction (Table 1) tests. In the social interaction test, we followed previously published protocol (Kalueff *et al.* 2006) enabling a direct comparison of the two extreme phenotypes

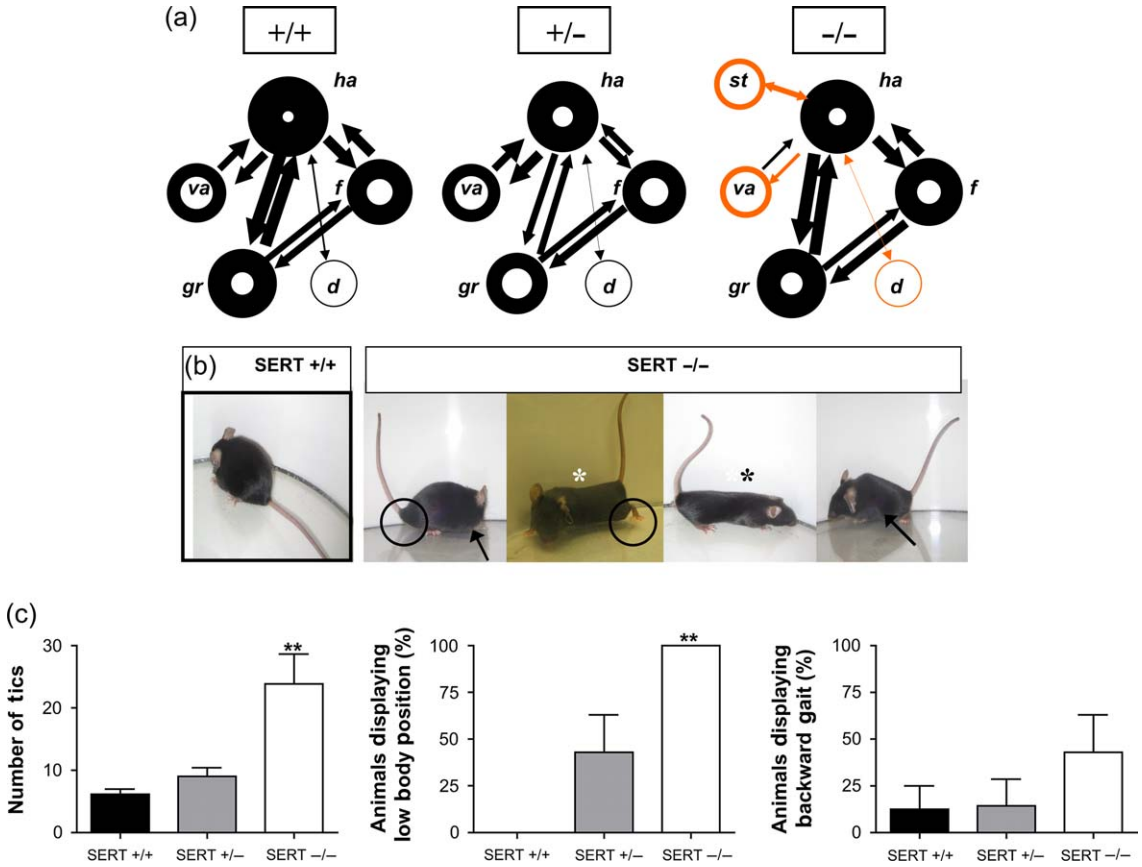

Figure 2: Frequencies (number of episodes) of specific behavioral activities and their transitions in SERT +/+, +/− and −/− mice. (a) Ethograms (5 min, observation cylinder): ha, horizontal activity (number of ha episodes); va, vertical activity (protected rears); f, freezing episodes; g, grooming bouts; d, defecation; st, Straub tail. Line width reflects frequency of behaviors (circles) or their transitions (arrows). SERT −/− mouse behaviors/transitions significantly different from those of SERT +/+ and +/− mice are marked by a different color. Note that only frequencies > 1 are shown in this diagram. For differences between genotypes in horizontal activity scores (e.g., total distance traveled) in another experiment see Fig. 3A. (b) Straub tail commonly seen in SERT −/− mice but not in SERT +/+ or +/− mice; also note body position low to the ground (arrows), hind leg dragging (circles) and flat back (asterisks) in SERT −/− mice. (c) Tics, body position and backward gait in SERT +/+, +/− and −/− mice observed for 3 min (mean ± SEM, $^{**}P < 0.01$ vs. SERT +/+ mice, Dunn's test for significant Kruskal–Wallis data).

by confronting SERT +/+ mouse with their SERT −/− counterparts. Although not allowing to examine the third (SERT +/−) group, this experiment confirmed marked behavioral differences between SERT +/+ and −/− mice. Overall, these findings are consistent with previous reports on reduced aggression and lower 24-h home cage activity in these mice (Holmes *et al.* 2002b). Likewise, SERT −/− mice showed hypoactivity in the sticky label and the chewing tests (Figs. 1 and 4), thus raising the question of correct interpretation of SERT −/− behaviors in anxiety and all other tests because of hypoactivity confounding the results (see also Kalueff *et al.*, in press, for discussion).

Although hypoactivity does appear to affect their phenotype, our results indicate that the behavioral profile of SERT −/− mice cannot be simply explained by non-specific behavioral inhibition. For example, SERT −/− mice showed unimpaired nest-building, horizontal rod performance, rope climbing, wire hanging, self-grooming, unaltered latency to approach food (Table 1) and relative and maximal velocity (Fig. 3A). Taken together, these data indicate that in some tests these mice are as active as their wild type controls. In addition, motor co-ordination and sensorimotor integration also appear to be unaltered in SERT +/− and −/− mice, as assessed by normal self-grooming patterning (sequencing) and horizontal rod performance (missteps and falls), respectively. In contrast, our study confirmed high anxiety behavior in the SERT −/− mice, including higher thigmotaxis (Fig. 3 A,B) and neophobia (fewer contacts with food and a novel object; Table 1). Fewer attempts to remove the sticky label in the sticky label test (Fig. 1) are also likely to reflect a neophobic response in SERT −/− mice, collectively suggesting that both anxiety and hypolocomotion (accompanied by no overt sensorimotor deficits) contribute to the complex behavioral phenotype of SERT −/− mice.

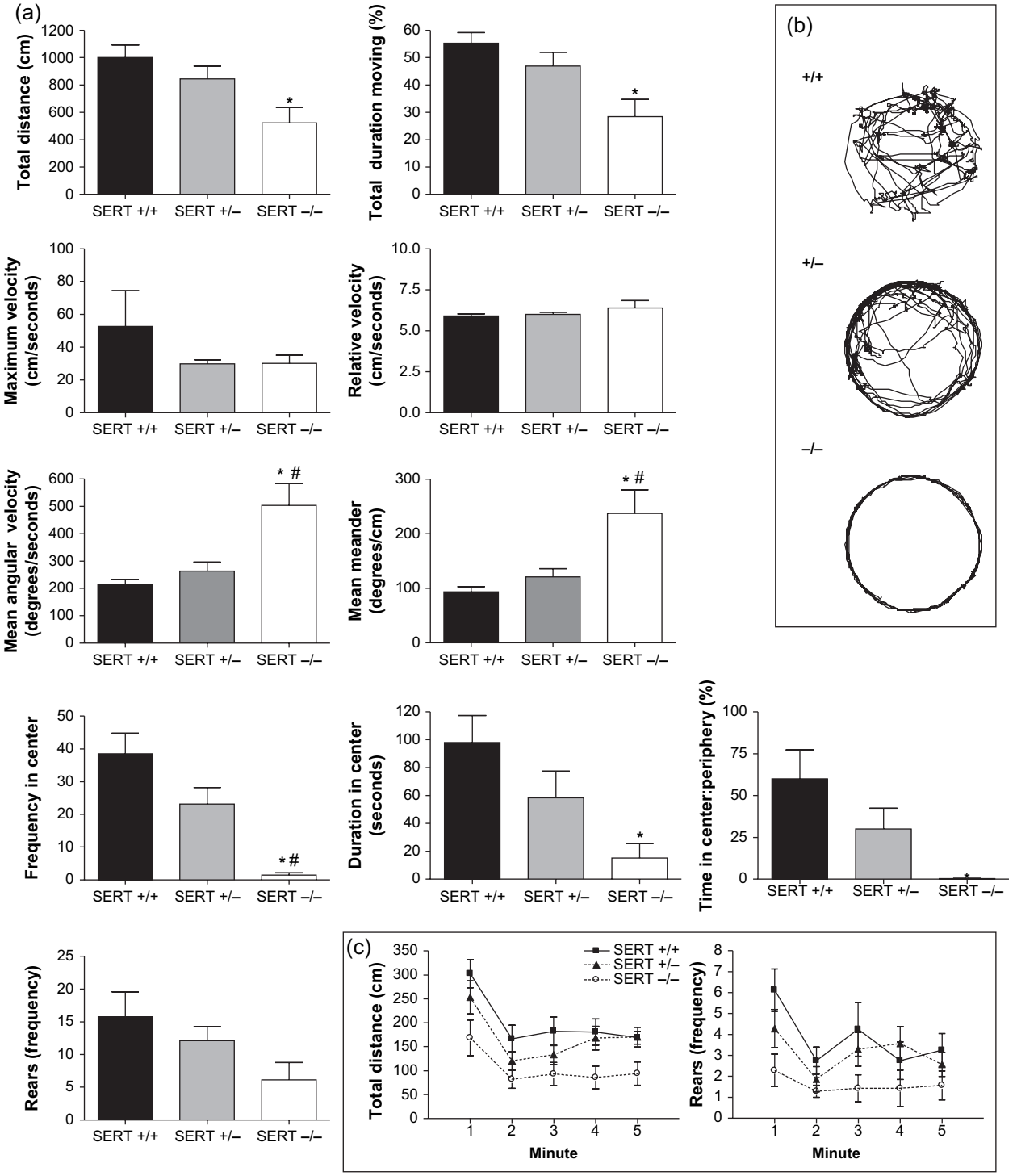

Figure 3: Behavioral performance of SERT +/+, +/− and −/− mice in an observation cylinder (mean ± SEM; 5-min test). (a) Behavioral scores: $*P < 0.05$ vs. SERT +/+ mice; $\#P < 0.05$ vs. SERT +/− mice (Tukey's *post hoc* test for significant ANOVA data). (b) Representative patterns of movements (selected based on mean total distance data in panel a). (c) Similar temporal (per minute) distribution of horizontal and vertical activity in all three genotypes.

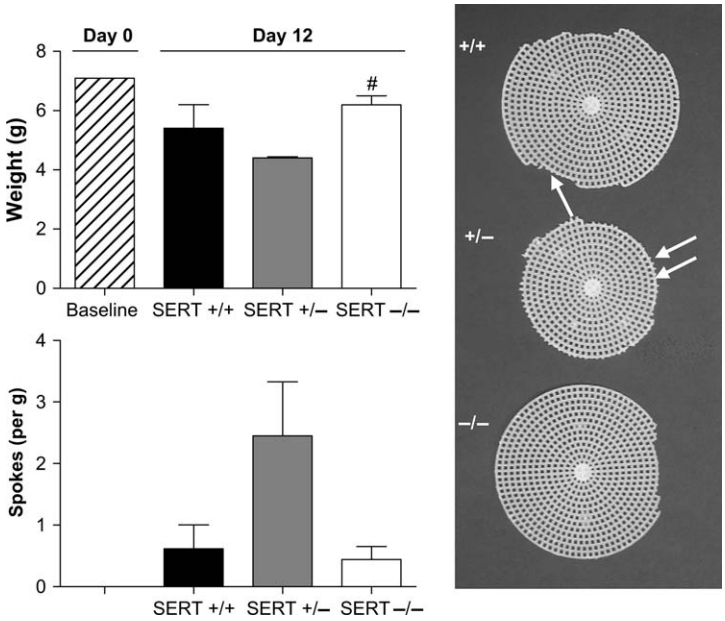

Figure 4: Chewing activity of SERT mutant mice. (a) Chewing of plastic canvas for 12 days (mean ± SEM; #$P < 0.05$ vs. SERT +/− mice, Dunn's test for significant Kruskal–Wallis data). (b) Representative patterns of chewing activity (spokes are indicated by arrows).

Interestingly, except for vertical activity and Straub tail indices (consistently altered in this and several other tests), ethograms showed similar behavioral patterning in all three genotypes, including the number of horizontal activity episodes and of freezing episodes (Fig. 2A). Given overall reductions in total distance and total duration of moving in SERT −/− mice (assessed by Ethovision in another experiment), mice of all three genotypes seem to initiate similar numbers of horizontal locomotion bouts, with SERT −/− mice traveling less during each bout. In line with a robust avoidance of open areas (Fig. 3A,B), this pattern is generally consistent with an anxious, hypoactive phenotype of SERT −/− mice reported here and in previous studies.

Because there are known problems with anxiety tests, such as their validity and reliability (Geyer & Markou 2002; Van der Staay & Steckler 2002), it was important to compare behavioral profiles obtained in different contexts, as well as with other published data using these mice. Overall, our findings are consistent with reduced activity and increased anxiety observed here in several different tests, and also reported in other studies using SERT −/− mice on different genetic backgrounds (Holmes *et al.* 2002b, 2003a; Zhao *et al.* 2006). Further supporting this notion, reduced social interaction in SERT −/− mice also may reflect their increased anxiety (File 1980; File & Seth). Notably, this interpretation does not preclude a contribution of hypolocomotion and low aggression to this profile, as has already been mentioned. Although social interest was unaltered in the resident-intruder test in male SERT −/− mice (Holmes *et al.* 2002b), gender and experimental differences between the two studies may explain this discrepancy.

Another interesting possibility may be altered sociability in these mice. Because reduced mouse sociability has been recently suggested to represent a model of autistic-like behavior (Crawley 2004; Moy *et al.* 2004, 2006), this also needs to be considered for SERT −/− mice. Indirectly supporting this notion, human data show genetic linkage between the chromosome 17q region (where SERT is located) and also associations of SERT and SERT polymorphisms and haplotypes with autism (Bacchelli & Maestrini 2006; Conroy *et al.* 2004; McCauley *et al.* 2004; Sutcliffe *et al.* 2005; Yonan *et al.* 2003). Clearly, further studies are needed to dissect in detail different aspects of altered social behavior in SERT −/− mice, and its possible relation to autism, social anxiety and/or aggression.

Another important question is how reduced (but not absent) SERT expression affects behavior in SERT +/− mice. Although SERT +/− mice display 50% SERT binding and somewhat altered serotonergic neurotransmission (Bengel *et al.* 1998; Kim *et al.* 2005; Murphy *et al.* 2001), it has sometimes been concluded that these mice produce only a mild phenotype (Lesch *et al.* 2003). Several observations can be made based on the results of the present study. While in some tests used here SERT +/− mice were indeed similar to SERT +/+ mice (significantly differing from the SERT −/− group), in other tests SERT +/− mice tended to display an intermediate phenotype (Table 1, Fig. 3). In line with recent data (Montanez *et al.* 2003; Mathews *et al.* 2004), this suggests that the SERT +/− mouse behavioral phenotype possesses further behavioral complexity, which requires experimental dissection. For example, because these SERT +/− mice are devoid of hypolocomotory problems and have

reduced (rather than abolished) SERT activity, these mice seem to be more relevant to model human serotonergic dysfunctions associated with the several different SERT genetic polymorphisms (Lesch *et al.* 1996; Murphy *et al.* 2001, 2004; Wendland *et al.* 2006).

Furthermore, we note that SERT −/− mice displayed frequent Straub tail, tremor, tics, hind leg dragging, backward gait and flat back/low posture (Fig. 2). Although further studies are needed to assess these findings in detail [e.g., assessing a possible role of stress/anxiety in the Straub tail response (Katz 1979)], all of these behaviors strikingly resemble classic serotonin syndrome (SS) behavior (Borsini *et al.* 2001; Izumi *et al.* 2006; Nisijima *et al.* 2000; Shioda *et al.* 2004), typically observed in rodents with pharmacologically elevated serotonin levels. Because SS (sometimes termed serotonin toxicity) is commonly observed in humans and is a life threatening disorder associated with disturbed serotonin functions (Boyer & Shannon 2005; Darmani & Ahmad 1999; Gillman 2006; Goitz 2002; Insel *et al.* 1981; Isbister & Buckley 2005), the possibility of using SERT +/− and −/− mice as a genetic model of SS merits further elaboration.

Because SERT −/− mice are well known to have increased serotonergic tone (Murphy *et al.* 2001, 2004), it is indeed possible to hypothesize that they may represent a model of SS. Consistent with this notion, tremor, tics and muscular hypertonicity frequently seen in SS patients (Boyer & Shannon 2005; Isbister & Buckley 2005) seem to parallel behavioral observations in SERT −/− mice reported here. In addition, hyperthermia, commonly observed in SS (Shioda *et al.* 2004), has also been reported in SERT −/− mice (Holmes *et al.* 2003a). In recent studies in our lab using serotonin-enhancing drugs (Fox & Murphy 2006), SERT −/− mice showed markedly different temperature responses and significant increases in some serotonin syndrome-like behaviors, while SERT +/− mice displayed a somewhat intermediate phenotype. These behavioral responses (including exaggerated hind limb abduction and low body posture) were similar to those observed spontaneously in the present study, and ongoing studies are investigating the underlying receptor mediation of these enhanced responses. Collectively, these pharmacological findings support the concept that enhanced serotonergic function in SERT −/− mice contributes to the serotonin syndrome-like behavioral phenotype reported here.

Several other SERT −/− mouse behaviors merit further investigation. For example, SERT −/− mice showed more and faster turning (Fig. 3A), which may reflect altered spatial patterning due to increased anxiety and disorientation, commonly seen in humans with SS (Goitz 2002). As such, altered spatial strategies in SERT −/− mice (Fig. 3B) may reflect either factor, or a combination of both. Analysis of individual behavioral differences and environmental influences (Ferrari *et al.* 1998; Hossain *et al.* 2004; Lathe 2004; Izidio *et al.* 2005) in SERT −/− mice may also be a promising direction of research. Finally, as the test history may influence mouse performance (McIlwain *et al.* 2001; Paylor *et al.* 2006), it will be interesting to assess in detail the effects on the SERT +/− and −/− mouse behaviors produced by repeated testing in various batteries of behavioral tests (Crawley 2000; Crawley & Paylor 1997; Sousa *et al.* 2006).

In conclusion, our study suggests that the complex behavioral profile observed in SERT −/− mice in this and in previous studies (Holmes *et al.* 2002a, 2002b, 2003a, 2003b, 2003c) may be determined by an interplay between three overlapping factors—hypoactivity, anxiety and SS-like behavior.

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