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Spermidine/spermine N^1-acetyltransferase overexpression in mice induces hypoactivity and spatial learning impairment

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Abstract

The present work addresses the role of polyamines in learning and general behavior by subjecting transgenic mice overexpressing polyamine catabolic enzyme, spermidine/spermine N^1-acetyltransferase (SSAT) and their syngenic littermates to neurobehavioral profiling assessment (SHIRPA) and to radial eight-arm maze. The general health and physiological conditions as well as the entire behavioral battery comprising of 34 parameters were recorded. The eight-arm radial maze (8-RAM) task included an initial acquisition task for 9 days followed by a 2-day retention test after a 2-week break. In addition, blood samples were taken for hormone analysis. Transgenic mice, which showed reduced motor activity, aggression and muscle tone, spent more time in the radial maze during initial acquisition and retention tasks as compared with syngenic mice. Moreover, the learning performance of transgenic females was significantly inferior to syngenic females. Interestingly, the levels of several hormones were significantly altered in SSAT transgenic mice; circulating adrenocorticotropic hormone (ACTH) and corticosterone levels were markedly increased while testosterone and thyroidal hormone levels were decreased.

These changes may be related to the dramatic increase in brain putrescine levels in SSAT-overexpressing (SSAT-OE) mice, but it is likewise possible that the behavioral changes and learning impairment are attributable to more peripheral mechanisms (such as alterations in steroid hormone metabolism), which in turn, could be a consequence of the disturbed polyamine homeostasis.

Keywords: Eight-arm radial maze; Polyamine; Putrescine; Spermidine; Spermine; SHIRPA; Spatial learning; SSAT

1. Introduction

Polyamines are positively charged small molecules that are involved in protein synthesis, cell growth, and regulation of several metabolic processes in a cell (Jänne et al., 1991; Kremzner, 1970; Seiler and Bolkenius, 1985; Tabor and Tabor, 1984). The interactions of polyamines with the *N*-methyl-D-aspartate (NMDA) receptor (Gallagher et al., 1997; Ransom and Stec, 1988; Williams et al., 1990, 1991) and with the inward rectifying K^+-channel (Ficker et al., 1994; Oliver et al., 2000) and α-amino-3-hydroxy-5-methylisoazole-4-propionate (AMPA; Bowie et al., 1998; Kamboj et al., 1995; Washburn and Dingledine, 1996; Williams, 1997) have been described in detail as well as their contribution to glutamate induced excitotoxicity (de Vera et al., 1997; Gilad and Gilad, 1992; Gimenez-Llort et al., 1997; Halonen et al., 1993; Kaasinen et al., 2000; Lukkarinen et al., 1998; Paschen, 1992; Rao et al., 2000). However, relatively few studies have addressed the influence of polyamines on animal behavior and learning and memory functions.

One of the first studies (Sakurada et al., 1977) on behavioral effects of polyamines indicated that intracerebrally administered spermidine enhances, and spermine inhibits, spontaneous motor activity in mice, but with a 24-h delay. A later study (Hirsch et al., 1987) demonstrated that spermidine and spermine dose-dependently inhibit spontaneous climbing and wheel-running behavior in mice. In addition, these polyamines antagonized stereotypic hyperactivity induced

Abbreviations: ACTH, adrenocorticotropic hormone; AMPA, α-amino-3-hydroxy-5-methylisoazole-4-propionate; CRH, corticotropin-releasing hormone; HPA, hypothalamic–pituitary–adrenal; LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartate; ODC, ornithine decarboxylase; 8-RAM, eight-arm radial maze; SSAT, spermidine/spermine N^1-acetyltransferase; TSH, thyroid-stimulating hormone; T4, thyroxine.

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by apomorphine when injected into the nucleus accumbens, suggesting an interaction with mesolimbic dopaminergic pathways. A recent study (Gimenez-Llort et al., 1997) showed a correlation between putrescine concentration and hyperactive behavior induced by NMDA while no correlation was observed with spermidine or spermine concentrations.

Studies assessing the role of polyamines in learning and memory have yielded controversial results. An impaired learning of the 14-unit T-maze induced by the NMDA-receptor antagonist (±)-3-(2-carboxypiperazine-4-yl)-propyl-1-phosphonic acid (CPP) was attenuated with intraperitoneally administered spermine in one study (Meyer et al., 1998), but an impaired learning of the same task by another NMDA antagonist, dizocilpine, was potentiated by intraperitoneal spermidine in another study (Shimada et al., 1994). In the latter study, spermidine alone was without an effect. Correspondingly, acute spermine at a moderate dose did not affect spatial learning in the water maze, while administration of a higher dose for 5 days resulted in hippocampal neurotoxicity and learning impairment (Conway, 1998). Spermidine injections into the hippocampus were also without any effect on a three-panel runway task for working memory, but antagonized the impairing effect of the muscarinic antagonist scopolamine, the NMDA antagonist dizocilpine, or a Class I metabotropic glutamate receptor antagonist (Kishi et al., 1998). However, posttrial injection of spermidine into the hippocampus or the amygdala (Rubin et al., 2001) has been reported to enhance passive avoidance learning in rats, an effect that was abolished by concurrent administration of arcaine. In addition to direct pharmacological administration of polyamines, spatial learning has been tested in transgenic mice overexpressing ornithine decarboxylase (ODC; Halonen et al., 1993). These mice showed impaired learning in the water maze, supposedly due to the partial block of NMDA receptors by putrescine.

We have developed a transgenic mouse line that overexpresses spermidine/spermine N^1-acetyltransferase (SSAT; Pietila et al., 1997), the rate controlling enzyme of polyamine catabolism. The SSAT transgenic mice have distorted polyamine homeostasis in all tissues. They have elevated SSAT activity, increased putrescine and acetylated spermidine levels, whereas spermidine and spermine levels are lower than normal. The influence of SSAT overexpression in peripheric systems have been reported in numerous studies (Alhonen et al., 1998; Jänne et al., 1999; Pietila et al., 2001; Suppola et al., 1999), but relatively few studies have focused on CNS. We have shown earlier that SSAT-overexpressing (SSAT-OE) mice are partially resistant to both kainate-induced and pentylenetetrazol (PTZ)-induced seizures (Kaasinen et al., 2000, 2003) and appear likewise to be protected from the provoked neuronal death induced by these excitotoxins. Because putrescine content in SSAT-OE mice is substantially increased in the cerebral cortex and hippocampus, which are, besides the amygdala, the most vulnerable brain areas to the kainate and PTZ excitotoxicity, we suggested that this neuroprotection is a result of dramatically expanded putrescine pool.

The present study aims to further elucidate the role of polyamines in learning and memory. Because of the pivotal role of the hippocampus in learning and memory functions (Rolls, 2000) and the altered balance between spermine, spermidine and putrescine pools in the hippocampus of the SSAT-OE mice, we focused on a spatial task, eight-arm radial maze (8-RAM) working memory task, which is very sensitive to hippocampal dysfunction (Olton et al., 1979). Another standard hippocampus-dependent task, the Morris water maze, was less suitable because SSAT-OE mice are hairless (Pietila et al., 1997) and thus sensitive to hypothermia. In addition, to elucidate overall changes in the behavior in these mice, we adopted the SHIRPA test battery (Rogers et al., 1997). It consists of comprehensive general health and behavior observations as well as the evaluation of motor and sensory functions.

2. Materials and methods

2.1. Animals

Adult (3–4 months) SSAT syngenic and transgenic BALBc × DBA/2 mice of the line UKU165b (Pietila et al., 1997) used in this study were housed one mouse per cage under controlled conditions (temperature +21 °C, lights 0700–1900 h). All testing took place between 0800 and 1600 h. Altogether, 69 mice were used in the behavioral study (25 syngenic males and 11 syngenic females, 22 transgenic males and 11 transgenic females). At the end of experiment series, the mice were anaesthetized with pentobarbiturate (40 mg/kg) and perfusion fixed with 4% *para*-formaldehyde for the histological analysis. The experiments were approved by the Institutional Animal Care and Use Committee of the University of Kuopio and by the Provincial Government of Eastern Finland.

2.2. Behavioral phenotyping

A comprehensive behavioral characterization of SSAT syngenic and transgenic mice was conducted according to the SHIRPA protocol (Rogers et al., 1997) and using the equipment described on the SHIRPA Web page: www.mgu.har.mrc.ac.uk/mutabase/shirpa-summary.html. Mice were tested twice, before and after the 8-RAM task, without familiarization to the testing equipment. In addition, we recorded the general health and physiological condition of the mice, such as body weight, body length, temperature and skin color. The entire behavioral battery comprised 34 recording parameters. To avoid incidental findings and to better manage these large data, we first tested which parameters were interrelated using Spearman rank correlations for the pooled data of all animals. This first screening yielded six groups of intercorrelated ($P < .05$) parameters that were named as follows. (1) *Activity* (body position, spontaneous activity and locomotor activity); (2) *Aggression*

(aggression, provoked biting and irritability); (3) *Defense* (trunk curl and limb grasping); (4) *Grip strength*; (5) *Muscle tone* (touch escape, toe pitch, body tone and limb tone); (6) *Rear elevation* (pelvic and tail elevation). The remaining parameters were ignored for this study. Scoring of each test parameter was normalized to a scale from 0 to 4 to allow parameter grouping.

2.3. Eight-arm radial maze

The 8-RAM used was a design similar to that developed by Olton et al. (1979) for rats and adapted to the mouse by Crusio et al. (1993). The eight arms radiated out from an octagonal Plexiglas center, 22 cm in diameter. The Plexiglas arms were 25 cm long, 6 cm wide and 6 cm high. The arm entrances could be blocked by guillotine doors. Rice Crispies (Kellogg's) were placed at an inaccessible recess at the end of each arm behind a perforated wall to give all arms a uniform scent. A single food reward was placed immediately in front of the perforated wall, but behind a low visual barrier (1 cm). The mice were trained to collect food rewards from every arm of the radial maze. The mice were removed from the 8-RAM after retrieval of all rewards, or after a total of 16 arm entries had been made or after 20 min

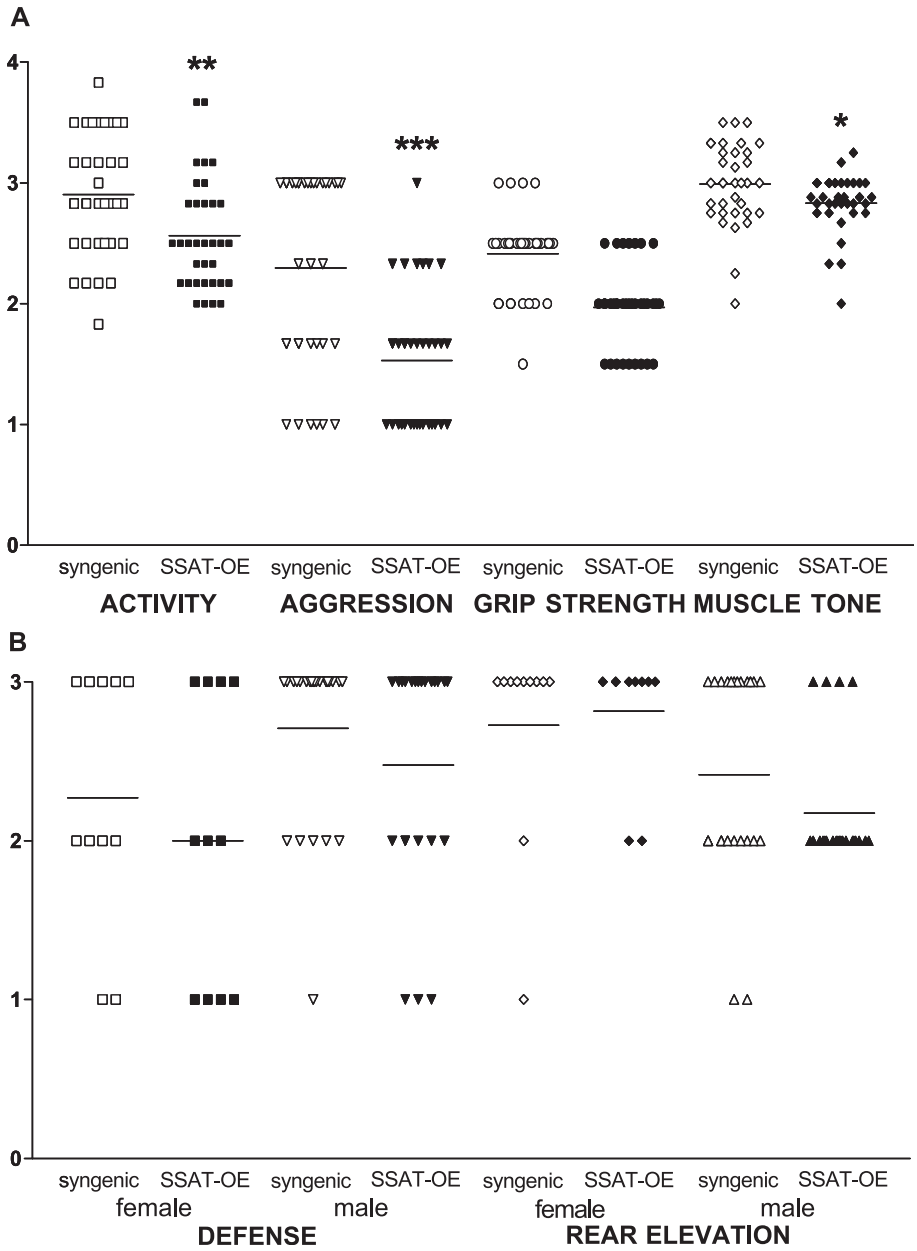

Fig. 1. The results of SHIRPA assessment were grouped (see Materials and Methods) into six categories of behavioral parameters, which present activity, aggressive, defense behavior, grip strength, muscle tone and rear elevation. Scatter blot (A) presents the data between syngenic and SSAT-OE mice. Two of the categories, defense behavior and rear elevation, were sex dependent and are presented in the separate scatter blot (B). Bars present values as mean ± S.E.M., * $P < .05$; ** $P < .01$; *** $P < .001$.

had passed, whichever came first. An entry was defined as all four paws entering the arm. Between male and female mice, the maze was cleaned with mild detergent to remove the odors of the opposite sex. Prior to the experiment, a food restriction schedule was initiated to reduce and subsequently maintain the body weight of the mice at 80–85% of their free-feeding level throughout testing. Two days of pretraining allowed the mice to explore the baited 8-RAM for 10 min at a time. During the experimental phase, all the eight arms were baited. After each return to the center from an arm, the doors were closed for 5 s. This discouraged the mouse from utilizing non-memory based strategies. The mice were allowed 9 days for the initial acquisition of the task. After a 2-week break, the mice were again tested in the 8-RAM for 2 days. By baiting all eight arms, an entry into an arm from which it had already retrieved the food pellet was deemed an error. Because of large interindividual variability in the number of errors before finding the most difficult eighth reward, the performance was evaluated on the basis of total number of errors made before the seventh correct choice.

2.4. Hormone analysis from blood samples and tissue weight

Separate groups of mice (20 syngenic and 20 transgenic) of both sexes were used for hormone analysis. Blood

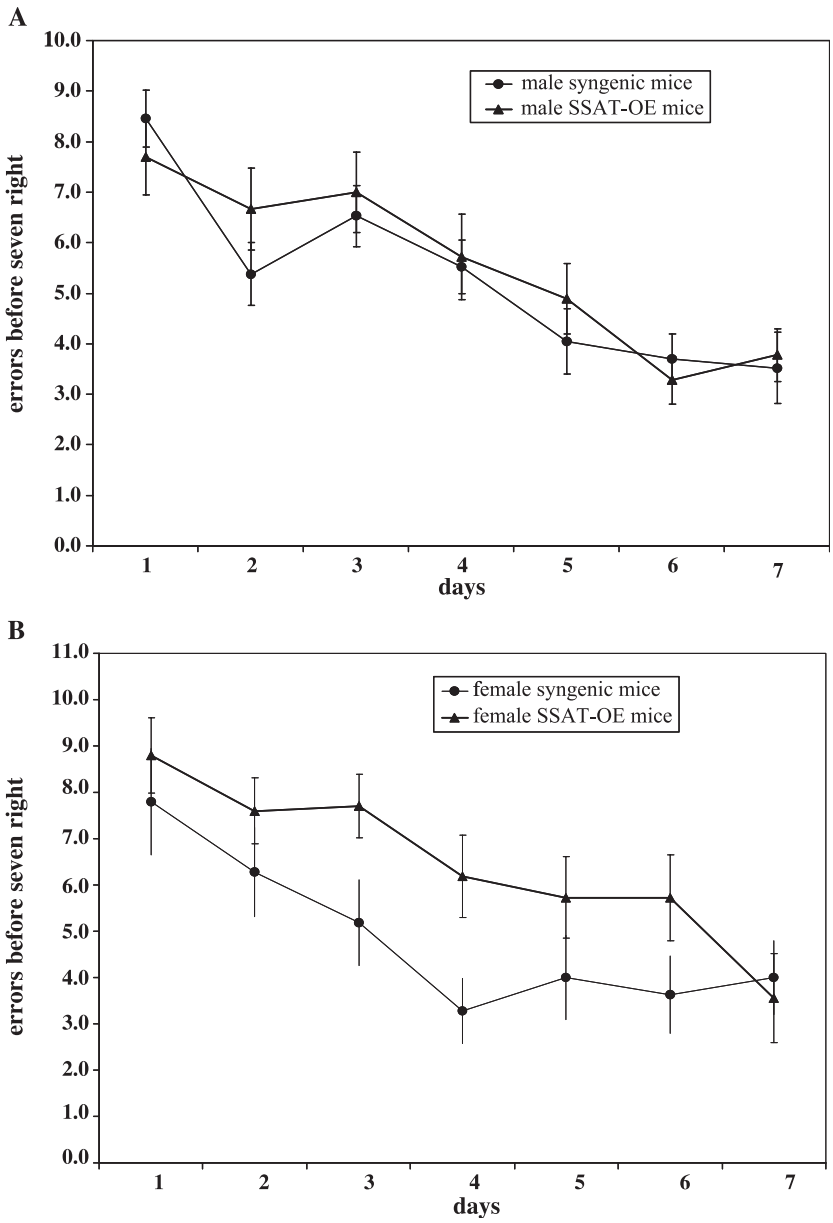

Fig. 2. The learning curves of SSAT-OE and syngenic mice in the 8-RAM. The *y*-axis values represent number of errors before mice obtained seven correct arm entries. The results of males (A) and females (B) are shown in separate boxes. SSAT-OE females were inferior to syngenic female mice [$F(1,15) = 14.3$, $P < .002$; ANOVA for repeated measures] but the male genotypes did not differ. ●, SSAT-OE mice and ▲, syngenic mice. Bars present values as mean ± S.E.M.

samples were taken from anaesthetized (midazolamine 0.6 mg/kg and fluanisone 1.25 mg/kg) mice through the heart with Microtainer K2E and SST tubes (Becton–Dickinson, USA). The blood samples were cooled down to room temperature and centrifuged for 10 min at 3000 rpm. The collected supernatant fractions were stored at −20 °C before analyses. Hormone analyses were carried out either in a commercial laboratory (Medix, Helsinki, Finland) or by us. We used ^{3}H-radioimmunoassay (RIA) for rats and mice (ICN Biomedicals, USA) to determine the concentration of corticosterone in plasma. The assay was made according to the protocol of the manufacturer.

After blood sampling, the mice were sacrificed for the tissue preparation. Testicles or uteri of both transgenic and syngenic mice were collected and weighed.

2.5. Statistical analyses

The statistical analyses were carried out by using SPSS software for Windows (SPSS, Chicago, USA). The behavioral data and hormonal analyses, when possible, covered both genotypes and genders. The SHIRPA test data were compiled into six parameter groups and the body weight was analyzed by using nonparametric Kruskall–Wallis Test. The data from 8-RAM were analyzed with ANOVA for

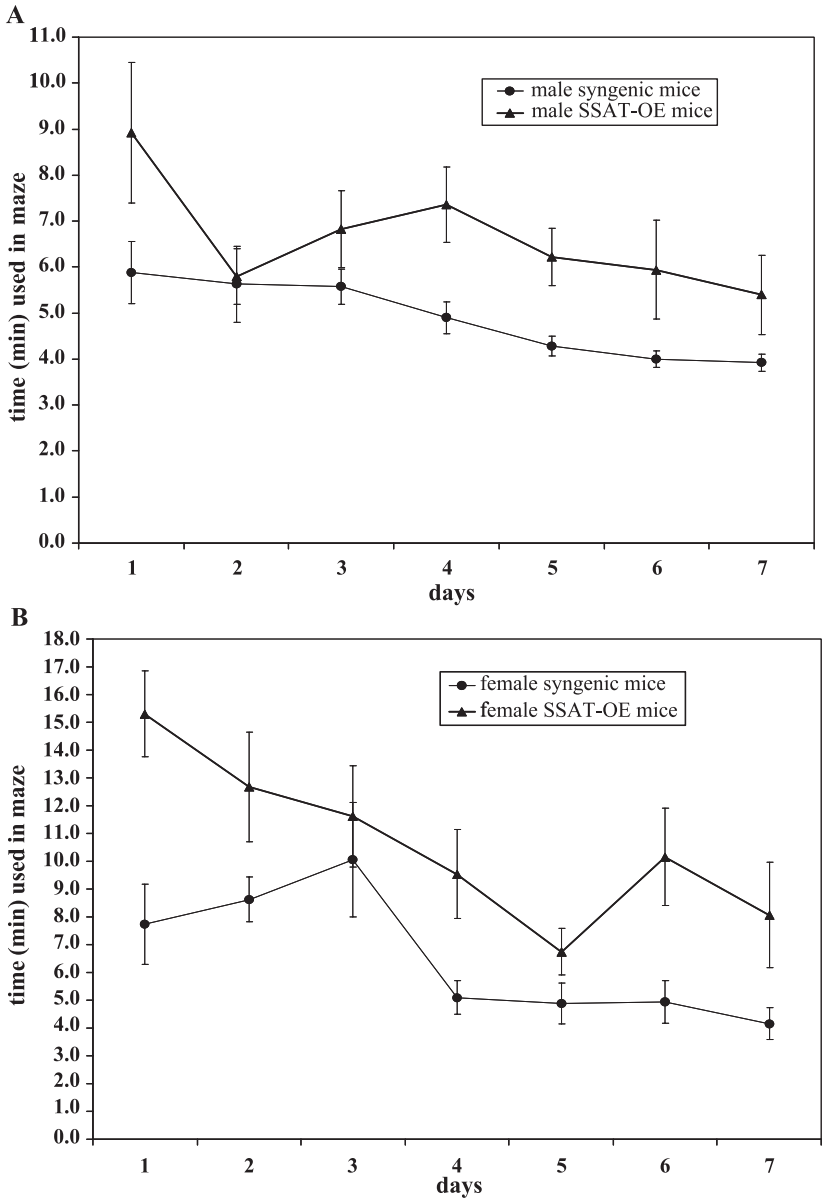

Fig. 3. The time to complete daily trials differed significantly between the genotypes ($P<.001$, ANOVA). Both SSAT-OE males (A; $P=.03$) and females (B; $P=.04$) spent more time in the acquisition phase of the task (ANOVA for repeated measures). ●, SSAT-OE mice and ▲, syngenic mice. Bars present values as mean ± S.E.M.

repeated measures. Finally, when appropriate, Student's *t* test or Fisher's Protected Least Significant Difference was used in evaluation of hormone results. All the data of this experiment are presented as mean ± S.E.M.

3. Results

3.1. Behavioral findings

SSAT-OE mice did not differ from syngenic controls in their body weight (mean ± S.E.M.). SSAT-OE: females ($n = 11$) 22.8 ± 0.8 g, males ($n = 23$) 26.0 ± 0.8 g; syngenic: females ($n = 11$) 21.1 ± 0.7 g, males ($n = 24$) 26.2 ± 0.7 g; all $P > .78$. However, their behavior was distinctly different as compared with their syngenic littermates. While syngenic mice were vivid and eager in the jar and the arena, SSAT-OE mice appeared almost phlegmatic, occasionally slumping and keeping long pauses between movement episodes. They also expressed little aggression during the handling. To quantify these observations, we conducted statistical analysis on six categories of behavioral parameters (see Materials and Methods). The outcome of this analysis is summarized in Fig. 1.

Activity [mean ± S.E.M., SSAT-OE ($n = 34$): 2.56 ± 0.08; syngenic ($n = 35$): 2.90 ± 0.08], aggression [SSAT-OE ($n = 34$): 1.53 ± 0.10; syngenic ($n = 35$): 2.30 ± 0.14], grip strength [SSAT-OE ($n = 34$): 1.97 ± 0.06; syngenic ($n = 35$): 2.41 ± 0.06] and muscle tone [SSAT-OE ($n = 34$): 2.83 ± 0.04; syngenic ($n = 35$): 2.99 ± 0.06]. Categories did

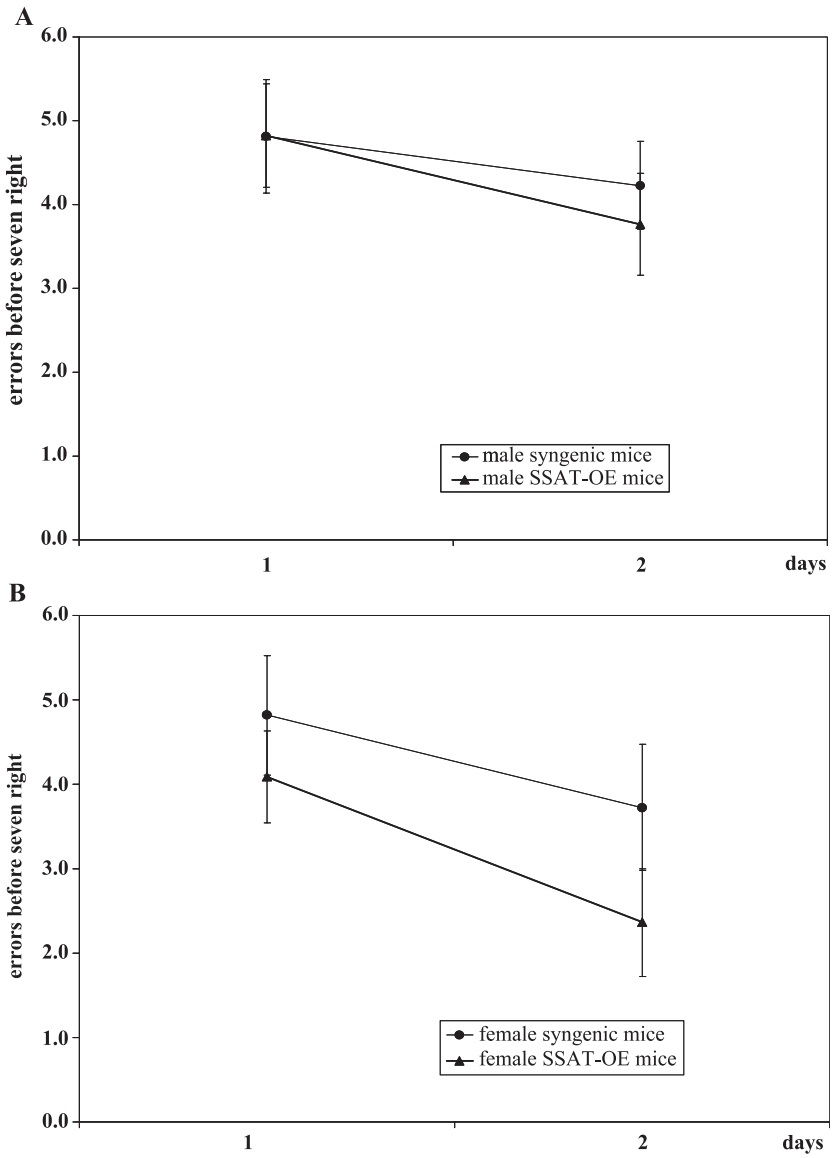

Fig. 4. To examine the long-term memory of both SSAT-OE and syngenic mice, they were retested after 2 weeks of rest. There was no difference between the genotypes [$F(1,57) = 1.6$, $P < .2$] or a genotype by sex interaction [$F(1,57) = 0.6$, $P < .4$] in the number of errors before the seventh correct choice. The results of males (A) and females (B) are shown in separate boxes. ●, SSAT-OE mice and ▲, syngenic mice. Bars present values as mean ± S.E.M.

not differ between the sexes and the data were pooled and analyzed for each genotype. However, defense [SSAT-OE ($n=34$): 2.32 ± 0.14; syngenic ($n=35$): 2.57 ± 0.11] and rear elevation [SSAT-OE ($n=34$): 2.38 ± 0.08; syngenic ($n=35$): 2.51 ± 0.11] categories were sex dependent [in defense: females ($n=22$): 2.14 ± 0.18; males ($n=47$): 2.60 ± 0.09 and in rear elevation: females ($n=22$): 2.78 ± 0.11; males ($n=47$): 2.30 ± 0.08], and were analyzed separately for each sex. SSAT-OE mice differed from their syngenic controls in the activity ($P<.004$), aggression ($P<.001$), grip strength ($P<.001$), and muscle tone ($P<.040$) categories. In contrast, SSAT-OE mice showed no reduction in their defensive reactivity [females: SSAT-OE ($n=11$): 2.00 ± 0.27; syngenic ($n=11$): 2.27 ± 0.24; males: SSAT-OE ($n=23$): 2.48 ± 0.15; syngenic ($n=24$): 2.71 ± 0.11], or change in rear elevation [females: SSAT-OE ($n=11$): 2.82 ± 0.12; syngenic ($n=11$): 2.73 ± 0.19; males: SSAT-OE ($n=23$): 2.17 ± 0.08; syngenic ($n=24$): 2.42 ± 0.13].

3.2. Learning and memory in the 8-RAM

3.2.1. Acquisition

All mice reached an asymptotic level of performance in the 8-RAM win–shift task by the seventh day of testing (Fig. 2). Therefore, only the first 7 days were included in the analysis of the task acquisition. In the number of errors, the ANOVA revealed a significant effect of genotype [$F(1,45)=6.6$, $P<.01$], and also a significant genotype by sex interaction [$F(1,45)=6.4$, $P<.02$]. In the

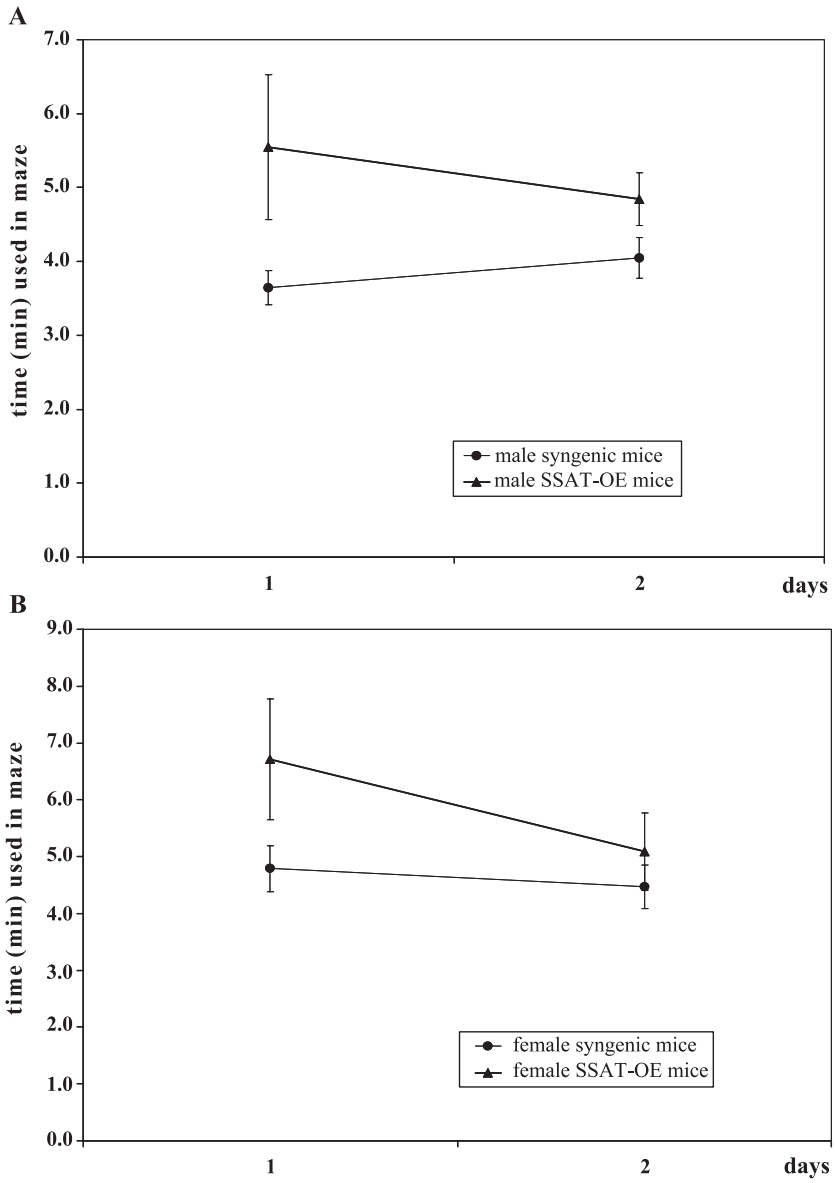

Fig. 5. In the memory retention phase of the 8-ARM, SSAT-OE mice spent significantly more time to complete the task [$F(1,57)=6.7$, $P=.01$], but there was no genotype by sex interaction [$F(1,57)=0.006$, $P<.9$], (A) males and (B) females. Bars present values as mean ± S.E.M.

subsequent analysis with the sexes separated, no difference between SSAT-OE and syngenic controls was found among the males. In contrast, the female SSAT-OE mice performed significantly inferior to female syngenic mice [$F(1,15) = 14.3$, $P < .002$].

Time spent in the radial arm also differed significantly between the genotypes [$F(1,45) = 12.6$, $P < .001$; Fig. 3]. In addition, the effect of sex was significant [$F(1,45) = 25.8$, $P < .000$], but no genotype by sex interaction was observed [$F(1,45) = 2.8$, $P < .10$].

3.2.2. Retention

After 2 weeks of rest, the mice were tested again during 3 days. As shown in Fig. 4, all mice reached their previous best performance level by the second testing day. The ANOVA revealed no difference between the genotypes in the number of errors [$F(1,57) = 1.6$, $P > .20$] or a genotype by sex interaction [$F(1,57) = 0.6$, $P > .40$]. However, the SSAT-OE mice continued to take longer to complete the task (Fig. 5). The effect of genotypes was significant [$F(1,57) = 6.7$, $P < .01$], whereas no genotype by sex interaction was detected [$F(1,57) = 0.006$, $P > .90$].

3.3. Circulating levels of certain hormones in syngenic and transgenic mice

The results of blood hormone analysis are summarized in Table 1. The adrenocorticotropine (ACTH) concentration was significantly increased nearly twofold in both sexes of transgenic mice. The corticosterone concentration was also significantly increased in SSAT transgenic mice. In contrast, the levels of thyroid-stimulating hormone (TSH) and thyroxine (T4) were markedly and highly significantly decreased in transgenic mice. Furthermore, the testosterone concentration of SSAT-OE mice was reduced to a level below detection.

3.4. Weight of reproductive tissues in syngenic and transgenic mice

Tissue weights were calculated in proportion to body weights (not shown). The ratio of transgenic uteri were half of that found in syngenic mice [SSAT-OE ($n = 5$): 2.84 ± 0.64; syngenic ($n = 5$): 5.61 ± 0.49, $P < .01$], whereas the ratio of testicles did not differ between transgenic and syngenic males [SSAT-OE ($n = 5$): 6.54 ± 0.32; syngenic ($n = 5$): 6.99 ± 0.39].

Table 1
Levels of some hormones in syngenic and SSAT-OE mice

	Syngenic mice	SSAT-OE mice	*n* (sg,tg)	*P* value
ACTH (ng/l)	153.7 ± 28.1	242.4 ± 41.7	(9,9)	0.038
Corticosterone (ng/ml)	53.9 ± 3.7	89.3 ± 6.4	(10,10)	0.001
Testosterone (nmol/l)	0.5 ± 0.0	< 0.4 ± 0.0	(5,5)	0.008
TSH (mU/l)	0.015 ± 0.0	0.003 ± 0.0	(5,5)	0.010
T4 (pmol/l)	17.8 ± 1.5	9.6 ± 1.1	(5,5)	0.002

Results are expressed as mean ± S.E.M.

4. Discussion

The present study revealed many behavioral abnormalities in the SSAT-OE mice as compared with their non-transgenic littermates (syngenic mice). The SSAT-OE mice were less active than syngenic mice and showed reduced aggressive behavior. Furthermore, SSAT-OE mice had reduced muscle tone and grip strength, although they did not differ from syngenic mice in several agility tasks (wire manoeuvre, negative geotaxis and contact righting reflex). SSAT-OE mice were also slower than syngenic mice in the radial arm maze, supporting the impression of their phlegmatic appearance. They also showed impaired learning, although this was sex dependent, as only SSAT-OE females were impaired in their acquisition of the maze task. However, memory retention also appeared normal in the SSAT-OE females, as they made no more errors than syngenic females in the retention phase 2 weeks after the initial learning.

Transgenic mice overexpressing SSAT (Pietila et al., 1997) have high concentrations of putrescine (from 10- to 200-fold in comparison with syngenic mice) in all brain areas, whereas spermidine concentration is reduced in the cerebral cortex, hippocampus and thalamus, and spermine in thalamus (Kaasinen et al., 2000). Hence, the increased brain levels of putrescine, the reduced levels of spermidine and spermine, or the markedly increased molar ratio of putrescine to the higher polyamines may contribute to the observed behavioral findings in SSAT-OE mice. Unfortunately, the available literature contains only a few reports on the correlation of increased putrescine levels with behavior. Putrescine given systemically to male rats produces motor disorders at high doses (Camon et al., 1994). Furthermore, increased putrescine concentration in the frontal cortex and hippocampus after systemic administration of a massive NMDA dose (150 mg/kg) correlates with vigor of movement and stereotypic motor activity (Gimenez-Llort et al., 1997). In contrast, accumulation of putrescine in the SSAT-OE mice appears to induce hypoactivity and reduce irritability and aggression. Mice overexpressing the human ODC gene display an over 10-fold increase in the brain levels of putrescine with only modest changes in spermine and spermidine pools (Halmekytö et al., 1991). The only behavioral study ever conducted with these mice revealed increased escape latency in the water maze. However, because the swimming speed was not measured, it remained unclear whether this deficit was primarily due to slow swimming of ODC mice or a spatial learning deficit. Intracerebrally administered spermidine has been found to stimulate, and spermine to inhibit, spontaneous motor activity in

mice (Sakurada et al., 1977). Furthermore, another study indicated that the systemic administration of spermidine and spermine, which dose-dependently inhibit spontaneous climbing and wheel-running behavior in mice, antagonize pharmacologically induced hyperactivity (Hirsch et al., 1987). In light of these studies, it is quite unlikely that reduced levels of spermine and spermidine would have contributed to the hypoactivity of SSAT-OE mice.

The use of Morris water maze, commonly used in standardized hippocampus-dependent performances, is apparently unsuitable for our experiments as the SSAT-OE mice are hairless and in all likelihood sensitive to hypothermia. In addition, Iivonen et al. (2003) have recently shown that, unlike rats, mice suffer from hypothermia in water maze that may distort the test results Therefore, the radial arm maze, also a model for hippocampal-related learning and memory tasks, was chosen to test SSAT-OE mice. According to the available literature, reduced levels of spermidine and spermine may have contributed to the impaired learning of SSAT-OE mice in the radial arm maze. Although direct administration of spermidine and spermine alone did not influence learning in several studies (Conway, 1998; Kishi et al., 1998; Shimada et al., 1994), one study reported enhancement of short-term recognition memory by spermidine (Mikolajczak et al., 2002). The impaired acquisition of the 8-RAM task by SSAT-OE mice could have also resulted from increased brain putrescine levels (Kaasinen et al., 2000). However, impaired learning was only observed in female SSAT-OE mice. We have not observed differences in polyamine levels between females and males. Therefore, the altered brain polyamine levels may not impact the RAM learning performance, but may influence noncognitive aspects. Two factors may explain this sex difference. Firstly, female SSAT-OE mice are infertile while male have only slightly impaired fertility. This may imply that the phenotype is more severe in females. Secondly, female SSAT-OE mice have atrophied ovaries (Min et al., 2002) and concomitantly reduced levels of circulating estrogen. Notably, lack of estrogen, as a result of ovariectomy, has been shown to impair acquisition in the 8-RAM (Daniel et al., 1999; Heikkinen et al., 2002). In addition, estrogen interacts with the NMDA-receptor function. Estrogen administration to ovariectomized rats increases the number of NMDA receptors in the hippocampus (Gazzaley et al., 1996) and renders mice more tolerant to the memory-impairing effects of an NMDA-receptor antagonist (Gurevicience et al., 2003). Furthermore, estrogen treatment that improves 8-RAM learning of ovariectomized rats also increases NMDA-receptor binding in the hippocampus (Daniel and Dohanich, 2001). Therefore, it is possible that the impaired 8-RAM acquisition by the SSAT-OE female mice resulted from a combined action of altered polyamine and estrogen levels on the NMDA-mediated neurotransmission. It remains to be elucidated whether SSAT-OE mice have alterations also in the induction of long-term potentiation (LTP) and NMDA-mediated neurotransmission.

Several studies have indicated that certain hormones, especially ACTH (Feige et al., 1986; Scalabrino and Lorenzini, 1991) and glucocorticoids (Ientile et al., 1988) stimulate polyamine biosynthesis. Both hormones are involved in hypothalamic–pituitary–adrenal (HPA) axis that is primarily activated by corticotropin-releasing hormone (CRH) in hypothalamus. The activation of CRH releases the ACTH in the pituitary that in turn triggers the secretion of glucocorticoid hormones (GC) from adrenal cortex (cortisol in humans and corticosterone in mice). In contrast, GCs suppresses the hypothalamic CRH expression and this negative feedback system maintains both the basal activity of HPA system and releases the stress-activated HPA system. The glucocorticoids are regulated via glucocorticoid receptors (GRs) or mineralocorticoid receptors (MRs), of which, GRs are located mainly in the hypothalamus and MRs in hippocampus and hypothalamus (Muller and Keck, 2002). Interestingly, hormonal analysis revealed a significant increase in the levels of ACTH and corticosterone in SSAT-OE mice, which may, in fact, contribute to the long-term activation of HPA in transgenic mice. HPA hyperactivity is demonstrated to be related to depression (Muller and Keck, 2002) whereas the deficiency of CRH and hence, decline in HPA activation, contributes to hypersensitivity and stress (Bale et al., 2000). Therefore, it is tempting to speculate that the upregulation of polyamine catabolism and putrescine overproduction contributes to the long-term hyperactivity of HPA, which in turn affects the behavioral abnormalities and learning disabilities. In addition, the concentrations of testosterone and thyroid hormones, TSH and T4, were markedly reduced in SSAT-OE mice. Hypothyroidism in humans is usually associated with general motor slowing and flaccidity. In addition, thyroid hormone manipulations early in the development may affect motor behavior and learning skills (Brosvic et al., 2002). Furthermore, prolonged (started at childhood) iodine deficiency, leading to decreased serum T4 concentration, may result in learning disability still in adults (Tiwari et al., 1996).

To our knowledge, the present study is the first behavioral characterization of animals with genetically targeted alterations in polyamine catabolism. We found a distinct reduction in the spontaneous activity and aggression in SSAT-OE mice of both sexes. The underlying mechanism remains elusive, but may be related to both a striking increase in the brain putrescine levels and to the altered hormonal levels, possibly resulting from the disturbed polyamine homeostasis in these mice. A study comparing ODC and SSAT-OE mice, which both share increased brain putrescine levels, would be able to test this hypothesis in the future. The finding of impaired spatial learning only in female SSAT mice may imply an interaction between polyamines, estrogen and NMDA receptors, which also warrants further studies. In fact, a recent article indicates that polyamines can modulate interactions between hormone receptors and their coregulatory proteins (Maeda et al., 2002).

Present findings may indicate that when the function and the role of polyamines in the brain are studied, the effects of polyamines to neuroendocrinology have to take into consideration.

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