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Lsamp$^{-/-}$ mice display lower sensitivity to amphetamine and have elevated 5-HT turnover

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ABSTRACT

In mice, the limbic system-associated membrane protein (*Lsamp*) gene has been implicated in locomotion, anxiety, fear reaction, learning, social behaviour and adaptation. Human data links the *LSAMP* gene to several psychiatric disorders and completed suicide. Here, we investigated changes in major monoamine systems in mice lacking the *Lsamp* gene. First, the locomotor and rewarding effects of amphetamine were studied in *Lsamp*$^{-/-}$ mice and *Lsamp*$^{+/+}$ mice. Second, monoamine levels in major brain regions in response to saline and amphetamine injections were measured and, third, the expression levels of dopamine system-related genes in the brain were studied in these mice. *Lsamp*$^{-/-}$ mice displayed lower sensitivity to amphetamine in the motility box. Likewise, in the place preference test, the rewarding effect of amphetamine was absent in *Lsamp*$^{-/-}$ mice. In all brain regions studied, *Lsamp*$^{-/-}$ mice displayed lower serotonin (5-HT) baseline levels, but a greater 5-HT turnover rate, and amphetamine increased the level of 5-HT and lowered 5-HT turnover to a greater extent in *Lsamp*$^{-/-}$ mice. Finally, *Lsamp*$^{-/-}$ mice had lower level of dopamine transporter (DAT) mRNA in the mesencephalon. In conclusion, *Lsamp*-deficiency leads to increased endogenous 5-HT-ergic tone and enhanced 5-HT release in response to amphetamine. Elevated 5-HT function and reduced activity of DAT are the probable reasons for the blunted effects of amphetamine in these mice. *Lsamp*$^{-/-}$ mice are a promising model to study the neurobiological mechanisms of deviant social behaviour and adaptation impairment observed in many psychiatric disorders.

1. Introduction

Limbic system associated membrane protein (LAMP) is a 64–68 kDa heavily glycosylated cell adhesion molecule of the IgLON family, structurally characterised by three immunoglobulin (Ig) domains [1]. LAMP protein has been shown to be specific to cortical and sub-cortical limbic-associated regions of the developing and adult brain [2–5] and is expressed on the surface of somata and proximal dendrites of neurons [6]. A 99% amino acid sequence identity between human and rodent LAMP [1] indicates strong phylogenetic conservation of the protein structure and associated functional properties. LAMP and the three other members of the IgLON family probably function predominantly as subunits of heterodimeric proteins [7].

Rodent studies indicate that increased level of *Lsamp* transcript in several brain areas is related with increased trait anxiety [8–10], acute fear reaction [11] and fear conditioning [12]. *Lsamp* gene deficiency has been associated with lower anxiety and deviant social behaviour as evidenced by decreased agonistic behaviour and lack of whisker trimming [13], lower sensitivity to stressful or challenging environmental stimuli [14], increased activity in novel environments [15] and deficit in spatial memory acquisition, probably related to poorly sustained CA1 long-term potentiation [16].

Human data link *LSAMP* with quite a wide spectrum of psychiatric disorders: polymorphisms in the human *LSAMP* gene have been associated with panic disorder [17], male completed suicide [18] and also major depressive disorder, panic disorder [19] and schizophrenia (our unpublished results). Furthermore, the levels of the LAMP protein have been found to be approximately 20%

Abbreviations: 3-MT, 3-methoxytyramine; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; DA, dopamine; DAT, dopamine transporter; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; LAMP, limbic system-associated membrane protein; *Lsamp*, limbic-system-associated membrane protein gene; NA, noradrenaline; NMN, normetanephrine; VMAT2, brain vesicular monoamine transporter.

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increased in postmortem frontal cortex both in patients with schizophrenia and bipolar disorder [20].

The links of the *LSAMP* gene to several major psychiatric disorders and the profound changes in the behavioural phenotype of *Lsamp*-deficient mice motivated us to study the relationship between *Lsamp*-deficiency and the major monoamine systems in the brain by means of *Lsamp* gene deficient homozygous ($Lsamp^{-/-}$), heterozygous ($Lsamp^{+/-}$) and wild-type ($Lsamp^{+/+}$) mice. We expected to see changes in at least some major monoamine systems in *Lsamp*-deficient mice. First, we measured the effect of a psychostimulant drug, amphetamine, in the motility boxes. Second, we studied the rewarding effect of amphetamine in the conditioned place preference test. Third, we measured the content of main monoamines and their metabolites in the dorsal striatum, ventral striatum and mesencephalon where dopamine-mediated neurotransmission plays a prominent role, and also in the prefrontal cortex and temporal lobe in response to saline or amphetamine (5 mg/kg) administration. Finally, we measured the expression level of the dopamine D2 receptor gene in the dorsal and ventral striatum, and the expression level of the dopamine transporter (DAT) gene and the brain vesicular monoamine transporter (VMAT2) gene in the mesencephalon.

2. Materials and methods

2.1. Animals and drugs

All experiments were performed in accordance with the EU guidelines (directive 86/609/EEC) and permit (No. 59, September 5, 2006) from the Estonian National Board of Animal Experiments. Generation of the *Lsamp* KO mouse line has been described previously [13]. All mice used in this study were male, had the genetic background 129S6/SvEvTac × C57BL/6, and were F2 hybrids derived from heterozygous F1 intercrosses as described in [13]. Mice were group-housed in standard laboratory cages (42.5 cm × 26.6 cm × 15.5 cm) 7–8 animals per cage at 22 ± 1 °C under a 12:12 h light/dark cycle (lights off at 19:00 h). 2 cm layer of aspen bedding and 0.5 l of aspen nesting material (Tapvei, Estonia) was used in each cage and changed every week. Tap water and food pellets (R70, Lactamin AB, Sweden) were available ad libitum. All mice were age matched littermates 2–3 months of age at the time of experiments. Amphetamine (amphetamine sulphate, Sigma–Aldrich, USA) was freshly prepared in sterile, pyrogen free, 0.9% solution of sodium chloride (B. Braun Melsungen AG, Germany). All drugs were injected intraperitoneally (i.p.) at a volume of 10 ml/kg.

2.2. Locomotor activity test with amphetamine

Mice were placed individually for 30 min into photoelectric motility boxes (44.8 cm × 44.8 cm × 45 cm) connected to a computer (TSE, Technical & Scientific Equipment GmbH, Germany). Computer registered distance travelled, the number of rearings, time spent and distance travelled in the central part of the box, and corner entries. Testing was carried out between 13:00 and 19:00 of the light phase. Before each experiment, mice were let to habituate with the experimental room for 1 h. $Lsamp^{-/-}$ and $Lsamp^{+/+}$ mice were randomly assigned to groups that received an i.p. injection of saline or 2.5, 5 or 7.5 mg/kg of amphetamine 30 min before testing in the motility box.

2.3. Conditioned place preference test

Conditioned place preference test was conducted with the amphetamine dose of 2.5 mg/kg in a two-chamber apparatus (TSE, Technical & Scientific Equipment GmbH, Germany) with two equal sized chambers that differed in wall colour and pattern and were separated by a doored wall. This dose was chosen based on a pilot study to avoid the behavioural stereotypies and motor activation, but to induce a measurable preference effect in wild-type mice. The details of the experimental design are described in online Supplementary Material.

2.4. Monoamine content measurements in response to saline and amphetamine

$Lsamp^{-/-}$ and $Lsamp^{+/+}$ mice were randomly divided into groups that received an i.p. injection of either saline or 5 mg/kg of amphetamine. After 30 min in isolation, the mice were decapitated. Brains were quickly dissected into five parts – the frontal cortex, ventral striatum (including the nucleus accumbens and olfactory tubercle), dorsal striatum, mesencephalon and temporal lobe (including the amygdala) – and frozen in liquid nitrogen. The brain dissection was performed according to the coordinates presented in the mouse brain atlas [21]. Monoamines–noradrenaline (NA), dopamine (DA) and serotonin (5-HT) – and their metabolites–normetanephrine (NMN), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA), and 3-methoxytyramine (3-MT) – were assayed by high performance liquid chromatography (HPLC) with electrochemical detection as described in online Supplementary Material.

2.5. Gene expression analysis by qRT-PCR

Wild-type and heterozygous and homozygous *Lsamp*-deficient mice were decapitated and their brains were quickly dissected. The dorsal striatum, ventral striatum (including the nucleus accumbens and olfactory tubercle) and mesencephalon were collected and frozen in liquid nitrogen. The brain dissection was performed according to the coordinates presented in the mouse brain atlas [21]. The expression level of the dopamine D2 receptor gene was measured in the mesolimbic area and striatum, and the level of the dopamine transporter (DAT) and brain vesicular monoamine transporter (VMAT2) gene were measured in the mesencephalon as described in online Supplementary Material.

2.6. Data analysis

The results of the amphetamine experiment in the motility box (genotype × dose) and monoamine measurements (genotype × treatment) were analysed by means of two-way ANOVA. Gene expression experiments and the conditioned place preference experiment were analysed by means of one-way ANOVA. In all experiments, $p < 0.05$ was considered statistically significant. Newman–Keuls *post hoc* test was used. Statistical analyses were performed using Statistica V10 (Statsoft Inc., Oklahoma, USA).

3. Results

3.1. Locomotor activity test

In the amphetamine dose curve study, distance travelled was significantly influenced by genotype ($F_{(1,40)} = 16.25$; $p < 0.001$), dose ($F_{(3,40)} = 22.07$; $p < 0.001$), and genotype × dose interaction ($F_{(3,40)} = 8.47$; $p < 0.001$), $Lsamp^{-/-}$ mice being significantly less sensitive to the stimulating effect of 5 mg/kg and 7.5 mg/kg of amphetamine (Fig. 1). We found no main effects for rearings, and time and distance in the central square. The number of corner entries was, like distance travelled, significantly influenced by genotype ($F_{(1,40)} = 15.98$; $p < 0.001$), dose ($F_{(3,40)} = 17.13$; $p < 0.001$), and

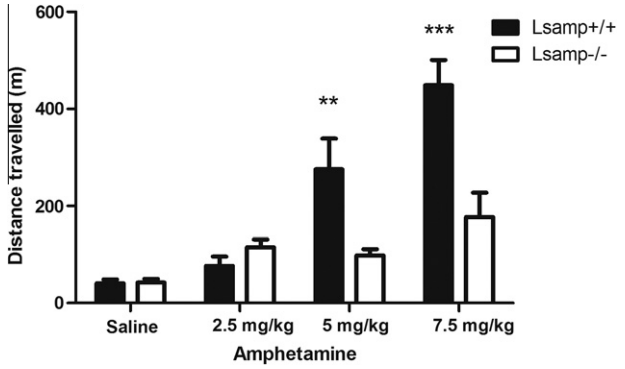

Fig. 1. The effect of amphetamine on distance travelled in the motility boxes in *Lsamp*$^{+/+}$ and *Lsamp*$^{-/-}$ mice. Data are presented as ± SEM, $n = 6$ for all treatment groups. $^{***}p < 0.001$; $^{**}p < 0.01$, *Lsamp*$^{+/+}$ mice vs. respective *Lsamp*$^{-/-}$ group.

genotype × dose interaction ($F_{(3,40)} = 7.31$; $p < 0.001$) and the results almost coincided with those for distance travelled.

3.2. Conditioned place preference test

Amphetamine (2.5 mg/kg) shifted preference for the conditioned chamber in *Lsamp*$^{+/+}$ mice by 388.5 ± 84.8 s and in *Lsamp*$^{-/-}$ mice by 86.4 ± 70.6 s and the difference between the genotypes was significant ($F_{(1,14)} = 7.5$; $p < 0.05$).

3.3. Monoamine content measurements

3.3.1. Dorsal striatum

In the dorsal striatum, there was a significant genotype effect on the turnover of 5-HT ($F_{(1,19)} = 5.36$; $p < 0.05$), and an almost significant genotype effect on the content of 5-HIAA ($F_{(1,19)} = 3.74$; $p = 0.07$). Amphetamine treatment had a significant effect on the content of NMN ($F_{(1,19)} = 17.85$; $p < 0.001$), DA ($F_{(1,19)} = 5.67$; $p < 0.05$), DOPAC ($F_{(1,19)} = 6.07$; $p < 0.05$), 5-HT ($F_{(1,19)} = 9.93$; $p < 0.01$) and 3-MT ($F_{(1,19)} = 12.52$; $p < 0.01$), and the turnover of NA ($F_{(1,19)} = 33.56$; $p < 0.001$), DA ($F_{(1,19)} = 55.41$; $p < 0.001$) and 5-HT ($F_{(1,19)} = 11.18$; $p < 0.01$). Genotype × treatment interaction had a significant effect on the turnover of 5-HT ($F_{(1,19)} = 4.6$; $p < 0.05$).

3.3.2. Ventral striatum

In the ventral striatum, there was a significant genotype effect on the content of NMN ($F_{(1,20)} = 5.26$; $p < 0.05$) and an almost significant genotype effect on the content of DA ($F_{(1,19)} = 3.74$; $p = 0.07$) and the turnover of DA ($F_{(1,19)} = 4.01$; $p = 0.06$). Amphetamine treatment significantly affected the content of NMN ($F_{(1,20)} = 46.27$; $p < 0.001$), HVA ($F_{(1,20)} = 10.51$; $p < 0.01$), 5-HT ($F_{(1,19)} = 5.89$; $p < 0.05$), 5-HIAA ($F_{(1,20)} = 6.25$; $p < 0.05$), and 3-MT ($F_{(1,19)} = 8.75$; $p < 0.01$), and the turnover of NA ($F_{(1,20)} = 21.6$; $p < 0.001$), DA ($F_{(1,19)} = 15.69$; $p < 0.001$), and 5-HT ($F_{(1,17)} = 7.1$; $p < 0.05$.

3.3.3. Mesencephalon

In the mesencephalon, there was a significant genotype effect on the content of DA ($F_{(1,19)} = 4.9$; $p < 0.05$), DOPAC ($F_{(1,17)} = 6.73$; $p < 0.05$), and 5-HT ($F_{(1,20)} = 6.91$; $p < 0.05$), and the turnover of 5-HT ($F_{(1,20)} = 9.35$; $p < 0.01$); also, an almost significant genotype effect on the content of HVA ($F_{(1,19)} = 3.84$; $p = 0.06$) was observed. Amphetamine treatment significantly affected the content of NA ($F_{(1,20)} = 22.49$; $p < 0.001$), DOPAC ($F_{(1,17)} = 8.16$; $p < 0.05$), 5-HT ($F_{(1,20)} = 31.73$; $p < 0.001$), 5-HIAA ($F_{(1,20)} = 22.49$; $p < 0.001$), and the turnover of 5-HT ($F_{(1,20)} = 18.24$; $p < 0.001$).

3.3.4. Prefrontal cortex

In the prefrontal cortex, amphetamine treatment significantly affected the content of DA ($F_{(1,20)} = 8.2$; $p < 0.01$), and 5-HT ($F_{(1,20)} = 10.74$; $p < 0.01$), and the turnover of NA ($F_{(1,20)} = 7.13$; $p < 0.05$) and 5-HT ($F_{(1,20)} = 6.33$; $p < 0.05$).

3.3.5. Temporal lobe

In the temporal lobe, amphetamine treatment significantly affected the content of NMN ($F_{(1,20)} = 8.67$; $p < 0.01$), and 5-HT ($F_{(1,20)} = 8.03$; $p < 0.01$), and the turnover of NA ($F_{(1,20)} = 11.42$; $p < 0.01$). Genotype × treatment interaction had a significant effect on the turnover of 5-HT ($F_{(1,19)} = 5.3$; $p < 0.05$).

For detailed data tables with *post hoc* analyses see online Supplementary Material. Most notably, in all five brain regions measured, *Lsamp*$^{-/-}$ mice had somewhat lower levels of 5-HT in response to a saline injection than wild-type mice (in the mesencephalon the difference was significant). Also, *Lsamp*$^{-/-}$ mice reacted with a stronger elevation in 5-HT levels than *Lsamp*$^{+/+}$ mice to 5 mg/kg of amphetamine in all five parts of the brain analysed (in the dorsal striatum, mesencephalon and temporal lobe the difference was significant). Wild-type mice had a significant increase in the level of 5-HT only in the mesencephalon and the magnitude of this increase was lower than in *Lsamp*$^{-/-}$ mice (Fig. 2).

Furthermore, *Lsamp*$^{-/-}$ mice had a significantly higher turnover of 5-HT compared to *Lsamp*$^{+/+}$ mice in the dorsal striatum and mesencephalon and somewhat higher in the ventral striatum, prefrontal cortex and temporal lobe. It is also remarkable that amphetamine lowered the turnover of 5-HT in *Lsamp*$^{-/-}$ mice significantly in the dorsal striatum, ventral striatum and mesencephalon, while none of these changes in *Lsamp*$^{+/+}$ mice were significant (Fig. 3). It is noteworthy that in all these brain regions dopamine-mediated neurotransmission plays a prominent role.

Other significant differences between the genotypes included a stronger increase in the level of DA and 3-MT in response to amphetamine administration in *Lsamp*$^{-/-}$ mice in the dorsal striatum, a stronger decrease in the level of HVA and DA turnover in response to amphetamine administration in *Lsamp*$^{-/-}$ mice and a stronger increase in 3-MT level in response to amphetamine treatment in *Lsamp*$^{+/+}$ mice in the ventral striatum. As for the noradrenergic system, both genotypes reacted with an elevation in the level

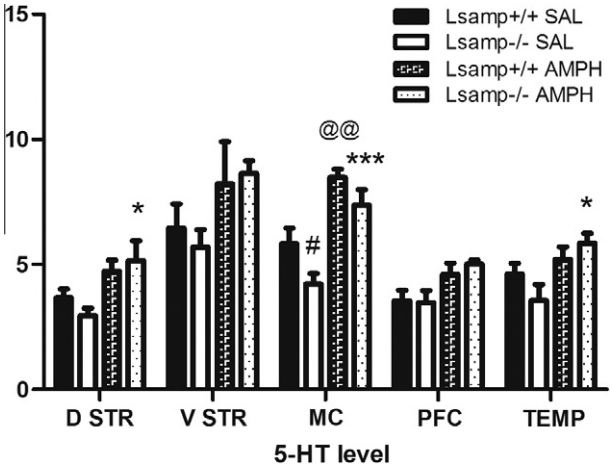

Fig. 2. 5-HT levels in the dorsal striatum (D STR), ventral striatum (V STR), mesencephalon (MC), prefrontal cortex (PFC) and temporal lobe (TEMP) in *Lsamp*$^{-/-}$ mice 30 min after saline (SAL) or 5 mg/kg of amphetamine (AMPH) administration. $n = 6$ per group. $^{\#}p < 0.05$, *Lsamp*$^{-/-}$ vs. respective *Lsamp*$^{+/+}$ group; $^{@@}p < 0.01$, *Lsamp*$^{+/+}$ amphetamine group vs. *Lsamp*$^{+/+}$ saline group; $^{***}p < 0.001$; $^{*}p < 0.05$ *Lsamp*$^{-/-}$ amphetamine group vs. *Lsamp*$^{-/-}$ saline group.

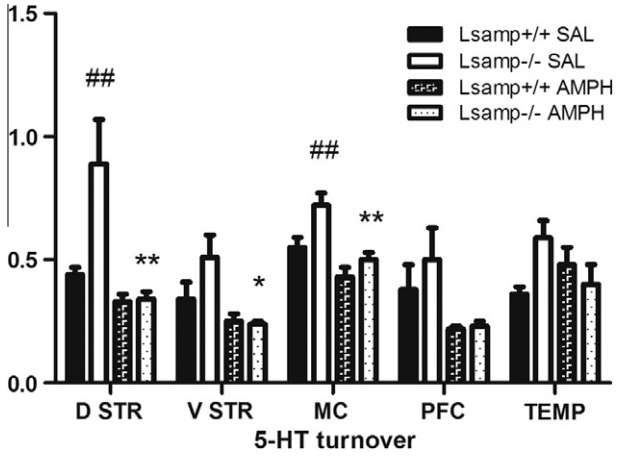

Fig. 3. 5-HT turnover in the dorsal striatum (D STR), ventral striatum (V STR), mesencephalon (MC), prefrontal cortex (PFC) and temporal lobe (TEMP) in *Lsamp*$^{-/-}$ mice 30 min after saline (SAL) or 5 mg/kg of amphetamine (AMPH) administration. $n = 6$ per group. $^{\#\#}p < 0.01$, *Lsamp*$^{-/-}$ vs. respective *Lsamp*$^{+/+}$ group; $^{**}p < 0.01$; $^{*}p < 0.05$ *Lsamp*$^{-/-}$ amphetamine group vs. *Lsamp*$^{-/-}$ saline group.

of NA and a decrease in NA turnover in response to amphetamine treatment and no clearcut differences between the genotypes could be observed (see Tables in online Supplementary Material).

3.4. Gene expression

In the ventral striatum, the expression level of dopamine D2 receptor in *Lsamp*$^{+/+}$ mice (0.26 ± 0.03) was not statistically different from that in *Lsamp*$^{+/-}$ (0.24 ± 0.03) and *Lsamp*$^{-/-}$ (0.21 ± 0.03) mice (Fig. 4(A)). In the dorsal striatum, the small differences between the expression levels of dopamine D2 receptor between *Lsamp*$^{+/+}$ (0.26 ± 0.03), *Lsamp*$^{+/-}$ (0.24 ± 0.03) and *Lsamp*$^{-/-}$ (0.21 ± 0.03) mice were not significant (Fig. 4(B)). In the mesencephalon, the expression level of DAT was dependent on genotype ($F_{(2,19)} = 3.69$; $p < 0.05$) and *post hoc* analysis revealed that the expression level of DAT was in *Lsamp*$^{-/-}$ mice significantly ($p < 0.05$) lower than in *Lsamp*$^{+/+}$ mice (Fig. 4(C)). In the mesencephalon, the differences in the expression level of VMAT2 between *Lsamp*$^{+/+}$ (0.055 ± 0.008), *Lsamp*$^{+/-}$ (0.044 ± 0.006) and *Lsamp*$^{-/-}$ (0.048 ± 0.009) mice were not significant (Fig. 4(D)).

4. Discussion

This study confirmed that profound changes in major monoamine systems, most notably in the 5-HT-ergic system, and also in sensitivity to the locomotor and rewarding effects of amphetamine are indeed observable in *Lsamp* gene knockout animals.

Lsamp$^{-/-}$ mice had a blunted response to the locomotor effect of amphetamine at higher dose levels compared to wild-type littermates. In the conditioned place preference test, amphetamine at dose level 2.5 mg/kg induced place preference in wild-type mice, but not in *Lsamp*$^{-/-}$ mice. This indicates that the partial loss of sensitivity to amphetamine in *Lsamp*$^{-/-}$ mice is probably not confined to locomotor effects, but rather is systemic, comprising also the reward-related mechanisms.

Monoamine measurements showed that the level of 5-HT was lower and the turnover of 5-HT higher in *Lsamp*$^{-/-}$ mice in all five brain regions measured; amphetamine raised the level of 5-HT and lowered the turnover of 5-HT in *Lsamp*$^{-/-}$ mice to a greater extent than in wild-type mice. Thus, *Lsamp*$^{-/-}$ mice seem to have an increased endogenous 5-HT tone that might readily explain their

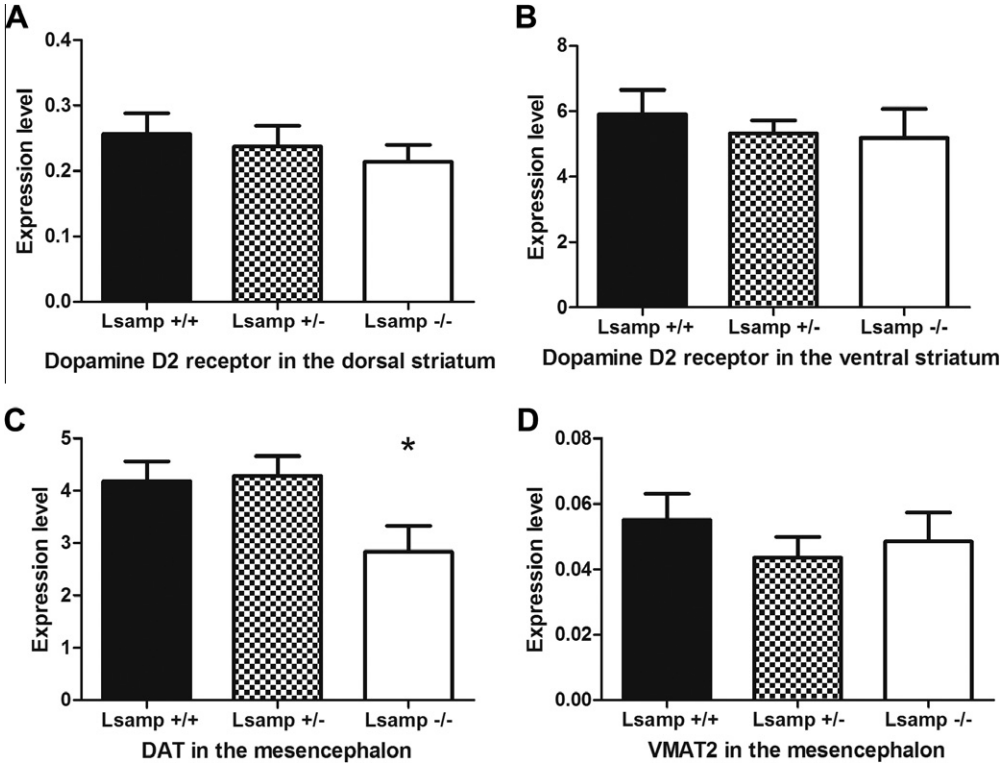

Fig. 4. The expression level of dopamine D2 receptor gene in the ventral (A) and dorsal striatum (B), and dopamine transporter DAT gene (C) and brain vesicular monoamine transporter (*vmat*2) gene (D) in the mesencephalon. $n = 6$–8 per group. $^{*}p < 0.05$ *Lsamp*$^{-/-}$ vs. *Lsamp*$^{+/+}$ group.

lower anxiety and a decrease in agonistic behaviour and aggression [13]. Slightly exaggerated behavioural activation observed in *Lsamp*$^{-/-}$ mice [13,15] is harder to explain by this increase, because, for example, increasing the 5-HT levels by using 5-HT transporter blockers leads to hyperactive behaviour, however, the increase of 5-HT due to gene knockout strategy (5-HT transporter knockout) induce hypoactivity, thus the effect of serotonin on locomotor behaviour is non-linear and might be dependent on secondary effects on other neurotransmitter systems, such as dopamine [22]. It has been shown that 5-HT neurons innervate both dopaminergic and non-dopaminergic neurons in the ventral tegmental area and may influence mesocortical and mesolimbic efferent systems through synaptic as well as non-synaptic mechanisms [23] and that central 5-HT system exerts a tonic and phasic inhibitory control on mesolimbic DA neuron activity [24], but the question, how and to what extent central 5-HT influences locomotor activity in rodents is open-ended. In this study, the acceleration of 5-HT turnover in several brain regions in *Lsamp*$^{-/-}$ mice is indicative of changes at the level of brainstem 5-HT-ergic neurons, i.e. the changes caused by the genetic invalidation of the *Lsamp* gene are rather presynaptic than receptor-related. However, in *Lsamp*$^{-/-}$ mice, amphetamine seems to release 5-HT more readily than in wild-type mice, suppressing its turnover rate. Blunted behavioural effect of amphetamine in *Lsamp*$^{-/-}$ mice could thus be explained by the antagonistic effect of 5-HT on the DA-ergic system, which leads to suppressed locomotor activity. For example, in the rat and monkey, elevated synaptic 5-HT level can dampen the behavioural effects, including locomotor activation, of DA-releasing agents [25]. The increase of tissue level of 5-HT in *Lsamp*$^{-/-}$ mice in response to amphetamine compared to wild-type mice could develop as a result of inhibiting the reuptake of 5-HT and this in turn would suppress its fast turnover rate. Since 5-HT is not removed from the synaptic cleft, and its production rate is not changed, the effect of 5-HT on the DA-ergic system is elevated in *Lsamp*$^{-/-}$ mice. This could account for the blunted locomotor activity in *Lsamp*$^{-/-}$ mice in response to amphetamine. In wild-type mice the concentration of 5-HT also increased at the tissue level in response to amphetamine compared to saline administration, but less than in *Lsamp*$^{-/-}$ mice.

For amphetamine, two alternative, but mutually not exclusive routes of action have been proposed: first, it exerts influence both at the vesicular level where it redistributes DA to the cytosol, promoting reverse transport, and DA release [26] and secondly, according to the "DAT hypothesis", amphetamine exerts its effect by binding to DAT and being transported into the terminals, resulting in DA efflux. Therefore, DAT expression level is one of the factors likely influencing amphetamine-induced locomotor stimulation [27]. This is in good accordance with the results of the present study where *Lsamp*$^{-/-}$ mice displayed both lower expression level of DAT in the mesencephalon and markedly blunted locomotor response to amphetamine.

Overall, the results indicate that the genetic invalidation of the *Lsamp* gene causes several major shifts in the activity of the monoamine systems in male mice. First, it leads to increased systemic 5-HT-ergic tone, which probably explains why *Lsamp*$^{-/-}$ mice display reduced anxiety and a decrease in agonistic behaviour. Second, it facilitates the release of 5-HT and suppresses the turnover rate of 5-HT in response to amphetamine administration, and third, *Lsamp*$^{-/-}$ mice display lower level of DAT mRNA in the mesencephalon. Either (or both) of the latter effects may be responsible for the markedly blunted behavioural response to amphetamine in *Lsamp*$^{-/-}$ mice. We propose *Lsamp*$^{-/-}$ mice as a promising model for studying the molecular mechanisms behind deviations in social behaviour and impaired adaptation observed in many psychiatric disorders.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.11.077.

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