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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.

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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

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