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Research report

# Deficit in emotional learning in neurotrimin knockout mice

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## HIGHLIGHTS

- Initial description of the phenotype of neurotrimin deficient mice is provided.
- Neurotrimin (Ntm) gene knockout mice have a deficit in emotional learning.
- There is no overlap in the behavioural phenotypes of Ntm−/− and Lsamp−/− mice.
- Despite interaction, the roles of IgLONs Ntm and Lsamp seem to be complementary.

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## ABSTRACT

Neurotrimin (Ntm) belongs to the IgLON family of cell adhesion molecules with Lsamp, Obcam and kilon that regulate the outgrowth of neurites mostly by forming heterodimers. IgLONs have been associated with psychiatric disorders, intelligence, body weight, heart disease and tumours. This study provides an initial behavioural and pharmacological characterization of the phenotype of Ntm-deficient mice. We expected to see at least some overlap with the phenotype of Lsamp-deficient mice as Ntm and Lsamp are the main interaction partners in the IgLON family and are colocalized in some brain regions. However, Ntm-deficient mice displayed none of the deviations in behaviour that we have previously shown in Lsamp-deficient mice, but differently from Lsamp-deficient mice, had a deficit in emotional learning in the active avoidance task. The only overlap was decreased sensitivity to the locomotor stimulating effect of amphetamine in both knockout models. Thus, despite being interaction partners, on the behavioural level Lsamp seems to play a much more central role than Ntm and the roles of these two proteins seem to be complementary rather than overlapping.



## 1. Introduction

Neurotrimin (Ntm), in humans also called human neurotrimin (Hnt), is a glycophosphatidylinositol (GPI)-anchored cell-adhesion molecule belonging to the IgLON protein family with Lsamp (also Lamp), Obcam (also Opcml) and kilon (also Negr1) [31]. It has been shown that Ntm, and other members of the IgLON family, regulate the development of neuronal projections via cell type specific attractive and repulsive mechanisms that are mediated by both homophilic and heterophilic interactions [6,7,17]. Reed et al. [29] have suggested that only Ntm might be both a homo- and a heterophilic cell adhesion molecule, whereas Lsamp and Obcam act only as heterophilic cell adhesion molecules. Lodge et al. [18] have shown that GPI-anchored Ntm has an alternatively-spliced isoform, possibly modulating the activity of all the IgLONs, that is secreted and co-expressed with the anchored version of the protein in the retina, cerebellum, and DRG neurons. In DRG neurons, Ntm promotes neurite outgrowth via Ntm forming noncovalent homodimers in the plane of the membrane. Conversely, Ntm inhibits neurite outgrowth in sympathetic neurons via heterophilic interactions because sympathetic neurons do not express Ntm [30,34]. McNamee et al. [21] have proposed that IgLONs may not have a primary role in axon guidance, but may be even more important for cell-cell adhesion and recognition.

According to the Eurexpress transcriptome atlas created by Diez-Roux et al. [5], there is a strong widespread expression of Ntm in mouse E14.5 embryonic brain, spinal cord, peripheral nervous system, ganglia, eye, skeleton and limbs. Gil et al. [7] have shown that in adult rat brain, Ntm protein is largely expressed in

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a complementary pattern to that of Lsamp in the nervous system, with co-expression at a few sites. Ntm is expressed at high levels in sensory-motor cortex and, of particular note, is transiently expressed in neurons of cortical barrel fields and corresponding thalamic "barreloids". Ntm is expressed also in the spinal cord [8] and is mediating estrogen-induced sympathetic pruning in some peripheral targets like myometrium; estrogen increases Ntm synthesis and secretion in myometrial smooth muscle cells, so Ntm seems to contribute to sympathetic myometrial neurodegeneration elicited by estrogen [13]. The spatial-temporal expression pattern of Ntm suggests that this adhesion molecule plays a role in axonal fasciculation of specific cerebellar systems and may also be involved in the formation of excitatory synapses and their stabilization into adulthood as it accumulates coincident with synaptogenesis [3], however, in hippocampal neurons *in vitro*, over-expression of Lsamp or Obcam increased synaptic number, while the over-expression of kilon reduced synaptic number and Ntm had no effects [9]. Ntm also mediates the inhibition of Schwann cell proliferation and migration following nerve injury repair through rapid regulation of a certain microRNA (miR-182) which targets FGF9 and Ntm [38].

According to Liu et al. [15], human neurotrimin (Hnt) shows high sequence similarity to the rat Ntm (97%) and has three different transcripts; it has a wider expression pattern than that of rat Ntm; furthermore, the expression of Hnt in fetal brain is higher than that in mature brain and is stronger in nervous tumors than that in normal brain tissues. Ye et al. [37] showed an expression of Hnt in the human fetal heart and a robust and selective expression in the right atrium with much weaker expression in the left atrium and ventricles in the human adult heart.

In 2015 two breakthrough articles were published on IgLONs that shed new light on the function and mechanism of action of these proteins and underline their importance in the nervous system. Sanz et al. [32] showed that metalloproteinase-dependent shedding of IgLON family members regulates neurite outgrowth from mature cortical neurons. The authors suggest that such proteolytic cleavage of IgLON family members could have critical roles in specific targeting and synaptogenesis of cortical neurons similar to roles of other major synaptic cell adhesion molecules like NCAM or N-cadherin. Sharma et al. [33] demonstrated that most IgLON family members were enriched in neurons and oligodendrocytes, Lsamp being the second-most enriched adhesion molecule in neurons and oligodendrocytes. The only exception was Ntm, which was only enriched in neurons.

During the last decade, IgLONs have been linked to several tumours. Ntm has been associated with tumour in two studies. Ntougkos et al. [23] found reduced Obcam, Lsamp and kilon expression and elevated Ntm protein level in human epithelial ovarian cancer relative to normal samples. Ulmer et al. [35] suggested that SNPs in Ntm may increase primary open-angle glaucoma susceptibility in a subset of cases.

Several studies have related Ntm to cognitive functions. Four SNPs in intron 1 of the Ntm gene have been shown to be associated with cognitive function and late-onset Alzheimer's disease [16] and in a family-based association study, Pan et al. [24] showed a link between Ntm polymorphisms and intelligence. The familial case study by Minhas et al. [22] suggested a role for Ntm and Obcam in developmental delay, autistic symptoms and cancer susceptibility, however, Maruani et al. [20] failed to establish Ntm as an autism susceptibility gene in a study including 1256 patients with an autism spectrum disorder.

Recently, intriguing associations between Ntm and heart/circulation have been found. Luukkonen et al. [19] revealed, by using genome-wide paired-end DNA sequencing, an association between a translocation in the Ntm gene and intracranial and thoracic aortic aneurysma, and Cao et al. [1] established Ntm as a novel biomarker for heart failure; in patients with heart failure that responded to long-term treatment with angiotensin-converting enzyme inhibitors and β blockers the expression of Ntm protein was very strongly upregulated compared to non-responders. However, deletion of the Ntm gene failed to induce congenital heart defects in mice [37]. Of relevant note, Li et al. [14] have revealed a link between Ntm and blood lipid levels.

To bring further light to the potential functions of Ntm, here we provide an initial behavioural and pharmacological screening of Ntm−/− mice. We have previously shown [10–12] that mice lacking Lsamp, the main interaction partner of Ntm [29], have decreased anxiety, deficiencies in social behaviour (impaired barbering behaviour, reduced aggressiveness), slight hyperactivity/disinhibition in novel environments, lower exploratory activity and slower swimming speed. Furthermore, Lsamp−/− mice are much less sensitive to the locomotor stimulating effect of amphetamine and much more sensitive to the anxiolytic and sedative effects of ethanol and benzodiazepines. In learning and memory tasks Lsamp−/− mice perform normally, however, in another Lsamp−/− model a spatial memory deficit in the Morris water maze was evident [27]. In this study with Ntm−/− mice, we used a similar behavioural test battery as in our previous studies with Lsamp−/− animals, and also carried out pharmacological screening with amphetamine and ethanol, as Lsamp−/− mice had displayed altered sensitivity to these substances. Besides pointing to possible roles of Ntm in the nervous system, the characterization of the Ntm knockout mouse model helps to bring light on the functional value of Lsamp-Ntm heterodimer forming interaction (which is very close *in vitro*) on the organism level. If the interaction is crucial, deletion of either Ntm or Lsamp should result in a similar or at least partially overlapping phenotype as in both cases no heterodimers are formed.

## 2. Methods

### 2.1. Animals

Ntm gene heterozygous mutants were procured from the Mutant Mouse Regional Resource Center at UC Davis. In the Ntm gene, consisting of 8 exons, coding exon 1b was targeted by homologous recombination. Complete deletion of functional Ntm transcripts encoded by both 1a and 1b promoters was achieved as the genomic structure and the assembly of the alternative transcripts of the Ntm gene is analogous to the Lsamp gene described in detail in Philips et al. [26]. Lexicon ES cell line derived from 129S5/SvEvBrd was used for making the construct. Mice were further bred at the animal facility of the University of Tartu. All studies were performed in male F2 hybrids [(129S5/SvEvBrd × C57BL/6) × (129S5/SvEvBrd × C57BL/6)]. Ntm deficient −/− and +/− animals and their wild-type (+/+) littermates were used in the study.

Mice were group-housed in standard laboratory cages measuring 42.5 (L) × 26.6 (W) × 15.5 (H) cm 6–8 animals per cage in the animal colony at 22 ± 1 °C under a 12:12 h light/dark cycle (lights off at 19:00 h). 2 cm layer of aspen bedding (Tapvei, Estonia) and 0.5 l of aspen nesting material (Tapvei, Estonia) was used in each cage and changed every week. No other enrichment was used besides nesting material. Tap water and food pellets (R70, Lactamin AB, Sweden) were available ad libitum. Unless noted otherwise, all experiments were performed with male mice aged 2–4 months.

### 2.2. Behavioural testing

Testing was carried out between 10:00 and 17:00 of the light phase. Before each experiment, mice were let to habituate to the