Reduced exploratory behavior in mice with altered expression of the extracellular protease inhibitor neuroserpin

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Summary

Neuroserpin (Ns), a member of the serpin family of serine protease inhibitors, is expressed in the central nervous system and is an inhibitor of tissue type plasminogen activator (tPA) and plasmin. Available evidence suggests that the balance between antiproteases and their target proteases in the extracellular space may be critical for neuronal function and pathology. Transgenic mice which overexpress neuroserpin in neurons (Thy/cNs) have decreased brain tPA proteolytic activity and show a reduced infarct size following focal ischemia. Mice genetically made deficient in neuroserpin (NsKO) have recently been generated. Western-blot and immunoprecipitation confirm that homozygous NsKO mice lack Ns protein completely, whereas heterozygous mice express a reduced amount. Interestingly, zymographic analysis of NsKO brain did not reveal increased tPA activity, suggesting that other inhibitors contribute to the regulation of tPA and may compensate for the defect. We analyzed NsKO and Thy/cNs mice in a preliminary behavioral screen. Both mutants are healthy and show normal spontaneous behaviour. Although available evidence suggests that the extracellular proteolysis may be critical for learning and memory, both NsKO and Thy/cNs mice perform normally in a water-maze task assessing spatial reference memory. However, homozygous NsKO mice exhibited reduced exploratory activity, in particular they were reluctant to investigate the open zones of an elevated maze, as well as a novel object introduced into a familiar arena. A milder form of this phenotype was observed also in heterozygous mice. Thy/cNs mice showed normal activity in most of these tests but displayed a neophobic reaction towards the novel object. These results implicate Ns in the regulation of emotional behaviour through a mechanism that are at least in part independent of tPA activity and shed new light on the regulation of extracellular proteolysis in the brain.

Introduction

- several Serine Protease Inhibitors (Serpins) are expressed in the central nervous system including Protease-Nexin-1 (PN-1) and the recently discovered Neuroserpin (Ns).
- Ns is secreted by neurons, and has inhibitory activity towards the extracellular proteases tissue type plasminogen activator (tPA) and plasmin.
- Strong evidence support a role of tPA in synaptic plasticity and learning. Transgenic mice that overexpress tPA within neurons have increased long-term potentiation (LTP) and better performance in spatial learning tasks.

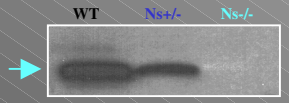
1- Neuroserpin overexpressing and deficient models

a- Thy/cNs mice overexpress Ns

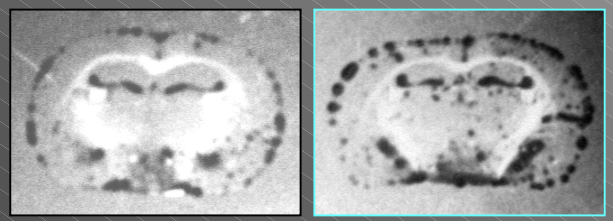
- Transgenic mice overexpressing Ns under the control of the neuronal promoter thy-1.2 have been generated. These mice, named (Thy/cNs), have decreased brain tPA proteolytic activity and show a reduced infarct size following focal ischemia (for details: Paolo Cinelli, MCN 2001 in press).

b- Generation and characterization of NsKO mice

- NsKO mice were generated by homologous recombination in ES cells. The first exon of the Ns gene was replaced by a neo resistance cassette (as described in thesis work of S. Kozlove). The genotype of mice was screened by PCR using specific primers to Ns and neo genes. Heterozygous (Ns+/-), homozygous (Ns-/-) and Wild-type (WT) were used in this study.

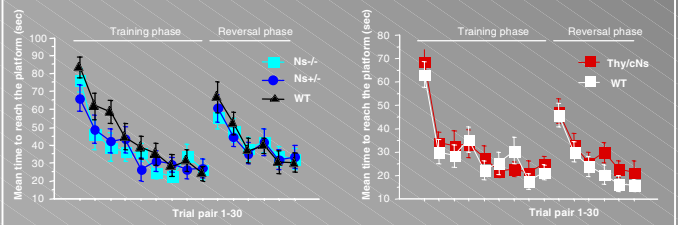


- Analysis of brain protein extract by Western-blot revealed a band at the level of 40 kDa in WT mice that corresponds to the endogenous Ns protein. This band is weaker in Ns+/- mice and is undetected in Ns-/- mice. The Western-blot was confirmed by immunoprecipitation.



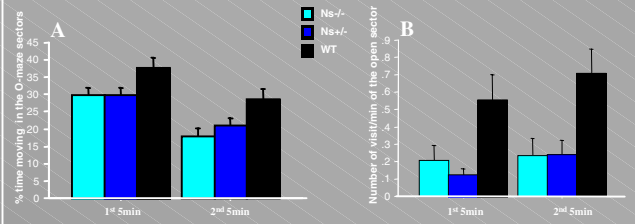
- To analyze the consequence of Ns deletion on tPA proteolytic activity, an histoenzymatic assay (in situ zymography) was performed on brain cryosections in the presence of plasminogen. We observed normal proteolytic zones in both Ns-/- and Ns+/-. Biochemical analysis, using SDS-PAGE and zymography underlay, confirms that Ns deletion did not provoke increasing in tPA activity.

2- Navigation of NsKO and Thy/cNs mice in MWM



- Since available evidence suggests that the extracellular proteolysis may be critical for learning and memory, both NsKO and Thy/cNs mice were tested in the Morris Water Maze (MWM). This task assesses the hippocampal-dependent spatial reference memory. NsKO and Thy/cNs mice performed normally; no difference was observed in the time to reach the hidden-platform and both mutants showed a normal reversal effect.

3- Exploratory activity of NsKO and Thy/cNs mice



- Exploratory activity was analyzed in several tests. In the elevated null-maze, both Ns+/- and Ns-/- exhibit significant reduced exploratory activity (A, F(1,83)= 6.9, P=0.0016) and are reluctant to investigate the open sector of the maze (B, F(1,83)= 5.1, P=0.007). A similar phenotype was observed in the open field and dark-light box.
- Thy/cNs mice have normal exploring activity in all the mentioned tests.

DISCUSSION

- While Ns overexpression in Thy/cNs mice lead to a reduced tPA activity, the Ns deficiency in NsKO mice did not cause an increase in tPA activity. This could be due to compensatino by PN-1 other inhibitors.
- Despite of the correct performance of Thy/cNs and NsKO mice in MWM, the influence of Ns on synaptic plasticity (LTP) is worth to be explored. Also, more a sensitive MWM protocol (e.g. the episodic MWM) may be able to detect a subtle modification of learning and memory.
- Normal MWM performance in Thy/cNs mice despite reduced tPA activity might suggest that the improved learning in thytPA mice may be independent of tPA proteolytic activity. Therefore, additional experiments are needed to analyze the alteration of extracellular proteases due to the absence of Ns.
- Both Thy/cNs and NsKO mice manifest striking anxiety-like traits. In particular, the novelty test reveled a neophobic reaction toward a novel object. Since NsKO mice do not have an alteration in tPA activity, this phenotype may be related to Ns effects that are independent of tPA inhibition.

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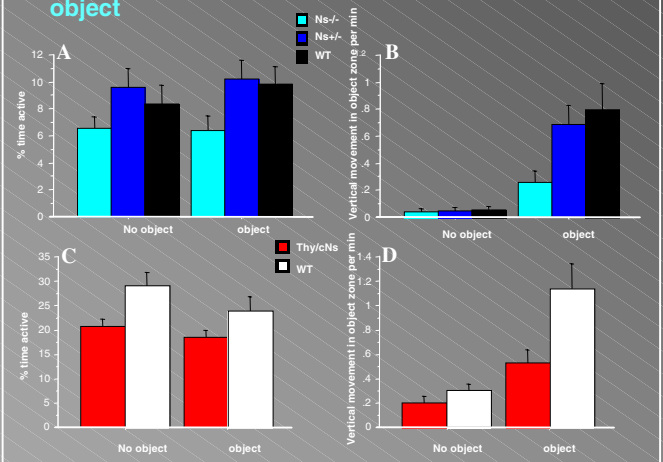
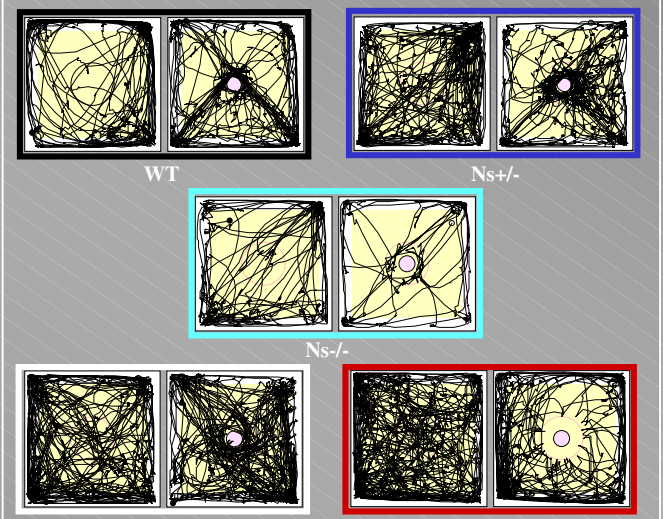
4- Reaction of NsKO and Thy/cNs mice toward a novel object



- The novelty test analyzed the reaction of mice toward a novel object introduced into a familiar arena. Ns-/- but not Ns+/- show an overall trend for reduced activity (A, F(2, 55)=2.51, P= 0.09). Ns-/- show a decrease in investigation of the novel object (B, WT versus Ns-/-, P= 0.01).
- Thy/cNs mice have reduced exploratory activity (C, F(1,28)= 5.7, P=0.02) and avoid the novel object (D, F(1,28)= 6.7, P=0.01).



- Novelty sessions before (right) and after introducing the object (left). Decreased investigation of the novel object in both Ns-/- and Thy/cNs mice compared to WT littermates is clearly evident despite overall greater activity in the Thy/cNs line.