Altered expression of the extracellular protease inhibitor neuroserpin interferes with exploratory behavior and reaction to novelty.

Rime Madani*, Serguei Kozlov‡, Alexander Akhmedov‡, Paolo Cinelli‡, Jochen Kinter‡, Hans-Peter Lipp*, Peter Sonderegger‡ and David-Paul Wolfer*.
*Institute of Anatomy and ‡ Institute of Biochemistry, University of Zurich CH-8057 Switzerland.

Summary

Available evidence suggests that the balance between antiproteases and their target proteases in the extracellular space may be critical for neuronal function and pathology. Neuroserpin, 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. Mice genetically made deficient in neuroserpin (NsKO) have been generated. Homozygous NsKO mice lack neuroserpin 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. These mice, together with transgenic mice which overexpress neuroserpin in neurons under the control of Thy1.2 promoter (Thy/cNs) have been subjected to analysis of activity in their home cages and to a battery of behavioral tests. Both NsKO and Thy/cNs mutants show normal levels and circadian patterns of home cage activity. However, homozygous NsKO mice exhibited reduced exploratory activity in novel environments, 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 neuroserpin in the regulation of emotional behaviour through a mechanism that is at least in part independent of tPA activity and they shed new light on the role of extracellular proteolysis in the brain.

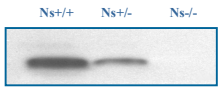
1- Neuroserpin overexpressing and deficient models

a- Thy/cNs mice overexpress Ns

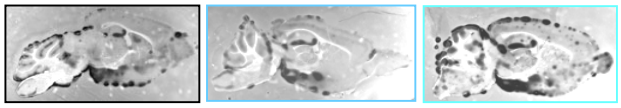
❖ Transgenic mice overexpressing Ns under the control of the neuronal promoter thy-1.2 have been generated. These mice, named (Thy/cNs), have decreased brain tPA proteolytic activity and show a reduced infarct size following focal ischemia (for details: Paolo Cinelli, MCN 2001 in press).

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.



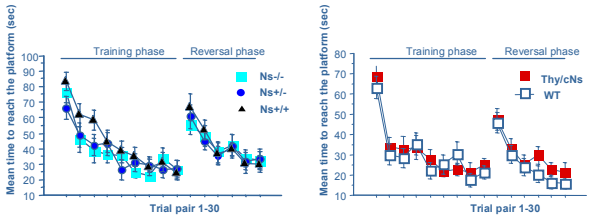
❖ Analysis of brain protein extract by Western-blot revealed a band at the level of 40 kDa in WT mice that corresponds to the endogenous Ns protein. This band is weaker in Ns+/- mice and is undetected in Ns-/- mice. The Western-blot was confirmed by immunoprecipitation.



Ns+/+ Ns +/- Ns -/-

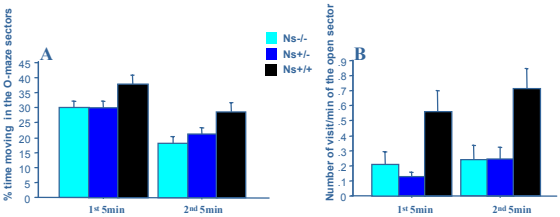
❖ To analyze the consequence of Ns deletion on tPA proteolytic activity, an histoenzymatic assay (in situ zymography) was performed on brain cryosections in the presence of plasminogen. We observed normal proteolytic zones in all genotypes. Biochemical analysis, using SDS-PAGE and zymography underlay,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

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



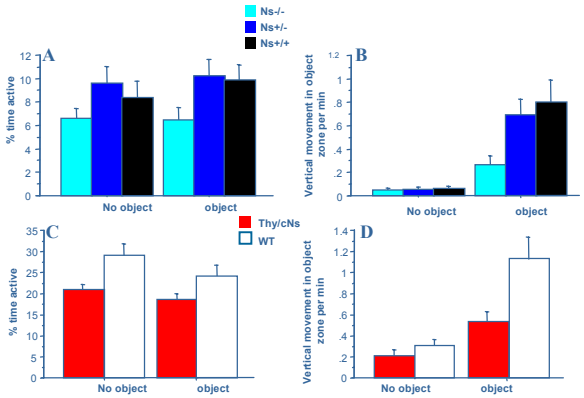
❖ Since available evidence suggests that the extracellular proteolysis may be critical for learning and memory, both NsKO and Thy/cNs mice were tested in the Morris Water Maze (MWM). This task assesses the hippocampal-dependent spatial reference memory. NsKO and Thy/cNs mice performed normally; no difference 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

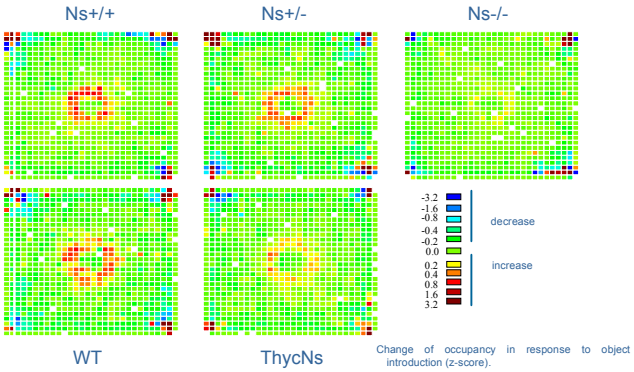


❖ Exploratory activity was analyzed in several tests. In the elevated null-maze, both Ns+/- and Ns-/- exhibit significant reduced exploratory activity (A, F(2,83)= 6.9, P=0.0016) and are reluctant to investigate the open sector of the maze (B, F(2,83)= 5.1, P=0.007). A similar phenotype was observed in the open field and dark-light box.
❖ Thy/cNs mice have normal exploring activity in all the mentioned tests.

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).



Change of occupancy in response to object introduction (z-score).

❖ Tracking analysis with Wintrack software (http://www.dpwolfer.ch/Wintrack) of novelty sessions. Decreased investigation of the novel object in both Ns-/- and Thy/cNs mice compared to WT littermates.

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 compensation 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.

Acknowledgments:
♥ We are grateful to Dr. Paolo Cinelli, Prof. Jean-Dominique Vassalli and Prof. Hans-Peter Lipp for their collaboration and helpful discussion. We wish to thank Rosemarie Lang and Inger Drescher for their excellent technical assistance.
Support:
❖ Supported by SNF 31-54184.98, BIO4CT980297/BBW98.0125, The Roche Reseach Foundation, Société Suisse de la Recherche Médicale, Hartmann-Müller Foundation and NCCR "Neural Plasticity and Repair".