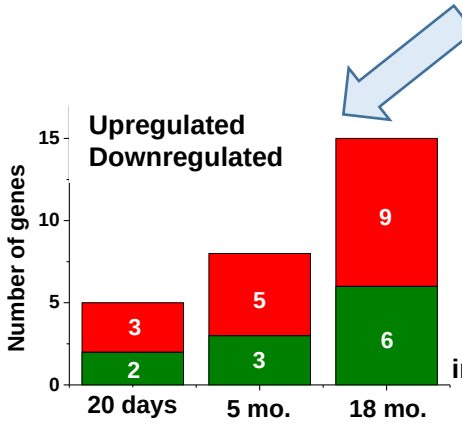
MAPK pathways and alphaB-crystallin phosphorylation in brain: a focus on aging and Alzheimer's disease

Natalia Muraleva
Molecular mechanisms of aging Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia

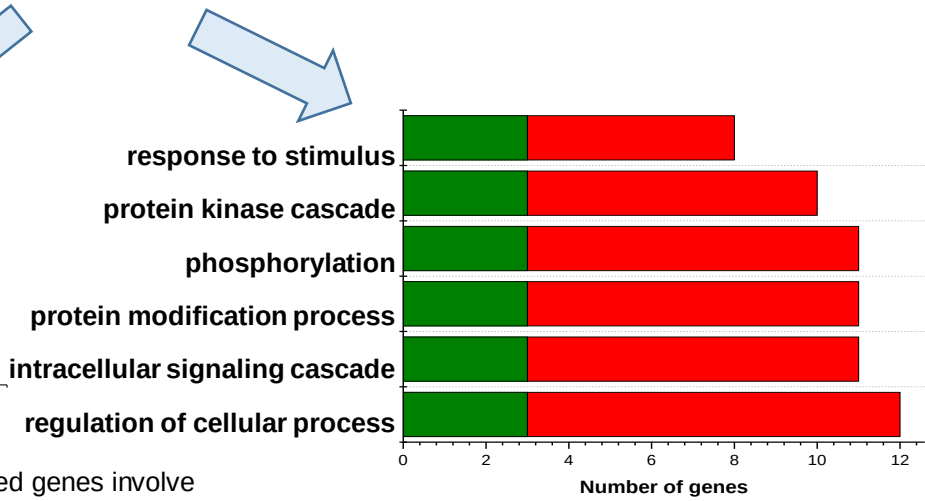
Motivation and Aim: Accumulation of intracellular damage and protein aggregates are an universal hallmark of aging. Maintenance of protein proteostasis is attained through precisely coordinated systems that must rapidly correct unwanted proteomic changes. The points of cross talk between the unfolded protein response and MAPK signaling pathways that may contribute to our understanding of the mechanisms of alteration of proteostasis processes with aging and with development of age-related diseases including Alzheimer's disease (AD). MAPK signaling network regulates cell survival and death responses following a variety of stresses including misfolded protein response stress. Two subfamilies of MAPKs (p38MAPK and ERK1/2) participate in this by regulating the activity of the alphaB-crystallin (CryaB) through its phosphorylation. CryaB as a molecular chaperone prevents aggregation of proteins (e.g. amyloid beta) and enables their correct refolding. Nevertheless, the link between changes in MAPK–dependent CryaB phosphorylation with the normal aging and the development of AD remains unclear. Here, we examined CryaB, phospho- (p-) Ser59-CryaB and p-Ser45-CryaB protein amounts in the brain of Wistar rats with normal aging and senescence-accelerated OXYS rats at different stages of the development of AD-like pathology. We compared this result with the changes in expression of genes involved in the p38 MAPK and ERK 1/2 signaling pathways and the content of key proteins of these pathways in brain of Wistar and OXYS rats.

Method and Algorithms: The work was carried out on male OXYS rats and Wistar rats (control) aged 20 days, 5, and 18 months. To detect changes in the expression of genes involved in MAPK- and ERK1/2 signaling pathways in Wistar and OXYS rats, we analyzed RNA-Seq data of hippocampus and prefrontal cortex. Standard techniques of western blott analysis and immunohistochemistry were used to examine of content of CryaB, p-Ser45-CryaB, p-Ser59-CryaB and key proteins of MAPK- and ERK1/2 signaling in the prefrontal cortex and hippocampus of Wistar and OXYS rats.

The alterations in the expression of the p38 MAPK pathway signaling genes precedes the development of signs of AD-like pathology in OXYS rats



The number of differentially expressed genes involve in the p38 MAPK signaling pathway in the prefrontal cortex of 20-day-old and 5- and 18-month-old OXYS rats compared to age-matched Wistar rats

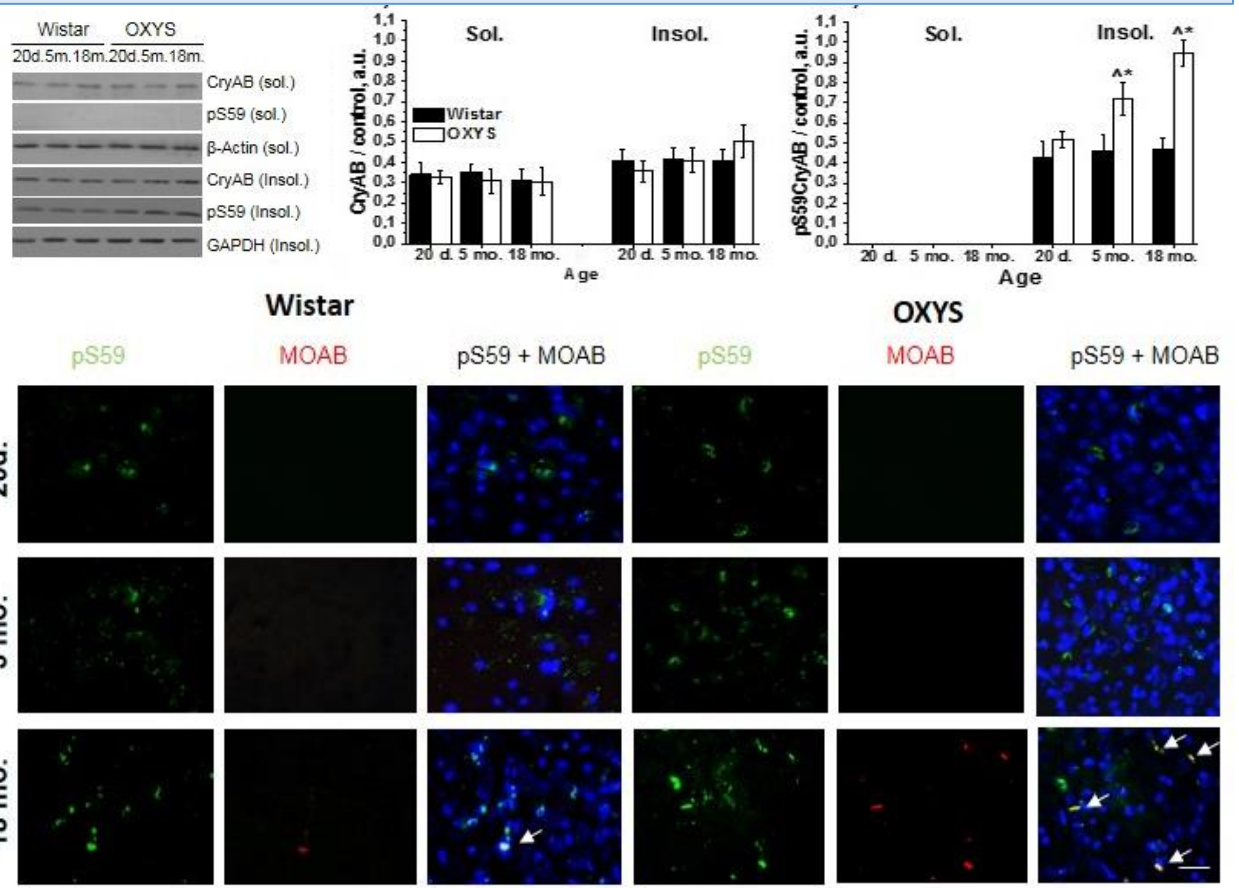


The GO terms according to DAVID for up- and downregulated differentially expressed genes from 5 to 18 months involve in the p38 MAPK signaling pathway in OXYS rats

According to the analysis of transcriptomes of the prefrontal cortex and hippocampus, the development of AD signs in OXYS rats is accompanied by an increase in the number of differentially expressed genes (DEGs) involved in the p38 MAPK signaling pathway. Changes in the mRNA level of genes in OXYS rats are associated with such categories of gene ontologies as the cascade of protein kinases, phosphorylation, calcium signaling, and the protein kinase activating signaling pathway.

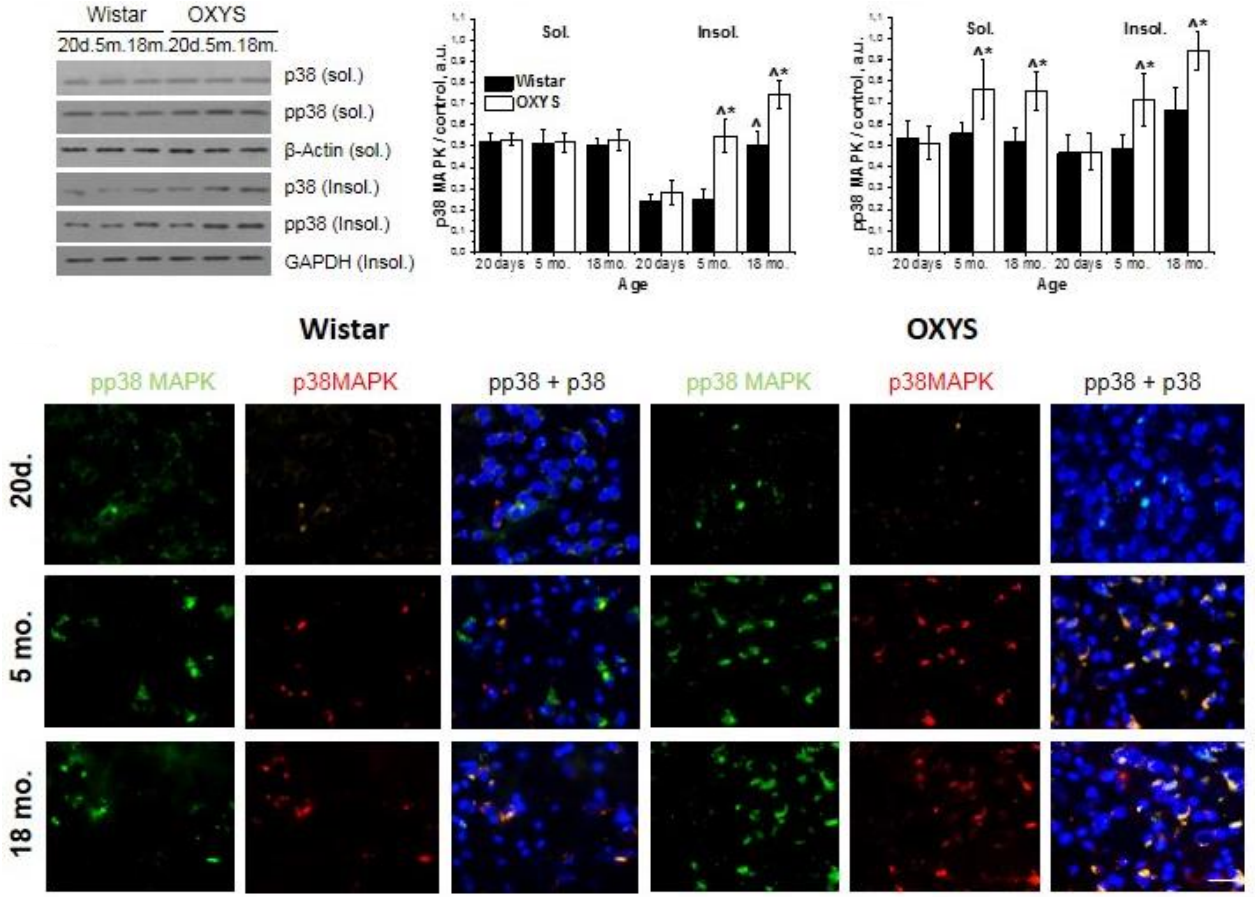
The alterations of protein contents of pS59-CryAB, p38 MAPK and pp38 MAPK precedes the development of signs of AD - like pathology in OXYS rats

The manifestation and progression of signs of AD in OXYS rats were accompanied by an increase in the level of p-Ser59-CryaB relative to Wistar rats



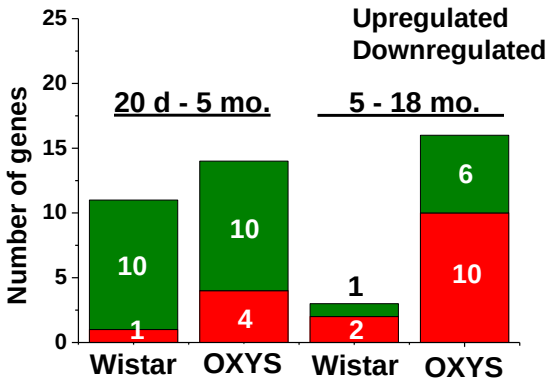
The protein content of CryAB in the cortex did not differ between OXYS rats and Wistar rats. pS59-CryAB absent in detergent – soluble protein fraction of OXYS and Wistar rats. It was detected only in detergent – insoluble fraction of rats and its level increased with age in the both strain. The pS59-CryAB was localized with Aβ and its colocalization was increased in OXYS rats at 18 months of age. Similar results were obtained in the hippocampus.

p38MAPK and pp38MAPK protein contents increase with the age in OXYS rats

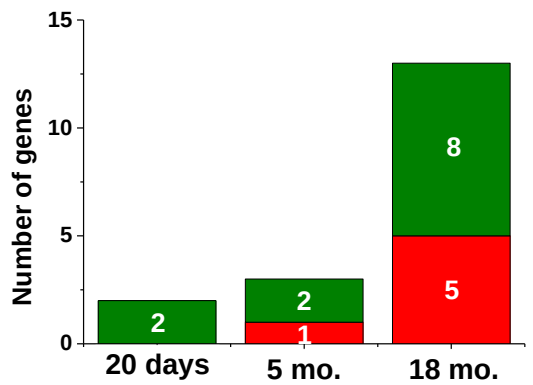


CryAB phosphorylation (S59) is mediated through activation of p38MAPK. A significant increase in the p38 MAPK protein content in the detergent-insoluble fraction was found in OXYS rats aged 5 and 18 months, while its level did not change in Wistar rats. In these age groups, OXYS rats showed an increase in the level of phosphorylation of p38 MAPK protein in both protein fractions. Similar results were obtained in the hippocampus.

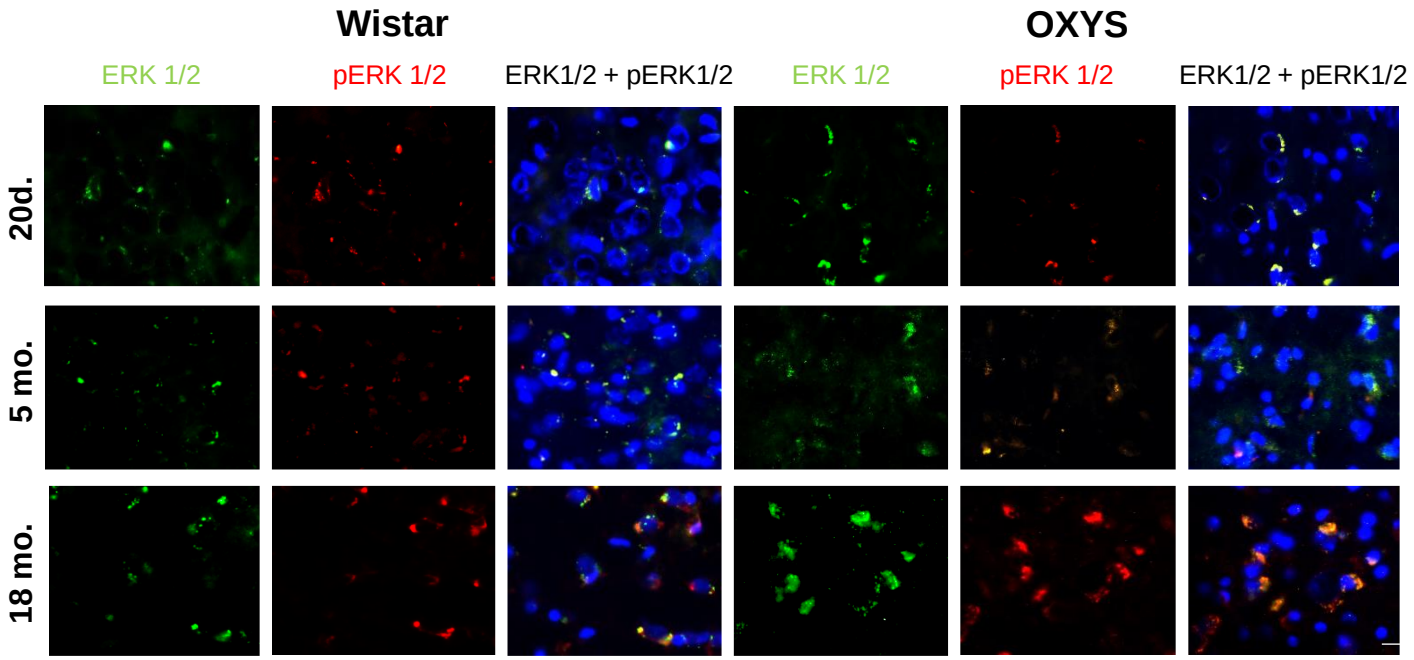
The progression of signs of AD in OXYS rats was accompanied by an activation of ERK1 / 2 signaling pathway



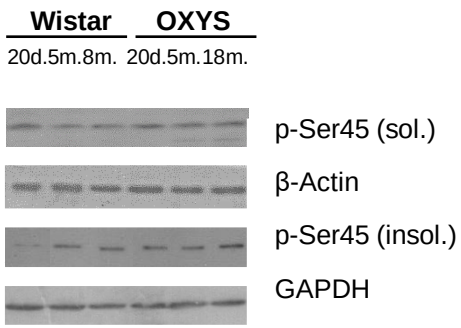
The number of differentially expressed genes involve in the p38 MAPK signaling pathway in prefrontal cortex of OXYS and Wistar rats with age

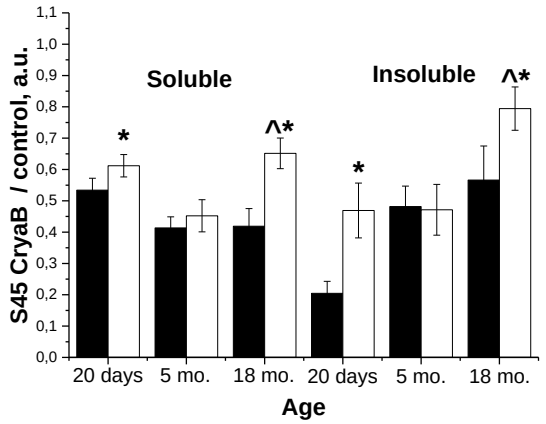


The number of differentially expressed genes involve in the p38 MAPK signaling pathway in the prefrontal cortex of OXYS rats compared to age-matched Wistar rats



CryaB phosphorylation (S45) is mediated through activation of the ERK1/2 signaling pathway controlled by ERK1/2 protein kinases. A significant increase in the protein content of ERK1/2 was found in the prefrontal cortex and hippocampus of OXYS rats at all studied ages groups.





The level of p-Ser45-CryaB in OXYS rats was higher than in the control Wistar rats at the age of 20 days and 18 months. It should be noted, that the accumulation of p-Ser45-CryaB increased already at the preclinical stage of AD - like pathology in OXYS rats.

CONCLUSION: Thus, alteration of MAPK-dependent phosphorylation of CryaB is happening with the normal aging. Activation of EPK1/2-dependent CryaB phosphorylation occurs with age and is characteristic for the preclinical and progressive stages of the AD-like pathology. Manifestation and progression of the signs of the AD occur against the background of activation of p38MAPK-dependent phosphorylation of CryaB and changes in gene expression of this signaling pathway. An increase in the level of p-Ser59-CryaB and its joint localization with Aβ1-42 can be considered as a response to the accumulation of toxic protein aggregates in the brain, which is an important part of the endogenous mechanism of AD development.

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