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Fund “Nauka” Project № 18016 Resume – Competitive-based Session 2018:

“The role of the transcription factor Zbtb20 for the development of the cortical interneurons in mammal brain”

Project leader: Prof. Anton Bozhidarov Tonchev, MD, PhD, DSc

The major idea behind the project is to quantify the number of interneurons marked by expression of different proteins like GABA, parvalbumin, somatostatin, calretinin and others during different developmental stages of the homozygous mutants (test group) and wild type (control group). In order to explain the deficits in Zbtb20 mutants we will look at the distribution and quantity of known regulators of neurogenesis in the subpallium in mice. In order to accomplish these goals we will use immunofluorescent stainings. The specimens will be micrographied and the images will be further analyzed. Additionally we plan to make a comparison between the homozygous and heterozygous mutant animals in order to see if Zbtb20 deficiency has a dose dependent effect on interneurogenesis.

Expected results:

1. Due to the serious defects in the hippocampus and neocortex, it is expected to find deficits in the interneuron as well.
2. A thorough map of Zbtb20 expression in the subpallium of wild type animals.
3. Offer a putative mechanisms of the effect of Zbtb20 on interneuron development.
4. Because heterozygous mutants have normal lifespans and less pronounced deficits, it is generally expected interneuron analysis to show more discrete disturbances. In case of severe deficiency in only one subpopulation, the heterozygous mutant can be used as a model animal in other studies.

Achieved results:

1. Analyzed the density of Somatostatin-positive and Parvalbumin-positive interneurons and prepared a preliminary figure for publication.
2. Immunofluorescent stainings were made for the common for CGE and MGE markers Rln and Cr. The already obtained preliminary results on the effect of Zbtb20 on MGE CINs allowed the evaluation of changes in CGE CINs despite the overlap of the expression of these proteins in both mentioned areas. Analyzed cell density, prepared preliminary figure for publication.

3. Analysis of the density of CGE interneuron by using the specific markers Sp8 and Prox1. Preliminary figure prepared for publication.
4. Counting proliferating cells marked with 40 minutes BrdU was performed. Due to the more difficult work with embryonic tissues, additional experimental animals will be needed. The results need to be confirmed.
5. Clarification of the expression of Zbtb20 in embryonic animals. Postnatal expression pattern in the neocortical neurons was clarified by double staining with the panneuronal marker NeuN. No overlap was observed.
6. Comparison of expression pattern of a gene panel was done. IF and ISH methods were used.
7. Comparison of the phenotype between the control and the mutant experimental animal was been started, but additional tissues are needed. There are preliminary results.
8. Methods used – The analysis was performed according to the following algorithm: Imaging of the prepared immunofluorescent stainings histological specimens was performed on Axio Imager 2 and a monochrome AxioCamMrm camera. Their manual counting was performed in Fiji, and the statistical analysis was performed in R.