The importance of polyamines for differential sensitivity of hippocampal subregions to excitotoxicity.

The hippocampus is a brain structure mainly responsible for learning and memory. It is composed of dentate gyrus and cornu ammonis (CA) regions. The CA is divided into regions CA1, CA2 and CA3. The CA2 region, due to its small size and poorly defined boundaries, is the least known structure of the hippocampus, although it is characterized by special properties among which strong resistance to damage (in contrast to CA1 which is very sensitive) is the most prominent one.

The preliminary analysis carried out in our Laboratory indicates that hippocampus may be characterized by a unique anatomical distribution of proteins related to the metabolism of amino acid arginine (Arg). Arg in the neurons can be utilized by an enzyme named neuronal nitric oxide synthase (nNOS) for the production of nitric oxide (NO), which is an important cellular signaling molecule, but which, if produced in excess, becomes toxic and can induce neuronal death. This mechanism is involved in neuronal injury in excitotoxicity, a state, where certain type of neuronal receptors is overstimulated for prolonged time. This excitotoxicity-induced NO overproduction is involved in neuronal dysfunction and death in numerous neurological disorders, including stroke, epilepsy or traumatic brain injury. On the other hand, Arg in neurons can be converted into polyamines (PAs) in a multistep metabolic pathway, that is initiated by a reaction competitive to NO synthesis, in which Arg is utilized by nNOS-competing enzyme, arginase 2 (Arg2) for the production of PAs precursor, ornithine (Orn). PAs play a variety of functions in the cell, and, importantly, are known for their neuroprotective properties. Our preliminary analyses suggest that CA2 region jest significantly enriched with Arg2 and several proteins related with PAs production and may be depleted of nNOS, in contrast to CA1 region which is depleted of Arg2 and PAs-producing proteins but enriched with nNOS. I hypothesize, that this can explain differential sensitivity of the neurons of these regions to excitotoxicity, where CA1 neurons die easily, and CA2 neurons usually survive. I observed the same pattern of injury in my experimental model of excitotoxicity, but only, when PAs production pathway is non-modified experimentally. Once PAs production is blocked with specific substance that stops the activity of a protein responsible for Orn conversion to first PA, putrescine, neurons in CA2 region become sensitive to excitotoxicity. This study appears to confirm my hypothesis, and in this project, I will attempt to explore in more details the relationship between PAs production in hippocampal neurons and response of these neurons to excitotoxicity with respect to hippocampal division to CA1-3 subregions.

First, I will develop and optimize a method to specifically visualize the region CA2 in my model. This region is located between CA1 and CA3, and its borders with these regions are normally not easily visible. Visualization of CA2 will allow me to accurately perform my further imaging experiments and to obtain, with high precision, separate data for CA1, CA2 and CA3 facilitating between-region comparisons. Next, I will measure the content of individual PAs and related compounds in hippocampal regions to confirm hypothesis that CA2 region is enriched in these metabolites compared to CA1. After this, I will check if neurons of individual regions respond to excitotoxic stimulus by modifying the PAs production. In the next experiment, by blocking individual steps of PAs production pathway, I will try to find which of the PAs is the most potent in neuroprotection of CA2 neurons. Finally, I will check if Arg2 may protect CA2 neurons from excitotoxicity by delivering of Orn for PAs synthesis or by depleting Arg, thus obstructing excessive synthesis of toxic NO by nNOS, or by combining both effects.

All my experiments will be performed using organotypic cultures of rat hippocampus, a model where tissue is cultured on the dish, in the conditions that allow it to live many days and retain its physiological functions. Compared to the experiments in vivo, where pathology would be induced in living animals, organotypic cultures allow to avoid animal stress and suffering, but still offer a model, where cellular mechanisms can be analyzed with respect to tissue anatomy. I expect that, after completion, my project will help to expand our understanding of brain functioning and will contribute to designing novel therapies against selected neurological disorders.