Report on the stay in Genova, Italy January 30- Aprile 28, 2011

The purpose of my visit to Genova, Italy was to attend a practical placement in the Department of Neuroscience and Brain Technologies of Italian Institute of Tehnologies (IIT) under the supervision of prof. Dityatev A.E. During my placement in IIT I had mastered some of the electrophysiological and biochemical methods, such as multielectrode registration in vitro, immunocytochemical analisis of cell cultures using confocal microscopy.

The main purpose of the study was the explanation of certain mechanisms of epileptogenesis using multielektrode registration (the method of multielectrode arrays, MEA). The experiments were performed on primary dissociated hippocampal cultures of 17-day-old mouse embryos. Neuronal cultures were planted and grown in parallel on multielektrode arrays for electrophysiological experiments - 6wellMEA (The culture chamber of the array is divided into six parts, each include 9 electrodes, so it can be possible to perform simultaneous recording from 6 neuronal cultures) and on coverslips for immunostaining using specific primary and secondary antibodies, and further analysis of the structure of cultures under the influence of the same factors that were used in experiments at the MEA, by confocal microscopy. The maintenance activity of cultures produced in CO_2 - incubator at 35.5^0C and high humidity in the atmosphere containing 5% carbon dioxide. Registration by MEA the spontaneous and modulated activity of cultures was performed with using of mini CO2 - incubator that kept the air temperature above the cultures at 35.5^0C and the concentration of carbon dioxide at 5%.

The experiments were aimed at studying the role of one of the main components of the extracellular matrix - heparan sulfates in the process of development of epileptogenesis and normal functioning of cultures. First of all has been made the comparing of different models of epileptogenesis such as low Mg model, model with using CTZ - blocker of desensitization of AMPA-receptors, model with high doses of one of the major excitatory neurotransmitter of the brain – glutamate, and model with potassium channel blocker 4-aminopyridine. Optimal for further experiments was selected model using 4-aminopyridine.

Next, have compared the activity at DIV 18-25 days of the 3 groups of cultures: without affecting, with application of the enzyme that destroys heparan sulfate - heparinase I, and with the inactivated enzyme as a control. Half of the cultures of these groups was exposed to epileptogenic factors a day after the application of enzymes, 50% of cultures was not affected. It was found that the destruction of heparan sulfate in the extracellular matrix under the influence of heparinase I leads to a decrease in spontaneous activity of the neuronal cultures. Also, used in the experiments a high concentration of cells led to the development of endogenous epileptogenesis at 19-20 days of development - activity of cultures became like epileptogenetic without influence of any factors apart from the natural structure of a neural network. Against this background, the application of 4-aminopyridine did not produce visible results.

In addition to disclosing the mechanisms of influence of heparan sulfates on the activity of the brain, experiments were performed using a number of blockers such as AP5 (effect on the NMDA-receptors) and diltiazem (L-type calcium channel). It was determined that the action of heparan sulfates is not associated with these cell components, as in all groups except group treated by enzyme was the increase in activity during development, and advance in the development occurred at 18-20 days. In the group with using of heparinase I development of spontaneous activity occurred more slowly. Cultures on coverslips were processed in parallel by the same substances as on MEA and were subjected to immunostaining using a variety of

primary antibodies such as anti-MAP2, anti-ΔHS, anti-parvalbumin, anti-calbindin and others to identify the structure of the matrix.

01.06.2011

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