The use of X-ray microprobe for the analysis of elemental abnormalities occurring in rat brain as a consequence of mechanical brain injury

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As a result of mechanical brain injury, the destruction of neurons, their connections and impairment in functional sub-systems are observed in nervous tissue [1]. According to clinical studies, epilepsy is a common outcome of brain injuries and almost 50% of patients with penetrating brain injuries (traffic accidents, missile wounds) develop seizures [2,3]. The present project is the continuation of our previous investigation concerning the role of trace elements in the pathogenesis and progress of epilepsy [4,5]. In the frame of it, we would like to verify if an increased susceptibility of injured brain to epileptic seizures is associated with the local and remote elemental anomalies in the nervous tissue.

Unfixed and non-embedded rat brains were cut frontally using a cryomicrotome into 15 µm thick slices. The sections were mounted on the Ultralene foil and freeze-dried. Typically, two sections of each brain were prepared for elemental analysis. The first section contained the area of injury and the second one the dorsal part of the hippocampus.

The topographic and quantitative elemental analysis of the tissues was done using X-ray fluorescence microscopy. The measurements were carried out at HASYLAB beamline L. The multilayer monochromator was applied and the primary photon energy was set to 17 keV. The polycapillary optics was used for the focusing of the beam and the obtained beam spot had the dimension of 15 µm x 15 µm. The X-ray fluorescence spectra were measured using the Vortex SDD detector from SII Nano Technology USA inc. and the time of single spectrum acquisition was 10 s. Measurements of NIST standard reference materials (SRM 1833 and SRM 1832) were performed for spectrometer calibration.

For each sample two-dimensional analysis of elemental distribution was performed. The results of such analysis obtained for selected animal with mechanical brain injury were shown in the Figure 1. The elemental composition of the cerebral cortex and white matter in injured and uninjured brain hemisphere was compared. Moreover, the differences between animals with mechanical brain injury as well as those with pilocarpine induced seizures and naive control rats were evaluated. In this case, besides the two areas mentioned previously, four sectors of the hippocampal formation (CA1 and CA3 regions of the Ammon's horn, the dentate gyrus (DG) and the hilus of the dentate gyrus (H)) were examined.

For all the analyzed tissue areas the mean masses per unit area of elements were calculated. Afterwards, the median values were evaluated for analyzed groups. The statistical significance of the differences in elemental composition was tested with the use of non-parametric U (Mann-Whitney) test.

We did not observe any statistically significant differences between injured and uninjured brain hemisphere. Elevated levels of Ca in the cortex and Fe in the white matter of both hemispheres were detected for animals with mechanical brain injury in comparison with the control group. For the hippocampal formation tissue, the lower masses per unit area of Cu in DG (p=0.02) and Zn in CA3 (p=0.02) and DG (p=0.03) areas were recorded for animals with brain injuries in comparison with control rats. It is necessary to mention that identical relations were previously observed for animals with pilocarpine-induced epilepsy. Decreased mass per unit area of Ca in CA1 (p=0.09) and DG (p=0.05) areas was observed for animals with mechanical brain injury in comparison with pilocarpine-treated rats, however such differences were not noticed for the control group.

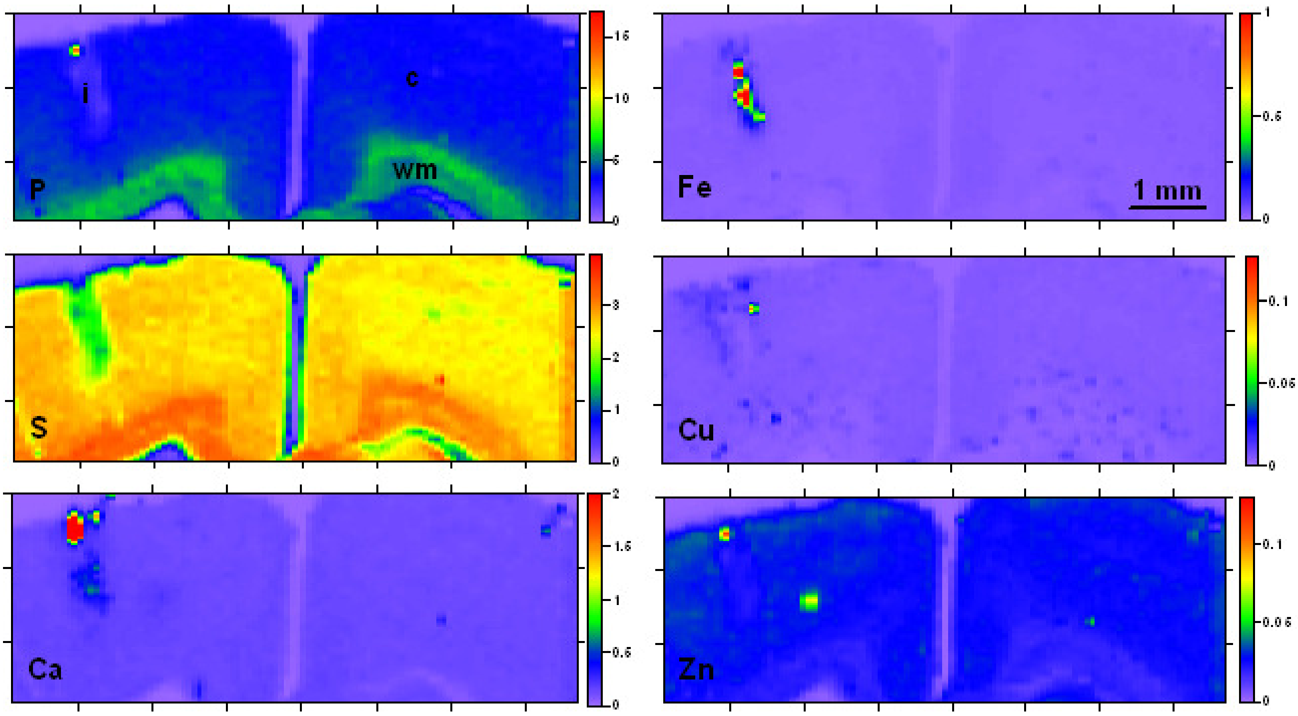

Figure 1. Maps of elemental distribution obtained for selected animal with mechanical brain injury; i - the area of injury, c - cerebral cortex, wm - white matter.

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