## INVESTIGATION OF DIFFERENCES IN FREQUENCY OF CREATINE INCLUSIONS WITHIN HIPPOCAMPAL FORMATION BETWEEN THE ACUTE AND LATENT PERIODS OF PILOCARPINE MODEL OF TLE-SRFTIR MICROSPECTROSCOPY STUDY

J. Kutorasinska<sup>1\*</sup>, Z. Setkowicz<sup>2</sup>, K. Janeczko<sup>2</sup>, C. Sandt<sup>3</sup>, P. Dumas<sup>3</sup>, and J. Chwiej<sup>1</sup>

 <sup>1</sup>AGH-University of Science and Technology, Faculty of Physics and Applied Computer Science, Krakow, Poland
<sup>2</sup>Jagiellonian University, Faculty of Biology and Earth Sciences, Department of Neuroanatomy, Krakow, Poland
<sup>3</sup>SOLEIL, St Aubin, France

Keywords: epilepsy, creatine, synchrotron radiation, SR FTIR microspectroscopy \*e-mail: Justyna.Kutorasinska@fis.agh.edu.pl

Epilepsy is one of the most common neurological disorders and despite of the longtime history of researches under this disease the etiology of almost 70% of its cases is still unknown. The investigation under the pathogenesis of epilepsy are rarely carried out based on the human tissues, which can be obtained only post mortem or during the resection of epileptic foci. Therefore, different animal models of epileptic seizures are used.

Epidemiological studies shows that the most frequently occurring type of epilepsy in adults is temporal lobe epilepsy (TLE) and the main features of TLE are [1]:

- 1. localization of epileptic foci in the limbic system,
- 2. a seizure-free time interval following the precipitating injury,
- 3. the presence of hippocampal sclerosis leading to reorganization of neuronal networks [2].

Highly isomorphic with human cases of TLE is animal model of epilepsy with pilocarpine induced seizures. After pilocarpine administration in animals three distinct periods can be distinguished in their behaviors. They are as follows:

- 1. an acute period occurring during the first 24 hours after pilocarpine injection,
- 2. a silent period with progressive normalization of EEG and behavior and lasting from around 4 to 44 days,
- 3. a chronic period in which spontaneous recurrent seizures may occur [3].

The present work is the continuation of our previous research in frame of which the frequency of creatine inclusions in hippocampal formation was examined for rats in the acute period after pilocarpine injection. The comparison of epileptic animals with controls showed an increased accumulation of creatine deposits which were localized mainly in the dentate gyrus hippocampal area and in the multiform cell layer. What is more, obtained data pointed to statistically important correlation between the number of inclusions and the total time of seizure activity within the observation period [4]. The purpose of the present study was comparison of the number of creatine inclusions in hippocampal formation for the acute and latent periods after injection of pilocarpine. As in our previous research, synchrotron Fourier-transform infrared microspectroscopy (SRFTIR) was used for the detection of creatine in tissues. The measurements were done at SMIS beamline of SOLEIL. The use of synchrotron source of infrared radiation allowed us to carry out the research with the spatial resolution of 10  $\mu$ m. Because SRFTIR microspectroscopy is noninvasive and nondestructive method we were able to verify obtained results with the use of Raman microspectroscopy [5].

Results of the present work showed that accumulation of creatine inclusions in the epileptic hippocampus is not only temporary but permanent effect of pilocarpine induced seizures.

Acknowledgments: This work was supported by Polish Ministry of Science and Higher Education and Polish National Science Centre grant: 2921/B/T02/2011/40. We acknowledge SOLEIL for provision of synchrotron radiation facilities (Proposal ID 20090749 and 20100664).

## References

- E.J, Pedley, *Epilepsy: A Comprehensive Textbook* (Williams & Wilkins, Philadelphia 2008).
- [2] G. Curia, D. Longo, G. Biagini, R.S.G. Jones, M. Avoli, "The pilocarpine model of temporal lobe epilepsy," J. Neurosci. Meth. 172 (2008) 143 – 157.
- [3] F.A. Scorza, R.M. Arida, M. Da Graca Naffah-Mazzacoratti, D.A. Scerni, L. Carderazzo, E.A. Cavalheiro, "The pilocarpine model of epilepsy: What have we learned?," An. Acad. Cienc. 81 (2009) 345 – 365.
- [4] J. Dulinska, Z. Setkowicz, K. Janeczko, C. Sandt, P. Dumas, L. Uram, K. Gzielo-Jurek, J. Chwiej "Synchrotron radiation Fourier-transform microspectroscopy study showed an increased frequency of creatine inclusions in the rat hippocampal formation following pilocarpine-induced seizures," *Anal. Bioanal. Chem.* 402 (2012) 2267 – 2274.
- [5] B. Stuart Modern Infrared Spectroscopy (ACOL University of Greenwich, UK 1996).