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Elemental changes of hippocampal formation occurring during postnatal brain development

J. Chwiej[a,*], M. Palczynska[a], A. Skoczen[a], K. Janeczko[b], J. Cieslak[a], R. Simon[c], Z. Setkowicz[b]

[a] AGH University of Science and Technology, Faculty of Physics and Applied Computer Science, Krakow, Poland
[b] Jagiellonian University, Institute of Zoology and Biomedical Research, Krakow, Poland
[c] Institut fur Synchrotronstrahlung, Research Centre Karlsruhe, Karlsruhe, Germany

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ABSTRACT

In this paper the elemental changes of rat hippocampal formation occurring during the postnatal development were examined. Three groups of animals were used in the study. These were naive Wistar rats at the age of 6-, 30- and 60-days and the chosen life periods corresponded to the neonatal period, childhood and early adulthood in humans, respectively.

For the topographic and quantitative elemental analysis X-ray fluorescence microscopy was applied and the measurements were done at the FLUO beamline of ANKA. The detailed quantitative and statistical analysis was done for four areas of hippocampal formation, namely sectors 1 and 3 of the Ammon's horn (CA1 and CA3, respectively), dentate gyrus (DG) and its internal area (hilus of DG, H).

The obtained results showed that among the all examined elements (P, S, K, Ca, Fe, Cu, Zn and Se), only the levels of Fe and Zn changed significantly during postnatal development of the hippocampal formation and both the elements were significantly higher in young adults comparing to the rats in neonatal period. The increased Fe areal density was found in all examined hippocampal areas whilst Zn was elevated in CA3, DG and H.

In order to follow the dynamics of age-dependent elemental changes, the statistical significance of differences in their accumulation between subsequent moments of time was examined. The obtained results showed statistically relevant increase of Zn level only in the first observation period (between 6th and 30th day of life). Afterwards the areal density of the element did not change significantly. The increase of Fe areal density took place in both examined periods, however the observed changes were small and usually not statistically relevant.

1. Introduction

The hippocampus is an important part of the mammalian limbic system. It plays a pivotal role in the behavioral, emotional and memory processes and therefore has an important contribution to functional properties of the whole brain [1]. Moreover, the hippocampus is the brain region presenting extraordinary capacity for structural reorganization [1]. The period in which its cellular framework is formed (neurogenesis and gliogenesis) and maturation of its internal connections are particularly elongated, comparing to other brain regions, extending from prenatal to advanced postnatal developmental stages [2,3]. Such structural plasticity probably participates in the hippocampal functions including learning, memory, anxiety and stress regulation. From the other side, such lifelong reorganization can be modulated by the different environmental and pathological factors [4,5].

This paper focuses on changes in the content and distribution of elements that are involved in molecular processes whose course is important for normal brain development but whose disorders may trigger different cerebral pathology, including epileptogenesis [6,7].

To realize the purpose of the paper three groups of normal rats were examined. These were the animals at the age of 6-, 30- and 60-days and this corresponds to the neonatal period, childhood and early adulthood in humans [8,2,3].

For highly spatially resolved elemental analysis X-ray fluorescence (XRF) microscopy was applied [9,10]. In the technique the focused X-ray beam with the energy from several to several dozen keV interacts with the inner shell electrons of the target atoms. The electronic vacancies being a result of this interaction are filled with the outer shell electrons what results in the emission of characteristic X-ray photons of energy being unique to the parent atom. The recorded XRF spectrum depends not only on the atomic species within the irradiated volume but, what is more, the intensities of the elemental fluorescence lines are proportional to the content of the elements within the sample.

* Corresponding author.
E-mail address: joanna.chwiej@fis.agh.edu.pl (J. Chwiej).

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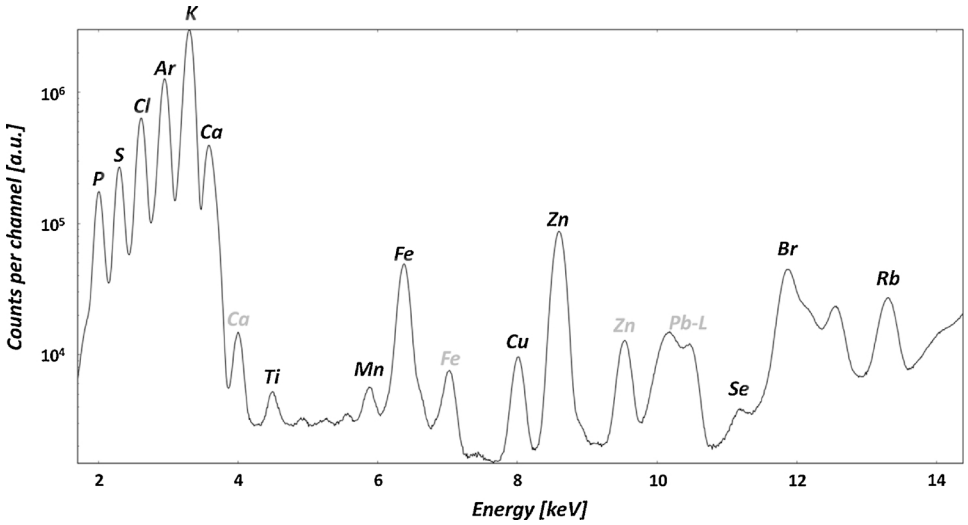

Fig. 1. The cumulative X-ray fluorescence spectrum recorded for hippocampal formation of the selected adult rat (N60). The analytical K-α lines of the elements were marked in black whilst K-b and L lines of elements were signed in grey.

Moreover, the high intensity X-ray sources and modern beam focusing systems allow for the 2D and 3D elemental imaging of samples at sub-micrometer or even nanometer spatial resolution [11,12]. Among other advantages of XRF microscopy non-destructivity and high sensitivity should be taken into account and make the technique very useful for wide range of scientific research including those carried out tissues as well as cells [13–16,11,12].

In the literature one can find many examples of the use of XRF microscopy for the elemental analysis of human and animal nervous tissue. The method has allowed the investigation of the roles of metals in aging [13], brain tumors and other neurological disorders [17–19,15] as well as the study of the toxic influence of different metals on the brain [20]. The technique has also been used in our previous research for the analysis of elemental anomalies of hippocampal formation occurring in different animal models as a result of seizures [21,14,22], neuroprotection [23] and the treatment with the ketogenic diet [24,25].

2. Materials and methods

2.1. Animals

Male Wistar rats came from an animal colony of the Department of Neuroanatomy (Institute of Zoology, Jagiellonian University). All animal-use procedures were carried out there and were approved by the Bioethical Commission of the Jagiellonian University in accordance with international standards. The animals were maintained under conditions of controlled temperature (20 ± 2°C) and illumination (12 h light:12 h dark cycle).

2.2. Sample preparation

On the 6th, 30th and 60th day of postnatal development, the rats from N6, N30 and N60 groups, respectively, were perfused with physiological saline solution of high analytical purity. Afterwards, their brains were quickly excised, frozen and cut using a cryomicrotome into 12 µm thick sections. The slices of the dorsal part of the hippocampus were mounted on the ultrapure, ultrathin and transparent for X-rays Ultralene® foil and freeze-dried. The number of rats in each group was equal to 6 and one slice per each animal was subjected to two dimensional elemental analysis.

2.3. Measurements

For analysis of the distribution and accumulation of selected elements (P, S, K, Ca, Fe, Cu, Zn and Se) the X-ray fluorescence microscopy was applied. The measurements were done at the FLUO beamline of ANKA Synchrotron Facility as a part of the proposal A2014-024-006393 [26].

The energy of the beam was set to 16 keV using double multilayer monochromator with W-Si multilayers at 2.7 nm period. The beam was focused with the polycapillary and the spot size on the sample was 13 µm × 18 µm (vertically × horizontally). The fluorescence radiation from the sample was detected by SDD. The detector was positioned at the angle of 45° in respect to the sample and 90° in respect to the exciting beam. The measurements were done in air at room temperature. The samples were mapped in two dimensions and X-ray fluorescence spectra were recorded with the live time of 8 s. The step size used during raster scanning was equal to 100 µm.

For spectrometer calibration and elemental sensitivities calculation MICROMATTER XRF calibration standards (GaP, CuS_x, KCl, CaF_2, Ti, Cr, Fe, Cu, Ge, Se, CsBr, RbI and SrF_2) were used.

3. Results

Each slice of hippocampal formation was subjected to raster scanning using the X-ray beam. As a result of this process X-ray fluorescence spectra were recorded for each examined tissue point.

As one can see from the Fig. 1 presenting the exemplary cumulative XRF spectrum recorded for selected N60 sample, the following elements were recorded in the hippocampal formation: P, S, Cl, K, Ca, Ti, Mn, Fe, Cu, Zn, Pb, Se, Br and Rb. However, some of them were excluded from further quantitative analysis. Cl was not taken into account because physiological saline solution was used for animal perfusion, whilst Ti and Mn were below the detection limits for some of the examined samples/areas. The L lines of Pb in the spectra came from the experimental setup whilst Ar line originated from the air.

For both the analysis of individual spectra and batch processing of large data sets PyMCA software (freely available for noncommercial use) was applied [27]. The measurements of calibration standards allowed for determination of the elemental sensitivity curve. The elemental sensitivities together with the net peak areas of K-α lines obtained from PyMCA for the analyzed elements were used to calculate their areal densities for each examined tissue point and the details of the computations were described elsewhere [22].