V. 3. Measurement of Elemental Distributions in Mouse Brain by Using Submilli-PIXE Camera

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Introduction

In a biological body, it is well known that trace elements including metallic elements play important roles such as component of hemprotein, detoxication, methabolism and so on. Therefore, knowing their spatial distribution and amounts, we can find out the relation among a physiological role of the trace element *in vivo*, the function, and the disease appearance. We have been analyzing the mouse brain in cell level by using micro-PIXE. In this research, we administered 5'-bromodeoxyuridine (5-BrdU) that was the analogue of the thymidine as a marker to detect a new born cell. 5-BrdU is taken into new born cells in especially the dentate gyrus of the hippocampus where the neuronal cell appears newly in the brain[1]. Because of this, we made mental disease model mice, and investigated the difference of the element distribution in comparison to control mice. We administered Methamphetamine (METH) to mice to make mental disease model. METH is a kind of a typical stimulant. A stimulant acts on a central nerve of a brain, and causes various impaired consciousness, hallucinatory disorder, and the deterioration of memory. The stimulant mental disease is assumed to be a model with animal's schizophrenia[2]. Averaged elemental concentrations of Ca, Fe and Zn over the brain of the mice to which Methamphetamine (METH) had been administrated were higher than those of control mouse. On the other hand, elemental concentration of Br of subject mouse was lower[3].

Due to these facts we needed to investigate, not only the cell level but more wide-ranging images over the brain. In this study, we investigated a method to obtain

elemental distributions in whole brain slice taken from the subject mice, using in-air submilli-PIXE camera at a Dynamitron laboratory of Tohoku University[4,5]. The submilli-PIXE camera is able to measure elemental distribution in the region of several cm^2 with a resolution of submilli-meters.

Sample Preparation

We prepared four mice in this study. Seven weeks old male C57BL6 mice were obtained from the Japan SLC (Hamamatsu, Japan). METH was administered intraperitoneally at a dose of 1 mg/kg/day to the mice, following the schedule as shown in Fig. 1. Saline was injected intraperitoneally to the control mice. 5-BrdU was administered 50 mg/kg to the mice intraperitoneally for 5 consecutive days, as shown in Fig. 1, before the brain was taken out.

After the brain was taken out, it was quickly frozen with dry ice, and it was sliced by a cryomicrotome. While the thickness of the slice is 16 μm in micro-PIXE analysis to get elemental distributions in a single cell, thicker slice is appropriate in this application. In this study, the slice thicknesses were 50, 100 and 200 μm. Thickness of 200 μm is the maximum in our cryomicrotome. The brain slices were put on a Myler film of 2 μm-thickness and dry-fixated on it. The 2 μm-thickness Mylar film used in this study contains less amount of Br compared to the polycarbonate film which is used in the micro-PIXE analysis.

Experiments

Analysis was carried out by using in-air PIXE camera at Tohoku University. Proton beams of 3 MeV were extracted into air through a kapton film of 12.5 μm and irradiated on the target. Samples were fixed to the target holder, and set just after the beam exit window. X-rays were detected two Si(Li) detectors[4,5]. Beam was scanned ca. 10×10 mm^2 on a surface of samples, which covered whole brain slice. Average beam current was around 3 nA and total accumulated charge was around 20-40 μC.

Figure 2 shows a typical photo and elemental images of mouse brain to which METH was administered. Figure 3 shows those of control. Figure 4 shows X-ray spectrum of a sample. Elemental images were produced by using GeoPIXEII software[7]. Brain contains a lot of light elements, such as P, S, Cl and K, which were uniformly distributed over the brain. Brains also contain Fe, Cu, and Zn as a indispensable element.

As shown in Fig. 4, Fe is accumulated in the specific area. Elemental concentration in the area was 10 times higher than that in the other. While some other samples from the subject mice showed similar tendency, accumulation of Fe was not observed in those of control mice. It is suspected that a part of neuronal cells were poisoned by an excitatory action of METH. The similar symptom appears to Alzheimer's disease etc.[4] Zn is accumulated in the vicinity in hippocampus and the side of the brain as shown in Figs. 2 and 3. Other samples showed similar tendency too. Since Zn in neuronal cells of hippocampus is indispensable element to transmit information, it might be related to their activity[5]. Cu is accumulated in the vicinity in third ventricle as shown in Fig. 3. Bromine is uniformly distributed over the brain in all samples.

Conclusion

In order to know the relation among a physiological role of the trace element *in vivo*, the function, and the disease appearance, investigation not only on the cellular level but also on the organ level was carried out. In this study, we investigated a method to obtain elemental distributions in whole brain slice taken from the subject and control mice, using in-air submilli-PIXE camera. Sample thickness more than 200 μm is appropriate in this study. We obtained the elemental distributions of the whole brain of the mice of mental disease model and control mice. Trace elements such as Fe and Zn are accumulated in the brain. Elemental concentration in the area where Fe was accumulated is 10 times higher than that in the other. The accumulation was not observed in the brain of the control mice. It is suspected that a part of neuronal cells were poisoned by an excitatory action of METH. Zn is accumulated in the vicinity in hippocampus. Br was uniformly distributed over the brain for both the administrated and the control mice. In this study, we could obtain the elemental distributions of whole brain slices. The submilli-PIXE camera will provide a powerful tool for this research.

Acknowledgement

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References

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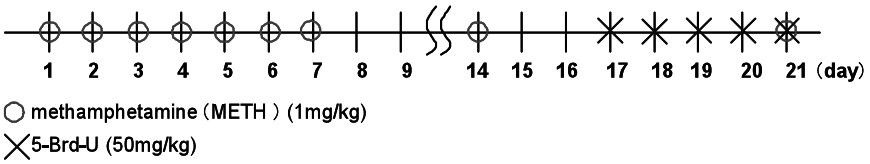

Figure 1. Schedule of administration.

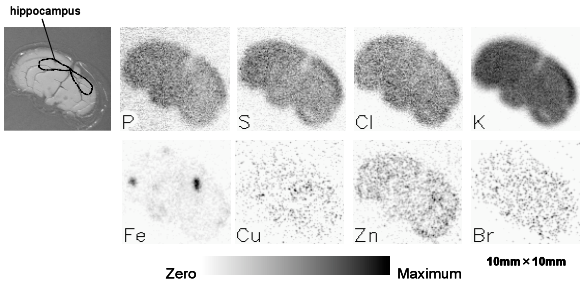

Figure 2. Photo and elemental distributions of the brain of subject mouse.

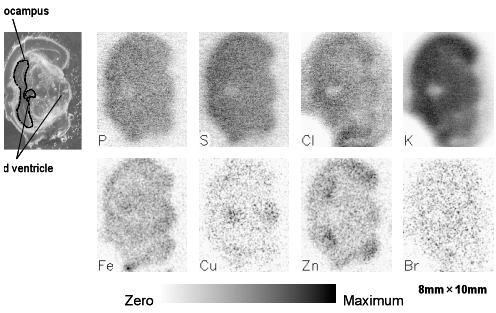

Figure 3. Photo and elemental distributions of the brain of control mouse.

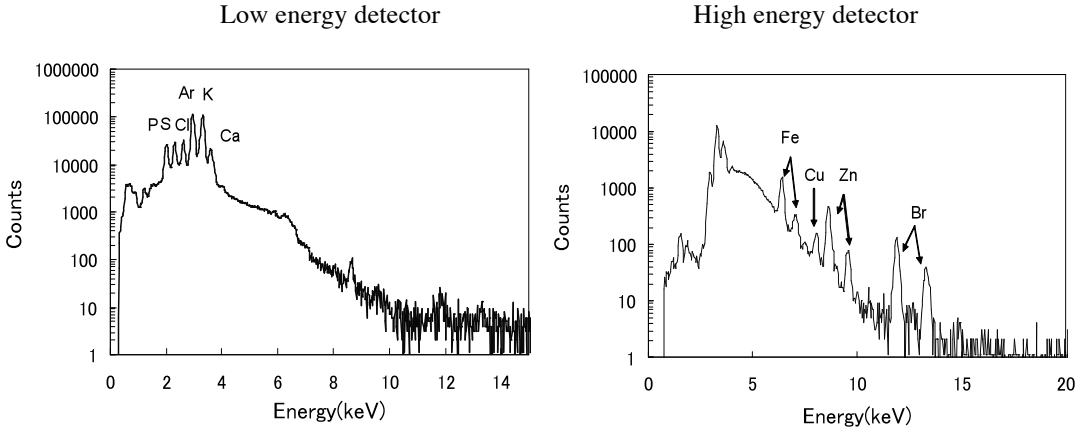

Figure 4. Typical X-ray spectra from a mouse brain slice by the detector for Lowand High energy. Total accumulated charge was 38 μC.