Spatial Distribution of Elements in the Brain of Rats Measured by Synchrotron Radiation X-Ray Fluorescence*

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Abstract Spatial (two-dimensional) distributions of inorganic elements such as chlorine, potassium, calcium, iron, copper, and zinc in the brain slices of Wistar rats were measured by synchrotron radiation X-ray fluorescence (SRXRF) analysis. Differences in two-dimensional distribution of these elements were observed. Chlorine, calcium, and zinc were primarily concentrated in hippocampus, while the potassium concentration was relatively higher in both cerebral cortex and hippocampus. However, the distribution of copper was comparatively ruleless in the three brain regions. The cluster analysis of the elemental results shows that the spatial distribution of chlorine was correlated well with the distribution of potassium. Since SRXRF is possible to obtain a pictorial representation of the elemental concentrations in tissue sections of brain, this nuclear method may be useful to evaluate the ionic changes in injured brain tissues in relation to histological observations.

Key words spatial distribution, trace elements, synchrotron radiation X-ray fluorescence

1 Introduction

Brain is extremely complex and it is a highly specialized organ in body. Specific regions of the brain have been shown to have unequal distribution of metabolic enzymes, neurotransmitter substances and trace elements. Any change of elemental distribution in brain can alter the activity of enzymes in brain, which can possibly influence the learning and memory abilities[1].

A number of studies have demonstrated significant changes in the ionic levels of brain in Alzheimer's disease, Parkinson's disease, and other brain injury models[2, 3]. Due to the increasing heavy metal pollution caused by anthropogenic activities, neurological studies of trace elements are of paramount significance, since it will not only shed knowledge on the appropriateness of the environment toward human and animals habitation, but also on possible causes for neurological disorders[4].

Many types of techniques have been used to assess the ionic levels in tissues. One method employs the ion-selective microelectrodes introduced in early 1970's[5]. However, this method only provides information about the extracellular or intracellular ionic activities, which may differ greatly from the total tissue ionic levels. X-ray fluorescence (XRF) analysis has the ability of multi-elemental analysis and positional distribution analysis. A previous study reported the two dimensional distributions of some

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important elements such as chlorine, potassium, phosphorus and sulphur in brain slices by XRF[5]. However, only a poor image of calcium distribution was obtained, since the X-ray intensity was so weak, the detection limits were poor. Another approach is to measure elemental concentrations in total tissues. Chemical analysis such as neutron activation analysis (NAA) and inductively-coupled plasma mass spectroscopy (ICP-MS) have been available to quantify major and trace element concentrations in biological samples[6, 7]. However, it is generally difficult to determine the spatial distribution or variation of elemental concentrations in samples such as tissue sections or slices.

Synchrotron radiation (SR) as one of X-ray sources has superior properties of high-intensity (10^3 to 10^6 stronger than the conventional X-ray sources), highly collimated and linearly polarized in the electron orbital plane. Its advantages greatly improve the analytical sensitivity and space resolution of XRF. An absolute detection limit of 10^{-12} to 10^{-15}g, and a relative detection limit at several μg/g, even as low as 10 ng/g, can be achieved with only micrograms of samples required[8]. Therefore, synchrotron radiation X-ray fluorescence analysis (SRXRF) is characterized of multi-elements, high sensitivity, microanalysis and positional analysis. It makes possible to measure the element distribution and to get the elemental maps in different regions of brain sections[9].

In the present work, synchrotron X-ray microprobe was used to measure the elemental distributions in brain slices of normal rats, which may help elucidate the mechanisms and effects of trace elements on brain functions.

2 Materials and methods

Female Wistar rats (80—100g body weight) were supplied by the Animal House of the Beijing Medical University. In the experiment, three rats were sacrificed rapidly and decapitated painlessly. The brains were quickly removed and frozen at −80℃. The 150μm coronal sections of the brains were cut with microtome and put onto polycarbonate films and then dried in air. Sections located at approximately −4.30mm from bregma were selected[10]. All the operations were done in a clean room.

The spatial distribution of elements in the cerebral cortex, hippocampus and thalamus areas of the slices was measured for 250—300 points with approximately 200μm × 200μm in each half brain area by SRXRF at Beijing Synchrotron Radiation Facility (Fig. 1). Since many points had to be measured for mapping the elemental concentrations, only the right hemisphere was studied. Each point was counted for 90 s. An aluminum absorber-foil was used to reduce the lower energy intensity of the white light. The spectra were analyzed by the AXIL program[11, 12]. The detection limit of X-ray peak area was $3(B)^{1/2}$ (B =the background area underlying the peak). The concentration was calculated by means of the normalization to the Compton scattering intensity, collecting time and counting time of the ion chamber respectively. We considered the counts after normalization as the comparative concentration of the element. In order to obtain detectable elements as many as possible, and to reduce the error of the calculation, the scattering from the materials around the detector should be avoided; the thickness of the film for supporting brain sections should be as thin as possible; and the thickness of brain sections should be thicker than the film.

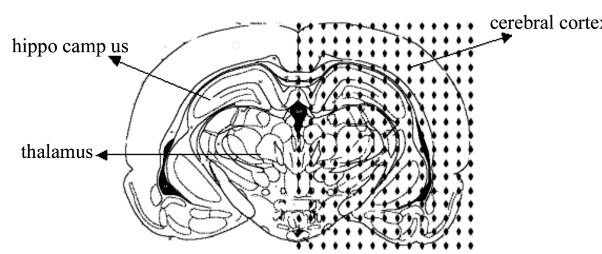

Fig. 1. The brain section scan area; Each point: 200μm×200μm. Step size: 500μm; measuring time: 90s.

All the experimental data were analyzed by the SPSS/PC statistical software. The elements as variables in rat brain including cerebral cortex, hippocampus and thalamus were classified using Centroid Method and measured the interval of Pearson correlation.

3 Results and discussions

The X-ray spectra of rat brain sections by SRXRF are shown in Fig. 2. The contour maps of the fluorescent X-ray intensities of chlorine, potassium, calcium, iron, copper, and zinc in the brain sections are shown in Fig. 3. In this figure, the ranges of elemental concentrations, i.e. X-ray intensities, are represented by different grey scale, black the highest; and white the lowest.

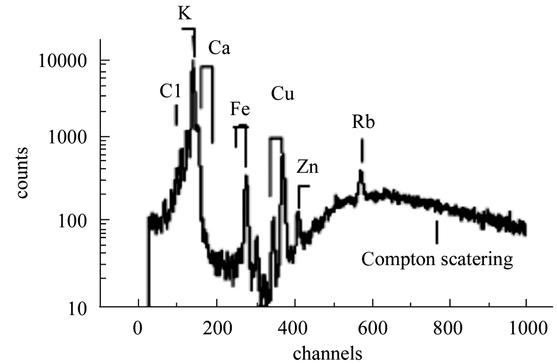

Fig. 2. X-ray spectra of normal rat brain collected by SRXRF.

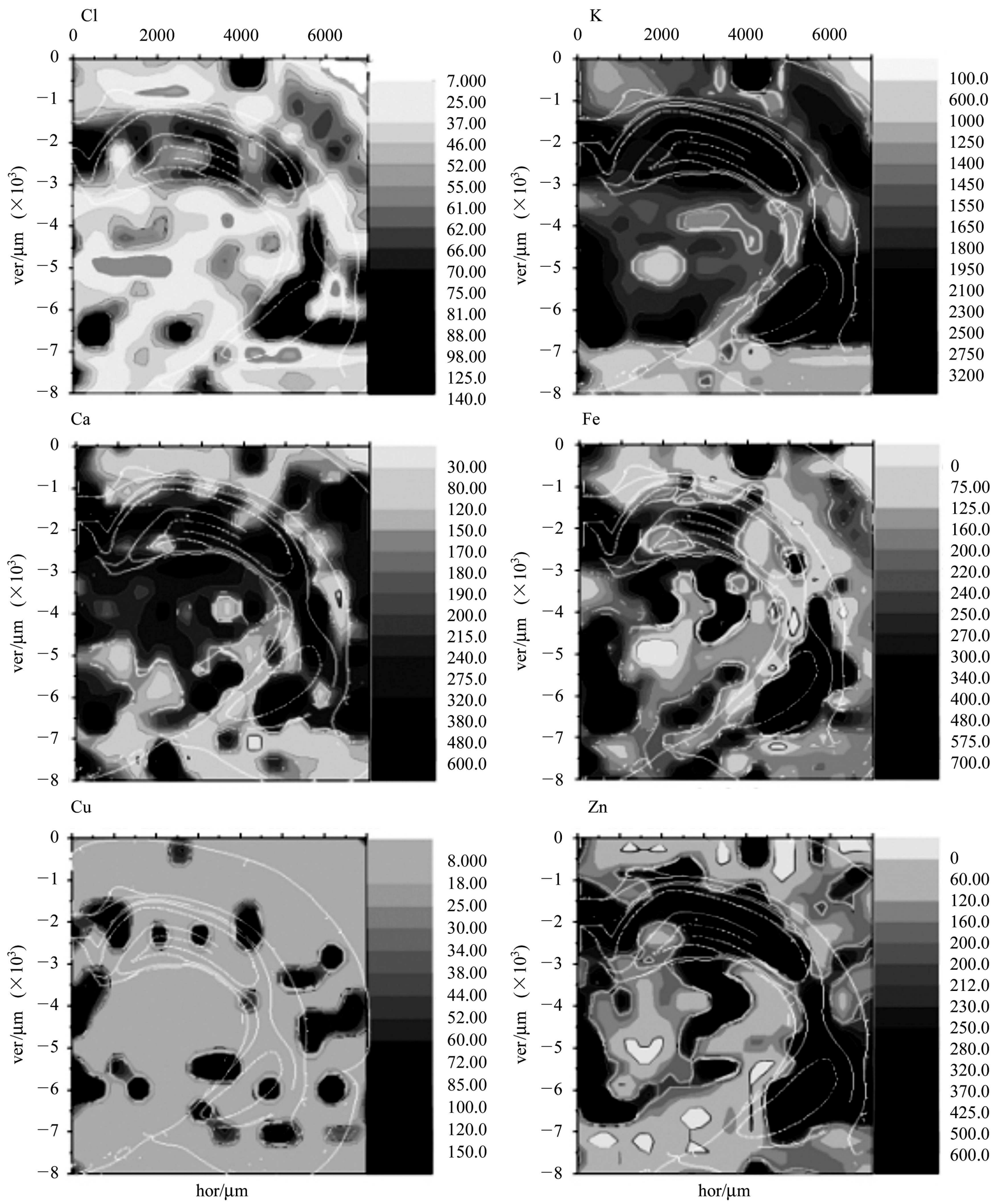

Fig. 3. Elemental maps of half brain section area.

It can be seen from Fig. 3 that all the elements were distributed heterogeneously in the brain section. Chlorine, calcium, and zinc were primarily concentrated in hippocampus, while potassium concentration was relatively higher in both cerebral cortex and hippocampus. Iron was mainly concentrated in some areas of hippocampus and thalamus. The distribution of copper was characterized by some high concentration dots in the brain section.

It has been known that all the six elements are necessary for the growth and function of the brain. Along with sodium, chlorine and potassium act as electrolytes and maintain the balance of pH and stabilize osmotic pressure. Potassium also plays a vital role in nerve function and cellular integrity by regulating the transfer of nutrients into the cell[13]. Calcium is involved in numerous signaling processes, like the control of presynaptic neurotransimitter release and regulation of membrane excitability, and also serves directly as the second and the third messengers[13]. Copper is involved in brain neurotransmitters (dopamine hydroxylase and peptidyl alpha amidating monoxygenase)[14]. Among the six elements, only two elements, iron and zinc, the regional distributions and physiological functions in brain have been well documented.

Iron is highly localized in dopaminergic-peptidergic regions, such as the globus pallidus, substantia nigra, red nucleus, thalamus, caudate nucleus and nucleus accumbens. Nonheme-Fe concentrations in brain and the number of dopamine D2 receptors are decreased by Fe deficiency in experimental animals, and learning is decreased[15]. Numerous studies have shown that zinc is one of the most prevalent trace elements found in the brain, where it is primarily retained in the hippocampus. Much of the zinc in the hippocampal area is associated with the axons of the mossy fibres projecting from the granule cells in the fascia dentata to the apical dendrites of the pyramidal cells. By virtue of its limbic connections, the hippocampus is able to influence many brain activities, thus zinc depletion in mossy fibres may contribute to a wide variety of cerebral physiopathologies[14]. The results of the present study may be useful to elucidate the mechanisms and effects of these elements on brain functions. Moreover, changes in the concentrations of these elements under normal and pathological conditions can provide useful information on physiology and pathology of the brain.

Correlations between one element and another are investigated by hierarchial cluster analysis. Fig. 4 is the dendrogram of the measured six elements as variables in the rat brains.

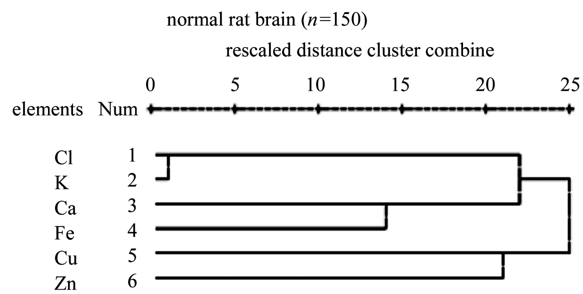

Fig. 4. Dendrogram of the measured elements as variables in the brain of normal rats (*n*–total number of measured points) by hierarchial cluster analysis with centroid clustering.

It can be seen that chlorine and potassium are grouped together. The close correlation between K and Cl indicates their close relationship in some brain functions.

4 Conclusions

In this study, two-dimensional contour maps of six inorganic elements in the brain sections of normal rats were obtained by SRXRF. All the elements were heterogeneously distributed in different brain regions. And the positional distribution of chlorine correlates well with that of potassium. These elements are important building blocks of cell and play important roles in cell and tissue physiology. SRXRF may determine the changes in the concentrations of these elements under normal and pathological conditions, which provides useful information on physiology and pathology of the brain.

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同步辐射X荧光测量大鼠脑中微量元素的分布*

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摘要 采用同步辐射微探针技术研究了正常大鼠脑中的微量元素的分布, 并给出了二维空间分布图. 结果显示: Cl, Ca, Zn主要集中分布在海马区, K主要分布在海马和皮层, 而Cu的分布则相对无规律. 元素的聚类分析结果表明, Cl和K的分布相关性较好. 由于同步辐射X荧光技术可以更直观地给出脑组织的二维元素分布, 因此这种方法可用于研究发生病变的脑中微量元素的变化.

关键词 空间分布 微量元素 同步辐射X射线荧光

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