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TRACE ELEMENT DISTRIBUTION IN THE RAT CEREBELLUM¹

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ABSTRACT

Spatial distributions and concentrations of trace elements (TE) in the brain are important because TE perform catalytic and structural functions in enzymes which regulate brain function and development. We have investigated the distributions of TE in rat cerebellum. Structures were sectioned and analyzed by the Synchrotron Radiation Induced X-ray Emission (SRIXE) method using the NSLS X-26 white-light microprobe facility. Advantages important for TE analysis of biological specimens with X-ray microscopy include short time of measurement, high brightness and flux, good spatial resolution, multielemental detection, good sensitivity, and non-destructive irradiation.

Trace elements were measured in thin rat brain sections of 20-micrometers thickness. The analyses were performed on sample volumes as small as 0.2 nl with Minimum Detectable Limits (MDL) of 50 ppb wet weight for Fe, 100 ppb wet weight for Cu, and Zn, and 1 ppm wet weight for Pb. The distribution of TE in the molecular cell layer, granule cell layer and fiber tract of rat cerebella was investigated. Both point analyses and two-dimensional semi-quantitative mapping of the TE distribution in a section were used. All analyzed elements were observed in each structure of cerebellum except mercury which was not observed in granule cell layer or fiber tract. This approach permits an exacting correlation of the TE distribution in complex structure with the diet, toxic elements, and functional status of the animal.

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SAMPLE PREPARATION

Two groups with a total number of 8 rats were put on two different diets. The rats from first group were put on American Institute of Nutrition (AIN) diet [3] marked as AIN-76TM and called here "AIN Diet". This diet is intended for rats growth and maintenance during the first year of life. The rats from the other group were put on Ralston Purina Company diet [4] called here "Purina Diet". This diet is designated especially for rats, hamsters and most mouse strains. The Purina Diet is a high-energy and high-protein diet. Table 1 compares the elemental composition of those two diets.

Those rats were fed for 28 days and then sacrificed. For this experiment the selected tissues were removed quickly from the animal and placed on dental wax. Then, with a clean sharp scalpel the tissues were trimmed to provide access to the structures and orientation required by the experiment. Usually samples were cut down to <5 mm in at least one dimension. The trimmed tissues were placed in a cryomold, containing a drop of Tissue Tek. The surfaces to be cut into sections were faced down. The whole sections were then covered with Tissue Tek, and the mold frozen in a dry ice - methanol slurry. The sample prepared that way was ready for cutting into 20 μ m sections when completely frozen.

The sections were placed onto 7.3 μ m Kapton support film mounted in a cardboard 5 cm x 5 cm slide mount. The adjacent sections were taken and used for histochemical purposes and for the identification of the structures and regions analyzed.

EXPERIMENTAL ARRANGEMENT

A white photon beam from the National Synchrotron Light Source of effective energy range upto 30 keV was used in this study. The experiment was performed at X-26C beam line. The beam is delivered through a vacuum beam pipe and the size of the beam is defined by four tantalum slits of 160 μ m thick.

The apertured beam passes through a helium filled Ion Chamber which provides a normalization of the X-ray spectra to the number

of incident photons. The characteristic X-rays produced were detected with a Si(Li) detector with an energy resolution of 15 eV for an ^{55}Fe source for X-ray of the 5.9 keV. In order to minimize the background in the spectra the detector was placed at 90° to the beam in the plane of the electron orbit. Samples were mounted at 45° to the beam on a stepping driven X, Y, Z, Θ stage. During the measurement sample was observed through a TV camera mounted on the microscope. Fig. 2 shows a diagram of an X-26C apparatus.

All samples were irradiated in the same experimental conditions for 300 s life time. After selecting experimental conditions [5] the filters were established to be 100 μm thick Aluminum foil for a beam and 150 μm thick Kapton foil for the detector. The beam size was 100 μm x 100 μm for both the point analysis and the two-dimensional semi-quantitative mapping. Fig. 3 shows a typical spectrum of a rat cerebellum section.

RESULTS

The concentrations of a total number of 11 elements (Ca, Mn, Fe, Cu, Zn, Se, Br, Rb, Sr, Pb, and Hg) were determined. Those elements were studied in three structures of the sample: Molecular Cell Layer (MCL), Inner Granule Cell Layer (IGL), and Fiber Tract (FT).

In order to obtain an average value of the element concentration each structure was measured in three different points. Fig. 4 shows the graphs of the average values of TE concentrations in three different structures for two diets and the average concentration in the whole tissues an appropriate label for a diet. Table 2 shows the average values of the TE concentrations for the whole animal section.

The map of iron distribution in one of the samples is shown in fig. 5 as an example of a two-dimensional semiquantitative mapping. The composed figure also shows a photograph of an analyzed section. The lighter pixels correspond to higher iron concentration.

DISCUSSION AND CONCLUSIONS

From tables 1 and 2 one can easily notice that increased TE concentrations of Ca, Mn, Cu, and Zn, for Purina diet correspond to increased concentrations of those elements in the diet itself. Such a correlation seems to be normal and was expected.

Although the Fe concentration in the Purina diet is higher than in that the AIN diet there is no significant difference between the average concentrations of this element in the samples.

The case of Se proves different. Increased Se concentration in the diet resulted in decreased concentration of it in the sample. This was not expected and needs further study. There must be some biological interactions between elements responsible for that phenomena.

As for the other elements analyzed but not present in those diets, one can see that the Pb concentration in the samples for the Purina diet is higher than that for the AIN diet, while for Hg the relation is opposite.

Br concentration for the AIN diet is lower than that for the Purina diet. There is no significant difference between Sr and Rb concentrations for those two diets.

A more detailed study may explain the origin of significant difference for mean values. Table 3 shows the result of the Student t-test on mean values for the structures and whole sections. The comparison was made between the same structure types. The difference between the mean values were regarded significant with $\alpha = 0.05$.

An interesting case is the strontium structural distribution for two diets. Although there is no significant difference between the mean values for the whole section there are significant differences for the structures. The Sr concentration in FT-structure for the AIN diet is higher than in FT- for the Purina diet while Sr concentration in MGL-structure for the AIN diet is lower than in MGL- for the Purina diet. There is no significant difference between Sr concentration in IGL structure for both diets. Therefore, the mean values for the sections are not different. This case seems very interesting and needs further study.

The graphs in fig. 4 show the distribution of the TE in each structure. It is obvious that the diet has significant influence to TE distribution in the cerebellum. The calculated correlations for each structure for both diets confirm that significance.

The potassium analysis would be important due to biological significance of this element but it was not possible to perform any quantitative or qualitative analysis of potassium because of the experimental setup. All the samples were kept in the air during the measurements and that caused an appearance of an Ar peak in the spectrum which invalidated the potassium analysis.

The correlation coefficients were tested with a test of significance assuming a significant level $\alpha = 0.1$. Comparing the structures between themselves one can observe some interesting dependences. For diet A the following correlations have been found. A strong positive correlation is observed between Ca and Zn for FT and IGL while for MCL that correlation is rather negative. For all three structures a positive correlation is observed between Fe and Br. The correlation between Cu and Zn is positive only for IGL but for FT is rather negative. The same relation is observed for: Ca and Cu, Fe and Zn, and Ca and Fe.

For diet P the other elements are significantly correlated than those for diet A. For all three structures a positive correlation is observed between: Cu and Zn, Br and Ca, and Rb and Se. Also positively correlates Pb and Cu. A negative correlation is observed between Zn and Mn for FT while for IGL it is positive. Zn and Ca show a positive correlation for IGL and MCL but for FT they show rather negative correlation. Also the negative correlation is observed between Zn and Fe for FT and MCL. For IGL there is a positive correlation between Zn and Mn. Tab. 4 summarizes the results of the test.

From the above observations one can conclude that the concentration level of TE in a structure depends on the structure itself that means it depends on the function of a structure. Comparing tables 1 and 3 it is obvious that diet and the concentration level of TE in the diet have significant influences on the TE distribution in the brain. It has also been found that the increased concentration of some element in the diet does not have to result of its increasing in analyzed tissue. Such a fact

was observed in case of Se.

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FIGURE CAPTIONS

- Fig. 1. Illustration of a rat cerebellum section.
- Fig. 2. Diagram of an X-26C apparatus.
- Fig. 3. Typical SRIXE spectrum of a rat cerebellum section.
- Fig. 4. Graphs of the average values of TE concentration in three different structures for two diets and the average concentration in the whole tissue.
- Fig. 5. Composed picture of a rat cerebellum section photograph and semi-quantitative two-dimensional mapping.

Schematic Diagram of X-26 XRF Beam Line

1 mm

Table 1. Comparison of elemental composition of AIN-76TM and Purina diets

Ingredient	AIN-76 TM	Purina
Ca	0.52 %	1.0 %
P	0.40 %	0.65 %
K	0.36 %	1.1 %
Mg	0.05 %	0.2 %
Na	0.10 %	0.28 %
Cl	0.156 %	0.48 %
F	---	30.0 ppm
Fe	35.0 ppm	200.0 ppm
Zn	30.0 ppm	73.3 ppm
Mn	54.0 ppm	70.7 ppm
Cu	6.0 ppm	16.0 ppm
Co	---	0.4 ppm
I	0.2 ppm	0.8 ppm
Cr	2.0 ppm	1.4 ppm
Se	0.1 ppm	0.2 ppm
Sulfate	0.1 %	---

Table 2. Average TE concentrations for the whole animal section for two diets. (values are in ppm)

	AIN	Purina		AIN	Purina
Ca	35.5 ±3.3	46.8 ±15.5	Se	0.42 ±0.11	0.20 ±0.07
Mn	0.3 ±0.1	0.7 ±0.3	Br	0.13 ±0.04	0.32 ±0.12
Fe	16.3 ±1.2	16.2 ±1.3	Rb	0.19 ±0.06	0.15 ±0.08
Cu	1.8 ±0.1	2.0 ±0.3	Sr	0.83 ±0.14	0.82 ±0.14
Zn	9.4 ±0.8	12.3 ±1.8	Pb	2.0 ±0.3	2.5 ±0.4
			Hg	1.0 ±0.2	0.6 ±0.2

Table 3. Students t-test results with a significance level $\alpha = 0.05$.

Element	A,P - FT	A,P - IGL	A,P - MCL	A, P - All
Ca	NSD	A SL P	A SG P	A SL P
Mn	A SL P	A SL P	NSD	A SL P
Fe	NSD	NSD	A SL P	NSD
Cu	A SG P	NSD	A SL P	A SL P
Zn	A SL P	A SL P	A SL P	A SL P
Hg	A SG P	A SG P	NSD	A SG P
Se	NSD	A SG P	A SG P	A SG P
Br	A SL P	NSD	A SL P	A SL P
Rb	NSD	NSD	A SG P	A SG P
Sr	A SG P	NSD	A SL P	NSD
Pb	A SL P	NSD	NSD	A SL P

where: A - mean for the AIN diet.

P - mean for the Purina diet.

SL - significantly less (than).

SG - significantly greather (than).

NSD - no significant difference.

Table 4. List of significantly correlated elements.

Correlation type	FT		IGL		MCL	
	A	P	A	P	A	P
positive	Ca & Zn	Ca & Br	Ca & Zn	Ca & Zn	Ca & Mn	Ca & Cu
	Fe & Br	Cu & Rb	Ca & Cu	Ca & Cu	Ca & Fe	Cu & Pb
		Se & Sr	Fe & Zn	Ca & Br	Mn & Hg	Se & Rb
			Cu & Zn	Cu & Zn		Br & Rb
				Cu & Br		
				Cu & Pb		
				Zn & Br		
				Se & Rb		
				Mn & Zn		
				Mn & Br		
negative	Cu & Mn	Cu & Mn				
	Cu & Hg	Mn & Rb				
		Se & Pb				