ESRF	**Experiment title:** Metals at the postsynaptic density: implications for neural plasticity and toxicity	**Experiment number**: LS-2366
Beamline: ID16A-NI	**Date of experiment**: from: 19/11/2014 to: 25/11/2014	**Date of report**: March 3rd 2015
Shifts: 18	**Local contact(s)**: Yang Yang and Sylvain Bohic	*Received at ESRF:*

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Aims

Our aim was to answer to the following questions: 1) is Zn, a known modulator of neural plasticity, located at the PSD (Post-Synaptic Density)? That would confirm the expected Zn function on protein architecture at the PSD; 2) are Cu and Fe, two metals suspected to be involved in neural plasticity, located at the PSD? That would indicate for the first time their functional role at the PSD; 3) are Pb and Co, two known neurotoxic elements, located at the PSD? That would reveal a new site for metal neurotoxicity; 4) is Zn quantitative distribution at the PSD altered by Pb and Co? That would indicate a new mechanism of neurotoxicity.

Sample Preparation

Rat primary hippocampal neurons were prepared in collaboration with the Interdisciplinary Institute of Neurosciences, Bordeaux university, as adapted from the Banker's method (Kaech & Banker, 2006) and from our previous work on neuroblastic cells to perform nano-SXRF (Synchrotron X-ray Fluorescence). Many critical steps were succesfully addressed such as: 1) the long-term (2 to 3 weeks) co-culturing of neurons onto 100 nm silicon nitride membranes and of glial cells (onto the culture disches) to obtain mature neurons with functional PSD and with a correct cell density; 2) since the 100 nm Si_3N_4 membranes are very fragile they were mounted onto intermediate holders to be manipulated more easily; 3) neurons are very sensitive to osmotic pressure changes of the culture medium, we compared different soulutions of rinsing (to remove the extracellular cellular elements including the usual buffer salts); the best results indicated by the highest K/Na intracellular ratios were obtained with a pH 7.2 solution of ammonium acetate at 230 mOsm/L. This complex protocol was validated before the LS-2366 experiment using micro-PIXE analysis in our institute. A methodological paper with full details will be submitted soon (Perrin et al., Journal of Analytical Atomic Spectrometry). To identify the PSD, cells were electroporated with PSD95-GFP 'intrabodies' (Gross et al., 2013), a state-of-the-art technology for vizualizing endogenous synaptic proteins in living neurons. Cells were exposed to metals (10 µM Fe, Cu, Zn, Co and Pb), observed by epifluorescence microscopy for PSD95-GFP imaging, and cryogenically processed using Leica CPC plunge freezing and Quorum K775X freeze drying, the ultimate technology for preservation of trace element distribution in freeze dried cells (Roudeau et al., 2014). Samples must be freeze-dried to be analyzed at room temperature under vacuum.

Experimental analytical conditions

The beam size obtained at ID16A-NI beamline was 21 nm x 37 nm at 17.0 keV. Nano-SXRF was carried out at room temperature, under vacuum, using a six elements silicon drift diode detector. SXRF data treatment was performed using Pymca software to provide fitted element distribution images. Quantitative results of element concentraions can be extrapolated from the analysis of thin film XRF reference sample from AXO.

Results

Cell exposed to 10 µM Fe, Cu, Zn, Co or Pb and controls could be analyzed. No cobalt was detected in cells expowed to $CoCl_2$. Results from cells exposed to Fe and Cu were too complex to be interpretated from this first single experiment.The following three conditions were investigated in detail: controls (representative examples shown in Fig. 1), Zn 10 µM (similar results to controls were obtained, with higher Zn signals within the PSD), and Pb 10 µM (representative example shown in Fig. 2).

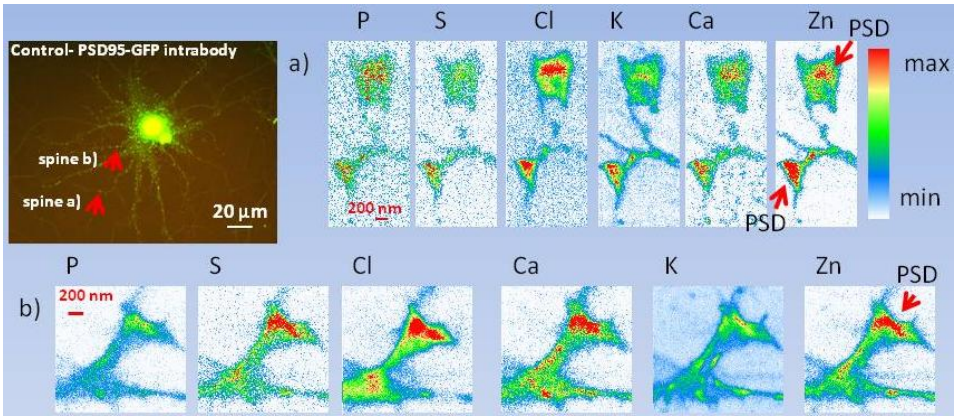

Fig. 1. Example of nano-SXRF element distributions within a control post-synaptic spine. *Cell were labeled with PSD95-GFP intrabody to identify functionnal PSD (spines a and b). The PSD contains very high levels of Zn even in control cells. The size of the PSD is about 500 nm x 200 nm in these examples (red arrows).*

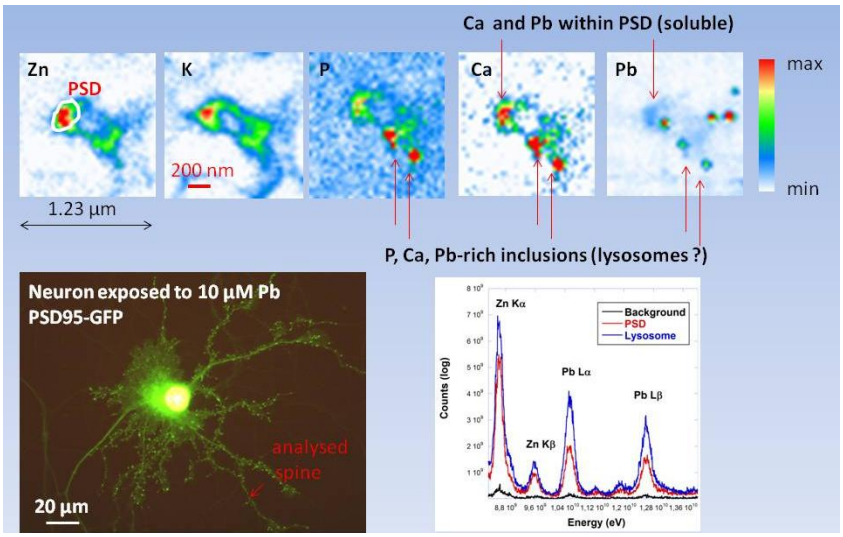

Fig. 2. Example of nano-SXRF element distribution within a post-synaptic spine after exposure to Pb*. PSD contains Zn, K, P, Ca and traces of Pb. Lead also accumulates within structures, possibly lysososomes as suggested by the P and Ca rich content. XRF fluorescence spectra from selected regions of the image were extracted to confirm spectroscopically the distributions: oustisde the spine no Pb is detected (background, black spectrum), within the PSD Pb is present (red spectrum), and also within structures hypothesized as lysosomes (blue spectrum).*

Conclusions

1) Zn is located at the PSD confirming the expected Zn function on protein architecture at the PSD;
2) are Cu and Fe, two metals suspected to be involved in neural plasticity, located at the PSD? We could not fully answer to this question during the allocated beamtime, a new proposal will be submitted ;
3) are Pb and Co, two known neurotoxic elements, located at the PSD? Cobalt was not detected at the PSD. Pb was detected at the PSD revealing a new site for this metal neurotoxicity. Moreover Pb accumulation in Ca and P-rich structures suggest a lysosomal distribution. Precipitation of Pb and Ca through lysosomial acid phosphatase coud be a way to detoxify soluble lead. Pb precipitates are usually detectable by electron density contrast using TEM. New samples have been prepared for transmission electron microscopy to investigate such Pb accumulation in lysosomes, or in other structures to be determined.
4) is Zn quantitative distribution at the PSD altered by Pb and Co? That would indicate a new mechanism of neurotoxicity. The quantitative analysis of the data is currently under way. Results on Zn and Pb synaptic distributions will be published as soon as the quantitative data and the TEM data will be avalaible.

References

Gross G.G. et al., (2013) Recombinant probes for visualizing endogenous synaptic proteins in living neurons, Neuron 78, 971–985.

Kaech S. & Banker G. (2006) Culturing hippocampal neurons, Nature Protocols, 1, 2406-2415.

Roudeau S., Carmona A., Perrin L., Ortega R. (2014) Correlative organelle fluorescence microscopy and synchrotron X-ray chemical element imaging in single cells. Analytical and Bioanalytical Chemistry, 406, 6979–6991.