ESRF	**Experiment title:** Sub-cellular imaging and speciation of trace elements in single neuronal cells using a new cryostage	**Experiment number**: MD556
Beamline: ID22	**Date of experiment**: from: 23/02/2011 to: 01/03/2011	**Date of report**:
Shifts: 18	**Local contact(s)**: Sylvain BOHIC	*Received at ESRF:*

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Objectives (as in proposal):

In dopamine depleted cells, we expect to observe a re-distribution of iron from storage compartments to protein aggregates into the cytoplasm, and/or diffusion into the nucleus due to altered vesicular storage as suggested by Lashuel & Hirling (2006). Such observation would support the hypothesis of dopamine-Fe interaction in the etiology of Parkinson's disease suggested since several years (Smyties et al., 1999) but never evidenced experimentally.

Experimental conditions

Four cell exposure conditions have been compared: control cells, cells exposed to excess iron (100 µM), cells depleted in dopamine using 6-hydroxydopamine (6-OHDA 100 µM) with or without excess of iron (100 µM). SXRF chemical nano-imaging has been performed on ID22 beamline at 17 keV in order to image Fe but also important trace elements such as K, Zn and P (Figure 1). About 10 cells were analysed for each experimental conditions.

Results

Element imaging. High spatial resolution chemical maps were obtained showing a specific distribution of Fe within 'hot spots' in the cytosol, neurite outgrowths and distal ends in all tested conditions (Fig. 1). Fe is also present in the nucleus but in a more diffuse distribution. From images of element distributions, there were no differences in the number of hot spots from contriols and 6-OHDA exposed cells, and from Fe exposed cells and Fe + 6-OHDA exposed cells. Fe hot spots correspond both to ferritin localization and dopamine vesicles as shown by dopamine immunofluorescence imaging.

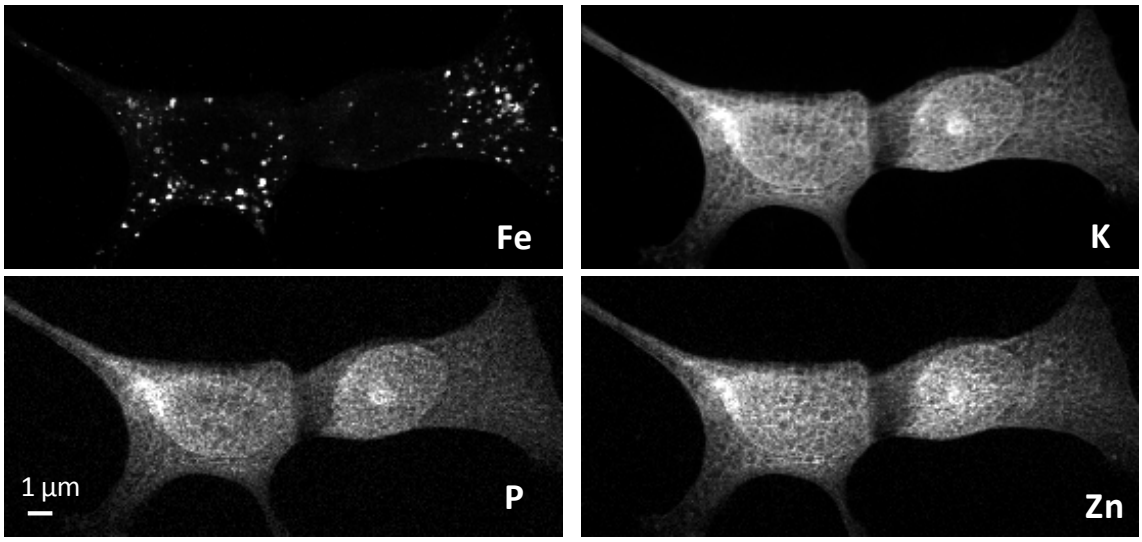

***Figure 1**. Fe, K, P and Zn distribution in PC12 cells exposed to 100 µM Fe and 100 µM 6-OHDA.*

Quantitative analysis. We used a micromatter set of standards to determine the Fe content (in ng/cm^2) in the cytosol and nucleus of PC12 cells. When control cells are compared to 6-OHDA dopamine depleted cells, Fe content is not changed either in the cytosol or the nucleus (Figure 2A). When cells are exposed to Fe (both conditions, Fe alone or with 6-OHDA) there is an increase in total Fe content with a modearte increase in the nucleus. Fe content is higher in 6-OHDA + Fe exposed cells than in cells exposed to Fe alone (Figure 2B). Fe increases in the cytosol without modification of Fe content in the nucleus in 6-OHDA exposed cells.

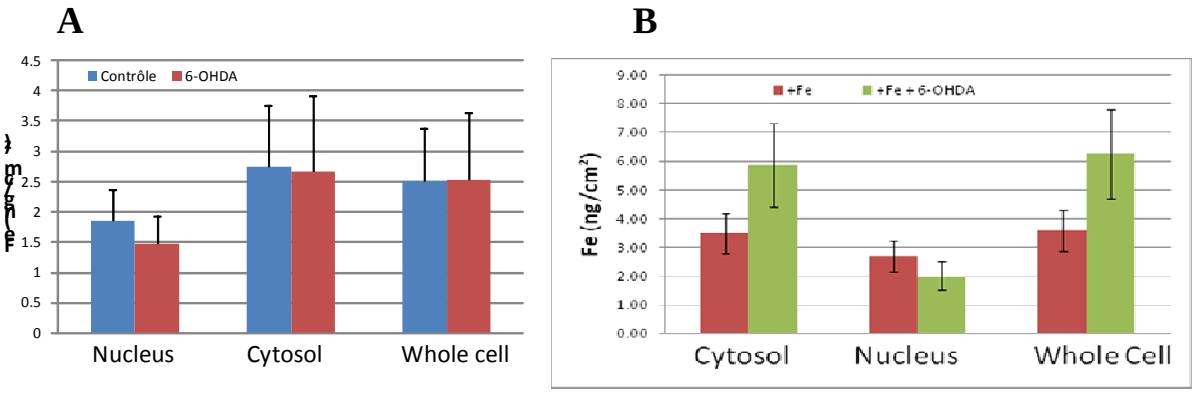

***Figure 2.** Fe content (ng/cm^2) in subcellular compartments of PC12 cells (nucleus, cytoplasm) for the 4 analysed conditions : (A) controls and 100 µM 6-OHDA; (B) 100 µM Fe and 100 µM Fe + 100 µM 6-OHDA).*

Conclusions

These results indicate that 6-OHDA, a neurotoxic molecule known to trigger Parkinson's disase symptoms in animal models, increases intracellular Fe content in PC12 cells in case of Fe overload (+ 100 µM Fe) compared to Fe exposure alone. This Fe increase is mainly located in the cytosol, not in the nucleus, suggesting that Fe content in the nucleus is strictly regulated in order to prevent oxidative damage in this cell compartment.