ESRF	**Experiment title: Nanoscopic chemical imaging of neurotransmitters and neurotoxics at the synapse**	**Experiment number**: MD178
Beamline: ID22	**Date of experiment**: from: 22 February 2006 to: 27 February 2006	**Date of report**: 01/09/2006
Shifts: 15	**Local contact(s)**: Dr. Sylvain BOHIC	*Received at ESRF:*

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Abstract. *A role for iron-dopamine complexes in neural physio-pathology has been suggested. We investigated the relationship of dopamine and iron in dopamine producing neurons using a setup for X-ray fluorescence nanochemical imaging with 90 nm spatial resolution. Iron accumulates into 100-200 nm catecholamine vesicles and the inhibition of dopamine synthesis results in a decrease of these iron-rich structures. Dopamine is therefore involved in iron vesicular storage indicating a physiological role of iron-dopamine complexes, and suggesting that the altered distribution of such highly reactive oxidant compound may contribute to the aetiology of neurological disorders such as Parkinson's disease.*

There is increasing evidence that abnormal iron handling in the brain may be involved in neurodegenerative disorders such as Parkinson's disease (PD). It has been suggested that catecholamines, such as dopamine, can form stable complexes with iron and this system may be faulty in PD. However this hypothesis remained to be confirmed experimentally. Very little is known about the role of iron in PD at the subcelullar level because of the lack of analytical techniques with sufficient detection sensitivity and spatial resolution. Here we determined the subcellular distribution of iron in dopaminergic cells using synchrotron nano-chemical imaging. An original setup for high spatial resolution X-ray fluorescence analysis was developed at ESRF with a 88 nm X-ray beam of very high flux (up to 10^{12} photons/s). The characteristics of this unique nanoprobe fulfill the requirements for mapping biological trace elements in the µg/g range, at a sub-micrometer scale, size of cellular ultrastructures such as the nucleus, cytosol, or dopamine vesicles in neurite outgrowths and distal ends (Fig. 1).

PC12 (rat pheochromocytoma) dopaminergic neurons were exposed *in vitro* to sub-cytotoxic concentrations of iron and/or methyltyrosine (AMT), an inhibitor of tyrosine hydroxylase (TH) and catecholamine synthesis. The iron profile distribution, as retrieved for example from the examples in figure 1, shows that iron is localized nearly exclusively in structures of 100-200 nm in size in the cytosol, neurite outgrowths, and distal ends (Fig. 1). When control cells are compared to cells exposed to an excess of iron, the subcellular distribution of iron is similar but with a smaller number of iron-rich structures in control cells. These structures are identified by epifluorescence microscopy as catecholamines granules and vesicles in PC12 cells. Another very important result of this study is that inhibition of TH results in a decrease of the iron content in PC12 cells (Fig. 2). It is interesting to note that AMT had no effect on the cellular concentration of zinc (Fig. 2A), indicating that AMT affects specifically the distribution of iron.

Our results indicate that functional dopaminergic cells are able to modulate iron cellular concentrations, and presumably protect themselves and neighbour cells from iron cytotoxicity, through iron-catecholamine storage into appropriate vesicles. It also suggests that the clinically observed elevation of iron concentration in the brain of PD patients, and the concomitant loss of TH hydroxylase activity, may lead to a lack of iron-catecholamine binding capability in PD rendering the dopaminergic cells more prone to iron toxicity.

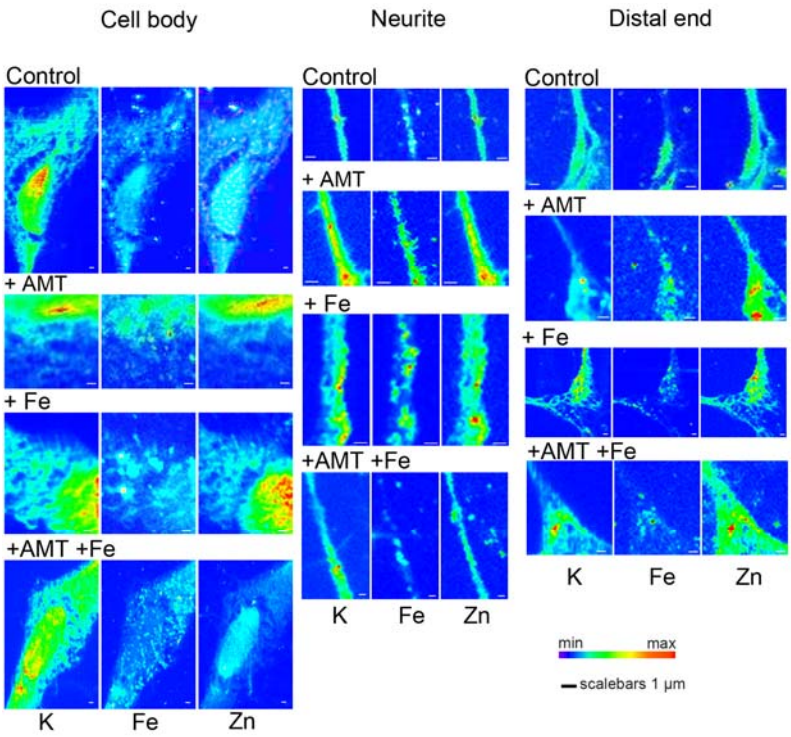

Figure 1. **Synchrotron hard x-ray fluorescence maps of potassium, iron, and zinc in single dopamine neurons**. Each series of images of cell bodies, neurite outgrowths, and distal ends, are representative of the entire cell population for each condition (control, 1mM AMT during 72 h and/or 300 µM $FeSO_4$ during 24 h). Iron is located within 100-200 nm structures identified as catecholamine granules by epifluorescence microscopy. In cell bodies of cells exposed to AMT alone, only a basal level of diffused iron is observed and almost no iron-rich structures. This observation confirms the role of dopamine in iron homeostasis. Min-max range bar unit is arbitrary. Scale bars = 1 µm.

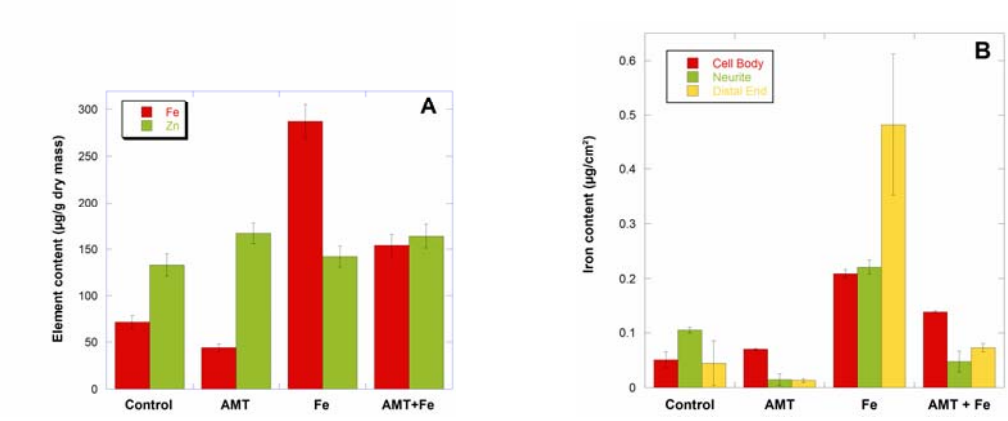

Figure 2. Iron concentration in dopamine cells is decreased after inhibition of catecholamine synthesis. (A) Total iron and zinc content obtained through quantitative analysis of a few hundred cells. (B) Iron content in sub-cellular compartments: cell body, neurite, and distal end. AMT exposure resulted in a decrease of total iron concentration in all studied cellular compartments, particularly in neurite outgrowths and distal ends suggesting a direct role of catecholamines in iron homeostasis.