ESRF	**Experiment title:** **Manganese and iron in dopaminergic neurons in Parkinson's disease: distribution and oxidative state**	**Experiment number**: MD 501
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Report:

Transition metals with high redox potential have been suggested to play a pivotal role in the pathogenesis of Parkinson's disease (PD) and other neurodegenerative disorders. We used an advanced high resolution analytical study based on the recent progress in synchrotron based X-ray imaging at ID21 (ESRF) to map the elemental distribution in primary dopaminergic neurons (PND) under cryo-condition, by using X-ray fluorescence (XRF) spectroscopy, including information on the redox state by spectro-microscopy. We report elemental mappings on the sub-cellular level in PDN- mouse neurons, after treatment with $Fe^{2+/3+}$ and $Mn^{2+/3+}$. The special interest was given to distribution and oxidative state of Fe, Mn, in respect to P, S, Cl, Na, K. In addition, we show correlative microscopy of X-ray fluorescence and visible light fluorescence.

Parkinson's disease (PD) is the second most frequent neurodegenerative disease with symptoms such as rigidity, tremor and bradykinesia [1, 2]. The current understanding of the pathophysiology of PD is polyetiologic, involving oxidative stress, neuromelanin, mitochondrial dysfunction, calcium-binding protein deficiency and nitric oxide in the development of the disorder. Trace elements like Mn, Fe Cu and Zn, essential for metalloenzymes throughout the brain and central nervous system, by chronic exposure can cause a neurodegenerative disease [3]. On the other hand, metals accumulate in the brain as a function of aging, and misregulation of brain metal ion pools is linked with PD, where oxidative stress and damage as well as protein misfolding and aggregation are a characteristic of their pathology [3]. Redox active metal ions like Fe, Mn and Cu can produce reactive oxidative species by means of the Fenton's or similar reactions. The valency of metal ions has a key role in their toxicity; for example the presence of Mn^{3+} even in trace amounts, may extensively modify the capacity of manganese or iron to generate free radicals [4]. If Mn^{3+}, the first oxidation product of Mn^{2+}, is not stabilized by complexation, similar to the more reactive Fe^{2+}, it can act as a powerful oxidant of numerous organic molecules, leading to toxic effects.

Fe and Mn ions distribution in native primary dopaminergic neurons- the cell culture

Primary midbrain neurons, containing up to 10% dopaminergic neurons, from transgenic mice were expressing EGFP under the DAT promoter, thus was allowing us fluorescent identification of labelled dopaminergic neurons. The cell culture was prepared in laboratory of Institute for Neurology in Göttingen. Briefly, isolated PND were grown on silicium-nitrite membrane coated with polyornithine and cultured for four days. The treatment with metals were performed on day 4 *in vitro* for 3 hours. Using an upright light fluorescence microscope, living dopaminergic neurons in culture were identified using an EGFP filter (Fig.1A). Directly after neuron cultures were cryo-preserved in liquid ethane, and kept under liquid nitrogen up to measurements at ID21. The distribution of ions was studied in previously identified dopaminergic

neurons as well as in non-labelled (non-dopaminergic) neurons equally present in the culture (Fig.1 A, B and C). The summarized spectra of PND is shown in Figure 1D.

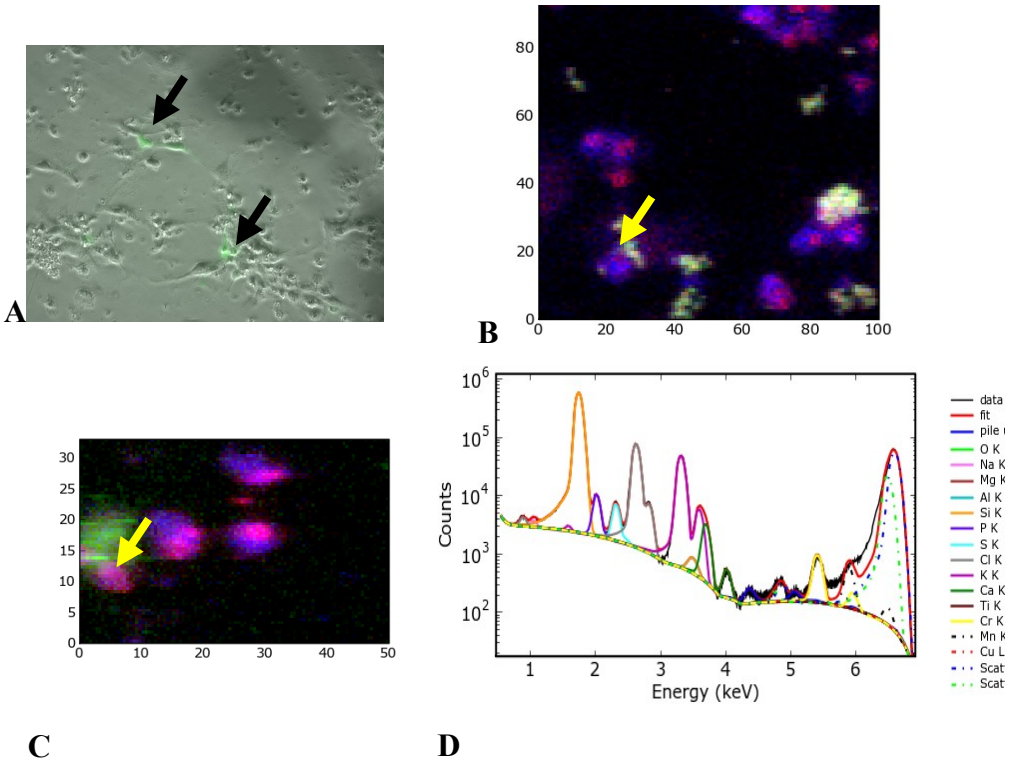
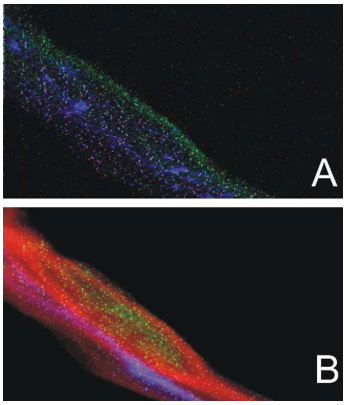


Figure 1: *Light fluorescence of primary dopaminergic neurons (PDN) marked with black arrows (A). B- XRF maps of cryo-fixed PDN at 7.2 keV- the overlapping map indicates Fe- green, P-red and S-blue after 50 µM Fe^{2+} treatment- the image 100x100 µm, pixel size 1µm, with dwell time 150 ms. The yellow arrows indicate the dopaminergic neurons, after identification in the light fluorescence microscopy. C- XRF maps of cryo-fixed PDN at 6.6 keV. Overlie map indicates Mn- green, P-red and S-blue after 50 µM Mn^{2+} treatment. The image 50X32 µm, pixel size 0.5µm, with dwell time 800 ms. D- a typical cumulative fluorescence spectrum at 6.6 keV with least-square fit-red.*

Using X-ray- fluorescence microscopy we were able to perform elemental and trace metals localization *in situ* of cryo-protected primary dopaminergic neurons after redox-metals treatments. The natural distribution of redox trace metals in wild type of sciatic neuron, without any exogenous treatment and without any additional staining, was previously performed under cryo-conditions (close to physiological state) at ID21 and published [5] (Fig. 2).



Additionally Mn and Fe oxidative status in PND with the highest metal accumulation was measured by using XANES-techniques (data not shown). The reported experiment gave us the unique opportunity to examine sub-cellular distribution of Fe and Mn in PND with both, high spatial and high spectral resolution. The main goal of this work was to provide an overview of different oxidative status of Fe and Mn and their induction of changes lead to Parkinson's disease.

Figure 2: *X-ray fluorescence of cryo-preserved myelinated neuron. Overlaying distribution of microelements: Mn- red, Fe- blue and Cu- green in situ (A) and the distribution of macroelements: P (red), Cl (green) and K (blue) (B) in the single isolated neuron. Image size 70x40 µm, 280 x 160 pixels with dwell time 150 ms per pixel. E = 7.2 keV (Ducic et al., 2011).*

References

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