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X-ray Phase Contrast Tomography for the investigation of ALS disease

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Summary. — Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease with unknown etiology characterized by progressive loss of the motor neurons in the cerebral motor cortex, brainstem and anterior horns of the spinal cord which determines the gradual loss of voluntary muscle function. Due to inadequate investigation tools, it is not easy to reach a qualitative and quantitative analysis of anatomical variations induced by the disease. In fact, conventional 2D imaging (histology or electron microscopy) produces an incomplete spatial coverage that leads to possible errors in data interpretation, while standard 3D tomographic imaging reaches insufficient resolution and contrast. High resolution X-ray Phase Contrast Tomography (XPCT) allows the simultaneous and three-dimensional visualization of the vascular and neuronal networks of the spinal cord on scales of length ranging from millimeters to hundreds of nanometers, without the use of contrast agent, sectioning or destructive sample preparation.

1. – Introduction

ALS incidence is 2 cases per 100000 persons every two years and the average life expectancy varies between 3 and 5 years after diagnosis.

Disease progression especially affects motor neurons in the spinal cord, responsible for transferring signals that control muscles contraction through the body [1]. ALS cases are often divided into two different forms: sporadic cases represent approximately 90% of the total (sALS), while in the remaining 10% a clear family history is present (fALS). This study will focus on the effects of familial ALS progression, using the G93A-SOD1 animal model [2] at different time points starting from a pre-symptomatic stage of the disease. This animal model mimics ALS inducing mutations in the superoxide dismutase gene 1 (SOD1), located on chromosome 21q22.

We exploit XPCT 3D images to investigate the alterations in vascular and neuronal networks in the lumbar region of the spinal cord. To characterize neuronal and vascular alterations, the entire spinal cord of 3 mice for each time point (Pre-symptomatic, 60 days old, 90 days old) has been analyzed and compared with control samples.

The analysis was extended to the peripheral nervous system, since recent works [3] exploit the correlation between the presence of denervation in peripheral nerves and neuron depletion in the spinal cord. In this sense, sciatic nerves were extracted from the same samples used for spinal cord analysis. A comparison between a 60 days old mouse and the control is shown.

2. – In-line phase contrast imaging and experimental setup

Phase contrast imaging is based on phase variations induced on the incident coherent X-ray beam by the sample. Since in-line free space propagation imaging (XPI) [4] is based on Fresnel's diffraction patterns after free space propagation, measuring the variations of the real part of the refractive index, a coherent light source like synchrotron radiation is needed. This technique allows the visualization of low absorbing materials thanks to its great contrast sensitivity when imaging details with similar densities [5].

The tomographic images were recorded at a single sample-detector distance and the phase retrieval algorithm proposed by Paganin [6] was applied to all the projections.

All the samples were measured at the ID17 beamline at ESRF in Grenoble, France. The experiment on ALS-affected mice spinal cord was performed with a monochromatic incident X-ray beam using an energy of 30 keV. The sample was set at 2.3 m from the CCD camera with a pixel size of 3.5 μm. The tomography has been acquired with 2000 projections covering a total angle range of 360° with acquisition time of 1 second per point. In this experiment we acquired the entire spinal cord.

The experiment on ALS-affected mice sciatic nerves was performed with a polychromatic, "pink", incident X-ray beam using an energy of 44 keV. The sample was set at 1.2 m from the CCD camera having a pixel size of 0.7 μm. Data pre-processing, phase retrieval and reconstruction were performed with the SYRMEP Tomo Project software [7, 8].

3. – Results

XPCT allowed a 3D reconstruction of the entire spinal cord at each time point (fig. 1). The analysis was concentrated on the lumbar region of the spinal cord to simultaneously visualize neuronal cells and the vascular network. For the neuronal network a gray-level based quantification of motor neuron cells was possible because motor neurons appear as bright object in the tomographic volume. We analysed the same portion of the anterior horn at different time points of the disease and we plotted the percentage of motor neuron depletion with respect to the control sample. We correlated these results with the detection of vasculature in the same mice, plotting the number of branches per mm^3, normalized with the control sample. We found a significant loss of detectability of motor neurons as the time point of the disease increases and a slight decrease of the number of branches in the vascular network at the same time points.

Sciatic nerves, belonging to the same mice used for the spinal cord analysis, where imaged at higher resolution to visualize nerve fibers structure. Fibers orientation was quantified from 0° to 180° in the 60 days old sample with respect to the control. The 60 days

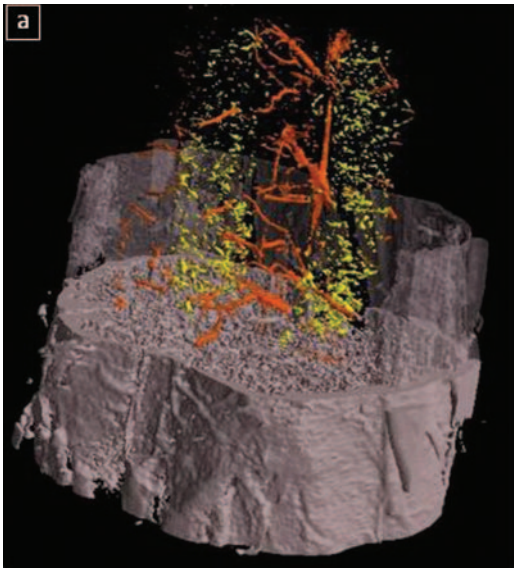

Fig. 1. – (a) 3D rendering of the lumbar region of a 60 days old mouse spinal cord, the motor neurons are rendered in yellow while the vascular network is rendered in orange. The detectability of motor neuron decreases while the disease progresses. In the vascular network a slight decrease is present in the number of branches from a pre-symptomatic to a 90 days old stage of the disease. The images were acquired with an isotropic pixel size of 3.5 μm at the ID17 beamline ESRF, Grenoble (France).

mouse presents a sparser distribution of fibers in the sciatic nerve with respect to the flat distribution of the control.

4. – Conclusions

Research on neurodegenerative diseases necessitates in-depth investigation from the whole organ to the single cell. Enabling the quantifying simultaneous visualization of micrometric structures inside tissue, XPCT is capable of quantifying the neuro-vascular damage at different stages of the disease. Mainly, it was possible to monitor the disease evolution even from an early pre-symptomatic stage. We demonstrated that XPCT enables a 3D reconstruction of the whole spinal cord without sample sectioning, without the use of any contrast agent or aggressive chemical preparation.

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