Neuronal and tissue damages in a mouse spinal cord models of injury imaged by means of High-Resolution X-Ray Phase Contrast Micro-Tomography

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Every year, more than 250 000 people, worldwide, are affected by a Spinal Cord Injury (SCI). As a consequence of the spinal cord damage, all the communications between brain and body (or part of it) are interrupted, resulting in a loss of movement and sensation.

From the clinical point of view, a better knowledge of the neuronal and tissue rearrangement inside the injured spinal cord is necessary in order to tune up tissue-repair strategies. To this aim, many studies focused on the investigation of the neuronal and vascular tissue rearrangement after a SCI are present in the literature.

To assess neurons and axonal integrity, retrograde neuroanatomical tracers were carried on the spinal horn, and further quantified via histological slides [1]. However, this morphological analysis did not allow visualizing the integrity of surviving neurons over long distances. This calls for studies aimed at a better understanding of the full 3D spatial organization of neurons and of their projections in the uninjured spinal cord. The evaluation and prognostication of SCI deficits is a highly demanding task, requiring the development of novel biomarkers or imaging methods. Although magnetic resonance imaging (MRI) is the best imaging modality for the evaluation of overall spinal cord parenchyma, conventional MRI techniques do not differentiate edema from axonal injury and do not generate sufficient spatial resolution or contrast to investigate neuronal cell bodies and their projections. Recent work [2] demonstrated the capability of synchrotron X-Ray Phase Contrast Micro-Tomography (XrPCuT) to visualize the 3D architecture of the neuronal network in mouse spinal cord, at scales spanning from millimeters to hundreds of nanometers, without contrast agent and without a destructive sample preparation, which could lead to data misinterpretation. In particular, single elements of the neuronal networks such as nerve fibers, axon-bundles and neuron soma can be imaged without sectioning or other destructive sample preparation procedures. Indeed, the high spatial resolution (order of micron) achievable with XrPCμT is, of course, attractive for the study of the neuronal organization as well as for the study of the fractal features (e.g., self-similarity and space-filling capacity, fractal dimension (FD)) characterizing CNS tissue [3]. Regarding the latter, XrPCμT is crucial as it enables to detect local variations of fractality induced by structural tissue alterations, including but not limited to glial activation, invading immune cells, or rearrangements of blood vessels. In this framework, we developed an experimental approach that consists in combining *ex vivo* XrPCμT technique with advanced analysis methods including neuronal counting and fractal analysis in order to evaluate the neuronal and tissue morphological alterations induced by a unilateral injury in a SCI mouse model, at different timing from the injury (30 minutes, 7 days). We investigated the SC neuronal loss due to the injury in both the contralateral and ipsilateral side (with respect to the lesion point) by imaging the 3D distribution of the neuronal-network with a spatial resolution of 640 nm at the TOMCAT beamline of PSI (CH). We found a significative reduction of the number of motoneurons (MN) in the ipsilateral side (Figure 1 region C) with the best reduction at 7 days post injury when compared to healthy spinal cord. In order to evaluate the tissue damage, we have developed a fractal analysis pipeline for the 7 days injured and healthy samples. Specifically, we estimated the fractal dimension (FD) [4]using the box-counting method on tomographic slices segmented at different threshold levels (spanning the entire range between 0–1) and observed an increased FD in the ipsilateral injured hemicord compared with the contralateral uninjured tissue, which was almost independent of the chosen threshold (Figure 2). The increase in FD for injured tissue of the spinal cord sample is likely the result of substantial structural changes taking place following the injury.

In particular, based on histology and other preliminary results, we suggest that a potential contribution to the increased FD in the injured hemicord is an altered neuron/glia ratio, possibly due to neuronal loss and/or recruitment or activation of glial cells (i.e., reactive astrocytes and microglia).

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Keywords: fractal dimension; high-resolution X-Ray phase contrast micro tomography; ex vivo mouse spinal cord

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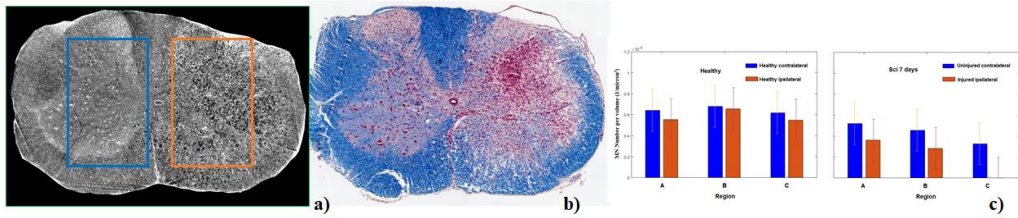

Figure 1. Representative slices from the tomographic reconstruction (a) and histology (b) of the unilaterally-injured mouse spinal cord. The rectangles in a) represent selected ROIs from contralateral uninjured hemicord (1, orange) and ipsilateral injured hemicord (2, cyan). In b): The spinal cord slice at the same rostrocaudal level as the tomographic reconstruction stained with Eriochrome R cyanine and Red neutral showing affinity respectively for myelin (in blue) and nervous cell bodies (in pink).**c)** MN counting inside contralateral(in blue) and ipsilateral(orange) grey matter of both healthy and injured 7 days) mouse spinal cord. For the computation of the number of motoneurons (MN), three region of interest, each of 350 μm extension, have been selected: Region A (away from the lesion), Region B(proximal to the lesion), Region C (inside the lesion).

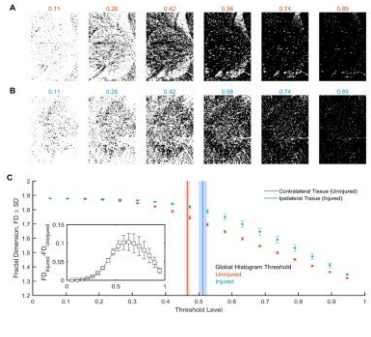

Figure 2: (A,B) Image binarization at different threshold levels for the contralateral and ipsilateral rois. (c) fractal dimension as a function of the threshold level. the vertical bars in red and blue represent the global histogram threshold the difference in FD between uninjured and injured tissue is shown in the inset.