X-ray Phase-Contrast multiscale-Tomography for the 3D quantitative investigation of the spinal cord neuronal arrangements for preclinical application

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Major efforts have been focused on the precise anatomical delineation of the neuronal network (NN), which is important for improving our understanding of the effects of pathological processes, such as spinal cord injury or neurodegenerative disease. In this framework, it appears essential to define morphological and topological quantitative parameters characterizing a healthy neuronal network architecture, to understand changes of these features in any part of the central nervous system (CNS) in diseased samples. Indeed, the quantitative analysis of the single elements in different districts is a crucial task. However, conventional 3D imaging techniques do not have enough spatial resolution and contrast to allow for a quantitative investigation of the neuronal network. In order to characterize quantitatively the neuronal network architecture, an improvement in the performance of 3D imaging tools at high spatial resolution is required. Multiple technologies [1-3] have been used to understand the 3D morphology of individual neurons, glia and axons within the brain and the spinal cord (SC). However, up to now, little progress has been made on understanding the arrangement of neurons in 3D space and the functional role of this arrangement, due to the limited field of view and/or low spatial resolution of currently available imaging tools. A recent work demonstrated that X-ray Phase-Contrast multiscale-Tomography allows for simultaneously image the 3D distribution of the small capillary network and of the neuronal network in an entire mouse spinal cord, covering a spatial range from millimeters to hundreds of nanometers without any aggressive sample preparation or sectioning [4]. However, this work provided only a qualitative description of the neuronal microanatomy. A tool for a 3D quantitative imaging of the SC neuronal network would considerably improve our knowledge of the effects of pathological processes, such as spinal SC injuries and neurological diseases. In the literature, several mathematical approaches have been used to quantify the neuronal patterning in 2D [5] and recently for neuroanatomical structures in 3D space [6]. The use of statistical analysis for the investigation of neuronal positioning has been explored extensively in the retina, where some classes of neurons are located with nearly crystalline order [7]. However, statistical analysis has not been applied to the CNS yet. We developed and applied a tool based on a spatial statistical analysis of the motor neurons to obtain quantitative information on their 3D arrangement in the healthy-mice spinal cord. Then, we applied this tool to different SC pathologies, such as injury and multiple sclerosis model. We investigated the healthy-mouse neuronal architecture by imaging the 3D distribution of the neuronal-network with a spatial resolution of 640 nm. Thanks to the high spatial resolution and large field of view, we were able to perform a detailed quantitative analysis of the neuronal network. We analyzed the reconstructed volume of the axial section of the SC ventral horn (about 1 mm thick), reported in figure1. This region includes groups of cells that form motor nuclei in the Lamina IX [8]. Figure 1B,C,D shows the potentiality of the technique: the neuronal network is imaged in sagittal cross section, showing a 500 µm thick volume relative to the selected Region Of Interest (ROI) in the ventral horn (red box in Fig.1A). The longitudinal distribution of white matter and the motor neurons pool (or motor nucleus) in the ventral horn are discernible [8]. Spatial statistical analysis was employed to obtain quantitative information about motor neurons arrangement at different levels of the spinal cord. To this end, we defined the following parameters, to effectively characterize the neurons spatial distribution: 1)*Clustering length and degree*, that tell us whether the neurons are aggregated (clustering) or dispersed (anti-clustering) in comparison to a Complete State of Randomness described by a homogenous Poisson process. 2)A *regularity factor*, given by the Voronoi tessellation, describing whether neurons are located in a more or less uniform way [5]. Since these characteristic parameters of the neuronal microanatomy are expected to change in pathological conditions, we applied the spatial statistical analysis to detect and quantitatively characterize the modification of the motor neurons networks in different pathological system.

1. Denk W. et al. J. Neurosci. Methods 54, 151–162 (1994).
2. Collins C. E., et al. PNAS 107, 15927–15932 (2010).
3. Ertürk A. et al. Nat. Med. 18, 166–171 (2012).
4. Fratini M. et al. Sci. Rep. 5 (2015).]
5. Diggle P. J. Statistical analysis of spatial point patterns. Vol. 2 (Academic-Press, 1983).
6. Leite-Almeida H. et al. J. Neurosci. Meth. 163, 17–23 (2007).
7. Reese B. In Eye, Retina, and Visual Systems of the Mouse (MIT Press 2008).
8. Watson C., Paxinos G. & Kayalioglu G. The spinal cord: a Christopher and Dana Reeve Foundation text and atlas. (Academic press, 2009).

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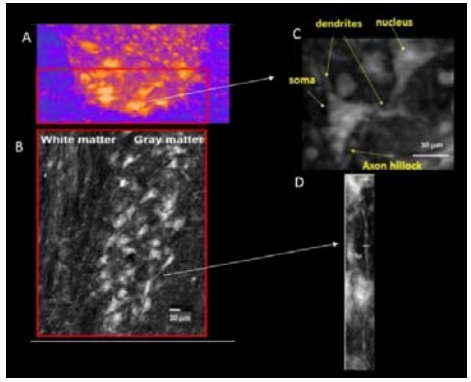

Figure 1. (A) Axial cross section of the spinal cord ventral horn at the thoracic level. The image was segmented to show the neurons (dark yellow) and the neuron fibers (light violet). (B) 500 μm thick volume in sagittal view, of the spinal cord relative to the selected ROI in (A) (red box). (C) A magnified image of one neuron fiber connection selected in a motor nucleus. (D) A magnification of the interaction between two motor neurons in the red box in A

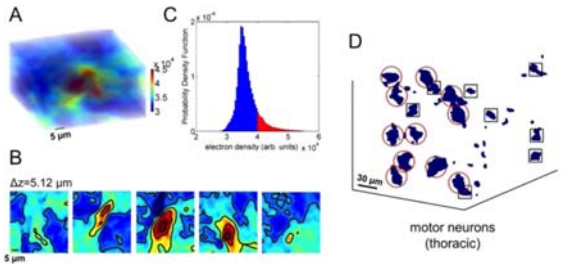

Figure 2. (A) Rendering of the electronic density of a typical single motor neuron in the thoracic region. (B) The contour plots identify the nucleus (region with higher intensity) in the (A) slabs sectioned at different levels. Each slice corresponds to a thickness of 5.12 µm. (C) The Probability density distribution of the motor neurons. The red areas represent values of density searched by the positioning algorithm (see Methods). (D) 3D spatial arrangement of motor neurons in a volume of about 0.5 × 107 µm3 in the thoracic spinal cord region. We note different size populations allowing us to distinguish the motor neurons (enclosed in red circles) from other cells whose size is compatible with glia (enclosed in black squares)