Quantitative 3D investigation of Neuronal network in mouse spinal cord model

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Supplementary Information

To confirm the validity of our approach we compare the results obtained by applying our algorithm on a tomographic reconstructed slice (20 micron thick) with the results obtained with a standard quantitative analysis on a histological section (20 micron thick), treated with a motor neurons marker (immunohistochemical analysis of SMI-32). As shown in the fig. S1 our approach locates the neurons in the tomographic image in the same region where they appear in the histology. The final neuron density in the histology, following the counting procedure described in Battaglia et al. (2015)[20], is about 6 $x10^3$ in the healthy mouse. In the x-ray tomography, where our approach has been applied, we found out 5 $x10^3$.

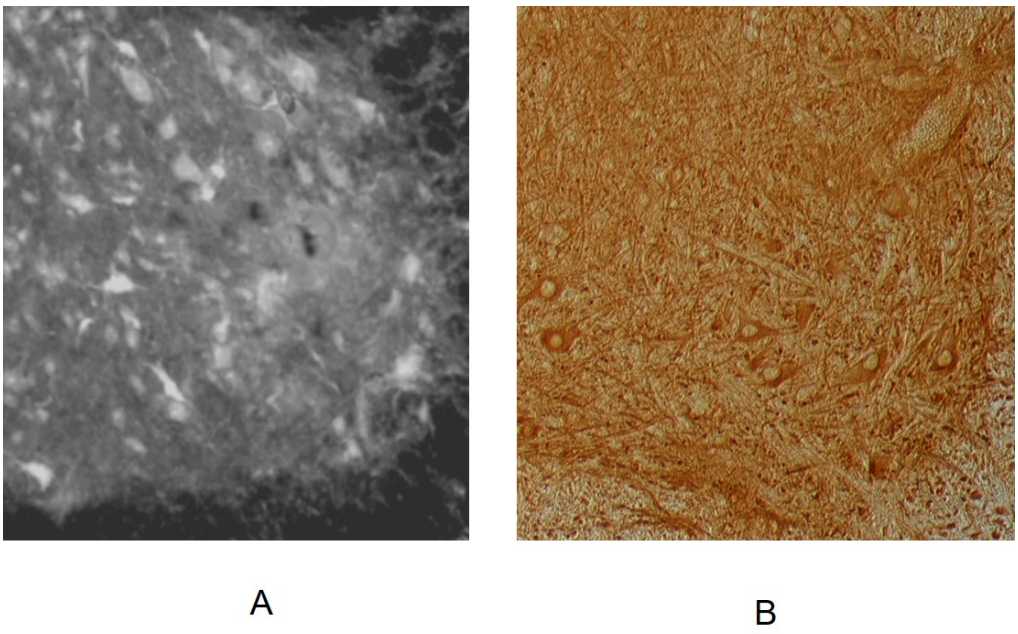

Figure S1: A) Tomographic reconstructed slice of the healthy mouse spinal cord in the ventral horn of the cervical region (20 micron thick). **B)** Immunohistochemical analysis of SMI-32, a marker of motor neurons, of a representative healthy mouse spinal cord in the ventral horn of the cervical region, where was applied the statistical analysis to

distinguish the motor neurons to confirm the potentially of our approach for the 3D reconstruction of imaged spinal cord**.**

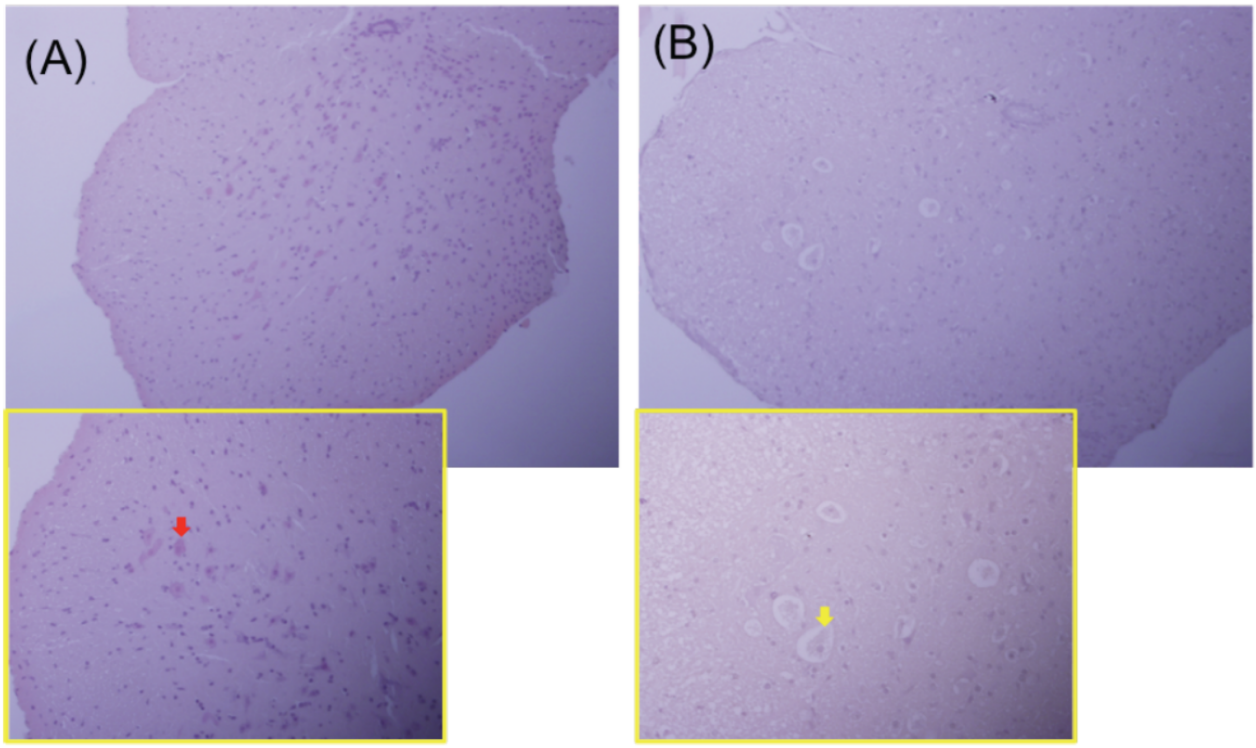

Figure S2: Population of cells in the histological sections (coloured with H/E) of the lumbar region in healthy **(A)** and EAE **(B)** mouse. The red arrow indicates neurons while yellow arrow indicates neurons with a pyknotic nucleus.

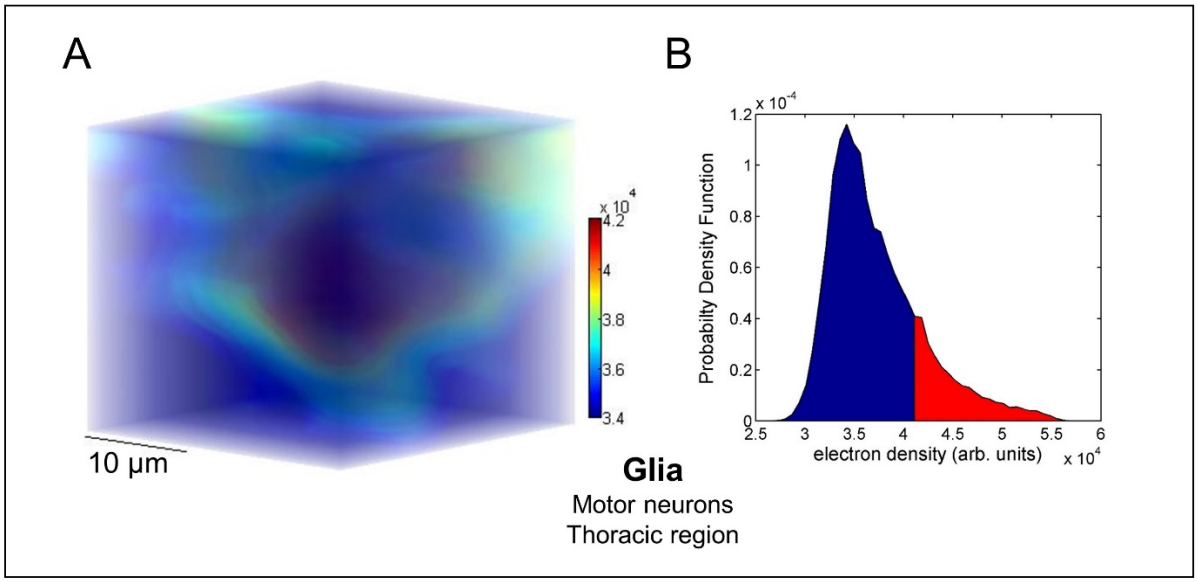

Figure S3 (A) 3D electronic density of a typical single glia in the thoracic region. **(B)** The probability density distribution of the glia. The red areas represent values of density searched by the positioning algorithm.