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Segmental demyelination of internodes triggers mitochondrial distribution

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Introduction

Oligodendrocytes, myelin sheaths and axons form a closely linked functional network, known as the axon-myelin unit. Neuronal cell bodies, which replenish the axon with mitochondria through anterograde transport, are directly implicated in the pathophysiology of progressive MS. While we know that the spatial distribution of intra-axonal mitochondria is severely disturbed in MS, underlying mechanisms are poorly understood. Specifically, to what extent loss of the myelin sheath per se regulates mitochondrial distribution is currently unknown. In this study, we took advantage of the cuprizone model, where oligodendrocyte injury and demyelination occurs in a highly predictive manner. We first examined whether oligodendrocytes die simultaneously or sequentially, and then investigated mitochondrial distribution.

Results

Oligodendrocytes die sequentially after metabolic stress induction

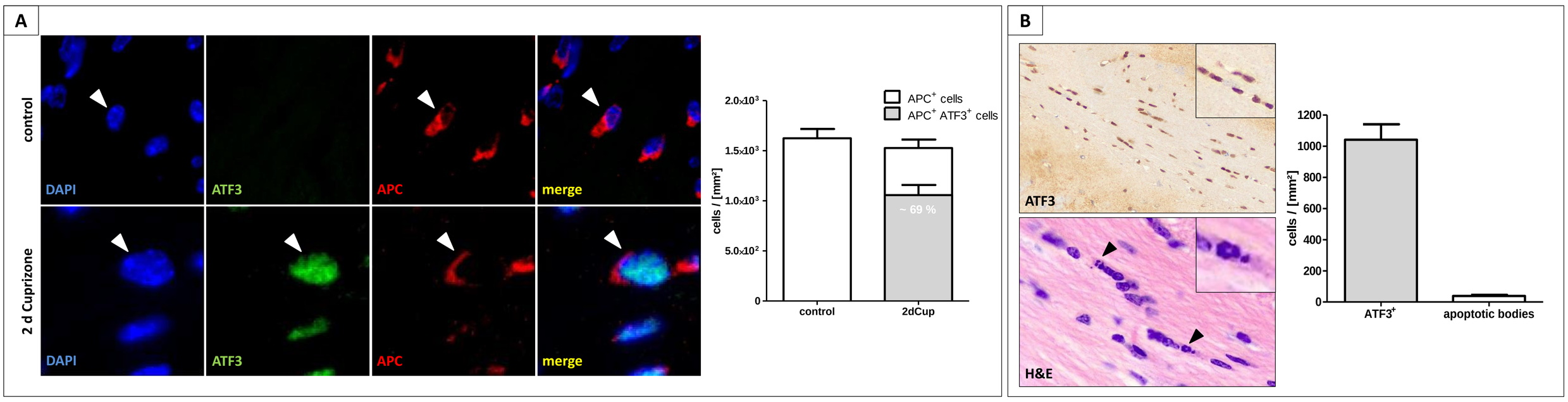

Figure 1: (A) ATF3/APC immunoflourescence double stain to vizualize activation of stress pathways (ATF3) in mature oligodendrocytes (APC). **(B)** Quantification of ATF3+ cells and apoptotic oligodendrocytes (H&E stain) after 2 days cuprizone intoxication. Note that during early cuprizone-induced oligodendrocyte degeneration, virtually all oligodendrocytes are stressed, while just a small percentage undergo apoptosis, indicating a graded oligodendrocyte vulnerability.

3-dimensional reconstruction of the axon-myelin-unit in controls

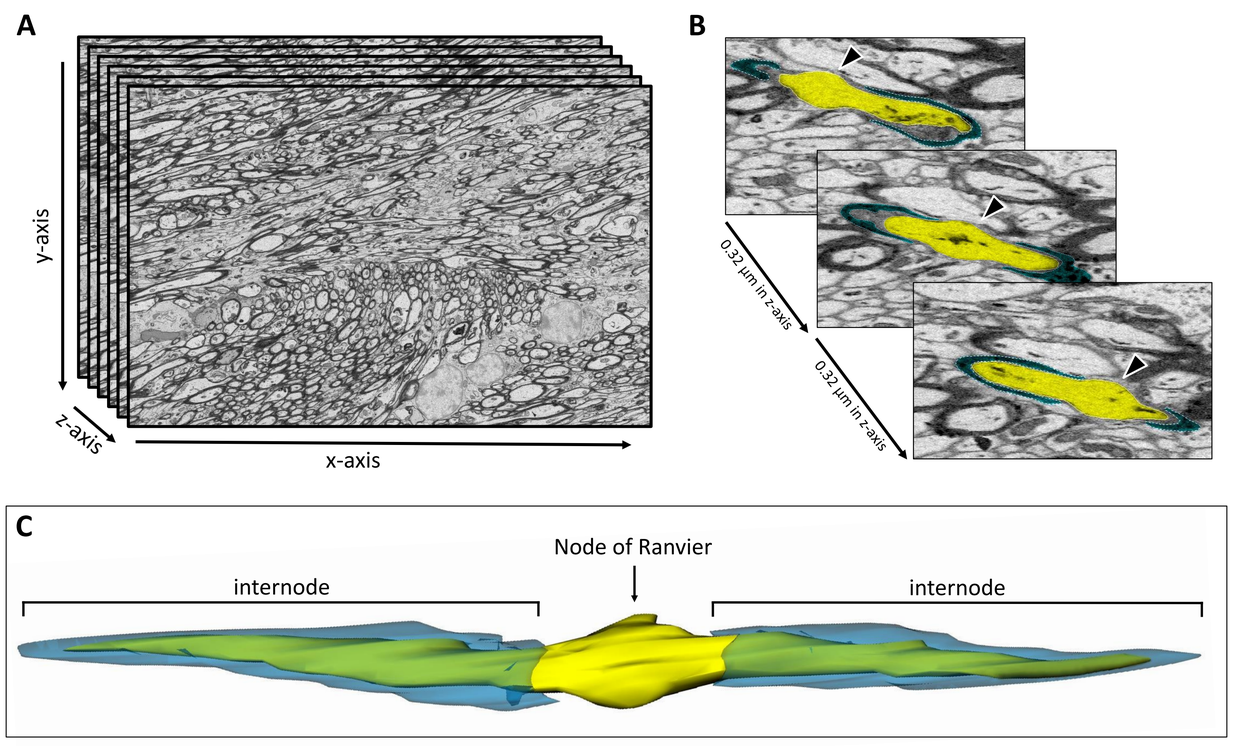

Figure 2: (A) Serial block-face scanning electron microscopy (3D-EM) of the corpus callosum was performed to reconstruct the axon-myelin unit. **(B)** Individual slices illustrating the axon (yellow) and its myelin sheath (blue) at the site of a Node of Ranvier (arrowheads). **(C)** 3D-reconstruction of the axon focusing on its Node of Ranvier flanked by normally myelinated axonal segments.

Demyelination of specific internodes after 3 weeks cuprizone intoxication

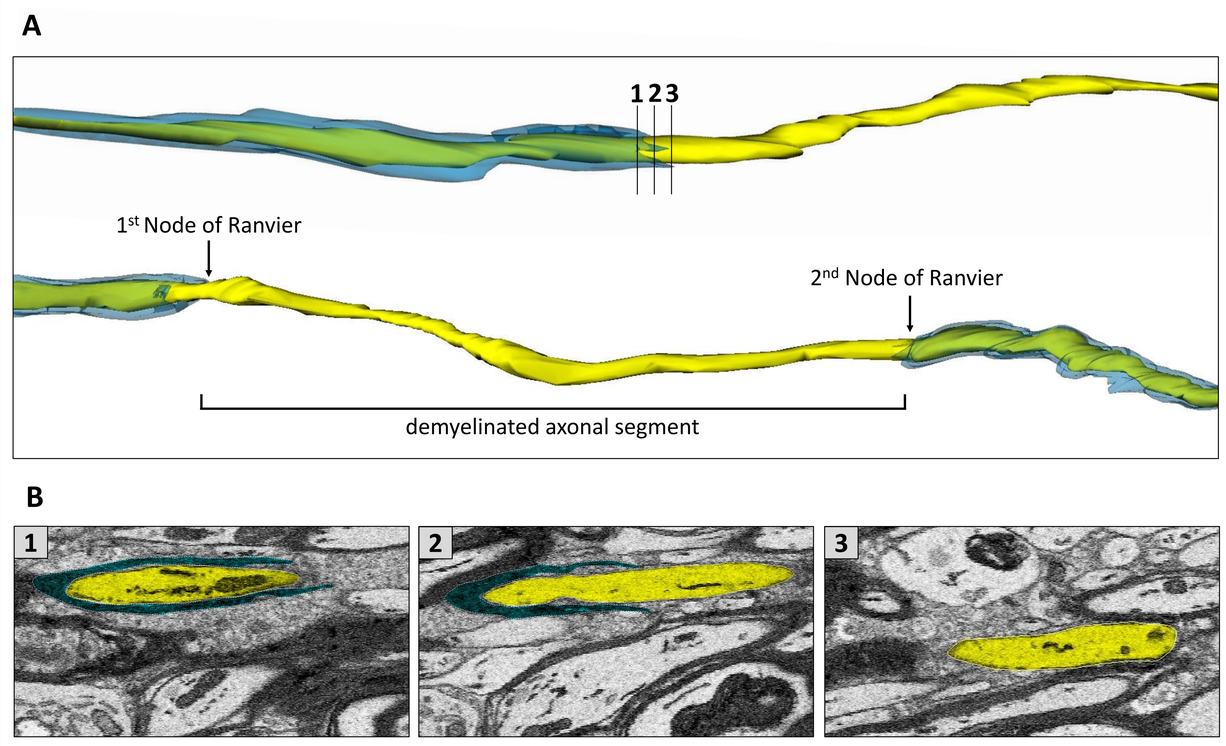

Figure 3: **(A)** illustrates cutouts of reconstructed axons affected by segmental demyelination. Demyelinated segments are flanked by normally myelinated axonal segments at both sites. Axonal sections highlighted with **(1-3)** are shown in **(B)** on the ultrastructural level. **(B)** Individual EM-slices illustrating **(1)** myelinated and **(3)** demyelinated axonal internodes as well as **(2)** Node of Ranvier.

Internode specific demyelination affects mitochondrial distribution in one and the same axon

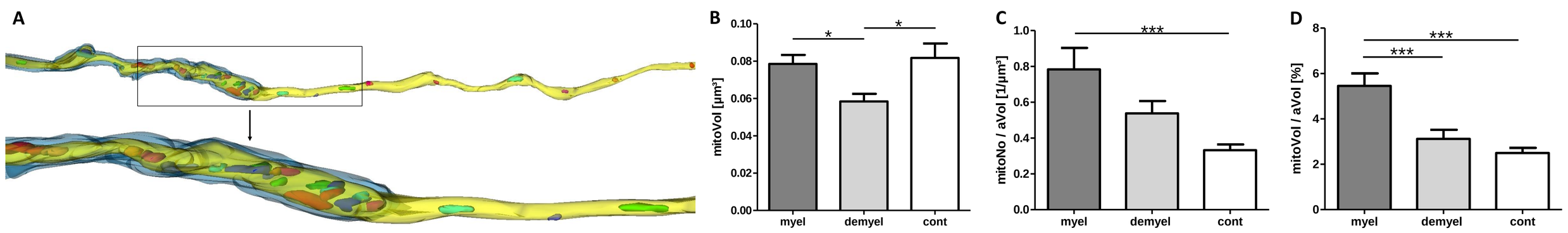

Figure 4: (A) Three-dimensional reconstructions of segmental demyelinated axons and individual axonal mitochondria (various colors) after three weeks cuprizone intoxication. **(B)** The individual mitochondrial volume was significantly higher in areas of preserved internodes and controls compared with demyelinated ones. The mitochondrial density **(C)** and the percent axonal volume occupied by mitochondria **(D)** were increased in myelinated axonal segments compared with demyelinated segments and controls.

Discussion

We showed that metabolic stress results in an “out of phase” degeneration of oligodendrocytes, indicating a graded – and probably regulated - oligodendrocyte vulnerability. During initial demyelination, mitochondria accumulate at internodes with a preserved myelin sheath. We conclude that loss of the myelin sheath critically contributes to mitochondrial pathology in MS. Our study, thus, provides an important functional link between oligodendrocyte pathology and neurodegeneration.

Literature

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