



Ultrastructural changes in diverticular disease

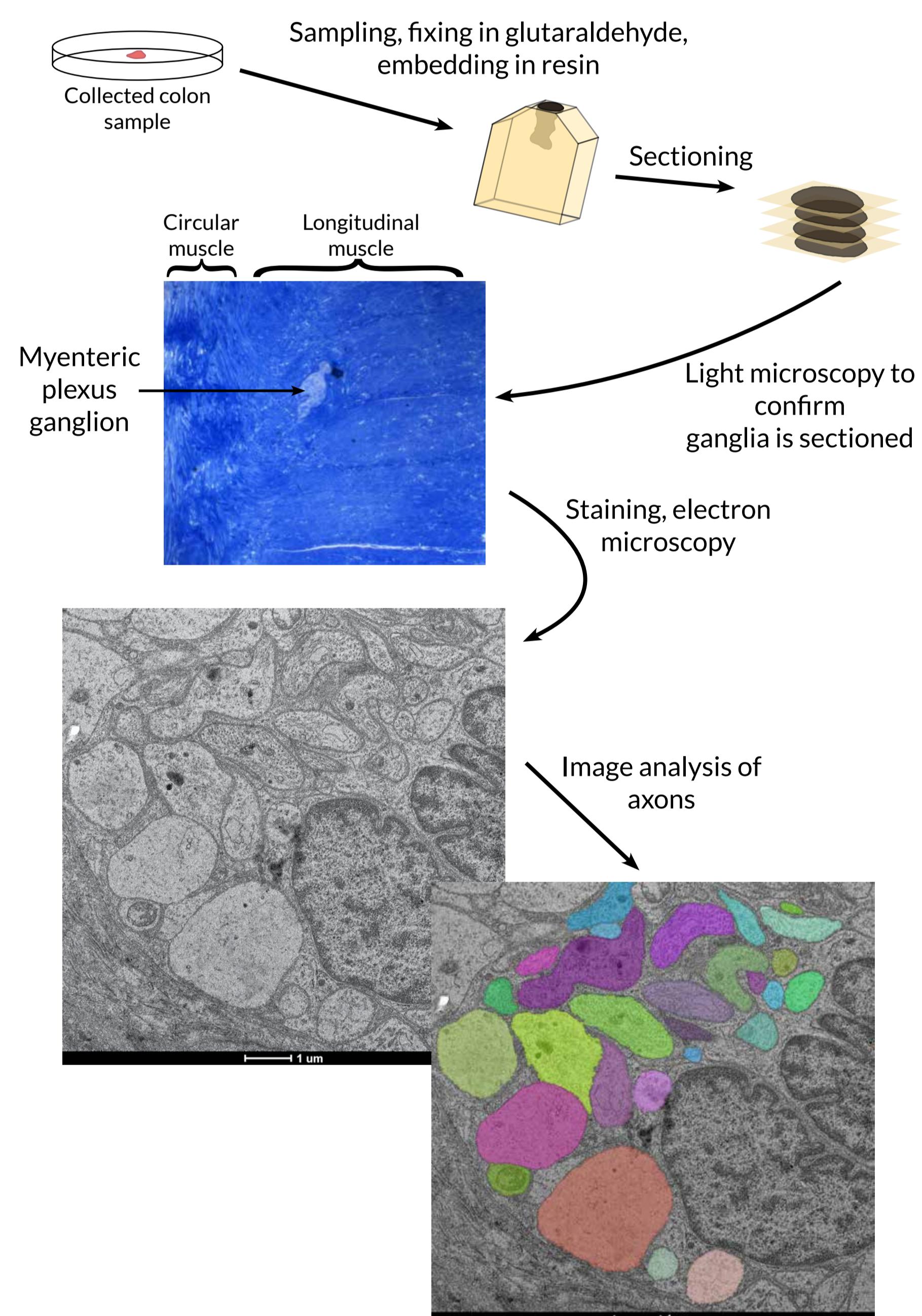
P. Alaburda¹, N. Paužienė¹, J. Lukošienė³, Ž. Saladžinskas², A. Tamelis²
¹Institute of Anatomy, ²Department of Surgery ³Institute of Digestive Research,
Faculty of Medicine, Lithuanian University of Health Sciences
Email: alaburdapaulius@gmail.com

Introduction

Diverticular disease (DD) is characterised by herniation of colonic mucosa and submucosa through points of weakness in smooth muscle. Current clinical and pathophysiological understanding of the disease suggests that disrupted neural control of normal colonic motility is important. However, no studies have been done to examine the fine structure of the enteric nervous system. The aim of this study was to study the ultrastructural changes of the myenteric and submucosal subplexuses that occur in DD.

Methods

Samples of the sigmoid colon were collected from 10 patients (2 controls and 8 patients with DD). Diverticular sigmoid colon samples were collected from patients who had undergone elective surgery after repeating attacks of diverticulitis. Specimens of control sigmoid colon were collected from non-obstructing colonic malignancies or polyps.



After fixation in glutaraldehyde and osmium tetroxide, sections were stained with uranyl acetate and lead citrate. Sections were observed with a transmission electron microscope. We photographed electron micrographs at 4800x and 6800x magnification. Axon area profiles were manually outlined and measured with ImageJ. Data of axon area profiles were log-transformed to normalise variance.

Means of control and DD-affected axons were compared with Student's t-test, proportions of swollen axons were compared with the test of equal given proportions. Data analysis was carried out in R v 3.3.1 (<http://www.r-project.org/>).

Acknowledgement

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Main findings

Patients with DD show structural alterations of the ENS characterized by axon remodelling and swelling. The morphometric data give evidence that the disease is associated with ultrastructural changes and further findings might prove important in understanding the disease. We will continue the study collecting patient colon tissue samples, as well as evaluating the effect DD has on Interstitial cells of Cajal (ICC).

Results

Patients with DD exhibit remodelling as well as damage of the myenteric plexus. We found patches of fragmented axons, as well as a higher percentage of swollen axons in DD-affected samples (controls 4.11%, DD 9.52%, 95% CI [2.09, 8.70], $p = 0.006$). The mean area of healthy axon profiles was higher in controls (Figure 1). The morphology of the submucosal plexus remained unchanged: the percentage of swollen axons (controls 4.49%, DD 4.35%, 95% CI [-3.9, 3.6], $p = 1$) as well as the size of the axons (Figure 1) did not change.

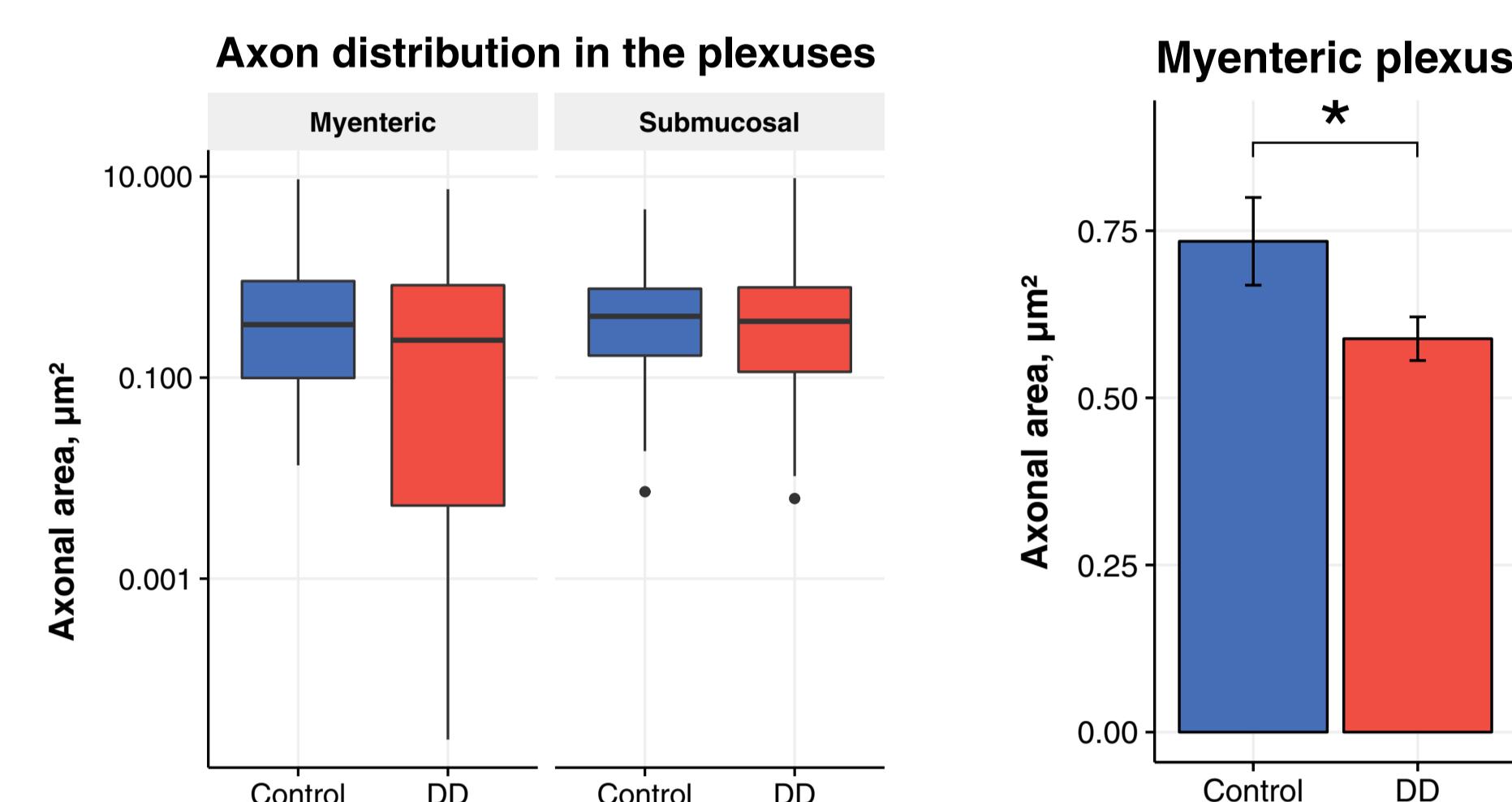
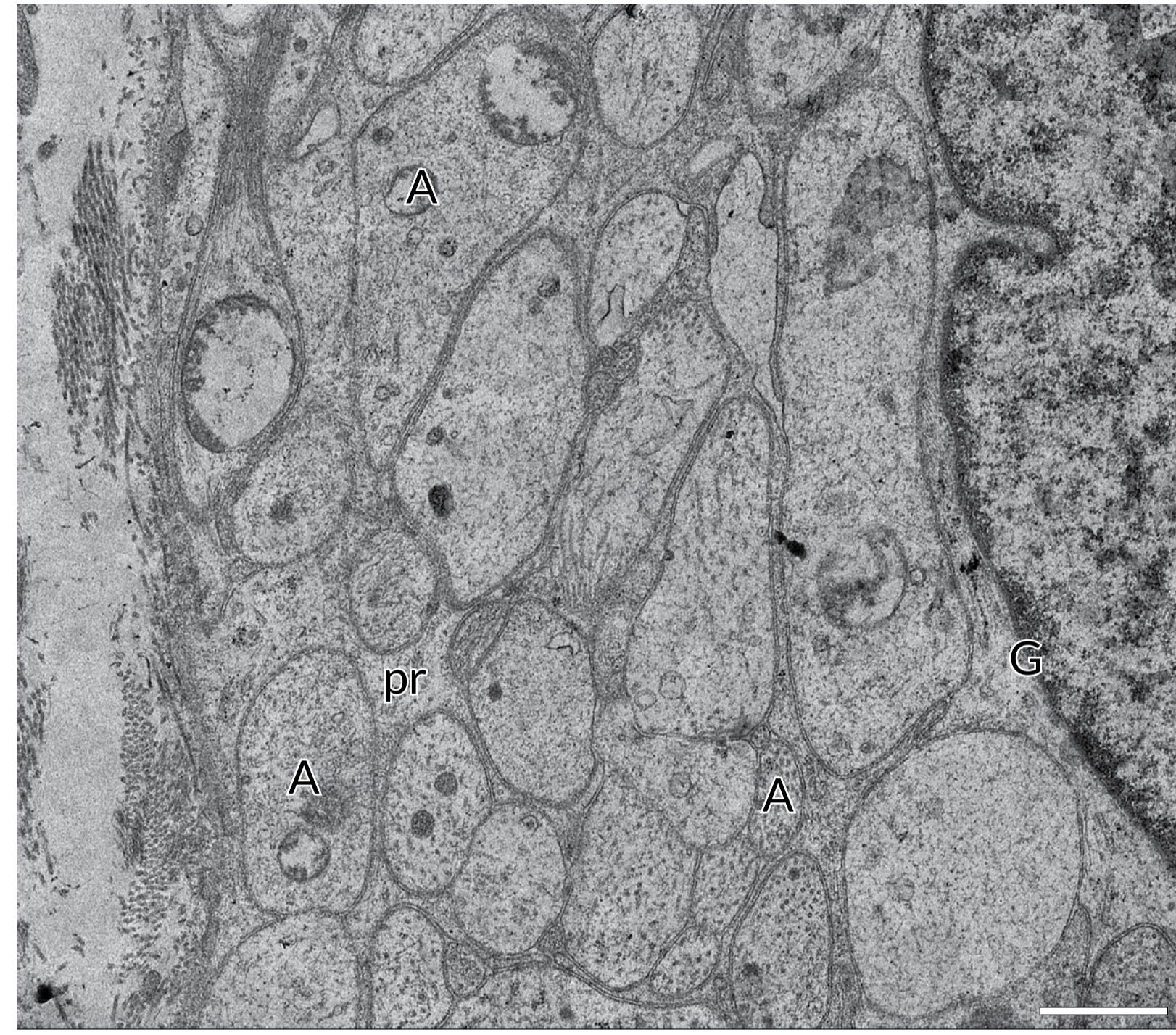


Figure 1. Mean axonal area in the myenteric plexus. Boxplot bands show medians, boxplot whiskers represent values within 1.5*IQR. Bar chart whiskers represent standard error of the mean. * $t = 7.465$, $p < 0.0001$, $d = 0.088$.

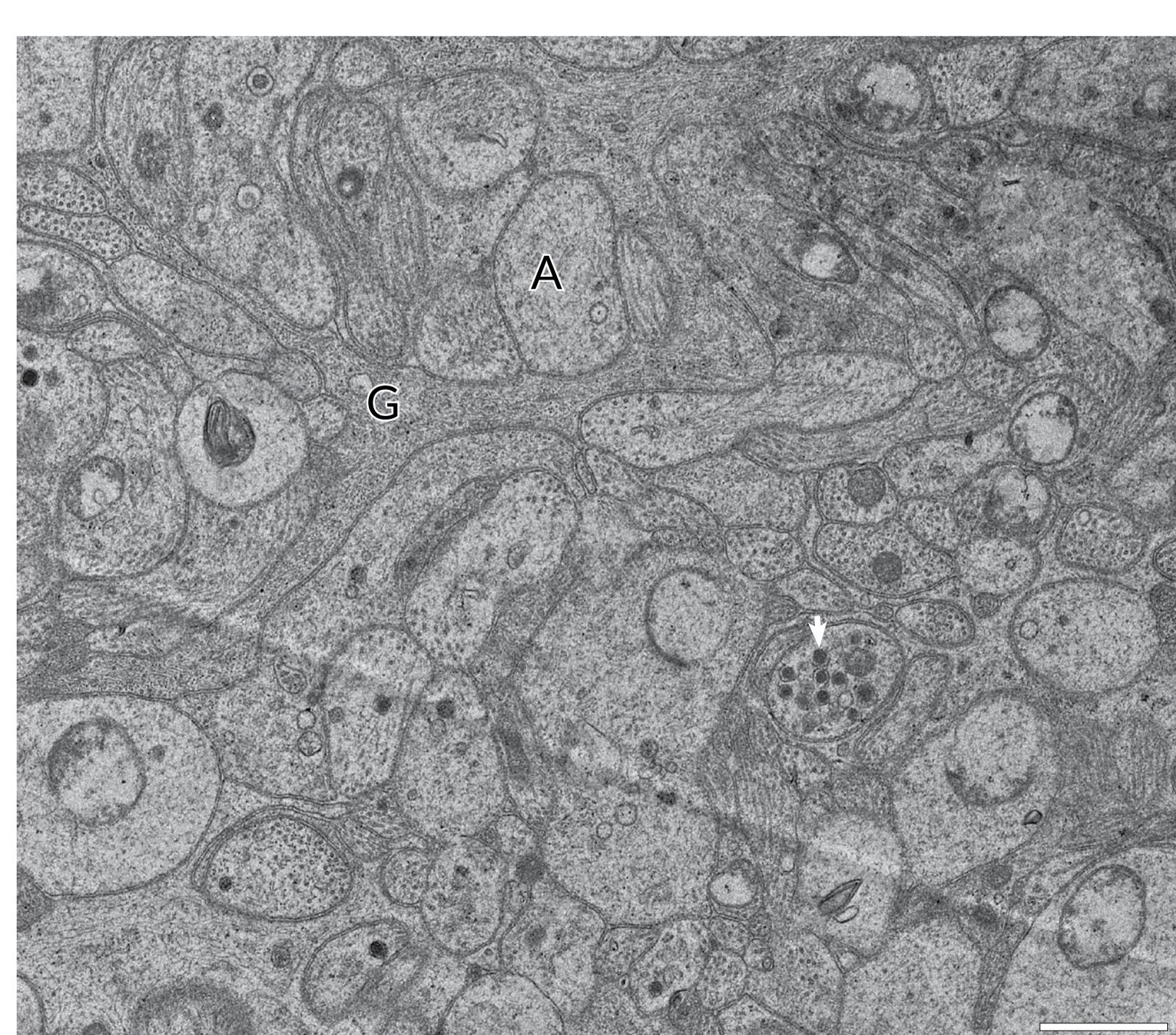
Control



Electron micrograph of the myenteric plexus. The healthy human myenteric plexus assumes a typical neuropil-like arrangement: the axons are closely arranged with thin glial processes in between. A – axon, G – glial cell, pr – glial process. Scale bar = 1 μm

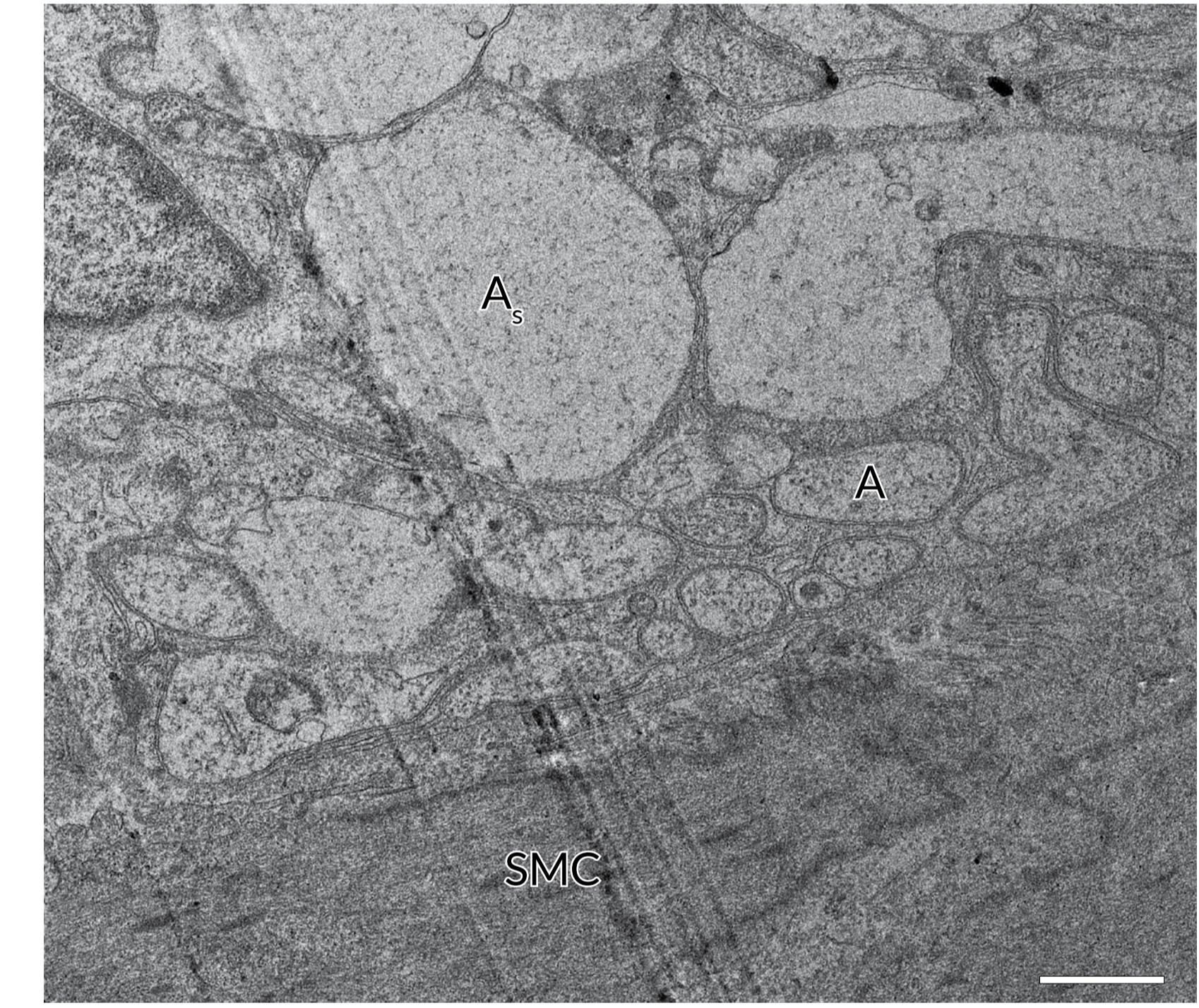


Electron micrograph of the submucosal plexus. The axons are surrounded by glial cell processes. Note that some of the axons contain vesicles. A – axon, arrow – vesicles in axon, G – glial cell. Scale bar = 1 μm

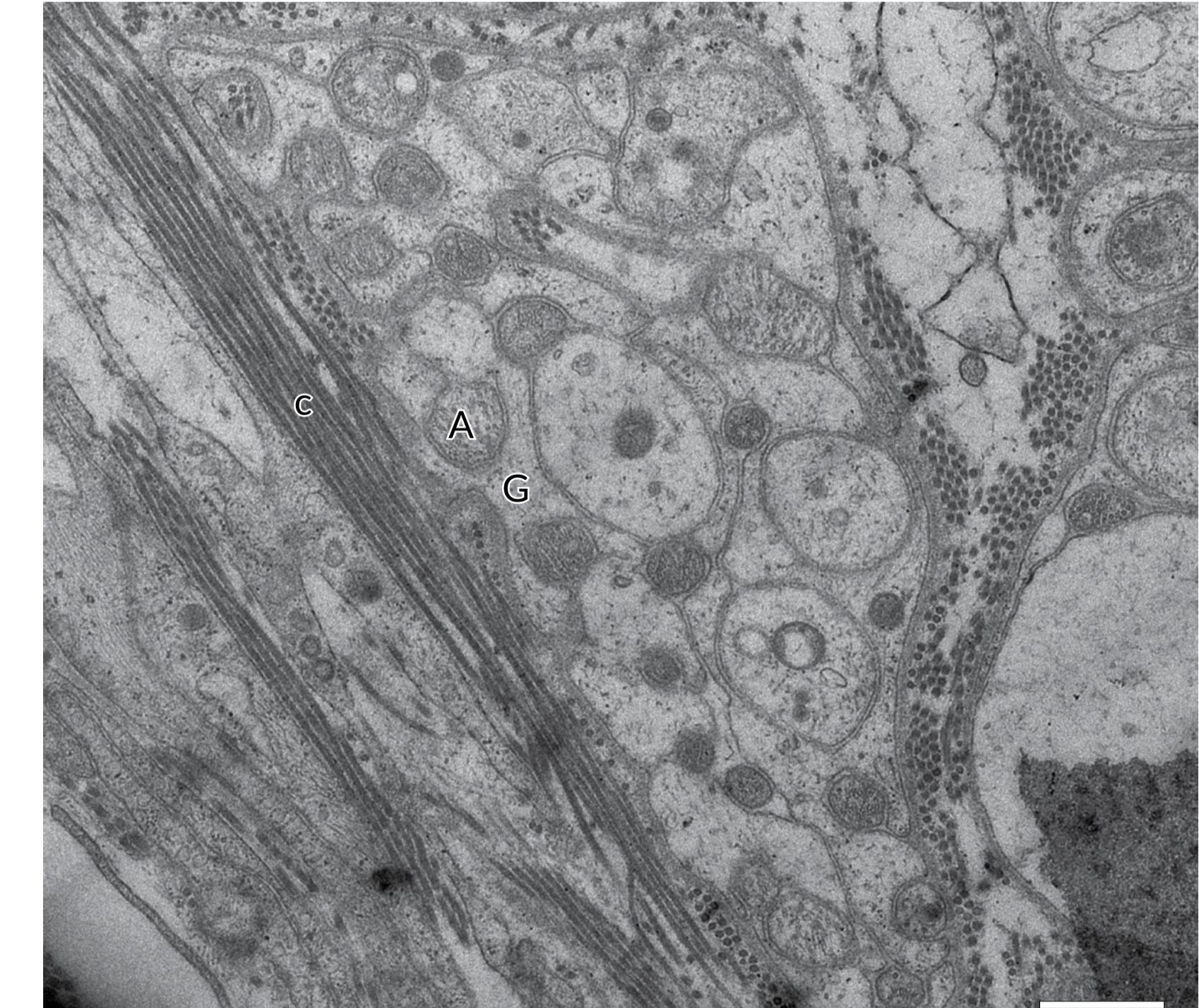


Electron micrograph of the myenteric plexus. The healthy myenteric plexus exhibits various axons without noticeable signs of swelling. A – axon, arrow – vesicles in axon, G – glial cell. Scale bar = 1 μm

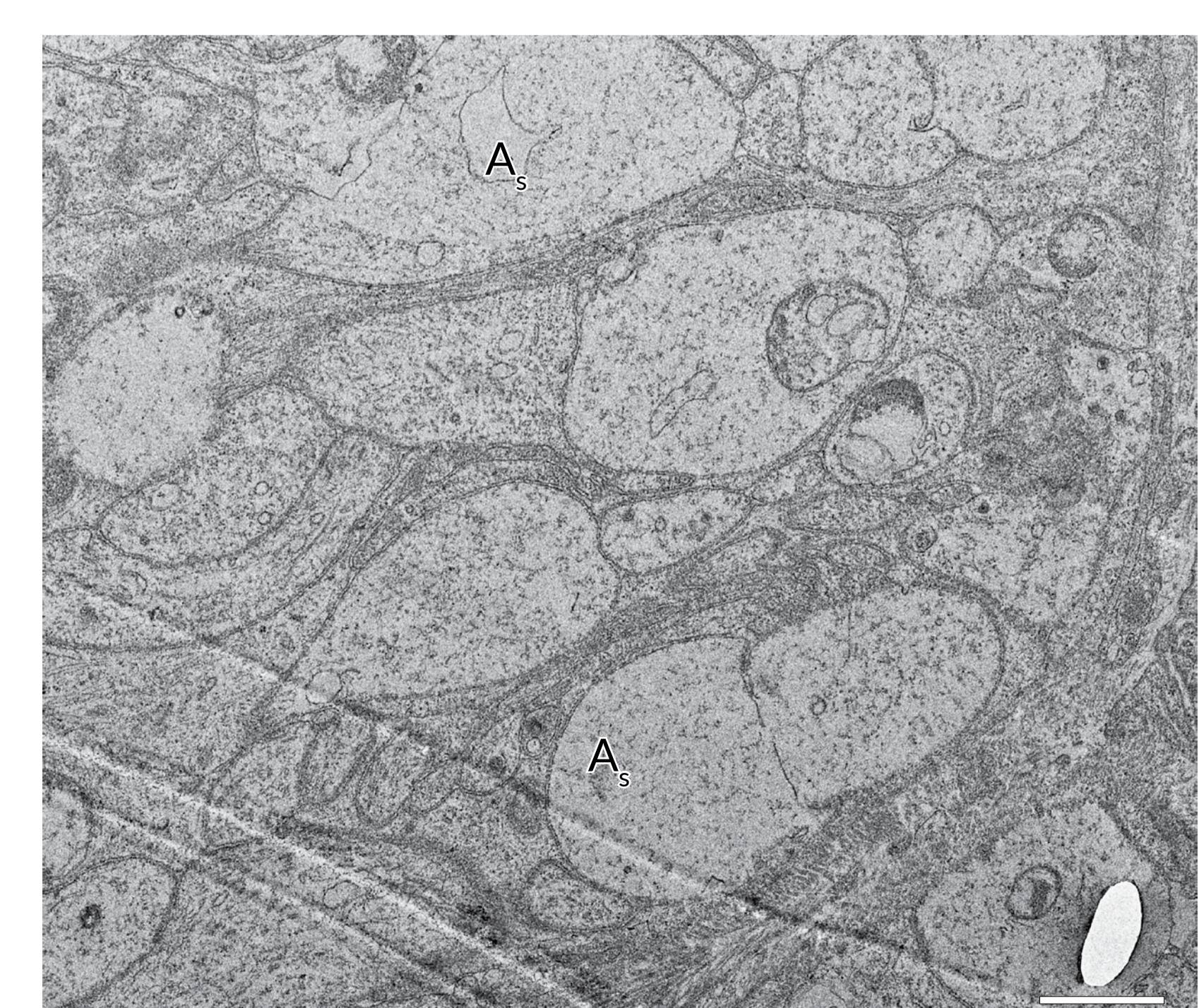
Diverticular disease



Electron micrograph of the myenteric plexus. Note the swollen axons at the top of photo as well as the decreased size of the axons on the border of the plexus. A – axon, As – swollen axon, SMC – smooth muscle cell. Scale bar = 1 μm



Electron micrograph of the submucosal plexus. While still enveloped by glial cell processes, axons are visibly smaller. A – axon, G – glial cell process, c – collagen. Scale bar = 1 μm



Electron micrograph of the myenteric plexus. Note the abundance of swollen axons. As – swollen axon. Scale bar = 1 μm