



# Quantitative analysis of the innervation of pig cardiac ventricles

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## Introduction

The porcine heart is a popular experimental model in cardiac electrophysiology. However, innervation of cardiac ventricles of this species remains insufficiently examined so far. Therefore, the present study immunohistochemically analysed the distribution of three intracardiac nerve plexuses – the epicardial, myocardial and endocardial plexuses – in the porcine heart.

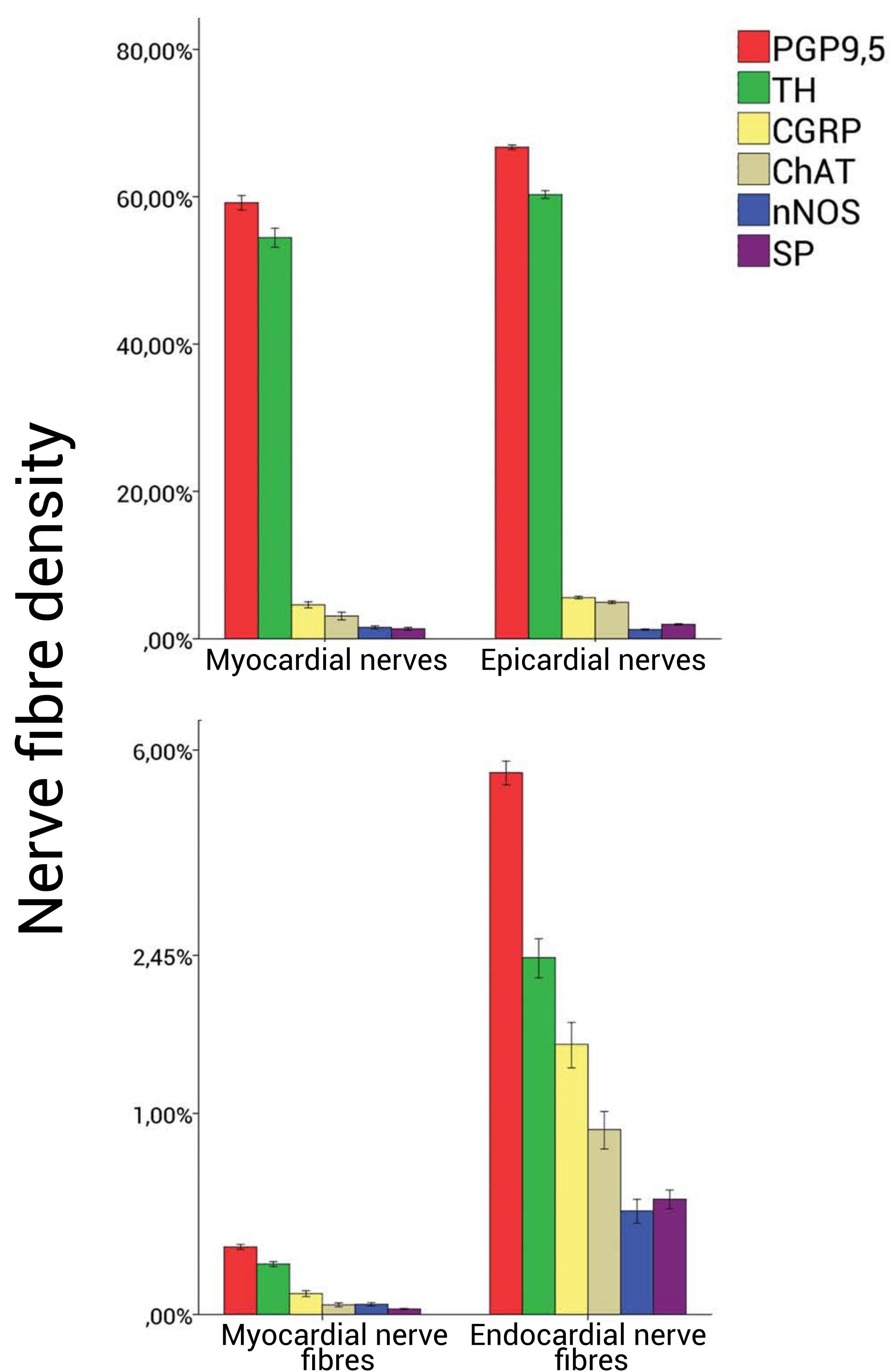


Fig. 1. Phenotype distribution of neural markers within the plexuses. Note that the left-hand charts show fibre density within nerves and the right-hand charts show nerve fibre density within the myocardium and the endocardium.

## Methods

Juvenile porcine hearts ( $N = 4$ ) were perfused with PBS and prefixed with a 4% PFA solution. The ventricles were sectioned into three blocks: the upper third or the base, the middle third or the middle and the lower third or the apex. Tissue samples were stained immunohistochemically for protein gene product 9,5 (PGP9,5), tyrosine hydroxylase (TH), choline acetyltransferase (ChAT), nitric oxide synthase (nNOS), calcitonin gene related peptide (CGRP) and substance P (SP).

The area of epicardial and myocardial nerves was measured manually and the density of nerve fibres within each nerve was expressed as the ratio of nerve fibres within the nerve to the nerve area. The density of myocardial nerve fibres was expressed as the ratio of nerve fibres within the counting frame to the area of the counting frame. The density of endocardial nerve fibres was expressed as the ratio of nerve fibres within the endocardium to the manually measured area of the endocardium.

## Results

### 1. Phenotype distribution of neural markers

Each plexus of the ventricles exhibited a similar distribution of neuronal phenotypes: PGP9,5 and TH were the most abundant phenotypes, with increasingly smaller amounts of CGRP, ChAT, SP and nNOS positive structures (Fig. 1).

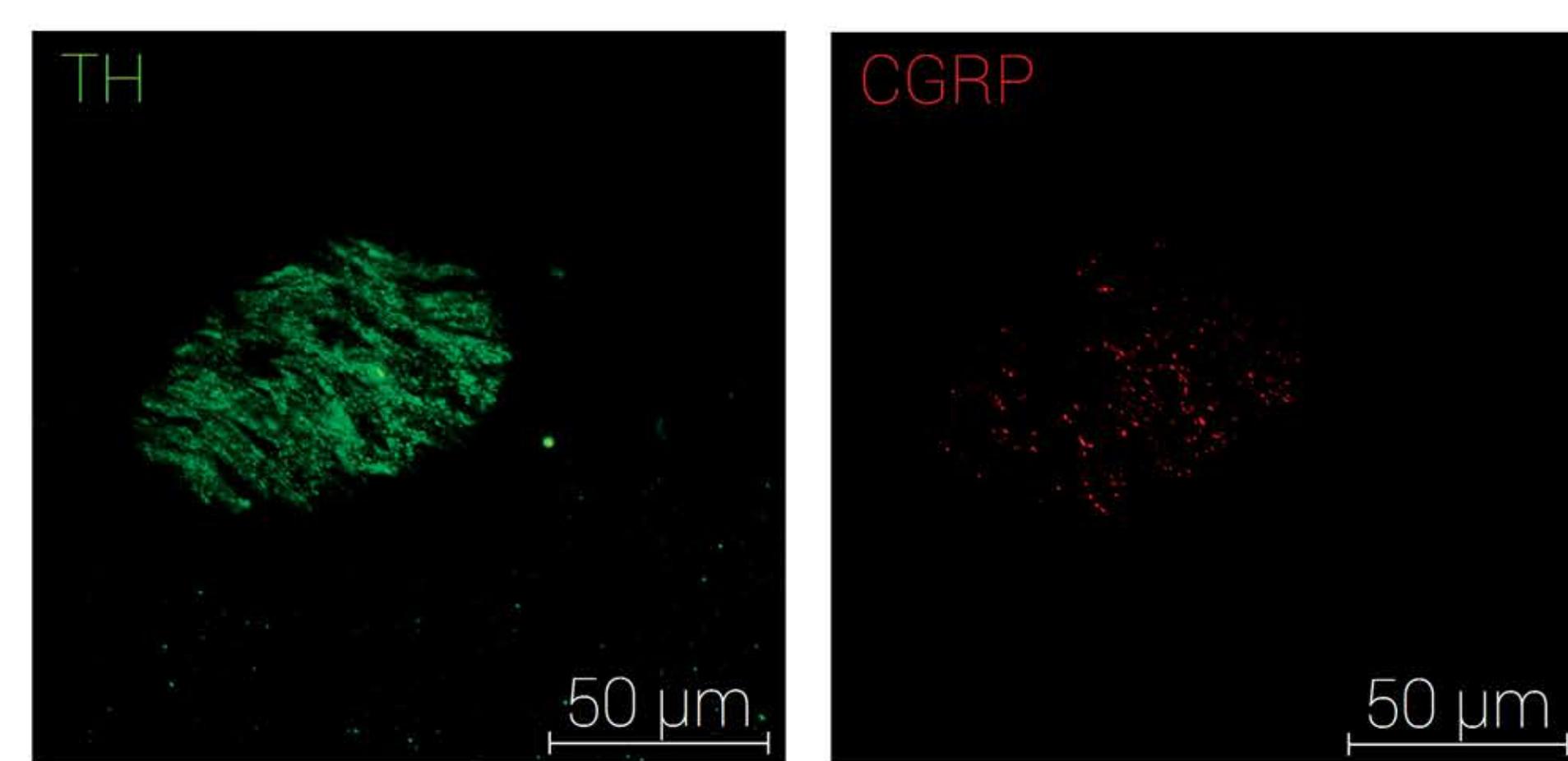


Fig. 2a. An epicardial nerve stained for TH and CGRP.

### 2. Epicardial and myocardial nerves

The epicardial nerve plexus contains a large number of nerves ( $N = 1400$ ), ranging from  $41.95 \mu\text{m}^2$  to  $14748.05 \mu\text{m}^2$ , with an average of  $917.96 \pm 22.49 \mu\text{m}^2$  (Fig. 2a and b). Myocardial nerves were smaller in number ( $N = 522$ ), yet were larger in size ( $1182.68 \pm 48.56 \mu\text{m}^2$ ) (Fig. 3).

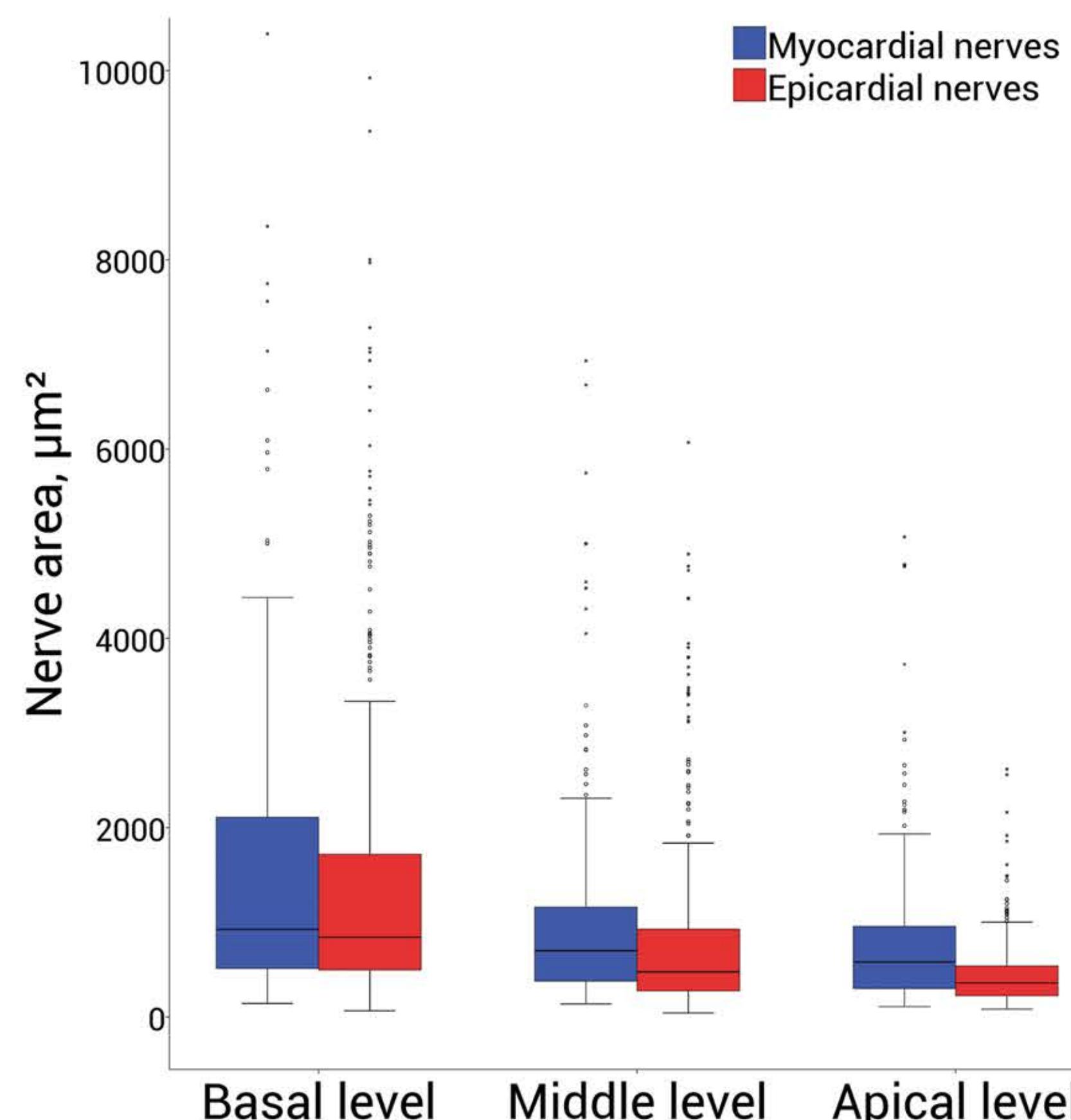


Fig. 3. Cross-sectional area of myocardial and epicardial nerves. The area of myocardial nerves is bigger at every level of the ventricles ( $p < 0.05$ ).

### 3. Myocardial and endocardial nerve fibres

Myocardial nerve fibres exhibited no strong innervation gradients across the myocardium ( $p > 0.05$ ). The endocardial nerve fibre network was much more dense than the one found in the myocardium (Fig. 4). The network itself shows denser innervation in the left ventricle than in the right ventricle (PGP9,5-positive structure density 4.48% vs 5.83%,  $p < 0.05$ ,  $r = 0.663$ ).

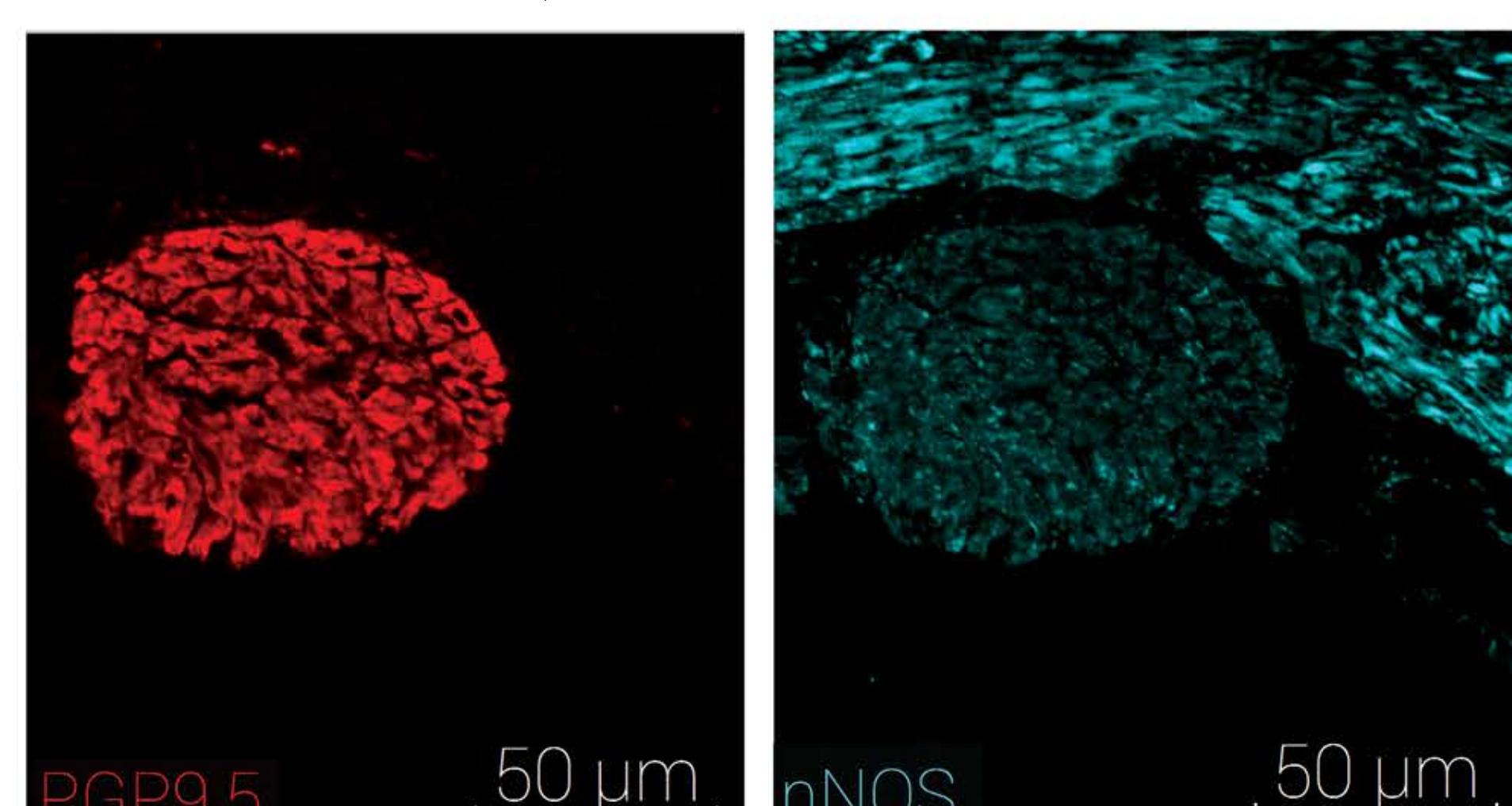


Fig. 2b. An epicardial nerve stained for PGP9,5 and nNOS.

## Conclusions

1. The epicardial nerve plexus contains a large number of nerves. However, nerves of the myocardial nerve plexus are bigger yet fewer in number.
2. The endocardial nerve fibre network is much denser than the one found in the myocardium. The endocardium of the left ventricle is comparatively more innervated than the right ventricle.
3. TH-positive fibres are dominant in each ventricular plexus. CGRP, SP, ChAT and nNOS-positive nerve fibres are substantially more scarce.

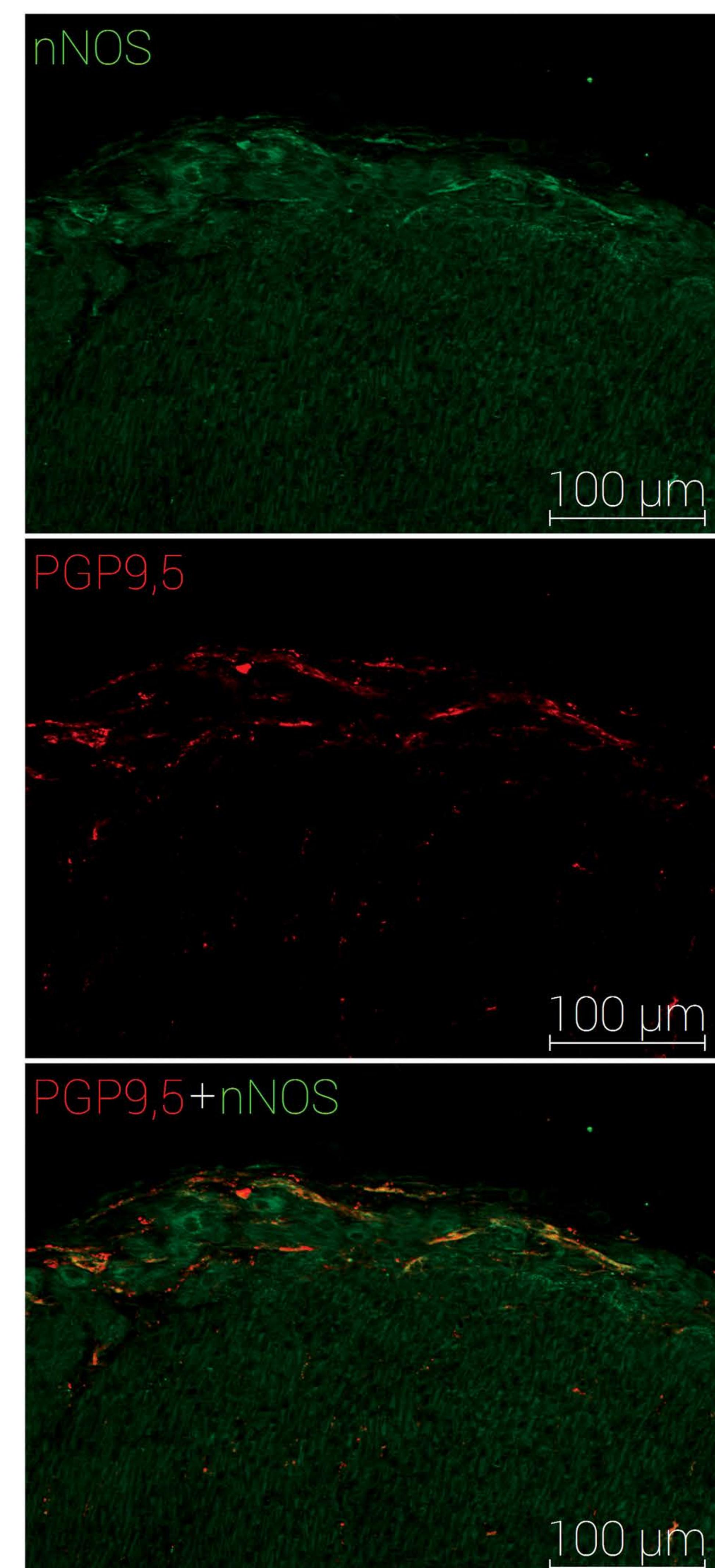


Fig. 4. Endocardial and myocardial nerve plexuses

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