

# NEUROCHEMICAL CHARACTERISATION OF SENSORY AND AUTONOMIC ENDINGS IN THE MOUSE BLADDER

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## INTRODUCTION

The bladder is innervated by spinal afferent neurons with cell bodies in the lumbosacral and thoracolumbar dorsal root ganglia (DRG) which project to the bladder via splanchnic-pelvic and lumbar-hypogastric nerves. However, there is still a gap in our understanding with regards to comprehensive classification of the of major functional classes of primary afferent neurons in the bladder.

## AIMS

The objective of this study was to characterize anatomically and immunohistochemically the terminal endings of sensory and autonomic neurons in the wholemounts of the mouse bladder.

## METHODS

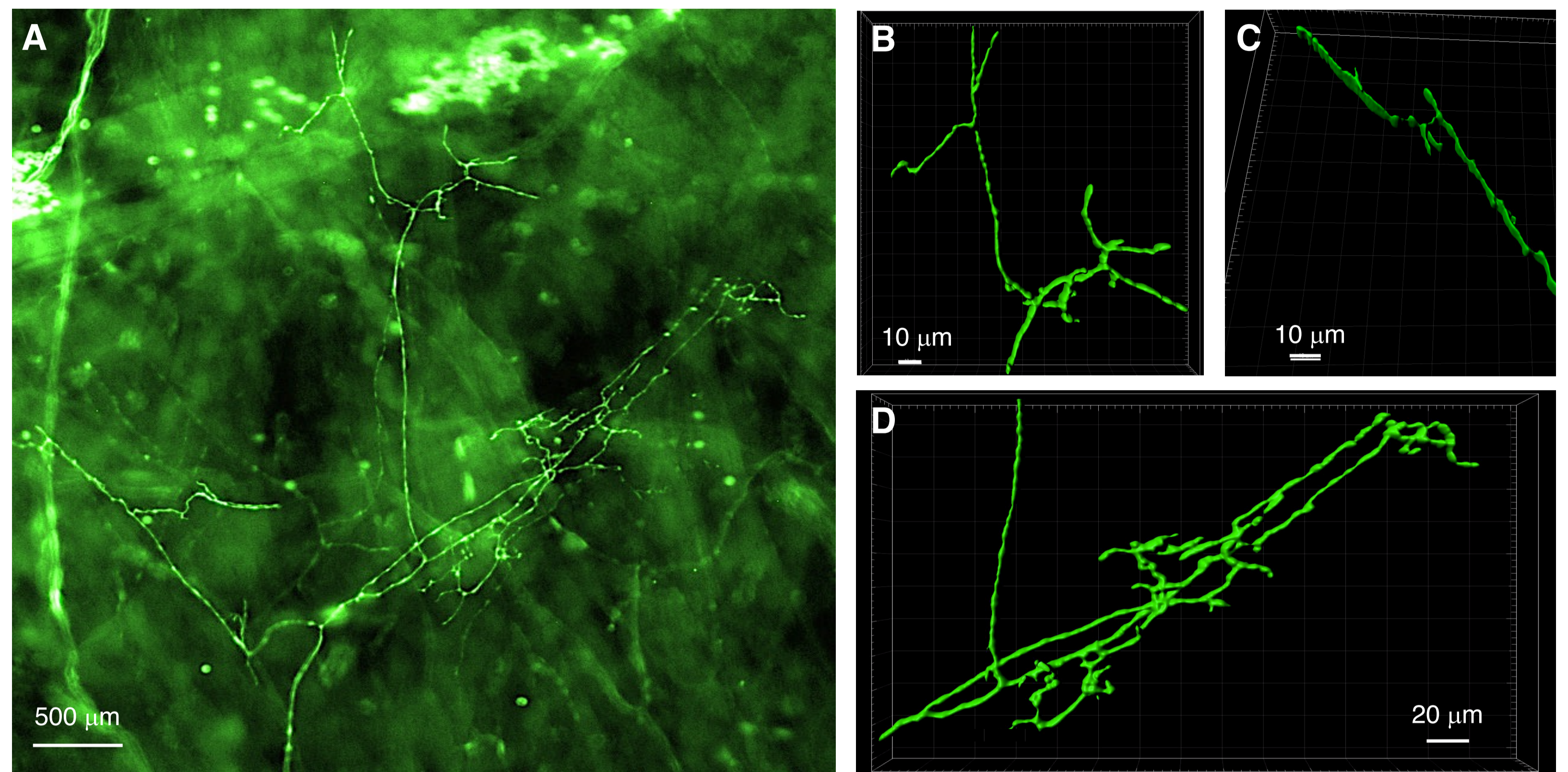
We combined anterograde labelling of pelvic and hypogastric nerves *ex vivo* and anterograde labelling from lumbosacral dorsal root ganglia (DRG) *in vivo* with immunohistochemistry for major markers of sensory (calcitonin gene-related peptide, CGRP), sympathetic (tyrosine hydroxylase, TH) and parasympathetic (vesicular acetylcholine transporter, VAcHT) nerves. In *ex vivo* anterograde tracing experiments (Zagorodnyuk & Brookes, 2000), biotinamide was applied to to nerve trunks of the vesical plexus. In *in vivo* experiments, dextran biotin was injected in L5-S2 DRG of C57BL/6 mice, and a week later was visualised with streptavidin CY3 (Spencer et al, 2018). For triple labelling immunohistochemistry, neurofilament 200 (NF200), CGRP and either vesicular glutamate transporter 2 (VGLUT2) or substance P (SP) was applied to fixed bladder tissue samples.

## REFERENCES

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- Spencer N, Greenhaigh S, Kylloh M, Hibberd TJ, Sharma H, Grundy L, Brierley S, Harrington A, Beckett E, Brookes S, Zagorodnyuk V (2018). Identifying unique subtypes of spinal afferent nerve endings within the urinary bladder of mice. *J Comp Neurol* 526: 707-720.
- Zagorodnyuk V & Brookes S (2000). Transduction sites of vagal mechanoreceptors in the guinea pig esophagus. *J Neurosci* 20: 6249-55.

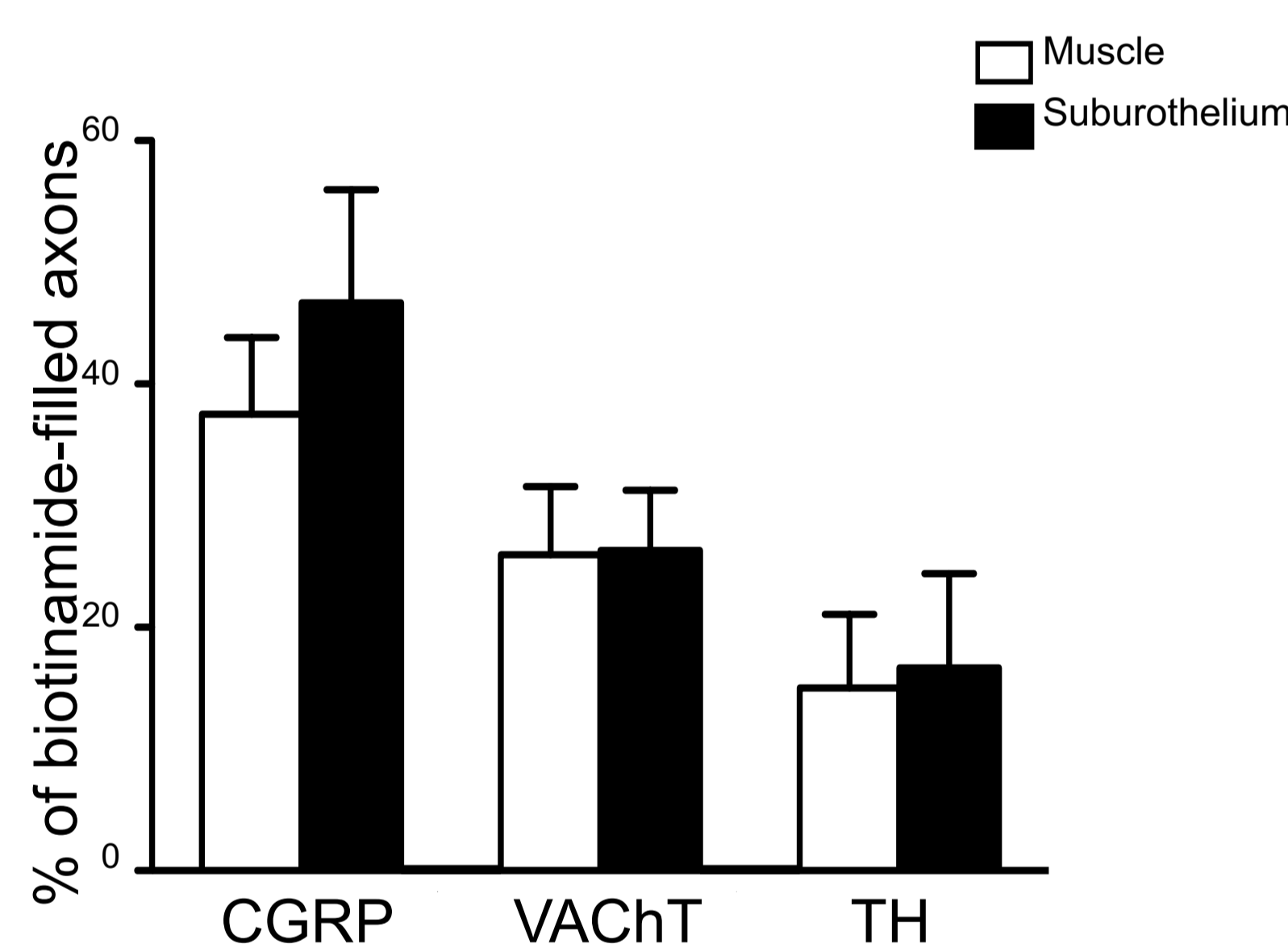
## RESULTS

### Anterograde labelling (*ex vivo*) and different types of endings in the muscle layer of mouse bladder

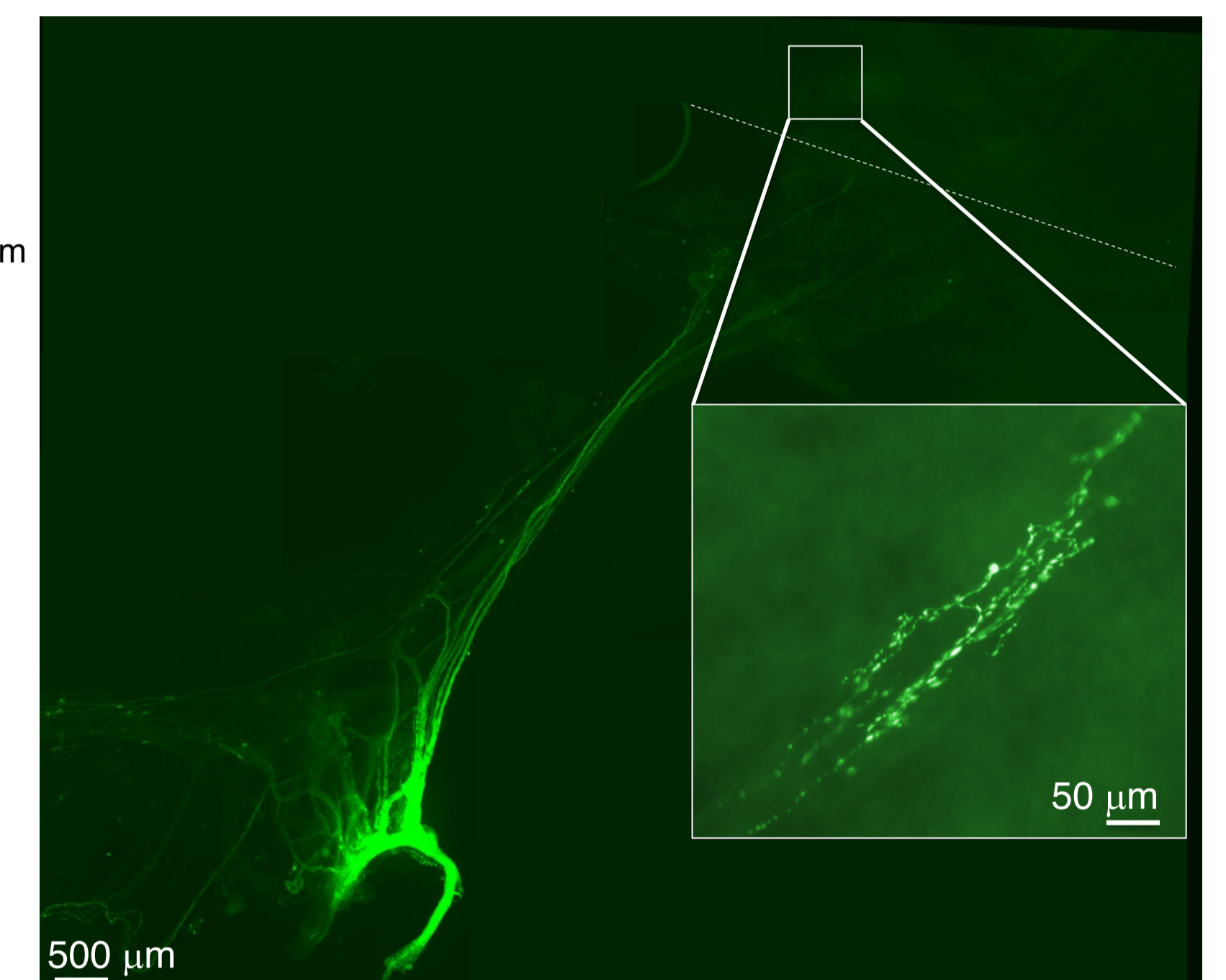


A- Fluorescence microscopic image at low magnification, showing the biotinamide labelled parent axon and the associated different types of endings in the muscle layer of the bladder. 3-D reconstruction of z-stacks using laser scanning confocal microscopy of B- simple type ending C- complex type ending and D- branching type ending as shown in A.

### Analysis of co-localisation of biotinamide labelled fibres with different markers



Group data of biotinamide-filled nerve fibres in the muscle and suburothelium layers combined with three immunohistochemical markers to identify different populations. Values are expressed as percentage of biotinamide-filled axons.  $n = 3-6$  animals



Montage of biotinamide labelled hypogastric nerve, projecting into the muscle layer of the bladder, edge of the bladder shown by the white dashed line. Expanded region in the white box shows a branching type ending in the muscle layer.

## CONCLUSIONS

- Immunohistochemical labelling was combined with anterograde labelling *ex vivo* and *in vivo* to identify extrinsic nerve endings in the bladder.
- This approach distinguished spinal afferent and autonomic efferent (motor) nerve endings in the bladder.
- Morphology alone (without immunohistochemistry), could not reliably distinguish simple and branching endings of spinal afferent and autonomic efferent neurons.