Characterization of viscerofugal neurons

A thesis submitted in total fulfilment of the requirements of the degree of doctor of philosophy

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Summary

The aim of the work presented in this thesis is to characterize enteric viscerofugal neurons. This population of neurons have their cell bodies in the gut wall and axons that project to prevertebral ganglia, via mesenteric/colonic nerve trunks. Their connections with sympathetic neurons form inhibitory reflex loops that can control gut motility and secretion.

Identified electrophysiological recordings were made from the axons viscerofugal neurons, for the first time. These were accomplished by maintaining isolated preparations of guinea pig distal colon in organ culture. Over 4-6 days, organ culture resulted in a selective loss of extrinsic nerves. This allowed unimpeded recordings of viscerofugal neurons from colonic nerves. Viscerofugal neurons were also identified in fresh preparations using the nicotinic agonist, DMPP. Sites on preparations where focal DMPP-ejections evoked bursts of firing recorded from colonic nerves were strongly associated with viscerofugal nerve cell bodies, revealed by neuronal tracing.

Recording from axons enabled detailed studies of the mechanosensory function of single viscerofugal neurons. All viscerofugal neurons were directly mechanosensitive. This implies that they are primary sensory neurons. Gut distension in the circumferential and longitudinal directions activated viscerofugal neuron firing during synaptic blockade and muscle paralysis. Studies of combined circumferential and longitudinal stretch, applied at the same time, indicated that viscerofugal neurons are sensitive to the total change in length of the gut, regardless of direction. Pharmacological stimulation of smooth muscle contractions revealed that enhancement of muscle tension, while holding gut wall length constant, did not affect viscerofugal neuron firing rate. However, enhancement of contractions under constant tension showed that firing was strongly predicted by gut wall length. Thus,

viscerofugal neurons behave like in-parallel stretch receptors, consistent with their anatomical location, in parallel to the muscle layers of the gut.

Viscerofugal neurons are also synaptically activated. Thus, they may be considered primary sensory neurons and interneurons. Nicotinic synaptic activation of viscerofugal neurons evoked bursts of firing. Sometimes these bursts involved synchronous activation of multiple individual neurons. This suggests that pools of viscerofugal neurons can be driven by a common neural pathway in the myenteric plexus. Viscerofugal neurons were sometimes strongly activated a few seconds before phasic contractions, suggesting they receive inputs from pathways that also activate enteric motor neurons.

Targeted intracellular recordings were made from retrogradely-labelled viscerofugal nerve cell bodies. All uniaxonal viscerofugal neurons were electrophysiologically S-neurons and received fast EPSPs; a single multipolar viscerofugal neuron with AH-neuron characteristics was recorded. Focal activation of myenteric neurons with DMPP revealed the location of neurons that provided synaptic inputs to recorded viscerofugal nerve cell bodies. Viscerofugal neurons equally received ascending and descending projections from cells locally (<3mm). This suggests that viscerofugal neurons receive input from multiple enteric pathways.

Immunoreactivity for choline acetyltransferase (ChAT) and nitric oxide synthase (NOS) was identified in viscerofugal nerve cell bodies. A population containing ChAT but not NOS differed from those which contained both ChAT and NOS, neurochemically, morphologically, and in their spatial distribution.

This work has advanced the detailed knowledge of viscerofugal neurons, revealing they are unique class of mechanically-sensitive interneurons.

Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Signed:

Publications arising from this thesis

Hibberd TJ, Zagorodnyuk VP, Spencer NJ, Brookes SJH (2012) Identification and mechanosensitivity of viscerofugal neurons. Neuroscience 225:118-129.

Hibberd TJ, Zagorodnyuk VP, Spencer NJ, Brookes SJH (2012) Viscerofugal neurons recorded from guinea-pig colonic nerves after organ culture. Neurogastroenterology and Motility 24:1041-e1548.

Hibberd TJ, Spencer NJ, Zagorodnyuk VP, Chen BN, Brookes SJH (2014) Targeted electrophysiological analysis of viscerofugal neurons in the myenteric plexus of guinea pig colon. Neuroscience (Accepted, in press)

Chen BN, Sharrad DF, Hibberd TJ, Zagorodnyuk VP, Costa M, Brookes SJH (2014) Neurochemical characterisation of extrinsic nerve fibres in myenteric ganglia of the guinea-pig distal colon. Journal of Comparative Neurology (Submitted)

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Figures and tables