Extracellular recording of viscerofugal neurons in guinea-pig colon

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Viscerofugal neurons have cell bodies in the myenteric plexus of the gut wall and project out of the gut to synapse with postganglionic sympathetic neurons in the abdominal prevertebral ganglia. Noradrenergic sympathetic neurons in turn project back into the gut, where they inhibit transmitter release from enteric neurons. This reflex circuit, when activated by mechanical and chemical stimulation of the intestine, causes inhibition of gut motility and secretion. Much of our current understanding of viscerofugal neurons has been deduced from intracellular recordings of cholinergic synaptic input onto symapthetic nerve cell bodies, where many viscerofugal terminals synapse. Direct extracellular recordings from axons of viscerofugal neurons, from mesenteric nerves, would make possible more detailed investigation of their physiology. However, this has not been possible to date because of the presence of spinal sensory neurons within the same nerve trunks. Previously, we have reported that maintaining preparations of guinea pig distal colon in organotypic culture for a period of 3-5 days causes degeneration of extrinsic nerve fibres which have been severed from their cell bodies. In these preparations, viscerofugal neurons and their axons survive and we were able to make direct extracellular electrophysiological recordings from identified viscerofugal axons. Our current aim was to determine whether axons of viscerofugal neurons could be recorded in mesenteric nerves in acute preparations (that had not been organ-cultured).

Methods: Close extracellular recordings from colonic nerve trunks were made from flat sheet preparations of guinea pig distal colon freshly removed from humanely killed guinea pigs. Preparations were studied *in vitro*, after removal of the mucosa, sub-mucosa and circular muscle layers. The nicotinic receptor agonist, DMPP, was ejected onto myenteric ganglia through a micropipette (5-10μm tip) using nitrogen pulses (100kPa, 10-40ms). Putative viscerofugal nerve cell body locations were identified when DMPP-stimulation of a ganglion evoked a burst of action potentials in the recorded colonic nerve. Ganglia were classified as responsive or non-responsive (to DMPP) and the results were mapped onto a printed micrograph of the preparation. The recorded nerve trunk was then filled with biotinamide and locations of viscerofugal nerve cell bodies projecting into the recorded nerve trunk were mapped.

Results: DMPP-sensitive sites were significantly associated with the presence of viscerofugal nerve cell bodies. In ten mapped preparations, 16 of 24 ganglia containing viscerofugal nerve cell bodies were DMPP-sensitive. Conversely, 158 of 162 ganglia without viscerofugal nerve cell bodies were non-responsive to DMPP. This association was highly significant ($p<0.001$). In responsive ganglia, spritzes of DMPP evoked bursts of action potentials from 100-1000ms in duration, typically of small amplitude (<200μV peak-to-peak). In many cases, single units could be discriminated with firing rates at up to 50Hz. None of the DMPP-responsive units were activated by capsaicin.

Conclusion: Single unit recordings of viscerofugal neurons can be made using standard intracellular recording techniques *in vitro*. Identified axons of viscerofugal neurons are abundant in colonic nerves and can be distinguished with high reliability by their responses to localised application of a nicotinic agonist. This result paves the way for detailed characterisation of viscerofugal neuron activity.