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# Not just there to fill space: profound observations on interstitial cells of Cajal in the gastric fundus

**Simon J. Gibbons**
*Enteric Neuroscience Program, Mayo Clinic, Rochester, MN 55905, USA*
Email: gibbons.simon@mayo.edu

Edited by: Peying Fong & Keith Sharkey

In common with other regions of the gastrointestinal tract, the stomach generates multiple patterns of motility that serve to accommodate, break down and mix food. Although these motility patterns can be controlled by an abundant innervation from extrinsic nerves, the motor responses on filling of the stomach are substantially preserved without an extrinsic nervous system. This preservation of autonomous function can be ascribed to an interesting assortment of cell types in the gastric tunica muscularis with roles that are becoming better defined as researchers map the distribution of myocytes, interstitial cells of Cajal (ICC), other mesoderm-derived mesenchymal cells (such as the platelet-derived growth factor-$\alpha$-positive (PDGFR$\alpha^+$) cells, fibroblast-like cells), macrophages and glia in relation to nerve endings from the extrinsic and intrinsic nervous system. The intrinsic innervation consists of as many as 10 subtypes of neurons based on morphology and/or chemical code but the majority of these appear to be motor neurons that alter phasic or tonic contractile responses in smooth muscle.

The gastric fundus has been a useful focus of studies on the regulation of gastric tone. In small mammals, the fundus relaxes on gastric filling and contracts as the stomach empties. It does not exhibit the sustained rhythmic contractile patterns generated by myenteric ICC in the gastric body and antrum. Furthermore, two major neurotransmitter systems in the stomach account for the bulk of inhibitory (nitric oxide-dependent) and excitatory (cholinergic) inputs to fundic smooth muscle. However, as illustrated by a lively exchange of views in a recent CrossTalk debate in these pages, the 'little brain' of the gut is not necessarily less complicated than that other brain. Indeed, it has taken several detailed studies over 20 years, to establish that a major component of neuro-muscular signalling in the gut relies on ICC and/or PDGFR-$\alpha^+$ cells as intermediaries between nerve endings and myocytes. The most recent of these, published in this issue of *The Journal of Physiology*, is a monumental and detailed demonstration by Sung *et al.* (2018) of the role of a $Ca^{2+}$ activated $Cl^-$ channel, Ano1 in cholinergic neuro-muscular excitation.

This study demonstrates that contractile responses activated by cholinergic nerves are mediated by Ano1 expressed in intramuscular ICC in smooth muscle of mouse fundus. It uses pharmacological tools and genetically modified mice to block Ano1 in multiple experiments and clearly demonstrates that muscarinic receptors on intramuscular ICC couple to Ano1, whereas muscarinic receptors on smooth muscle cells couple to non-selective cation channels with characteristics of Trpc4 or Trpc6 proteins. However, functional responses, namely fast excitatory junction potentials, or mechanical responses to electrical field stimulation in the presence of an inhibitor of nitric oxide synthesis are predominantly dependent on expression of Ano1 in intra-muscular ICC. These observations neatly identify the functional consequences of the abundant nerve terminals closely associated with intramuscular ICC in the fundus. As Ward and colleagues have previously demonstrated; these terminals express the vesicular acetylcholine transporter, express proteins characteristic of active neuronal synapses and are closely apposed to post-junctional specializations on ICC. The paper also confirms studies on mice with loss-of-function mutations in the Kit receptor tyrosine kinase that have depleted intramuscular ICC in the fundus where nitrergic (Burns *et al.* 1996) and cholinergic (Ward *et al.* 2000) junction potentials were reduced or absent. By demonstrating the role in cholinergic signalling of Ano1 on intramuscular ICC, the authors identify a robust signalling response that cannot be ascribed to direct neuro-muscular signalling, since Ano1 is expressed, if at all, at minimal levels in smooth muscle (Gomez-Pinilla *et al.* 2009) and activation by muscarinic agonists of $Ca^{2+}$ activated $Cl^-$ channels on smooth muscle cells isolated from the fundus was not detected.

In the past, this model has been questioned, by comparison with direct nerve-target signalling, either through nerve–smooth muscle junctions or by virtue of diffusion of some neurotransmitters or neuro-modulators from free nerve terminals or boutons to smooth muscle (so-called volume transmission). However, it seems highly unlikely that a major component of cholinergic neuromuscular signalling is dependent on the relatively low numbers of nerve terminals that are closer to smooth muscle cells than ICC in gastric fundus or that volume transmission due to diffusion of acetylcholine is relevant. There are circumstances under which extra-synaptic receptors play important roles in neuronal signalling throughout the body, including development, especially in a tissue like visceral smooth muscle that exhibits dynamic plasticity. Perhaps it is unsurprising that complex mechanisms maintain gastrointestinal function, considering the need for resilience in an evolutionarily conserved organ that sustaiyns nutrition and life.

In common with most studies that significantly advance a field, this study brings up new questions. The authors have demonstrated the principle that, in intact fundus, ICC are intermediaries in cholinergic neuromuscular signalling; using other genetic models to study nitrergic signalling in the colon revealed a similar but more complex result (Sanders *et al.* 2016). This raises the possibility that ICC and/or PDGFR-$\alpha^+$ cells in differing tissues integrate diverse neuronal signals in various ways to generate the assorted patterns of motility in the gastrointestinal tract. By demonstrating this complex link between nerve terminals, ICC and myocytes, the authors provoke questions about both the roles of co-transmitters and how the activity of nerve fibres might be regulated to produce, for example, receptive relaxation in an intact system. Encouragingly, there is reason to believe that we can address these questions on several levels, particularly with the development of increasingly sophisticated animal models for activating or recording from specific pathways or cell types.


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## Additional information

### Competing interests

The author has no conflicts of interest on this submission

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