Chapter 7
Extrinsic Sensory Innervation of the Gut: Structure and Function

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Extrinsic Afferents 30 Years Ago

It was known since the early 1800s that the dorsal roots largely contain sensory fibres, whereas ventral roots are primarily motor. In fact, some visceral afferents had been shown to project in the ventral roots in the 1970s (Ryall and Piercey 1970; Clifton et al. 1976; Coggeshall and Ito 1977). The first recordings of visceral afferent neurons were from vagal afferents to the stomach by Iggo and Paintal in the early 1950s (Paintal 1954; Iggo 1955). Their recordings identified a class of low-threshold, tension-sensitive afferents to the upper gut. A few years later, a distinct class of mucosal, chemosensitive vagal afferent fibres to the stomach was identified (Clarke and Davison 1978). This indicated that multiple functional classes of extrinsic visceral sensory fibres might exist, each encoding different types of mechanical and chemical stimuli. Early recordings from mesenteric nerves indicated that the spinal afferent innervation of the gut contained sensory units with properties that differed from vagal afferents (Bessou and Perl 1966). Many of the high threshold spinal fibres had branches associated with mesenteric arteries (Morrison 1973; Floyd and Morrison 1974). Further studies showed that these same fibres were responsive to hypoxia (Longhurst and Dittman 1987) and to a wide range of mediators released during damage and inflammation (Blackshaw and Gebhart 2002). Vagal

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and spinal afferent neurons were directly compared in the opossum oesophagus (Sengupta et al. 1992), showing clear differences in mechanosensitive responses, with many vagal afferents being saturating mechanoreceptors, while splanchnic afferents tended to have higher thresholds and a wider dynamic range (Sengupta 2000). A range of similar studies led to a widespread acceptance that spinal afferent pathways contain more neurons with nociceptor-like responses than vagal pathways (Berthoud et al. 2004; Beyak et al. 2006; Grundy et al. 2006; Brierley et al. 2012).

Classes of Visceral Afferents: The Last Three Decades

Studies in the last 30 years have added considerably to our understanding of the structure-function relationship of extrinsic sensory nerves to the gut. Molecular biological techniques have driven a revolution in understanding of the ion channels, receptors, second messenger systems and genetics of sensory neurons. However, this review will be restricted to a few key papers that have improved our understanding of structure-function relationships, specifically.

Vagal and Sacral Sensory Pathways

Anatomical studies in the early to mid 1990s, using tracers injected into the nodose ganglion, revealed both the morphology and extent of vagal afferent nerve endings in the gut wall (Berthoud et al. 1995, 1997; Fox et al. 2000). Systematic recordings showed that vagal mechanoreceptors are not all low threshold saturating fibres: there are also wide dynamic range endings too, at least in the oesophagus (Yu et al. 2005). The chemosensory afferents in vagus nerve have been shown to be activated by release of mediators from entero-endocrine cells (Blackshaw and Grundy 1990; Eastwood et al. 1998). Different classes of spinal afferents can be distinguished by sensitivity to distension, mucosal stroking and strong compression (Lynn and Blackshaw 1999). During this period, it was shown that there are differences in the spinal afferents that innervate the rectum (via sacral/pelvic pathways) compared to the colon (via splanchnic pathways). For example, a large population of low threshold mechanoreceptors innervates the rectum: these are much sparser in the colon and splanchnic pathways (Lynn et al. 2003). Systematic studies extended these findings, showing that there were significant differences in both mechanosensitivity and chemosensitivity (Brierley et al. 2004, 2005) of spinal afferents in pelvic and splanchnic pathways to the mouse large intestine. The upper gut and the rectum both receive prominent parasympathetic efferent innervation—from vagal and sacral pathways respectively. Similarly, both upper and lower gut are innervated by specialised afferents (from vagal and sacral ganglia) which include many low-threshold mechanoreceptors. These are strongly activated during normal physiology and presumably are responsible for vago-vagal

and sacral parasympathetic reflexes involved in gastric accommodation and defaecatory behaviours respectively.

Vascular Afferents

One specific class of spinal afferents is particularly significant: these are higher threshold sensory neurons that have endings closely associated with mesenteric blood vessels (Bessou and Perl 1966; Morrison 1973; Floyd and Morrison 1974). Immunohistochemical studies showed that these neurons (and many other nociceptor-like cells) have a distinct chemical coding, containing immunoreactivity for the neuropeptides CGRP and a tachykinin (Gibbins et al. 1985). This fitted nicely with long-established finding that sensory neurons can cause peripheral vasodilation (Bayliss 1901), via the release of CGRP (Kawasaki et al. 1988). Studies tracing the pathways of these "vascular afferents" showed that they are not restricted to mesenteric vessels—they also innervate intramural blood vessels, particularly in the submucosa (Song et al. 2009). Their endings on blood vessels are sensitive to distortion of the vessel (Humenick et al. 2015) and to distension of the gut wall; these neurons appear to function as medium-to-high threshold mechanonociceptors (Song et al. 2009). Furthermore, they often have multiple receptive fields, spread over several centimetres of bowel (Berthoud et al. 2001) with the same neuron innervating both intramural and extramural blood vessels (Song et al. 2009). This provides a firm anatomical foundation for the observation that large distensions of the bowel cause upstream vasodilation of mesenteric arteries via an axon reflex (Meehan and Kreulen 1992). These same vascular afferents are sensitive to a wide range of mediators released by inflammation and by cell damage, thus they function as sophisticated polymodal nociceptors, alerting the central nervous system about actual or potential damage to the gut wall, while simultaneously triggering a protective hyperaemia.

In many organs, including the gut, populations of sensory fibres exist that cannot be activated by conventional mechanical and/or chemical stimuli; these are so-called "silent afferents". In the gastrointestinal tract, application of mediators associated with damage and inflammation acutely cause sensitisation of many visceral sensory neurons (Su and Gebhart 1998). In some cases "silent afferents" then become mechanically sensitive (Feng and Gebhart 2011). Experimental colitis also induces chronic hypersensitivity of some classes of visceral afferents, which outlasts the period of inflammation. These include vascular afferents with "serosal or mesenteric" endings (Hughes et al. 2009). Specialised low threshold rectal afferents are not sensitised to the same degree (Lynn et al. 2008). There is also evidence that experimental inflammation chronically activates "silent afferents" at least some of which are mechanically-insensitive vascular ("serosal") afferents (Feng et al. 2012). Potentially, this may explain the hypersensitivity associated with inflammatory conditions of the bowel, since more nociceptors become capable of responding to mechanical stimuli and each nociceptor's response is exaggerated. Low-level

inflammatory mechanisms may occur in functional bowel disorders, including Irritable Bowel Syndrome (IBS) (Wahnschaffe et al. 2001; Tornblom et al. 2002; Barbara et al. 2004). Responses to inflammatory mediators are likely to be important in generating pain in these conditions.

Morphological Studies of Afferent Nerve Endings

Some of the work in our laboratory in the last 15 years has characterised structure-function relationships of extrinsic visceral afferent neurons. Using a combination of rapid anterograde tracing (Tassicker et al. 1999) and in vitro afferent recording, we have identified the structure of some visceral afferent nerve endings and transduction sites in the gut wall. Using these techniques, the low threshold vagal mechanosensors in the stomach and oesophagus were shown to correspond to "intraganglionic laminar endings" in the upper gut (Zagorodnyuk and Brookes 2000; Zagorodnyuk et al. 2001). Comparable low threshold mechanoreceptors were also described in the guinea pig rectum and shown to have similar flattened "intraganglionic laminar endings" in myenteric ganglia to those of vagal tension receptors (Lynn et al. 2003). Studies on the high threshold mechanonociceptors associated with mesenteric blood vessels characterised their endings as branching varicose axons on both extramural (mesenteric) arteries and on intramural arteries in the submucosa (Song et al. 2009). This study also showed that there are few sensory endings in either the serosal membrane or the mesenteric membranes (apart from those on blood vessels) indicating that the terms "serosal" afferent and "mesenteric afferent" are not anatomically accurate. We have also characterised the enteric viscerofugal neurons that project out the gut wall via the mesenteric nerves, where their action potentials can be recorded alongside extrinsic afferent fibres (Cervero and Sharkey 1988). They project to sympathetic ganglia (Kuramoto and Furness 1989; Messenger and Furness 1993) and, in the distal colorectum, to the spinal cord (Doerffler-Melly and Neuhuber 1988). Combining dye filling with recordings from mesenteric nerves, it was shown that action potentials of viscerofugal neurons can be recorded from mesenteric nerves (Hibberd et al. 2012b) and that the cell bodies of these neurons are mechanosensitive (Hibberd et al. 2012a). They also receive synaptic drive from other enteric neurons (Hibberd et al. 2014). Other classes of extrinsic afferents have also been characterised using these techniques, including mechanoreceptors innervating the internal anal sphincter (Lynn and Brookes 2011).

Overall, in the last 30 years, structural and functional studies of extrinsic sensory nerves that innervate the gastrointestinal tract have made considerable progress. Discrete classes of neurons that encode specific combinations of mechanical and chemical stimuli and transmit this information to the central nervous system. Whether these "labelled lines" of afferents synapse onto different classes of second order neurons in the spinal cord seems likely, but has not yet been systematically investigated. The presence of multiple classes of extrinsic sensory neurons undoubt-

edly complicates analysis of sensory signalling from the gut. However, it also raises the possibility that specific classes of afferents may be targeted by future therapeutics to modify common disorders of intestinal functions.

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