Chapter 7
General discussion

Identification of viscerofugal neurons in recordings from mesenteric nerves

Extracellular electrophysiological recordings from mesenteric nerve trunks to the gut were first made in the 1930's (Adrian et al., 1932, Tower, 1933, Gammon and Bronk, 1935). In addition to viscerofugal neurons, mesenteric nerve trunks contain axons of extrinsic sensory neurons of both vagal and spinal origin (Aldskogius et al., 1986, Wang and Powley, 2000, 2007), as well as autonomic efferent neurons (Berthoud et al., 1990, Berthoud et al., 1991, Messenger et al., 1994). Classes of vagal and spinal sensory neurons have been physiologically and anatomically characterised in the gut using correlated electrophysiological recording and neuroanatomical tracing methods. These include low-threshold vagal mechanoreceptors (Zagorodnyuk and Brookes, 2000, Zagorodnyuk et al., 2001), low-threshold rectal mechanoreceptors (Lynn et al., 2003), medium/high-threshold perivascular afferents (Song et al., 2009), and low-threshold rectal afferents in the internal anal sphincter (Lynn and Brookes, 2011). Despite some attempts (Cervero and Sharkey, 1988), positive identification of the action potentials from viscerofugal neurons in mesenteric nerve recordings had not been achieved prior to this project. This raised uncertainty whether viscerofugal neurons contribute to firing detected in electrophysiological recordings from mesenteric nerve trunks (Booth et al., 2008).

Selective degeneration of extrinsic neurons

We first demonstrated that viscerofugal neuron action potentials can be recorded from mesenteric nerves by selectively ablating extrinsic nerves in organ culture (**chapter 2**). We showed that in isolated flat sheets of colon maintained for several days in organ culture most axons in colonic nerves that did not belong to

viscerofugal neurons degenerated; probably because their axons had been severed from their cell bodies. Supporting this interpretation, immunohistochemical markers of extrinsic nerves including CGRP and TH were either absent or markedly reduced in organ cultured tissue. Capsaicin, a drug that activates the majority of spinal sensory neurons in colonic nerve trunks (Song et al., 2009), caused robust firing in fresh preparations of guinea pig distal colon, but failed to evoke any changes in firing in organ cultured preparations. Consistent with degeneration of extrinsic nerves, biotinamide labelling revealed that the proportion of viscerofugal neuron axons within colonic nerves significantly increased after organ culture. Importantly, viscerofugal nerve cell bodies could still be labelled by biotinamide after organ culture. This showed viscerofugal neurons were still intact and viable in organ cultured tissue. Extracellular electrophysiological recordings made from colonic nerves in organ cultured preparations revealed firing activity. Previous studies had shown that viscerofugal neurons express nicotinic receptors (Ermilov et al., 2003). Consistent with this, firing rates in organ cultured preparations was increased by nicotinic receptor agonists, and inhibited by nicotinic receptor antagonists. Firing rates were also increased by gut distension, consistent with recordings of viscerofugal neuron synaptic inputs to sympathetic prevertebral neurons (Crowcroft et al., 1971b, Szurszewski and Weems, 1976). Taken together, these several lines of converging evidence strongly suggest that viscerofugal neurons could be selectively recorded from colonic nerve trunks in organ cultured preparations of guinea pig colon, after extrinsic sensory axons had degenerated.

Selective degeneration of extrinsic afferent neurons has been used previously to obtain recordings from viscerofugal neurons. Cervero and Sharkey (1988) attempted

to record viscerofugal neurons in mesenteric nerve trunks in rat small intestine. They treated rats at birth with capsaicin to lesion spinal afferent fibres, theoretically leaving mostly viscerofugal neurons to be recorded. Consistent with the properties of viscerofugal neurons recorded in the present study, the units they recorded after capsaicin treatment were mechanically activated by distension and by probing, and were strongly activated by acetylcholine (6 units). However, the current neuronal tracing techniques were not available at that time to correlate neuronal structures with the electrophysiological data, thus leaving uncertain the origin of the recorded firing activity. In addition, studies have since shown that several types of vagal and spinal afferents in the small intestine may not be excited by capsaicin (Berthoud et al., 2001b, Tan et al., 2008, 2009), and are thus likely to have survived capsaicin treatment.

Identification of viscerofugal neuron firing

In the present studies, recordings from colonic nerve trunks in fresh preparations revealed action potentials from spinal sensory neurons (**chapter 3**). Spinal afferent neurons could be differentiated from viscerofugal neurons by their activation by capsaicin. A bolus of capsaicin initially excited spinal afferent firing, and then subsequently abolished it. Viscerofugal neurons in the preparation could be activated by the nicotinic receptor agonist, DMPP. The sites on the tissue at which a focal application of a nicotinic agonist evoked detectable firing in colonic nerve trunks were significantly associated with viscerofugal nerve cell bodies, filled by biotinamide applied to the recorded nerve trunk. In addition, no viscerofugal neurons identified in this way were activated by capsaicin. This is consistent with the lack of firing evoked by capsaicin in organ cultured preparations. This suggests that

viscerofugal neuron firing can be identified in colonic nerve trunks by their combined sensitivity to a nicotinic receptor agonist and insensitivity to capsaicin. In addition, we demonstrated that the location of viscerofugal nerve cell bodies may be precisely mapped using focal DMPP application.

These studies thus provided the first identified recordings of viscerofugal neurons from colonic nerve trunks. They demonstrate that viscerofugal neuron firing activity probably contributed to conventional electrophysiological recordings from mesenteric nerves (Bessou and Perl, 1966, Richards et al., 1996, Lynn et al., 2003, Rong et al., 2007, Booth et al., 2008, Mueller et al., 2009, Zagorodnyuk et al., 2012). It is possible that viscerofugal neurons also contribute to recordings from the splanchnic or pelvic nerves, as some viscerofugal neurons may project centrally (Doerffler-Melly and Neuhuber, 1988, Neuhuber et al., 1993, Suckow and Caudle, 2008). This possibility will require further investigation.

The guinea pig distal colon is an ideal region of gut to make identified recordings from viscerofugal neurons, due to the relatively large numbers of viscerofugal nerve cell bodies in this region of gut (Messenger and Furness, 1992, 1993). Furthermore, the spinal afferent innervation of the outer muscle layers is relatively sparse, with few low-threshold, capsaicin-insensitive mechanoreceptors (Lynn et al., 2003). The most common type of mechanosensitive spinal afferent neurons in distal colon are "vascular afferent" neurons (Song et al., 2009, Brookes et al., 2013). This type of neuron corresponds to mechanosensitive endings that have been referred to as "mesenteric" and "serosal" in other studies (Brookes et al., 2013). These have transduction sites on blood vessels which are most prominent in the mesentery and in

the submucosa (Song et al., 2009). It was observed that removing the mucosa and submucosa from *in vitro* preparations leaves a very sparse spinal afferent innervation; probably because most vascular afferent endings are removed (Song et al., 2009). Thus, compared to full thickness preparations, our preparations lacking submucosa had an enriched proportion of viscerofugal neurons because of the highly reduced spinal afferent nerve supply.

Several factors may have contributed to the difficulty in identifying viscerofugal neuron firing in mesenteric nerve recordings, prior to this study. First, many viscerofugal neurons had low amplitude spikes, close to the noise levels. This sometimes made them relatively inconspicuous in extracellular recordings and difficult to discriminate as single units. For example, only at 15 of 24 sites where DMPP evoked detectable firing in colonic nerve trunks could single units be uniquely discriminated from noise (**chapter 3**). Secondly, we found that the firing of viscerofugal neurons was only altered by a nicotinic receptor antagonist when their firing occurred in a burst-type pattern. Firing in bursts was more common after organ culture than in fresh preparations. The regular spontaneous firing activity seen in most viscerofugal neurons in fresh preparations was not altered by either nicotinic receptor blockade using hexamethonium nor by synaptic blockade using a Ca^{2+}-free Krebs solution. This suggests that viscerofugal neurons are driven by spontaneous suprathreshold cholinergic synaptic inputs only under certain conditions. Finally, mechanical activation of viscerofugal neurons by either von Frey hair probing or by gut distensions typically evoked modest increases in firing rate. In our studies, the largest amplitude stretches typically doubled basal firing rates. Comparable increases in the frequency of fast EPSPs during large distensions (30mmHg) were observed in

indirect studies of populations of colonic viscerofugal neurons, carried out by recording their cholinergic synaptic outputs onto sympathetic prevertebral neurons (Anthony and Kreulen, 1990, Bywater, 1993, Parkman et al., 1993, Stapelfeldt et al., 1993, Ermilov et al., 2004b).

Activation of enteric viscerofugal neurons

Direct mechanosensitivity of viscerofugal neurons

Sympathetic nerves can be activated by gut distension, leading to inhibition of contractility (Kuntz, 1940, Kuntz and Saccomanno, 1944). Intracellular recordings have been made from nerve cell bodies of postganglionic sympathetic neurons in prevertebral ganglion preparations attached to the gut via mesenteric nerves (Crowcroft et al., 1970). These revealed nicotinic fast EPSPs from viscerofugal neurons (Crowcroft et al., 1971b). Nicotinic inputs increased in frequency during gut distension (Crowcroft et al., 1971b). Synaptic or nicotinic receptor blockade applied selectively to the gut reduced, but did not abolish, distension-evoked increases in the frequency of nicotinic inputs, or acetylcholine released into sympathetic ganglia (Bywater, 1993, Parkman et al., 1993, Stebbing and Bornstein, 1993). This raised the possibility that a proportion of viscerofugal neurons might be directly mechanosensitive. However, another explanation for these observations was that nicotinic inputs might arise from collateral axons of cholinergic spinal afferent neurons (Keef and Kreulen, 1990, Sann et al., 1995a).

Our ability to positively identify single viscerofugal neurons in extracellular recordings was exploited to investigate their mechanosensitivity (**chapter 4**). Identified viscerofugal neurons were readily activated by both circumferential and longitudinal distensions in preparations of guinea pig distal colon, even during synaptic blockade with a Ca^{2+}-free Krebs solution. Furthermore, all viscerofugal nerve cell bodies mapped by focal DMPP ejection (18/18 tested) could be activated by focal mechanical compression with light von Frey hair during synaptic blockade.

This suggests that all viscerofugal neurons are directly mechanosensitive, probably at their cell bodies and/or dendrites.

Direct mechanosensitivity of viscerofugal neurons parallels similar observations in other types of enteric neurons. Mechanosensitivity has been demonstrated in Dogiel type II AH neurons (Kunze et al., 1995, Kunze et al., 2000, Mao et al., 2006), interneurons (Spencer and Smith, 2004, Mazzuoli and Schemann, 2009) and motor neurons (Mazzuoli and Schemann, 2009). AH neurons have been reported to be tension-sensitive. Interneurons and motor neurons were reported by Mazzuoli and Schemann (2009) to be rapidly adapting in response to a stimulus that distorted enteric ganglia. Viscerofugal neurons recorded in the present study more closely resembled the mechanosensory interneurons characterized in guinea pig colon by Spencer and Smith (2004). Like viscerofugal neurons, these interneurons were uniaxonal enteric S-neurons that were both directly mechanosensitive and received fast synaptic inputs. Also like viscerofugal neurons, they fired tonically during maintained stretch and their firing rates were unaffected by muscle paralysis. This is consistent with sensitivity to gut strain rather than intramural tension.

During synaptic blockade, proximal process potentials were observed in a subset of the mechanosensory S-neurons recorded by Spencer & Smith (2004). However the ion channels underlying mechanically-activated currents in these neurons have not been identified. In viscerofugal neurons, amiloride partially blocked distension evoked firing during synaptic blockade in the gut (Ermilov et al., 2004a). Amiloride blocks the mechanosensitive epithelial sodium channel (Kleyman and Cragoe, 1988, Driscoll and Chalfie, 1991, Drummond et al., 2004), and transient receptor potential

channel (TRP) C6 (Inoue et al., 2001, Welsh et al., 2002), suggesting that these, or a similar type of channel, may be molecular mechanotransducers in viscerofugal neurons. Epithelial sodium channels have been reported on myenteric neurons in human and zebrafish intestine (Yiangou et al., 2001, Levanti et al., 2011), while TRPC6 is expressed in guinea pig cholinergic and nitrergic myenteric neurons that lacked calbindin (Liu et al., 2008). Whether amiloride blocks mechanosensitive firing in mechanosensory S-neurons is unknown and would be worth testing in future studies.

Activation by both circumferential and longitudinal tissue strain

Combined evidence from indirect recordings of populations of viscerofugal neurons suggest that their mechanosensitivity was closely related to changes in intraluminal gut volume, rather than pressure (Anthony and Kreulen, 1990, Miller and Szurszewski, 1997, 2002, 2003). Based on these data, the firing behaviour of viscerofugal neurons would be expected to reflect changes in the length, rather than the tension, of the gut wall. This was fully supported in the present studies of single viscerofugal neurons (**chapter 4**). No significant differences in viscerofugal neuron firing rate were observed when intramural tension of smooth muscle was increased by a Ca^{2+} channel agonist (BAY K8644) while the length of preparations was held constant. Furthermore, there was no significant effect of muscle inhibition by nicardipine or a Ca^{2+}-free Krebs solution on stretch-evoked viscerofugal neuron firing rate – despite significantly reductions in muscle tension. In contrast, significant changes in firing were observed when the length of preparations was allowed to vary during phasic contractions of the smooth muscle while tension was held constant (under isotonic conditions). We extended these findings by showing that

mechanosensitive firing of viscerofugal neurons was not dependent on the direction of strain; both circumferential and longitudinal distensions evoked increased firing rates and the composite length/strain of the tissue showed a stronger relationship with firing than strain in either single axis.

By analogy to muscle spindle afferents in skeletal muscle, viscerofugal neurons have been described as mechanoreceptors that are 'in-parallel" to the circular muscle (Szurszewski and Miller, 1994). This was based on viscerofugal neuron firing activity that was deduced from synaptic inputs to sympathetic neurons during phasic contractions in tubular preparations of colon (Szurszewski and Miller, 1994). Indeed, viscerofugal neurons are located in the myenteric plexus, which lies in-parallel to circular muscle. However, the myenteric plexus is also in-parallel to longitudinal muscle. While the neurites of Dogiel type II viscerofugal neurons have been described as preferentially orientated in the circumferential axis (Szurszewski et al., 2002, Szurszewski and Miller, 2006), there is no evidence for preferential orientation among the uniaxonal Dogiel type I viscerofugal neurons. Uniaxonal cells constituted almost all viscerofugal neurons revealed in the present studies. Consistent with a lack of preferential orientation is our finding that viscerofugal neurons lack a directional mechanosensitivity. Rather, they were sensitive to total tissue strain, regardless of the direction applied. It remains to be determined whether Dogiel type II viscerofugal neurons show directionality in their mechanosensitivity.

The intraganglionic laminar endings in the upper and lower digestive tract similarly lack specific direction sensitivity to mechanical stimuli (Zagorodnyuk et al., 2003, Lynn et al., 2005). Like viscerofugal neurons, intraganglionic laminar endings are

located in the myenteric plexus; in-parallel with both outer smooth muscle layers in the gut. However, it is interesting that viscerofugal neurons are sensitive to changes in gut wall length, yet intraganglionic laminar endings are sensitive to tension (Zagorodnyuk et al., 2003, Lynn et al., 2005, Zagorodnyuk et al., 2005). This indicates that viscerofugal neurons differ in their interaction with the smooth muscle layers of the gut, compared to intraganglionic laminar endings. It is unclear what underlying mechanisms account for this difference. Ultrastructural studies of intraganglionic laminar endings have shown they are located superficially in myenteric ganglia, immediately beneath the basal lamina (Neuhuber, 1987, Neuhuber et al., 2006). Fibrils of collagen attach to the basal lamina, adjacent to intraganglionic laminar endings (Neuhuber, 1987, Neuhuber et al., 2006). Additionally, the plasma membranes of intraganglionic laminar endings face and extend small processes into the periganglionic extracellular matrix (Neuhuber, 1987, Neuhuber et al., 2006). Whether these features confer tension sensitivity, and the differences in responses compared to viscerofugal neurons, is not known. Ultrastructural studies of viscerofugal neurons have been performed (Feher, 1982), but their relationship with surrounding structures was not described in detail.

Viscerofugal neurons and spinal afferent mechanoreceptors

Studies of neurotransmitter release in sympathetic ganglia suggest viscerofugal neurons have a lower threshold for activation than capsaicin-sensitive spinal afferent neurons (Parkman et al., 1993, Ma and Szurszewski, 1996a, b). The colonic distension pressure required to evoke increases in acetylcholine and VIP release (from viscerofugal neuron terminals) in inferior mesenteric ganglia was less than the pressure required to evoke CGRP and SP release from vascular afferent neurons.

This suggests that viscerofugal neurons have lower thresholds for firing than vascular afferent neurons. In addition, the length sensitivity of viscerofugal neurons contrasts with the mechanoreceptive properties of spinal afferent neurons. Phasic contractions of the muscle wall strongly inhibited viscerofugal neuron firing rate. However, muscle contractions potently activate firing of low threshold vagal mechanoreceptors (Zagorodnyuk and Brookes, 2000, Zagorodnyuk et al., 2001, Zagorodnyuk et al., 2003), rectal mechanoreceptors (Lynn et al., 2003, Lynn et al., 2005, Zagorodnyuk et al., 2005), and large contractions activate vascular afferents (Brookes et al., unpublished observations). This suggests viscerofugal neurons encode different information about the mechanical state of the gut than either low-threshold spinal afferents or medium/high-threshold vascular spinal afferents. These data should facilitate interpretation of firing activity detected in mesenteric nerves to whole preparations of gut.

Synaptic activation and gut contractility

Reports that mechanosensitivity is widespread among enteric neurons blurs the distinctions between sensory neurons, interneurons and motor neurons. All uniaxonal viscerofugal neurons in the present study (**chapter 6**), and the study by Sharkey and colleagues (1998) received spontaneous or evoked fast EPSPs. Together with the observation that they were all directly mechanosensitive (**chapter 4**) indicates that viscerofugal neurons function as interneurons as well as primary sensory neurons. During intestinal distension, in vivo, it would be expected that direct mechanical excitation, as well as synaptic input, jointly contribute to activation of viscerofugal neurons. Studies in organ cultured preparations (**chapter 2**) and in fresh preparations (**chapter 4**) demonstrated that viscerofugal neurons sometimes discharge large bursts

of firing, followed by phasic contractions of the circular muscle. Importantly, these were observed in viscerofugal neurons several seconds prior to the onset of shortening. A remarkably similar pattern of firing behaviour associated with phasic contractions was reported by Miller and Szurszewski (2002, 2003). They recorded from the mouse superior mesenteric ganglion connected to segments of colon, while measuring intraluminal volume and pressure of the colon. Large bursts of synaptic input occurred several seconds prior to changes in intraluminal volume and pressure (see figure 1 in Miller and Szurszewski 2002, for example). This has been attributed to the relaxation of the intestine prior to contraction, thus increasing the length of the gut wall (Miller and Szurszewski, 2002, Szurszewski et al., 2002). We speculate these large bursts of firing may have been driven by synaptic activation of viscerofugal neurons by enteric motor pathways that subsequently cause phasic contractions.

Physiological role of viscerofugal neurons: motility

Passage of content through the large intestine occurs more slowly than in any other region of gut. In humans, transit in the large intestine is typically measured in days, compared to seconds in the oesophagus, minutes in the stomach, and hours in the small intestine (Read et al., 1986, Madsen et al., 1991, Herbst et al., 1997, Omari et al., 2011). The increase in transit times along the gut parallel the increase in the density of viscerofugal neurons. The region of gut with the most viscerofugal neurons is the large intestine (Messenger and Furness, 1992, 1993). Inhibitory reflex arcs, formed by viscerofugal neurons and sympathetic neurons, have been suggested to facilitate intestinal storage (Szurszewski et al., 2002). These circuits may raise the distension thresholds required to evoke propulsive reflexes, ultimately inhibiting

smooth muscle cells that would otherwise contract against distending luminal contents (Szurszewski et al., 2002). Consistent with this hypothesis, viscerofugal neurons in the present study were mechanically sensitive to gut wall length. Thus, as volume of the intestine increases, activation of inhibitory reflexes through sympathetic ganglia would be expected to increase, leading to inhibition of motility. Intestinal filling also leads to elongation of the gut wall, inhibiting enteric motor pathways via nitric oxide release from enteric neurons – termed the "occult reflex" (Dickson et al., 2007). This reflex would add further to intestinal storage capacity by inhibiting motility. Thus, both enteric and sympathetic reflexes may contribute to slowing transit of contents and promoting storage in the large intestine. Storage may promote water and electrolyte reabsorption, as well as microbial fermentation to take place. Obviously at some point, distension must activate propulsive enteric reflexes, leading to clearance of the content. The relative contributions of intestino-intestinal and occult reflexes to inhibition of motility along the gut is yet to be investigated, and may be important for modelling intestinal motility. We speculate that the contribution of viscerofugal neurons to intestinal storage increases with their density.

Synaptic excitation of viscerofugal neurons can arise from excitation of both ascending and descending enteric pathways along the gut (**chapter 6**). As mentioned above, strong excitation of viscerofugal neurons often preceded spontaneous phasic gut contractions (**chapter 2**). This suggests that under certain conditions, viscerofugal neurons are excited by enteric motor circuits. It is possible that enteric motor circuits can use sympathetic inhibition to limit and segment excitation of motility along the gut via connections with viscerofugal neurons. Synaptic excitation of viscerofugal neurons tended to organize their firing behaviour into bursts of

activity. Bursts of firing often arose from the synchronized activity of several viscerofugal neurons (**chapter 2** and **chapter 4**). Summation of fast EPSPs arising from bursts of firing in viscerofugal neurons may be more likely to evoke action potentials in prevertebral sympathetic neurons. In addition, the synaptically driven bursts of firing activity in viscerofugal neurons evoked firing rates up to 50Hz (**chapter 2**). In contrast, direct mechanical excitation evoked modest firing rates: up to 5-10Hz with the largest amplitude stretches studied (**chapter 3**). Slow excitatory neurotransmission in the inferior mesenteric ganglion could not be evoked by stimulating colonic nerves at lower frequencies than 10Hz (Neild, 1978). Slow neurotransmission is mediated in part by VIP and PACAP released from viscerofugal neurons, leading to persistent depolarization and repetitive firing in sympathetic neurons (Love and Szurszewski, 1987, Parkman et al., 1993, Ermilov et al., 2004b). Thus, it is possible that viscerofugal neurons release different combinations of neurotransmitters depending on their mode of activation: mechanical activation alone may lead to release of acetylcholine, while synaptic activation may lead to release of acetylcholine and neuropeptides. The latter is expected to evoke longer lasting inhibition of motility via sympathetic neurons, however this requires investigation.

Physiological role of viscerofugal neurons: secretion and blood flow

To date there have been no studies investigating whether reflexes involving viscerofugal neurons can affect blood flow to the gut. Viscerofugal neurons make projections to the sympathetic prevertebral, but not paravertebral, ganglia. Within the prevertebral ganglia, viscerofugal neuron terminals target motor and secretomotor neurons, but not vasoconstrictor neurons (Macrae et al., 1986, Anderson et al., 2002). Thus, the intestino-intestinal reflexes involving viscerofugal neurons are unlikely to

affect the blood flow to the gut directly. On the other hand, viscerofugal neurons may affect local blood flow indirectly via projections onto secretomotor sympathetic neurons, which inhibit enteric submucous neurons (North and Surprenant, 1985, Bornstein et al., 1988). These submucous neurons include the VIP-IR secretomotor/vasodilator neurons, which may control both secretion and blood flow (Bornstein et al., 1988).

The role of viscerofugal neurons in controlling secretion is also largely unknown. A single study has investigated a possible role of viscerofugal neurons in secretomotor reflexes (Quinson and Furness, 2002). This study suggested that secretion could be activated in a transected segment of jejunum by applying hypertonic solution to the proximal colon (Quinson and Furness, 2002). Viscerofugal neurons containing NOS are located in the colon, and likely to be involved in secretomotor reflexes (Costa and Furness, 1984, Macrae et al., 1986, Anderson et al., 1995). All identified viscerofugal neurons in the present study were directly mechanosensitive (18 cells, n=6), and it is likely these included NOS-IR viscerofugal neurons. Thus, it is possible that secretomotor reflexes involving viscerofugal neurons can be activated by distension, in addition to chemical stimuli. This remains to be tested directly. It is not known whether projections made to secretomotor sympathetic neurons are specific to the class of NOS-IR viscerofugal neurons. If so, we speculate that secretomotor intestino-intestinal reflexes may be initiated only from the large intestine (Anderson et al., 1995).

Physiological role of viscerofugal neurons with central projections

Small subsets of viscerofugal neurons that project into the central nervous system may form the basis of centripetal communication from the enteric nervous system. If mechanosensitivity, identified in the present study in colonic viscerofugal neurons, generalizes to those with central projections, it is possible that primary afferent signalling from the gut may arise from viscerofugal neurons in addition to extrinsic sensory neurons. Indeed, centrally projecting viscerofugal neurons in the colon have been reported in the rat (Suckow and Caudle, 2008). However little is known about the targets of these cells, limiting definitive conclusions about their functional role and whether they could possibly feed into pathways that can give rise to sensation.

In addition to colonic viscerofugal neurons, some viscerofugal neurons project via vagal nerve trunks into the dorsal motor nucleus of the vagus nerve from the stomach and duodenum (Holst et al., 1997). Viscerofugal nerve cell bodies have also been labelled from vagal nerve trunks to the oesophagus and stomach, but their targets were not identified (Zagorodnyuk and Brookes, 2000, Zagorodnyuk et al., 2001). The vagal viscerofugal neurons may represent a pathway through which enteric neurons can supply inputs to modify activity of parasympathetic neurons. However, the specific targets of viscerofugal projections into the vagus nerve have not been identified.

Rectospinal neurons are a population of viscerofugal neurons whose cell bodies are confined to the terminal 1-1.5cm of the rectum (Neuhuber et al., 1993). The nerve cell bodies of rectospinal neurons lie in myenteric and adventitial ganglia, but not submucous ganglia (Neuhuber et al., 1993). Most rectospinal neurons project into the

L_6-S_1 spinal cord, entering via the dorsal roots (Doerffler-Melly and Neuhuber, 1988, Neuhuber et al., 1993). It is tempting to speculate that this population of neurons may interact with sacral parasympathetic neurons that can lead to activation of enteric neurons (Tamura, 1997). However, their targets have not been positively identified. Their nerve cell bodies in the myenteric plexus receive ultrastructurally-identified synaptic contacts, probably from other enteric neurons (Neuhuber et al., 1993). This suggests they may be interneurons that transmit input from enteric neurons to the central nervous system. It was debated by Neuhuber and colleagues (1993) whether rectospinal neurons may serve a primary afferent or interneuronal function, given their receipt of synaptic contacts. The findings of the present study suggest these functions are not mutually exclusive, and it is possible they can do both. If so, rectospinal neurons may contribute to mechanically-evoked firing in recordings from pelvic nerves (Brierley et al., 2004, Beyak et al., 2009, Feng et al., 2010).