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## Enteric nervous system: sensory transduction, neural circuits and gastrointestinal motility

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### Abstract

The gastrointestinal tract is the only internal organ to have evolved with its own independent nervous system, known as the enteric nervous system (ENS). This Review provides an update on advances that have been made in our understanding of how neurons within the ENS coordinate sensory and motor functions. Understanding this function is critical for determining how deficits in neurogenic motor patterns arise. Knowledge of how distension or chemical stimulation of the bowel evokes sensory responses in the ENS and central nervous system have progressed, including critical elements that underlie mechanotransduction of distension-evoked colonic peristalsis. Contrary to original thought, evidence suggests that mucosal serotonin is not required for peristalsis or colonic migrating motor complexes, although mucosal serotonin can modulate their characteristics. Chemosensory stimuli applied to the lumen can release substances from enteroendocrine cells, which could subsequently modulate ENS activity. Advances have been made in optogenetic technologies, such that specific neurochemical classes of enteric neurons can be stimulated. A major focus of this Review will be the latest advances in our understanding of how intrinsic sensory neurons in the ENS detect and respond to sensory stimuli and how these mechanisms differ from extrinsic sensory nerve endings in the gut that underlie the gut–brain axis.

### Introduction

Over the past decade, there has been extraordinary interest in how the gut and brain communicate with one another, in particular with regards to the microbiome and how substances within the wall of the gut could be responsible for changes in health and wellbeing. Understanding how the gut responds to different substances in the lumen requires an understanding how the different sensory nerves are activated. Discerning how the gut detects sensory stimuli is of supreme importance to understanding the control of the gut-to-brain axis. There have been important advances in our knowledge of the different types of

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intrinsic and extrinsic sensory neurons that communicate local reflexes within the gut and outside the gut to the spinal cord and brain.

It has been known since the mid-1700s that segments of intestine isolated from vertebrates can respond to particular stimuli, despite the intestine being disconnected from the brain and spinal cord<sup>1</sup>. Surprisingly, it was not until in the mid-1990s that unequivocal evidence was presented that the enteric nervous system (ENS) contained its own population of sensory neurons<sup>2</sup> and these neurons were capable of initiating reflex responses that were mediated entirely via the ENS alone<sup>3</sup>. Over the past 5–10 years, there have been important advances in our understanding of how chemical and mechanical stimuli applied to the gut wall can elicit neural reflex responses within the ENS. There have also been major advances in our understanding of how extrinsic afferents, with sensory nerve endings in the gut wall (but their cell bodies outside the gut) respond to certain stimuli. Interestingly, studies have shown that even within the same region bowel from the same species, the mechanisms that transduce mechanical or chemical stimuli of the bowel into neural reflexes can differ substantially between intrinsic sensory neurons (in the ENS) and extrinsic sensory nerve endings in the gut, with cell bodies outside gut wall. Understanding how the gut detects sensory stimuli is of supreme importance to understanding the control of the gut-to-brain axis. In this Review, we will discuss the major advances that have been made regarding the mechanisms underlying the transduction of sensory stimuli in the gut wall into neural activity within intrinsic and extrinsic sensory nerves.

### Enteric nervous system

Of all the hollow organs in the body, the gastrointestinal tract is the only organ to have evolved with its own complete nervous system, known as the ENS, which can function fully independently of any neural inputs from the central nervous system (CNS), that is the brain and spinal cord<sup>1</sup>. Perhaps the best evidence to support the autonomous nature of the ENS is exemplified when segments of bowel are removed from vertebrates and studied in isolation *ex vivo*. Under these conditions, the intestine continues to generate complex propulsive neurogenic motor patterns, despite all extrinsic nerves being severed<sup>4–6</sup>. The notion that the gut was capable of generating reflex responses independent of the CNS was actually first identified in 1755 by Albert Von Haller, who stated that after removing the intestine from the body “*. the intestines in this state after being deprived from all communication with the brain, preserve their peristaltic motion*”<sup>1</sup> (Figure 1). His discovery was further supported 135 years later by Carl Luderitz who provided the first clear description that local stimulation of the bowel could evoke polarized responses in isolated segments of intestine<sup>7,8</sup>. About 10 years later, Bayliss and Starling then confirmed the presence of polarized neural responses in exteriorized segments of dog intestine following local stimulation, leading them to formulate the ‘law of the intestine’, that stated: “Local stimulation of the gut produces excitation above and inhibition below the excited spot”<sup>9</sup>(Fig.1). However, it was work by Paul Trendelenberg during the first World War that the now common term ‘peristaltic reflex’ was coined, which he used to describe the actual propulsion of content along isolated segments of bowel<sup>4</sup>. Since these early seminal studies, there has been much progress in identifying the fundamental control mechanisms underlying neurogenic propulsion in the small and large intestine of small vertebrates<sup>5,6,10–17</sup>, including the small intestine<sup>18</sup> and colon<sup>19</sup> of humans,

confirming that the ENS alone is capable of generating complex neurogenic motor patterns without inputs from the CNS (Fig.2).

The ENS consists of many thousands of discrete small ganglia that retain neural continuity with each other forming two distinct ganglionated neuronal plexuses, called the myenteric and submucosal plexus<sup>14</sup>. Unlike skeletal muscle which is only innervated by excitatory neurons<sup>20</sup>, smooth muscle cells of the gastrointestinal tract are densely innervated by both excitatory and inhibitory motor neurons<sup>21–24</sup>. Within each plexus lies a heterogeneous population of individual neurons with distinct neurochemical coding, projections and functional roles<sup>14,25,26</sup>. Ganglia within the submucosal and myenteric plexuses are connected to neighboring ganglia via internodal strands that carry axons over substantial distances (even up to 13 cm) to facilitate the rapid conduction of neuronal signals along the bowel<sup>27,28</sup>. There is considerable redundancy in the ENS. Loss of large numbers of enteric neurons does not necessarily lead to loss of motility or function. Mice that lose at least half their ENS can still generate rhythmic propagating neurogenic colonic motor complexes and live a normal life span<sup>29</sup>. The evolutionary process that has led to the development of the ENS has not been restricted to vertebrates. Simple invertebrates in the cnidarian phylum with radial symmetry, such as *Hydra*, evolved an intrinsic nervous system without any central ganglia (that is, a CNS)<sup>30</sup> that is clearly capable of generating propulsive peristaltic-like movements<sup>31</sup> and expelling waste.

In general, the role of the myenteric plexus is to coordinate muscle movements underlying propulsion of content, while the submucosal plexus is broadly involved in secretion and absorption<sup>14</sup>. It is presumed that the myenteric plexus fulfills a similar role in invertebrates. The importance of the ENS for life is best exemplified in vertebrates that are born with genetic mutations that lead to complete loss of enteric ganglia in the terminal colorectum<sup>32,33</sup>. A variety of animal species with a complete loss of enteric neurons over a substantial length of colorectum lack neurogenic motility patterns and have improper expulsion of colonic contents. This defect usually leads to the development of megacolon and animals commonly die shortly after birth<sup>32,5</sup>.

There has been long-term interest in generating enteric neurons in aganglionic regions of bowel of a variety of mammals born with Hirschsprung disease<sup>32,34</sup>, or in mammals that develop enteropathies associated with a deficiency in number of enteric neurons (for example, Chagas disease). In the past few years, studies have demonstrated that transplanted neural progenitor-containing neurospheres from mouse and human can integrate into the aganglionic mouse colon both *in vivo*<sup>35–37</sup> and *ex vivo*<sup>38,39</sup>. However, because of the extreme phenotype of the aganglionic mouse model used, the vast majority of the offspring died within the first 1–2 months of birth (independent of the neurosphere treatment), making it difficult to determine the potential benefits of the treatment. It has now been demonstrated that ENS progenitors from human pluripotent stem (PS) cells can be efficiently derived and isolated leading to their differentiation into functional enteric neurons<sup>40</sup>. This research showed that ENS precursors derived *in vitro* display targeted migration in the developing embryo of chicks and colonization of the large intestine of adult rodents<sup>40</sup>. Exciting *in vivo* studies have demonstrated that enteric neural stem cells can be successfully transplanted and integrated *in vivo* to restore nitrergic neurons in nitric oxide deficient mouse colon<sup>41</sup>. This

study demonstrated that transplantation of enteric neural stem cells in vivo leads to a recovery of neuronal nitric oxide synthase positive enteric neurons and a restoration of motility. These effects were associated with the development of elaborate networks of transplanted cells<sup>41</sup>. This finding was a major advance because it demonstrated for the first time that enteric neural stem cell transplantation can lead to an improvement in colonic function.

There has been some controversy regarding the life span and turnover rates of enteric neurons. The controversy arises because original studies in mice showed that neurogenesis continues postnatally through to about P21, at which point neurogenesis declines<sup>42</sup>. However, a very different concept was proposed in 2017 by Kulkarni and colleagues where the rate of enteric neuronal turnover and neurogenesis in adult mice occurs at a rapid rate, whereby >85% of myenteric neurons in the adult mouse small intestine are less than 2 weeks of age<sup>43</sup>. If this is true, this rate of neuronal turnover would require substantial cell death and a concern with this concept is that apoptosis has been shown to occur infrequently<sup>44</sup>, if at all in the developing ENS of mice<sup>44,45</sup>. The study by Kulkarni and colleagues proposed that despite ongoing neuronal cell loss (due to apoptosis), the substantial turnover and neurogenesis of adult enteric neurons means that the total number of myenteric neurons remains constant in adult mouse small intestine<sup>43</sup>. This was suggested to occur because neurons formed from dividing precursor cells located within myenteric ganglia that express nestin and p75NTR<sup>43</sup>. This study suggested there was a loss of approximately 4–5% of myenteric neurons per day, or about 30% of myenteric neurons every 7 days<sup>43</sup>. If the majority of neurons are turning over at such a high rate, it is unclear how retrograde neuronal tracing studies can visualize labeled enteric neurons many days after application of tracers to autonomic ganglia<sup>46</sup>. It is also unclear how synaptic connections between neurons and neurotransmission can persist if nerve cell bodies are turning over so rapidly<sup>47</sup>. Future studies will be required to provide a better understanding of the rates of neuronal turn over and whether similar turnover rates occur throughout the full length of gastrointestinal tract.

Just like the heart, the gut contains non-neuronal pacemaker cells which generate rhythmic electrical rhythmicity in the smooth muscle cells. These electrical oscillations in the smooth muscle are called slow waves. The pacemaker cells of the GI-tract that generate slow waves have been identified as Interstitial Cells of Cajal (ICC)<sup>48</sup>. Slow waves cause phasic contractions of the gut without any requirement of activity from enteric neurons<sup>49</sup>. It is accepted that the ENS is required for propulsion along the gut. However, according to evidence in experimental models, pacemaker-type ICC (at the level of the myenteric plexus) and the electrical rhythmicity these cells generate is not required for normal gastrointestinal function, at least in the small intestine, where they can be selectively ablated<sup>50,51</sup>. By contrast, mice that fail to develop an ENS, but still develop ICC in the aganglionic smooth muscle region die<sup>52</sup>. These mice are unable to generate sufficient propulsion or polarized contractions that could propel content and animals die soon after birth<sup>53</sup>. This research showed that while ICC at the level of the myenteric plexus (referred to as Interstitial Cells of Cajal – Myenteric, termed “ICCMY”) were important for slow-wave-mediated peristalsis<sup>49</sup>, these pacemaker cells and the rhythmic electrical depolarisations they generate were unable to take over the critical role of the ENS. Interestingly, mutant mice that lack pacemaker-type ICC at the level of the myenteric plexus and consequently lack electrical slow waves in the

small intestine live a full life span with minor, or no obvious gastrointestinal deficits<sup>50,51</sup> and still generate propagating neurogenic contractions<sup>54</sup>. Evidence exists that another population of ICC lying adjacent to the smooth muscle cells (termed ICC-IM) are important for neurotransmission<sup>55,56</sup>, although this is still controversial and some remain unconvinced<sup>57-59</sup> as excitatory<sup>60</sup> and inhibitory<sup>61</sup> neurotransmission persists in animals without ICC-IM.

With the rapid increase in interest in sensory communication within the gut and between the gut and brain axis, our understanding of sensory innervation of the gastrointestinal tract has been the focus of much attention. Since the gastrointestinal tract is the only internal organ that has evolved with its own sensory neurons there is intense interest in understanding how the mechanisms of activation of intrinsic sensory neurons differs from extrinsic sensory nerve endings in the gastrointestinal tract (Fig.3). Also, understanding the relative contribution of intrinsic versus extrinsic sensory nerves to the generation of chemosensory and mechanosensory reflex responses is of supreme relevance to the development of future therapeutic interventions to modify gastrointestinal function.

### Intrinsic sensory neurons

**Mechanotransduction**—It was first demonstrated in the mid-1700s that the gastrointestinal tract is unique, in that it can respond to sensory stimuli *in vitro*<sup>1</sup>. Other visceral organs such as the bladder, uterus and lungs rely on sensory stimuli being conveyed to the CNS, via spinal or vagal afferents to elicit motor reflexes<sup>62</sup>.

There have been important advances in our understanding of the intrinsic sensory innervation of gastrointestinal tract. That there exists a population of sensory neurons intrinsic to the gut wall had been suspected for many years. In fact, in the mid-1700s, Von Haller seems to have been the first to identify that the isolated gut could retain an ability to respond to sensory stimuli, despite losing connectivity with the spinal cord. He stated that if he touched the intestines using a knife or corrosives, the intestine responded as if it were still connected to the spinal cord and brain<sup>1</sup>. This was the first documented evidence that the gut could respond to stimuli without any apparent neural pathways between the gut and central nervous system.<sup>1</sup> However, it was not until the first intracellular electrophysiological recordings were made from enteric neurons that evidence emerged for a class of sensory neuron actually within the ENS<sup>63,64</sup>. It was not until the mid-1990s that compelling evidence was presented that some enteric neurons in guinea-pig small intestine exhibited sensory properties<sup>3</sup>. These studies initially revealed that neurons with Dogiel type II morphology were indeed responsive to chemical<sup>2,14</sup> and mechanical stimuli<sup>65</sup>. Dogiel type II neurons have distinct characteristics consisting of large cell somas and multipolar processes that ramify extensively within the myenteric plexus<sup>66</sup> and have projections both into the mucosa (Table 1)<sup>2,14,67</sup> and circumferentially, or aborally along the intestine<sup>27,68</sup>. The notion that neurons with Dogiel type II morphology could function as mechanosensory neurons was also supported by the work of Mao and colleagues in the mouse small intestine<sup>69</sup>. They showed during patch recordings from Dogiel type II nerve cell bodies that were immersed in a low Ca<sup>2+</sup> solution (to block all synaptic transmission), that these neurons generated bursts of action potentials in response to mechanical compression of

neighboring nerve fibre tracts. Their findings provided additional evidence that mechanosensation might be a ubiquitous property of Dogiel type II neurons in the ENS of different species.

While it had been thought that Dogiel type II neurons were the only intrinsic sensory neuron in the ENS, research has shown that other populations of enteric neurons with Dogiel type I morphologies can also have mechanosensory properties (Table 1)<sup>70-72</sup>. Dogiel type I neurons were originally classified as having small to medium sized cell bodies with short, broad, flat dendrites and a single axon<sup>73</sup>. Later studies identified Dogiel type I neurons as predominantly being interneurons or motor neurons<sup>14</sup>. In mouse ileum, neuronal imaging of the ENS has revealed 22% of myenteric neurons are rapidly-adapting mechanosensitive myenteric neurons (RAMENs) while in the colon they represent 15% of myenteric neurons<sup>74</sup>. In guinea-pig intestine, 45% of cultured myenteric neurons responded with mechanosensory properties<sup>74-76</sup>. RAMENs have now been shown to be prevalent in the ENS of a variety of species and these neurons encode dynamic changes in force. This was demonstrated when action potential firing frequency was found to be proportional to the deformation applied to the ganglion in which the cell soma was located<sup>74</sup>. These neurons were confirmed in the small intestine of mice<sup>74</sup>, gastric corpus of guinea-pigs<sup>76</sup> and human colon<sup>75</sup>. Notably, strain forces activated all classes of mechanosensitive enteric neurons, whereas shear stress was less effective<sup>77</sup>. On the basis of these findings, there is sound reasoning that neurons with either Dogiel type I or Type II properties can not only respond to sensory stimuli as primary afferents, but also receive synaptic inputs from other neurons. Indeed, intracellular electrophysiological studies have revealed that a population of Dogiel type I neurons (interneurons) in the myenteric plexus of guinea-pig colon are mechanically sensitive and also receive fast synaptic inputs<sup>70</sup>. Curiously, there are major differences in the mechanisms of mechanotransduction of Dogiel type II neurons in the guinea-pig small intestine, compared with Dogiel type I neurons in the large intestine of the same species. For example, in the guinea-pig distal colon, Dogiel type I neurons are largely stretch-sensitive, which continue to fire action potentials when smooth muscle tension is reduced or abolished (that is when the muscles are paralysed)<sup>70</sup>. By contrast, in the small intestine of the guinea-pig, Dogiel type II neurons are sensitive to changes in muscle tension and when the muscles are paralysed, these neurons are markedly less responsive to changes in circumferential length than Dogiel type I neurons<sup>65,78</sup>. Why the mechanisms of mechanotransduction are so different between the small bowel and colon are not clear, but could be related to the different composition of the luminal contents and the fact that largely liquid is propelled in the small intestine, whereas largely solids are propelled in the distal colon. One of the major unresolved mysteries of Dogiel type II neurons is whether rapid release of substances (like serotonin) from enteroendocrine cells can dynamically activate the terminals of these neurons that project into the mucosa<sup>67</sup>. There has been an assumption that endogenous serotonin is released from enterochromaffin (EC) cells following mucosal distortion and that this can directly activate the terminals of mucosally-projecting Dogiel type II neurons terminals. Despite it being well accepted that exogenous 5-HT can activate the terminals of Dogiel type II neurons directly<sup>79</sup>, there is no direct evidence that endogenous 5-HT released from EC cells can rapidly activate their terminals directly (Fig.4). These neurons do form a

unique sensory circuit, at least in guinea-pig intestine, that involves preferentially activating ascending excitatory interneurons and excitatory motor neurons<sup>80</sup>.

Our understanding of the mechanisms underlying mechanotransduction of intestinofugal neurons in the ENS has progressed, although their precise functional role in the body remains somewhat mysterious. Intestinofugal neurons are neurons with cell bodies in the gut wall, typically in the myenteric plexus, which have axon projections out of the gut wall to sympathetic neurons in prevertebral ganglia (Fig.2&3)<sup>81,82</sup>. When activated by mechanical stimulation of the gut, these neurons give off fast synaptic potentials<sup>82-85</sup> and sometimes slow<sup>86</sup> synaptic potentials in sympathetic neurons. The current belief is that when intestinofugal neurons are activated in the ENS, they probably function to cause increased sympathetic inhibition of gut motility, via noradrenergic neurons in prevertebral ganglia. To support this theory, there is clear experimental evidence that when one segment of colon is distended, extrinsic sympathetic reflex pathways can inhibit neighboring isolated segments of colon in an organ bath<sup>87</sup>.

In contrast to what is known about intrinsic sensory neurons in the ENS, which directly respond to chemical or mechanical stimuli without any synaptic transmission<sup>28,70</sup>, intestinofugal neurons in the distal colon<sup>81-83,85</sup> and proximal colon<sup>84</sup> are largely second order neurons that are activated indirectly (Fig.3), by fast synaptic inputs from other enteric neurons that do respond directly to sensory stimuli<sup>81-85</sup>. However, in both regions of bowel, a population of intestinofugal neurons seem to be directly responsive to mechanical stimuli, since blockade of synaptic transmission in the colon does not prevent distension-activated firing of these neurons<sup>83,84</sup>. It is known that intestinofugal neurons, at least in the distal colon of laboratory animals, are potently activated by changes in circumferential stretch, but do not respond to longitudinal stretch<sup>88</sup>. Curiously, this finding is in direct contrast to the mechanotransduction of spinal afferent endings in the colon (whose cell bodies lie outside the gut wall, in dorsal root ganglion (DRG)), which are potently activated by both circumferential and longitudinal stretch<sup>89</sup>. At present, what is known about mechanosensation of intestinofugal neurons in the colon<sup>88,90</sup> is similar to our current knowledge of intrinsic sensory neurons in the colon, in so much as they are both largely insensitive to changes in muscle tension<sup>71,88</sup>. That is, paralysis of smooth muscle contraction and reducing muscle tone or tension in the colon of laboratory animals has no detectable effect on stretch-activated firing of either class of enteric neuron<sup>70,82,88</sup>. In addition, intestinofugal neurons actually decrease their firing during muscle contraction<sup>90,91</sup>. Miller and Szurszewski suggested that intestinofugal neurons respond primarily to changes in length, rather than as tension receptors; and function to monitor changes in intracolonic volume<sup>91</sup>. This hypothesis was based on their findings that clearly demonstrated that intestinofugal neurons increased their firing with increases in circumferential length; and decrease firing with contraction, that is when intraluminal volume decreases. Again, this aspect is vastly different from spinal afferent nerve endings that innervate the same region of colon, which show increased firing of action potentials with increases in muscle tension (under either isotonic<sup>92</sup> or isometric conditions<sup>92-94</sup>). The ionic mechanisms underlying the transduction of mechanical and chemical stimuli of intrinsic sensory neurons in the ENS are not well understood. It is known that mechanotransduction of spinal afferent endings is very rapid involving direct mechano-gated channels and does not require exocytotic release of

transmitters, since mechanotransduction persists in zero calcium solution<sup>93,95</sup>, and is not blocked by conventional stretch-activated ion channel or transient receptor potential (TRP) channel blockers<sup>95</sup>. This aspect suggests that stretch-activated ion channels involve direct physical mechanotransduction. Whether the Piezo ion channel family are involved, as they are for direct mechanotransduction of somatic afferents in skin<sup>96</sup> awaits further study. Intestinofugal neurons can also be directly mechanically-sensitive and respond to Von Frey hair probing (Fig.3)<sup>97</sup>, confirming early suggestions that a population of these neurons can indeed be directly mechanosensitive<sup>83,84</sup>. The level to which these neurons participate in dynamic control of gastrointestinal motility remains unclear *in vivo*.

**Generation of neurogenic motor patterns**—There have been some important advances in our understanding of the intrinsic neuronal mechanisms and pathways that underlie distension-evoked peristalsis. Originally, in the 1950s, work from Edith Büllbring's laboratory suggested that the release of 5-HT from the mucosa was important for distension-evoked peristalsis to occur, findings that were largely based on the observation that endogenous 5-HT was released when peristalsis occurred and that exogenous 5-HT could also elicit peristalsis<sup>98–100</sup>. Indeed, since then, others had also proposed that endogenous 5-HT release from the mucosa had an essential role in the generation of colonic peristalsis<sup>101,102</sup> or colonic migrating motor complexes (CMMCs)<sup>103</sup>. Since 5-HT antagonists also could block these motor patterns in the colon of laboratory animals<sup>101,102</sup>, there seemed to be a compelling case that endogenous 5-HT was important for distension-evoked peristalsis to occur. This notion was contradicted in the past decade when a selective small molecule inhibitor of the enzyme tryptophan hydroxylase-1 (TPH-1) was given to conscious mice to selectively block synthesis of mucosal 5-HT<sup>104</sup>. This drug was found to have no effect on gastric emptying, colonic transit or overall gastrointestinal-transit times, raising doubts about earlier suggestions that mucosal 5-HT was an essential player in gastrointestinal motility<sup>104</sup>. In support of these findings, work by<sup>105</sup> showed that in conscious mice genetic ablation of the gene *Tph1* also had no changes in gastrointestinal transit *in vivo*<sup>105</sup> and CMMCs still occurred<sup>15,106,107</sup>. Real-time amperometric recordings of 5-HT release have provided powerful insights into the role of mucosal 5-HT release. Studies showed that release of endogenous 5-HT that occurred at the same time as peristalsis or CMMCs was actually a consequence of the contraction underlying peristalsis<sup>108</sup> and not the underlying cause of peristalsis or CMMCs<sup>109</sup>.

Although pharmacological or genetic blockage of mucosal 5-HT synthesis does not reduce gastrointestinal transit *in vivo*, studies have shown that inhibitors of serotonin reuptake cause a reduction in the threshold to elicit propulsive contractions in the small intestine of guinea-pigs<sup>110</sup>. It was concluded that background release of endogenous 5-HT from the mucosa alone is inadequate to modify the threshold for propulsive motor activity. However, if exogenous 5-HT was applied to the lumen, or there was increased release of endogenous 5-HT this was found to lower the threshold of propulsive contractions in the small bowel of guinea-pigs, which was mediated by 5-HT<sub>3</sub> and 5-HT<sub>4</sub> receptors<sup>110</sup>. Indeed, others had shown some time ago that mucosally-applied 5-HT reduces the threshold for peristalsis in the small intestine of guinea-pigs, that is likely mediated by the 5-HT<sub>3</sub> receptor located on the mucosal, but not serosal surface of the bowel<sup>111</sup>. This aspect was determined when 5-



HT3 antagonists affected peristaltic threshold when lumenally applied, but not serosally-applied<sup>111</sup>. These findings supported earlier studies that showed distension-evoked peristalsis and the propulsion of natural fecal content did not cease when the mucosa was removed and all dynamic release of endogenous 5-HT from the mucosa was prevented<sup>112</sup>, leading to the conclusion that the mechanotransduction process underlying distension-evoked colonic peristalsis by natural fecal pellets required only the myenteric plexus and smooth muscle layers<sup>112,113</sup>. Similar results were obtained in the small intestine, where peristalsis was still shown to occur in intact isolated preparations where the mucosa and submucosa had been dissected away<sup>114</sup>. Taken together, these findings led to the inescapable conclusion that distension-evoked peristalsis involved an intrinsic neural reflex circuit in the myenteric plexus, whose stretch receptors must have been located outside the mucosa. The only remaining possibility was that the distension-evoked circuit lay in the myenteric plexus<sup>114</sup>. Further studies in the colon showed that the mechanosensory transduction process required for activation of distension-evoked ascending excitatory and descending inhibitory nerve pathways (at least in the colon) was critically dependent upon connectivity between the circular muscle and myenteric ganglia<sup>115</sup>. Removal of the circular muscle prevented distension-evoked ascending excitatory and descending inhibitory pathways, but removal of the longitudinal muscle from the myenteric plexus did not reduce mechanotransduction<sup>115</sup>. In addition, removal of the circular muscle from the myenteric plexus abolished the vast majority of ongoing fast synaptic potentials in the ENS of the distal colon<sup>116</sup>, but removal of the longitudinal muscle did not<sup>70</sup>. One possibility is that intrinsic sensory neurons in the colon have fine nerve projections that lie in the circular muscle layer that are length-sensitive and these are destroyed when the circular layer is removed. Indeed, both Dogiel type II neurons<sup>70,117</sup> and mechanosensitive Dogiel type I neurons have been shown to have fine processes in the circular muscle<sup>70</sup>.

Compelling evidence exists that populations of enteroendocrine cells in the gut are mechanosensitive and can release high quantities of 5-HT<sup>108,118,119</sup>. However, evidence from various laboratories has shown that this release of 5-HT is not required to initiate distension-evoked peristalsis, nor does it seem to be required for normal orderly transit of content *in vivo*<sup>17,104,105,113</sup>. There is no doubt that large quantities of 5-HT can be released into the blood stream in response to distension (much of which is taken up by platelets<sup>120</sup>). However, the final concentration of 5-HT that reaches the target effector cells is unclear. A major focus of future research will be to unravel the functional role of enteroendocrine cells in living animals. Indeed, there is evidence for major changes in expression of the serotonin reuptake transporter and increased expression of mucosal 5-HT in mammals with inflammatory bowel disease and it is possible that endogenous 5-HT is largely responsible for the changes in ENS excitability only in disease states<sup>121,122</sup>.

Direct recordings from isolated enterochromaffin (EC) cells revealed they are indeed electrically excitable<sup>119,123–125</sup> and express major ion channels for the generation of action potentials<sup>108,118,119,124,125</sup>. This expression includes L-Type  $Ca^{2+}$  channels, which are essential for contraction or distension-evoked release of 5-HT<sup>108,118</sup>.

The identification of the mechanosensitive ion channels that underlie distension-evoked neurogenic motor patterns in the gut, such as peristalsis, is of major interest, in particular the

Piezo family of mechano-gated ion channels and their role in stretch-evoked reflexes in visceral organs such as the gut<sup>126,127</sup>. A study published in 2018 has shown that in guinea-pig intestine, the mechanosensitive ion channel Piezo 1 is expressed in enteric nitrergic and some cholinergic enteric neurons, whereas Piezo 2 was rarely expressed in any enteric neurons<sup>128</sup>. We wait with excitement to learn if Piezo channels are involved in the stretch-activation of peristalsis. Interestingly, (EC cells have been shown to express the mechanosensitive ion channel Piezo 2, which is located in close apposition to vesicles containing serotonin<sup>119</sup>. These studies also showed that mechanical sensitivity of a subset of isolated EC cells relies on Piezo 2 ion channels<sup>125</sup>. This finding could be important because this channel has emerged as a major player in mechanotransduction in Merkel cells in skin to modulate the conversion of touch into itch sensation<sup>96</sup>. Also, studies suggest that some population of enteroendocrine cells also make synaptic-like connections with vagal afferent endings<sup>129</sup>, very much like the Merkel cell-neurite complex in the skin. It was concluded from these studies that synaptically released glutamate is used by an epithelial sensor cell in the gut to rapidly transduce luminal stimuli to the CNS<sup>129</sup>.

There are major differences in mechanotransduction processes that convert mechanical distension of the small intestine and colon into neurogenic motor patterns. In the small intestine, the mechanisms underlying distension-evoked peristalsis require muscle tone or tension in the smooth muscles<sup>130</sup>. This is supported by direct recordings from intrinsic sensory neurons in the small intestine that display substantially reduced stretch-sensitivity following muscle paralysis<sup>78</sup> (discussed earlier). By contrast, in the colon, paralysis of tone or tension in the smooth muscle has little or no effect on distension-evoked peristaltic reflex pathways<sup>131,132</sup>, nor the propagation of peristalsis<sup>133</sup>. This finding means that the sensory neurons underlying the activation of colonic peristalsis are primarily length-sensitive, rather than muscle tension or tone-sensitive. The precise ionic transduction process by which mechanical stretch activates enteric neurons in the colon to elicit peristalsis or CMMCs is not fully understood.

## ENS circuits and motility patterns

### Chemotransduction

Although endogenous 5-HT release from the mucosa is not required for normal gastrointestinal motility or transit in healthy bowel, there is evidence that endogenous 5-HT release from the mucosa can modulate ENS activity and gastrointestinal motor patterns, via particular chemosensory stimulants applied to the lumen<sup>134</sup>. For example, in the isolated small intestine, studies have suggested that endogenous 5-HT modulates activity in intrinsic neural circuits that underlies nutrient-induced segmental contractions<sup>134</sup>. Segmental contractions are different from classic peristaltic contractions in that segmental contractions have been long proposed to involve myogenic electrical rhythmicity in the smooth muscle and consist of<sup>135</sup>. In contrast, peristalsis is critically dependent upon the ENS and cannot occur when the ENS activity is absent. Interestingly, studies have shown that segmental contractions that are induced by nutrients, at least in guinea-pig small intestine, can occur when slow waves and electrical rhythmicity are not recorded<sup>136</sup>. This finding raises serious doubts about whether myogenic rhythmicity in the smooth muscle is in fact a prerequisite

for segmentation to occur<sup>136</sup>. Evidence supporting the notion that serotonin and CCK released from the mucosa may be involved in segmentation was provided when nutrient induced segmentation (induced by decanoic acid) was found to be depressed when the 5-HT receptor antagonists (granisetron and SB-207266) and CCK antagonist, devazepide were applied to the lumen. It was proposed that decanoic acid activated a novel intrinsic pathway in which segmentation involves 5-HT<sub>3</sub> and 5-HT<sub>4</sub> receptors and CCK-1 and CCK-2 receptors<sup>134</sup>.

Potent enterotoxins, such as cholera toxin, can also potently modify gastrointestinal motility by stimulating secretomotor neurons<sup>137</sup>, leading to the release of 5-HT from enteroendocrine cells<sup>137,138</sup>. Most commonly, cholera toxin causes diarrhoea by increasing secretion of water into the intestinal lumen<sup>139</sup>. Studies have shown that cholera toxin administered into the lumen of the jejunum *ex vivo* can have opposing effects on distinct motor patterns. For example, in organ bath experiments using isolated segments of guinea-pig small intestine, cholera has been reported to enhance propulsive contractile activity, in the same preparation it can also suppress nutrient-induced<sup>140</sup>. It was also proposed that cholera toxin is able to stimulate more than one intrinsic neural pathway in the ENS and that the segmenting pattern and propulsive motor patterns are differentially regulated. Support for the notion that ENS activity can be modulated by agents such as cholera exposed to the lumen comes from direct intracellular recordings that have shown increased excitability of myenteric Dogiel type II neurons<sup>141</sup>, which was proposed to occur via a mechanism that involved NK3 receptors but not 5-HT<sub>3</sub> receptors<sup>141</sup>. A particularly interesting finding was reported when cholera toxin was infused into the colon and effects on motility compared not only between male and female mice, but also between the stages of oestrus cycle in female mice. It was found that cholera toxin immediately and reversibly inhibited CMMCs in female mice that were in the oestrus stage of cycle, but not in female mice in pro-oestrus, or in male mice. The inhibitory effects of cholera toxin on CMMC frequency seemed to be mediated by the 5-HT<sub>3</sub> receptor and EC cells, since these effects of cholera toxin were absent from female mice in which the enzyme TPH1 was genetically ablated. These investigators showed that the number of EC cells that contain 5-HT was approximately 30% higher in female mice during the oestrus stage than with female mice in the pro-oestrus stage, or in male mice<sup>15</sup>. Taken together, while mutation of the gene *Tph1* that synthesizes mucosal 5-HT does not lead to changes in gastrointestinal transit *in vivo* in mice, there is still evidence that chemosensory activation of the mucosa can lead to changes in ENS circuits via 5-HT receptors and possibly EC cells. In support of the notion that intrinsic sensory neurons can also respond to chemosensory stimuli, direct recordings from Dogiel type II neurons have shown that these neurons do respond to electrical stimuli of the mucosa<sup>142</sup> or chemical stimulation of the mucosa with 5-HT or acetate<sup>79</sup>.

**Neurotransmitters in the ENS**—Despite the large number of different neurochemicals synthesized in the different classes of enteric neurons<sup>26</sup>, most neurochemicals shown to be expressed and synthesized in the ENS have not been demonstrated to behave as neurotransmitters. It is well established that the major excitatory neurotransmitter underlying enteric neuro-neuronal (a synaptic junction between two neurons) transmission is acetylcholine acting on nicotinic receptors on enteric neurons<sup>63,64</sup>. In most species, blockade

of nicotinic transmission abolishes all fast synaptic neurotransmission<sup>17,143,144</sup>. In a smaller population of neurons, there is evidence that after nicotinic receptor blockade, adenosine triphosphate (ATP) acts on P2X receptors and serotonin acts on 5-HT<sub>3</sub> receptors to elicit fast excitatory postsynaptic potentials<sup>145</sup>. Interestingly, while glutamate is a major fast excitatory neurotransmitter in the CNS<sup>146</sup>, evidence for glutamate as a neurotransmitter in the ENS is poor<sup>147</sup>. Indeed, glutamatergic receptors are expressed in some enteric neurons, but electrophysiological studies have found the evidence for glutamate as a neurotransmitter in the ENS is weak or absent<sup>148</sup>. Interestingly, a recent study using calcium imaging from myenteric neurons in mouse colon suggested that endogenous glutamate may mediate part of the slow EPSP, particularly in neurons that express calbindin<sup>149</sup>.

In addition to fast excitatory postsynaptic potential (EPSPs) in the ENS<sup>147</sup>, there is sound evidence for the existence of slow EPSPs in myenteric neurons. ATP has been shown to mediate slow EPSPs via P2Y<sub>1</sub> receptors and there is also compelling evidence that tachykinins can mediate slow EPSPs in myenteric neurons via the NK1 receptor<sup>150–153</sup>. In the submucosal plexus, there is evidence that acetylcholine, ATP and 5-HT mediate fast synaptic transmission mediated by nicotinic, P2X and 5-HT<sub>3</sub> receptors<sup>152</sup>. In addition, ATP can mediate an intermediate and slow EPSP in some submucosal neurons<sup>152</sup> and 5-HT can also mediate some slow EPSPs in myenteric neurons via an action on the 5-HT<sub>7</sub> receptor<sup>154</sup>.

Interestingly, in addition to a role for ATP as an excitatory enteric neuronal transmitter in the ENS, ATP can mediate the fast inhibitory junction potential at the neuromuscular junction in gastrointestinal smooth muscle<sup>155</sup>. It has been argued that  $\beta$ -nicotinamide adenine dinucleotide ( $\beta$ -NAD) is the inhibitory neurotransmitter instead of ATP at neuromuscular junctions in gastrointestinal smooth muscle<sup>156,157</sup>. However, this stance has not been accepted by all groups and other explanations have been proposed<sup>158,159</sup>. For example, others have not been able to find evidence for a physiological role of  $\beta$ -NAD acting on receptors on the smooth muscle to cause inhibitory neurotransmission<sup>158</sup>. Also, others have demonstrated credible evidence that  $\beta$ -NAD can act prejunctionally by acting on adenosine A<sub>1</sub> receptors to suppress the release of neurotransmitters at neuromuscular junctions; and this could account for reduced inhibitory neuromuscular transmission<sup>158</sup>. Hence, the notion that  $\beta$ -NAD is an inhibitory neurotransmitter is equivocal.

Compelling evidence indicates that vasoactive intestinal peptide (VIP) is a major inhibitory neurotransmitter released from secretomotor neurons<sup>160,161</sup>. Indeed, evidence suggests that 5-HT<sub>1A</sub>, SST<sub>1</sub> and SST<sub>2</sub> receptors mediate nonadrenergic inhibitory postsynaptic potentials (IPSPs) in the noncholinergic (VIP) secretomotor neurons<sup>162</sup>. GABA<sub>A</sub> receptors can also modulate synaptic transmission in the myenteric plexus, which was demonstrated when calcium transients elicited by brief trains of electrical stimuli were actually enhanced by antagonists of GABA<sub>A</sub> receptors in isolated mouse small intestine<sup>163</sup>. At present, there is no direct evidence that endogenous GABA is released following electrical stimulation of the ENS to elicit a synaptic potential in myenteric neurons. Interestingly, there is evidence that approximately one third of cell bodies in the myenteric plexus that are immunoreactive to GABA are also cholinergic. However, less than 5% of cholinergic neurons also contain GABA<sup>164</sup>.

**ENS circuitry during neurogenic motility patterns**—Although it is well accepted that activity from the ENS is required for the generation of neurogenic motility patterns in the small intestine<sup>165</sup> and colon<sup>5,11,102,130,133,166–168</sup>, one of the great mysteries of the ENS has been how such a large population of individual neurons are temporally activated over large distances to cause the propulsion of content. Understanding how the ENS is physiologically activated as an intact neural network during healthy conditions is essential if we are to fully understand the aetiology of neurogenic disorders of the ENS. Important advances have been made in this area.

It has been found that there is a distinct clustering of clonally related enteric neurons in the ENS. This important observation led to the suggestion that in the small intestine the ENS develops from postmigratory neural crest-derived progenitors which form overlapping clonal units<sup>169</sup>. A major finding of this study was that it revealed that enteric neurons that are clonally-related generate synchronous activity following local stimulation of internodal strands. This suggests the presence of an underlying hard-wired circuit, such that large populations of enteric neurons can be synaptically activated at the same time (Fig.5). Consistent with this discovery, another study showed that during neurogenic CMMCs that propagated along the colon, the ENS generated a highly organized, rhythmic firing pattern that involved the simultaneous synaptic recruitment of many thousands of myenteric inhibitory and excitatory neurons at the same time<sup>170</sup>. This fundamental polarized neural circuit involved the convergence and synaptic recruitment of ascending and descending interneurons (Fig.5). This firing pattern occurred at ~2Hz that led to rhythmic depolarisations (EJPs) and hyperpolarisations (IJPs) in the smooth muscle at the same rate<sup>170</sup>. As the faecal content had propelled along the bowel, past the site of imaging, these neurons became temporally less correlated into a ‘desynchronized state’<sup>17,170</sup>. Another study using neuronal imaging and tracing from the large intestine have shown topographic differences in the enteric neuronal wiring patterns between the proximal and distal colon<sup>171</sup>. An ascending inhibitory myenteric pathway exists in the proximal colon, which was driven by nicotinic transmission. Interestingly, this phenomenon was not identified in the distal colon<sup>171</sup>. Taken together, there is sound evidence for local changes in the neuronal circuits along the full length of colon which may be related to the differences in motility requirements of these different regions.

**Emerging technologies to study ENS neural circuits**—Optogenetics was first developed about 15 years and is an exciting technique to control the excitability of particular cells of interest<sup>172,173</sup>. However, only in the past 5 years or so has there been a rapid increase in the use of this technique with high fidelity. The basic principle of optogenetics is that light-sensitive ion channels are genetically encoded into specific cells of interest. This means that it is possible to excite or inhibit cells (e.g. neurons) of interest using different wavelengths of light, provided the cells of interest express the excitatory or inhibitory light-sensitive ion channels. One of the most exciting benefits of using optogenetics is the ability to selectively stimulate or inhibit particular neurochemical classes of neurons. This was not possible with electrical stimulation of the ENS. Indeed, this benefit is highly relevant to studying the ENS, because one of the major obstacles to study ENS neural circuits is that the ENS contains a highly heterogeneous population of neurons that are difficult to be

selectively targeted with conventional electrophysiological and imaging techniques. Advances in optogenetic techniques have provided unique tools for *in vivo* stimulation or inhibition of neuronal electrical activity, which is an efficient way to probe causal relationships between specific neuronal cells and behavior<sup>172,173</sup>. Optogenetic techniques have been extensively utilized in the CNS for a number of years<sup>174</sup>. But, only in the past 3 years has the field of optogenetics been demonstrated to control the excitability of the ENS<sup>17,34,175–177</sup>. To date, there have been two studies that have successfully demonstrated optogenetic technologies can potentially control activity in the ENS<sup>34,175</sup> and induce propagating neurogenic contractions in mice<sup>175</sup>.

One of the most exciting aspects of applying optogenetic technology to the ENS is the conspicuous advantage of being able to selectively stimulate or inhibit specific classes of neurons that lie only within the ENS. This can occur using cre/lox recombination technology which involves site specific recombination of light-sensitive ion channels (like Channelrhodopsin, ChR2) into specific neurochemical classes of enteric neurons<sup>17,175–177</sup>. This advance has been demonstrated using wireless optogenetic approaches whereby excitatory neurons were activated with micro-LEDs implanted onto the colon wall *in vivo* in mice, to cause an increased transit of colonic content in conscious freely-moving animals<sup>175,178</sup>.

There are some advantages and some disadvantages of using optogenetic tools to stimulate gastrointestinal transit in live animals (Box 1). The major advantages of using wireless optogenetics to stimulate gastrointestinal motility *in vivo*, as opposed to current orally-ingested agonists to stimulate the ENS (for example, 5-HT agonists) are that: focally applied light can be delivered instantaneously to target specific regions of gut, without requiring oral consumption of non-specific agonists that inadvertently stimulate other neural pathways in central or peripheral ganglia (Box 1). In *in vitro* experimentation using transgenic mouse models or Cre-inducible viruses, optogenetic approaches could provide a major advantage of being able to control ENS excitability via either excitatory or inhibitory light-sensitive ion channels, in specific neurochemical populations of neurons. This technical advance is a major step forward in the field. This approach would be impossible using conventional pharmacological agents, that do not act on only one class of neuron.

There are also some obvious downfalls using optogenetic techniques to control the ENS in live animals. Firstly, light-sensitive opsins must be expressed in the ENS. This step requires either the injection of viral vectors into the host animal, or the breeding of transgenic animals together. In either case, it involves genetically modifying specific cells of interest in the ENS. A second major disadvantage is that light is required to stimulate enteric neurons. Prolonged light stimulation can raise the temperature of the target organ, which has been shown to clearly alter function<sup>179</sup>.

A particularly exciting aspect of optogenetic technology is that it has now been demonstrated that it is possible to express sufficient levels of channelrhodopsin 2 (ChR2) in sensory neurons of non-transgenic vertebrates<sup>180</sup>, which was demonstrated using adeno-associated viruses that can be transported retrograde along nerve axons and effectively express ChR2 into nerve cell bodies. Conceptually, it could be possible to control gut

motility and transit in conscious non-transgenic mammals using wireless optogenetics after injection of adeno-associated viruses into the gut wall. However, to do so would require overcoming the technical challenge of implanting wireless micro-LEDs onto the gut wall and ensuring that sufficient levels of Chr2 are expressed in the particular classes of neurons of interest. This approach would almost certainly have to involve surgery and the pros and cons would need to be carefully evaluated before any procedure(s) were undertaken.

## Conclusions

This Review has discussed the current advances that have been made in the transduction of sensory stimuli into action potentials in intrinsic and extrinsic sensory neurons in the small and large intestine of vertebrates. There is substantial interest in improving our understanding the basis of gastrointestinal motility disorders and transit in a variety of conditions, such as slow-transit or idiopathic constipation, irritable bowel syndrome, or as occurs in inflammatory bowel disease. However, before we can hope to try to explain the causes of disordered neurogenic motility and transit in diseased or dysfunctional bowel, it is important that we first have a clear understanding of the fundamental control mechanisms underlying these neurogenic motor patterns in healthy bowel. This aspect has not yet been achieved. With the rapid improvements and advances in optogenetic techniques, it will be exciting to use these approaches to control the excitability of specific classes of neurons in the ENS to carefully dissect their functional roles.

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**Box 1****Pros and cons of optogenetics to control the ENS.****Advantages**

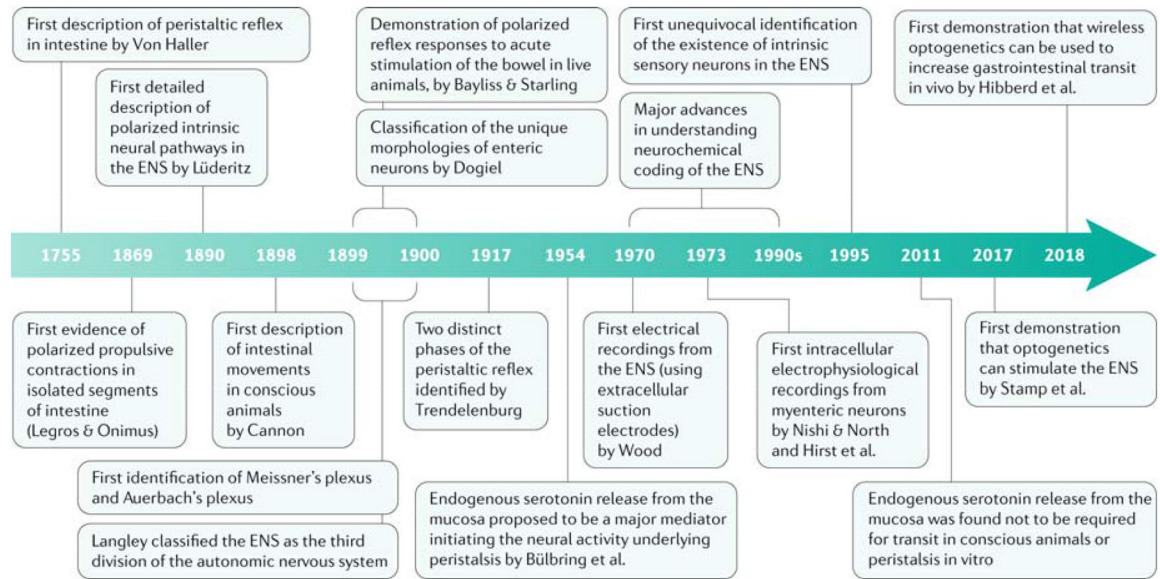
- Allows selective expression of light-sensitive ion channels in specific classes of neurons
- Light can instantaneously activate nerve pathways of interest
- No oral ingestion of agonists required to selectively stimulate specific populations of neurons
- Can identify the functional role of specific neural circuits

**Disadvantages**

- Light delivery generates heat, which can alter neuronal function
  - Requires genetic modification of cells
  - Takes many weeks to express light-sensitive ion channels
  - Low yield of expression can occur using viruses
  - Not clear how long opsins can retain expression in vivo
- ENS, enteric nervous system.

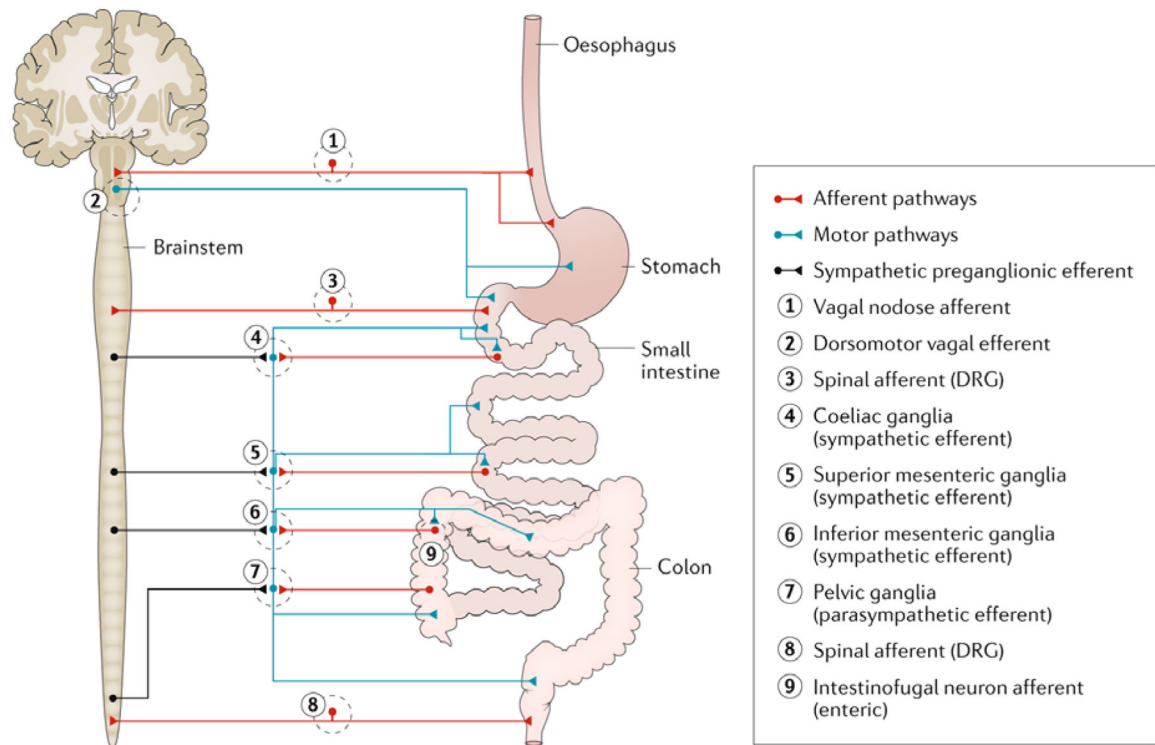
### Key Points

1. In vertebrates, the enteric nervous system (ENS) is critical for gastrointestinal function
2. There has been much progress in understanding the mechanisms by which mechanical or chemical stimulation of the gut is converted into neural activity with the ENS and propulsive motility.
3. Mechanosensory elements critical for distension-evoked colonic peristalsis have been identified to lie in the myenteric plexus and/or circular muscle of the gastrointestinal tract and do not require the mucosa or submucosal plexus.
4. Evidence suggests that substances released from cells in the mucosa (such as enteroendocrine cells) can modulate ENS activity, but release of mediators like serotonin are not required for distension-evoked peristalsis nor colonic migrating motor complexes.
5. Fundamental differences have been revealed in the mechanisms of activation of extrinsic spinal afferent nerves compared with intrinsic sensory nerves in the same region of bowel.
6. Recent refinements in optogenetic technologies can now permit stimulation of specific neurochemical classes of neurons in the ENS to elucidate function.



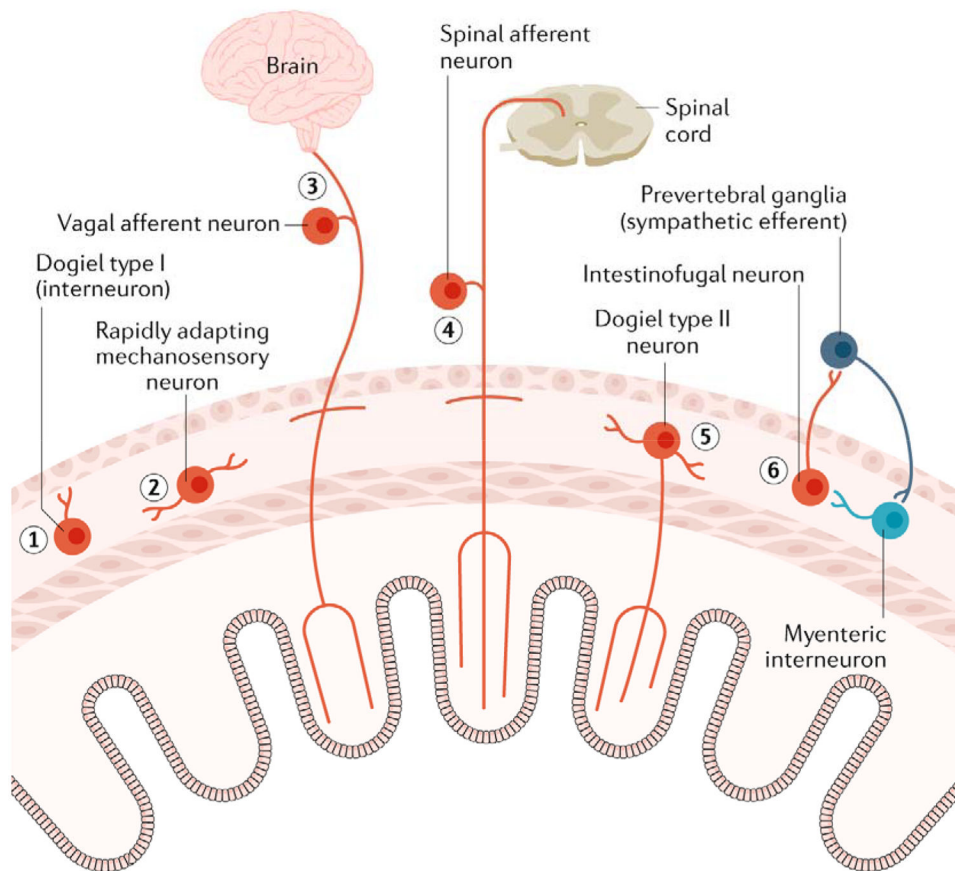
**Fig. 1: Key developments in the enteric nervous system.**

The figure shows the timeline of the major advances made in our understanding of the enteric nervous system (ENS) and the identification of motility reflexes. The first evidence of a reflex response in an isolated segment of the gut was provided in 1755 by Von Haller<sup>1</sup>. It was not until 1995 that the first unequivocal evidence that the ENS contained its own population of sensory neurons was presented<sup>2</sup>. For Cannon, Legros & Onimus and Langley see refs<sup>181,182,183</sup>



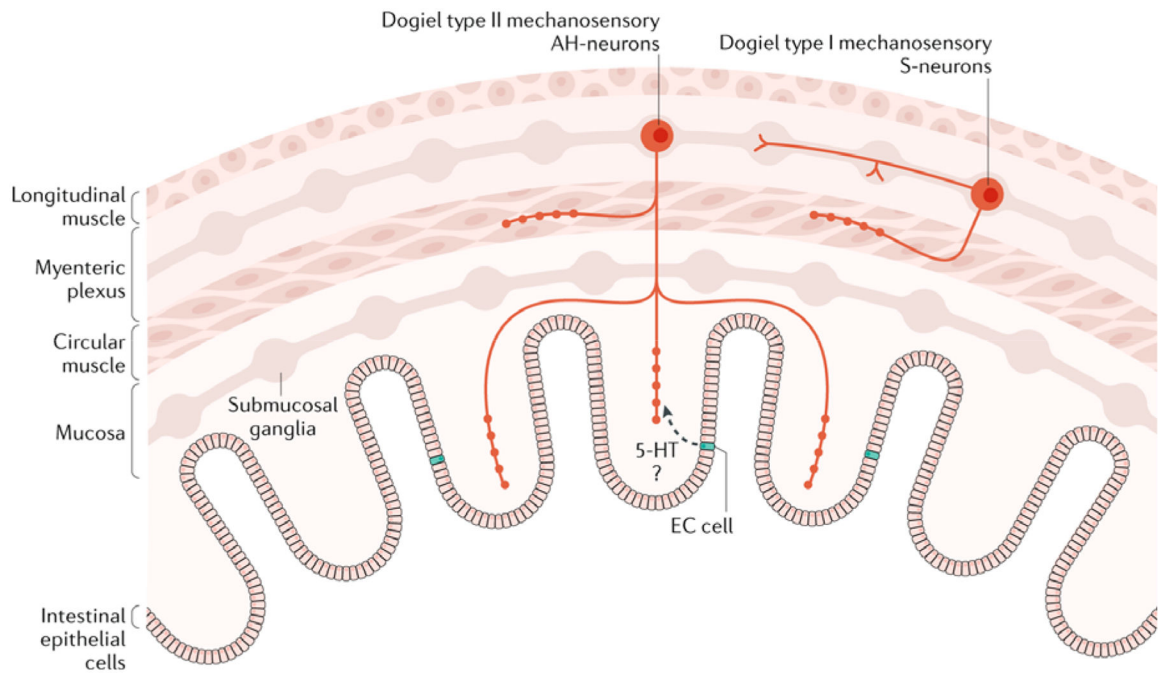
**Fig. 2: The major extrinsic neural pathways between the ENS and spinal cord and brain.**

The major extrinsic motor pathways between the enteric nervous system (ENS) and spinal cord and brain are shown in blue. These motor pathways constitute the parasympathetic (vagal motor) and sympathetic nervous systems (arising from the thoracolumbar spinal cord and synapsing on the coeliac, superior mesenteric ganglia and inferior mesenteric ganglia). In the gut, the sympathetic nerves are inhibitory, whereas the parasympathetic nerves are excitatory. In the upper gut (oesophagus and stomach), the major extrinsic sensory nerves arise from the vagus nerve, whereas in the lower gut (colon), the influence of the vagus is reduced and the major extrinsic sensory nerves to the colon arise from spinal afferent nerves, whose cell bodies lie in dorsal root ganglia (DRG).



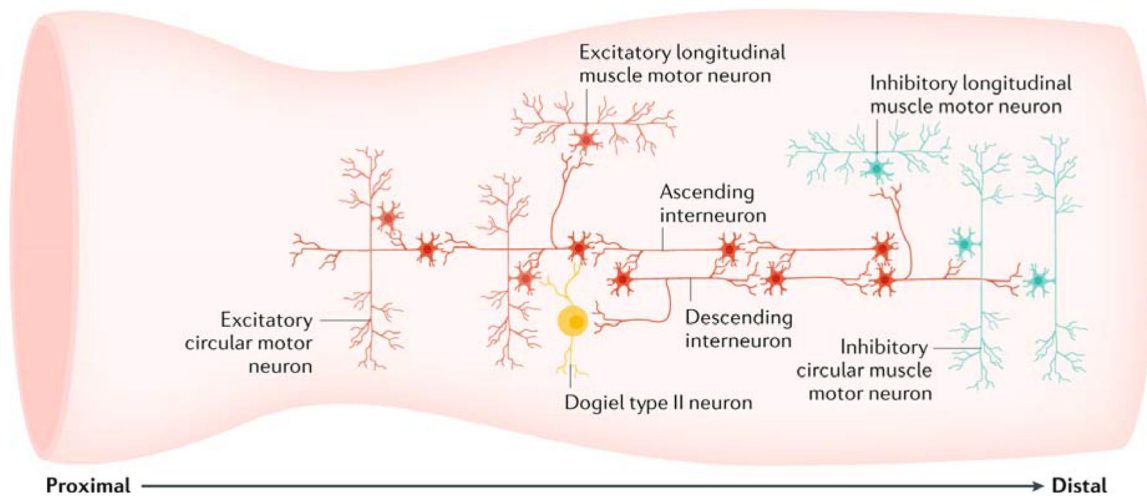
**Fig. 3: Different types of intrinsic sensory neurons and extrinsic sensory nerve endings in the enteric nervous system.**

A range of intrinsic sensory neurons and extrinsic sensory nerve endings are known to exist in the enteric nervous system (ENS). (1) Dogiel type I neurons represents a class of myenteric interneuron in the colon that have been identified as being largely length sensitive and tension insensitive. (2) At least two classes of cholinergic and nitrgergic myenteric neurons in the myenteric plexus have been demonstrated to be rapidly adapting myenteric excitatory neurons. (3) Extrinsic vagal afferent nerve endings innervate largely the upper gut and behave predominantly as slowly adapting tension receptors. (4) Spinal afferent nerve endings provide a very rich sensory innervation to the lower gut (distal colon) and are potently activated by stretch and increases in muscle tension. (5) Dogiel type II neurons in the myenteric plexus are both chemosensory and mechanosensitive and receive fast and slow synaptic inputs from other enteric neurons. (6) Intestinofugal neurons are usually thought of as second-order neurons but have been shown to be directly mechanosensitive and respond to direct mechanical compression stimuli.



**Fig. 4: Possible mechanisms underlying the activation of two major classes of intrinsic sensory neuron in the ENS.**

Dogiel type I neurons are mechanosensory and have been functionally identified as interneurons. These neurons also have fine nerve projections into the circular muscle, which could facilitate stretch sensitivity and connectivity with the circular muscle layer. Dogiel type II neurons have projections into the mucosa. They also have been shown to have fine varicose projections into the circular muscle layers. The functional role of enterochromaffin (EC) cells in the physiological activation of the nerve endings of these intrinsic sensory neurons is unknown but serotonin (5-hydroxytryptamine; 5-HT) could have a role.



**Fig. 5: Major intrinsic neuronal circuits in the large intestine active during neurogenic motor patterns.**

A fundamental pathway involves the synaptic convergence of ascending and descending interneurons, such that increased activation of ascending pathways leads to a corresponding increased activation of descending inhibitory pathways. Furthermore, common ascending interneurons simultaneously activate independent populations of excitatory motor neurons to the longitudinal muscle and circular muscle orally; at the same time, common descending interneurons simultaneously activate separate populations of inhibitory motor neurons to both the longitudinal muscle and circular muscle aborally. Thus, temporally coordinated firing of ascending and descending interneurons simultaneously activates cholinergic excitatory motor neurons and nitrenergic inhibitory motor neurons. These pathways underlie major neurogenic motility patterns in the large intestine. In the colon, calbindin-immunoreactive Dogiel type II neurons project to the mucosa and receive nicotinic inputs from some populations of myenteric interneurons. The Dogiel type II neurons (in the colon) preferentially provide synaptic outputs to local cholinergic excitatory motor neurons and cholinergic ascending interneurons, and rarely or never to nitrenergic neurons<sup>80</sup>.

**Table 1**

Comparison of two major types of enteric neurons.

Characteristic	Enteric neuron type	
	S neurons	AH neurons
Exhibit prominent Fast EPSPs	Yes	Less common
Exhibit slow EPSPs	Yes	Common
Projections into the mucosa	Rare or never from the myenteric plexus	Yes, common from the myenteric plexus
Mechanosensory properties	Yes	Yes
Chemosensory properties	None known	Yes
Morphology	Dogiel type 1	Dogiel type 2
Functional classes	Intrinsic sensory neurons, interneurons and motor neurons	Intrinsic sensory neurons and possibly some interneurons

Table shows the major differences in the characteristics of Myenteric S neurons and AH neurons in the myenteric plexus. EPSP, excitatory postsynaptic potential.