

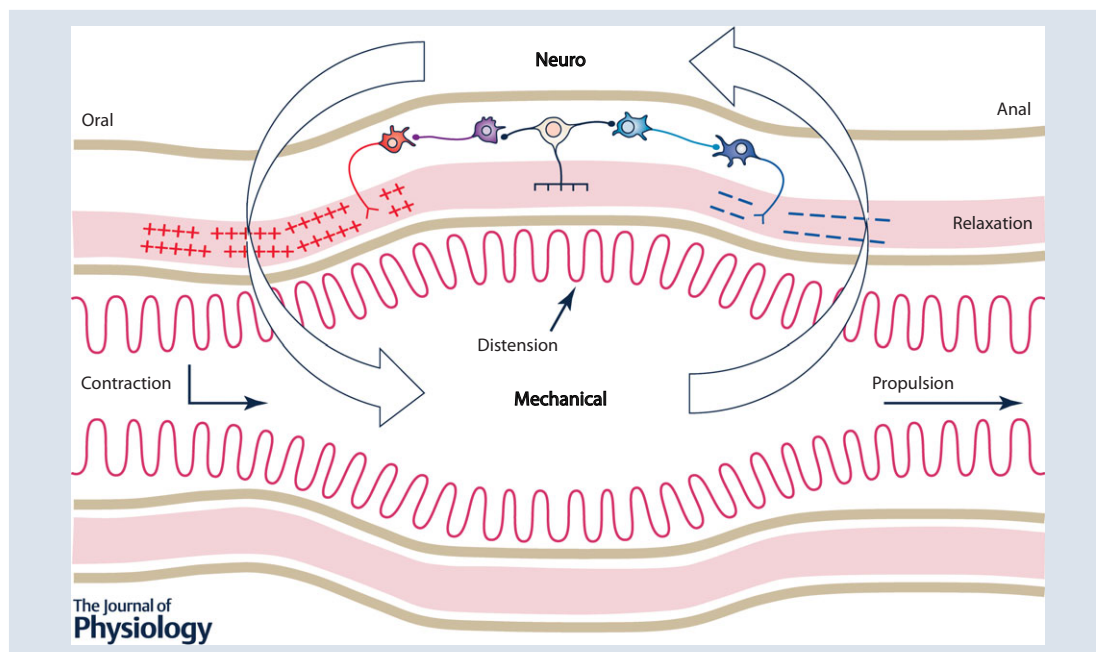
TOPICAL REVIEW

Insights into the mechanisms underlying colonic motor patterns

Nick J. Spencer¹, Phil G. Dinning^{1,2}, Simon J. Brookes¹ and Marcello Costa¹

¹Department of Human Physiology and Centre for Neuroscience, Flinders University of South Australia, Adelaide, Australia

²Departments of Gastroenterology and Surgery, Flinders Medical Centre, Adelaide, Australia



Nick Spencer completed his PhD in 1998 at Monash University, Australia in the field of enteric neurogastroenterology. After his PhD he spent 10 years at the University of Nevada School of Medicine, investigating neuronal mechanisms underlying gastrointestinal motility, where he obtained independent NIH funding. Nick has published >80 peer-reviewed research publications and in 2015 was promoted to Professor. Nick has had continuous NH&MRC funding since returning to Australia in 2008. **Phil Dinning**



is senior clinical scientist in the Department of Gastroenterology and Surgery at Flinders Medical Centre, Adelaide, South Australia. He has over 20 years' experience related to the physiological investigation of human lower gastrointestinal disorders. **Simon Brookes** studied insect nervous system for his PhD at Bristol University before moving to the London Hospital Medical College, then Flinders University where he has studied the nerves and motility of the gastrointestinal tract. He has studied primarily the enteric nervous system and the sensory innervation of upper and lower gut. He is currently the Head of Department of Human Physiology at Flinders University. **Marcello Costa** was born in Italy where he obtained his medical degree. He moved to Australia in 1970 to a postdoc position at the Department of Zoology in Melbourne, then a foundation lecturer in the School of Medicine at Flinders in 1975, where he established one of the first multidisciplinary laboratories in neurogastroenterology.

Abstract In recent years there have been significant technical and methodological advances in our ability to record the movements of the gastrointestinal tract. This has led to significant changes in our understanding of the different types of motor patterns that exist in the gastrointestinal tract (particularly the large intestine) and in our understanding of the mechanisms underlying their generation. Compared with other tubular smooth muscle organs, a rich variety of motor patterns occurs in the large intestine. This reflects a relatively autonomous nervous system in the gut wall, which has its own unique population of sensory neurons. Although the enteric nervous system can function independently of central neural inputs, under physiological conditions bowel motility is influenced by the CNS: if spinal pathways are disrupted, deficits in motility occur. The combination of high resolution manometry and video imaging has improved our knowledge of the range of motor patterns and provided some insight into the neural and mechanical factors underlying propulsion of contents. The neural circuits responsible for the generation of peristalsis and colonic migrating motor complexes have now been identified to lie within the myenteric plexus and do not require inputs from the mucosa or submucosal ganglia for their generation, but can be modified by their activity. This review will discuss the recent advances in our understanding of the different patterns of propagating motor activity in the large intestine of mammals and how latest technologies have led to major changes in our understanding of the mechanisms underlying their generation.

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Corresponding author N. J. Spencer: Department of Human Physiology, School of Medicine, Flinders University, South Australia, Australia. E-mail: nicholas.spencer@flinders.edu.au

Abstract figure legend The mechanism by which distension of the wall of the colon activates intrinsic neurons to generate peristalsis and the propulsion of colonic contents. Latest findings reveal that the mechanosensory enteric neurons (located in the myenteric plexus) have essential mechanosensitive nerve endings in the circular muscle. Distension or stretch of the colon activates these sensory neurons to initiate polarized neural pathways that result in oral contraction and anal relaxation. These pathways do not require the mucosa but can be modulated by sensory nerve endings that project into the mucosa. The polarized enteric circuits form the bases of a neuromechanical loop which ensures that polarized enteric neural circuitry can efficiently propel content with a wide range of physical properties.

Abbreviations CMMC, colonic migrating motor complex; DCMMC, distal colonic migrating motor complex; HAPCs, high amplitude propagating contractions; ICCs, interstitial cells of Cajal.

Colonic motility

The large intestine in all mammalian species performs a number of functions essential for the optimal handling of ingested material. Water, ions and bile salts are also extracted from the content, and storage, formation and expulsion of faeces occurs normally in a controlled fashion suited to the physico-chemical composition of the contents. The colonic microbiome ferments some indigestible materials, releasing nutrients (largely short chain fatty acids) that are absorbed and thus increase to a small degree the efficiency of digestion. The final common effectors of motility are longitudinally and circularly aligned smooth muscle cells. However, the key component of physiological motility is the temporal and spatial coordination of smooth muscle contractions and relaxations. These controlled movements of the intestinal muscle involve two fundamental mechanisms. One is responsible for the spontaneous 'myogenic' activity of the muscle and the second involves complex enteric neural circuits embedded within the gut wall that are influenced

by the extrinsic innervation of the large bowel. In humans, lesions to extrinsic nerve pathways or spinal cord damage leads to abnormal colonic motility and often constipation (Anderson, 2004; Coggrave & Norton, 2010), which can be improved by stimulation of defaecation centres in the spinal cord (Ferens *et al.* 2011; Ellis *et al.* 2015). Over the past three decades significant advances have been made in revealing the bases of these mechanisms and the findings suggest strongly preserved fundamental features across all mammalian species.

The identity of enteric neurons forming simple polarized reflex pathways has been studied in detail in several species (Fig. 1A). The major transmitter substances of excitatory and inhibitory motor neurons have been identified and the properties of intrinsic mechanosensitive enteric neurons analysed in detail (Bornstein *et al.* 2004; Spencer & Smith, 2004; Brookes & Costa, 2006; Costa & Brookes, 2008; Furness, 2012; Mazzuoli-Weber & Schemann, 2015). In parallel, an early study showed that the proto-oncogene *c-kit* and the tyrosine kinase receptor that it encodes, Kit, are expressed by pacemaker cells in

the gut wall and are essential for myogenic pacemaking functions (Maeda *et al.* 1992). This led to identification of several types of interstitial cells of Cajal (ICCs). Some of the ICCs function as the pacemakers and generate and coordinate myogenic rhythmicity. Some ICCs act as intermediaries in motor neuron to smooth muscle neurotransmission (see reviews by: Farrugia, 2008; Huizinga & Lammers, 2009; Sanders *et al.* 2014).

While the cellular components of these two systems have been individually studied in detail, how they interact to coordinate motility has only recently been actively explored at the whole organ level. Contraction at any point in the bowel can either propel content, or impede its progress, depending on the mechanical state of neighbouring regions. The dynamic nature of the movements at every point along the gut renders analysis of the movements of the intestinal wall and the luminal

content a significant challenge in biomechanics. Although understanding the basis of coordinated motility is in its infancy, the reality is that in all species contents in most cases appear to be propelled at a suitable rate with an appropriate degree of fluid absorption. The intrinsic neural and myogenic mechanisms operate in conjunction and extrinsic neural and hormonal influences do play critical roles.

Discoveries, techniques and methodologies that have advanced understanding of colonic motility

Two technical developments have also led to improvements in our understanding of intestinal motility. First, the description, display and analysis of complex patterns of motility recorded from isolated sections of intestine have been facilitated by the development of

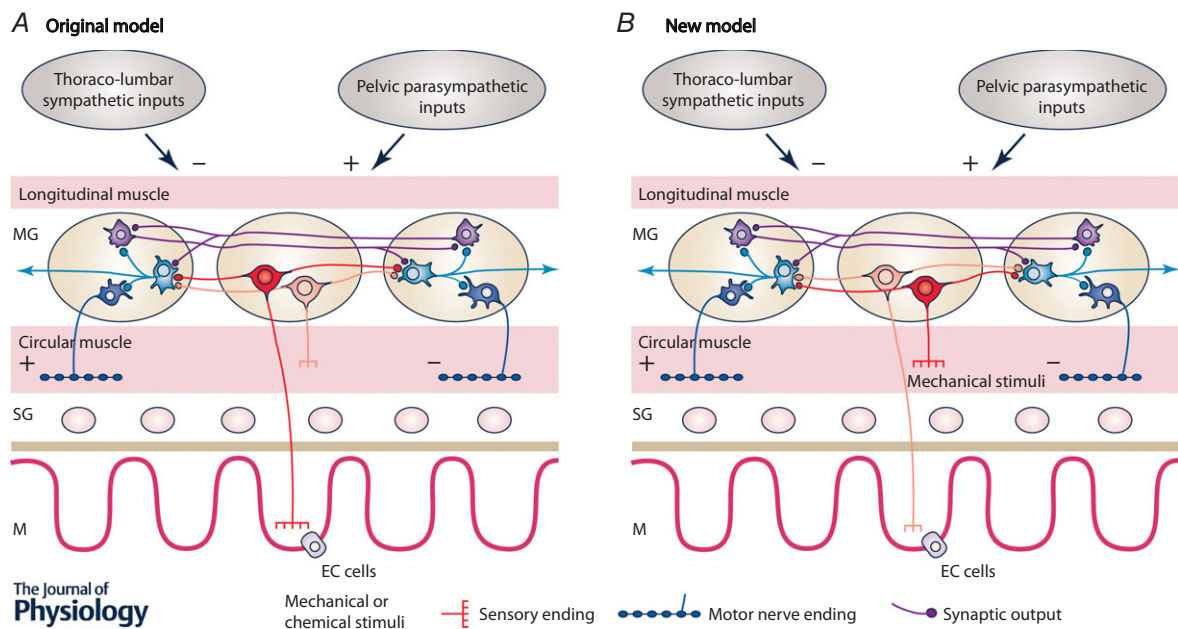


Figure 1. Originally proposed enteric neural pathway underlying distension-evoked reflexes (A) compared with latest understanding of this neural pathway (see B)

A, Originally proposed arrangement of enteric neural pathways in the intestine. Mucosally projecting sensory neurons (in red) respond to chemical and mechanical stimuli and are responsible for initiating the peristaltic reflex. Mechanosensory interneurons respond to circumferential stretch and have transduction sites in the circular muscle. They can initiate polarized neural reflexes to the circular and longitudinal muscle in the distal colon. Possible other enteric circuits of interneurons generate migrating motor complexes. Extrinsic inputs from sympathetic and parasympathetic pathways inhibit and excite motility by acting on the enteric circuits. Current knowledge of submucosal neurons and longitudinal muscle have been deliberately omitted to maintain simplicity. B, The new model describes how our understanding of these pathways has changed in recent years. We propose a refinement of the enteric circuits involved in colonic motility. The mechanosensory enteric neurons (located in the myenteric plexus) have essential mechanosensitive nerve endings located in the circular muscle (see Spencer *et al.* 2006) (now shown in red) which initiate polarized neural pathways that result in oral contraction and anal relaxation. These pathways do not require mucosal inputs and represent the bases of the neuromechanical loop responsible for propulsion adapted to the consistency of contents. However, stimulation of mucosal sensory nerve endings can initiate and modulate enteric neural activity. The polarized enteric circuits involved in the neuromechanical loop are modulated by underlying cyclic neural activity initiated by any maintained distension and providing the bases of the migrating motor complexes. Extrinsic excitatory inputs from both pelvic and vagal sources are involved in the greater or lesser permissive role depending on the degree of central control of colonic movements. EC cells, enterochromaffin cells, with permission.

spatio-temporal maps. Changes in intestinal diameter are measured from high resolution video imaging of the intestine. Maps can be constructed that detail the changes in diameter (DMaps) in real time, along the length of the isolated intestinal segments (Bouchoucha *et al.* 1999; Hennig *et al.* 1999; Bercik *et al.* 2000). Comparable maps can be created for longitudinal muscle contractions. These maps provide a simple, readily interpretable visual summary of motility over periods of seconds to many minutes, with automated quantitative analysis becoming increasingly feasible. Using these video-imaging methods detailed description of motor patterns have been made on isolated colon preparations of several experimental animals (Hennig *et al.* 1999; D'Antona *et al.* 2001; Lentle *et al.* 2008; Huizinga *et al.* 2011; Costa *et al.* 2013a).

A second major technical advance has been the development of high resolution manometry, for recording motor patterns in the human colon *in vivo*. For the most part these recordings have been made using fibre optic manometry catheters with 10 mm spacing between the 72 and 120 pressure sensors. This has increased the spatial resolution of intraluminal pressure sensing by almost an order of magnitude compared to many older catheters (Arkwright *et al.* 2009). Studies have shown that many previous colonic manometry studies, using more widely spaced sensors, misinterpreted a large proportion of propagating motility events (Dinning *et al.* 2013).

Combining these two technologies, high resolution manometry with spatio-temporal mapping of diameter, in isolated sections of intestine has also provided a valuable type of analysis (Dinning *et al.* 2011). The separate pressure traces of high resolution recordings can be interpolated to create spatio-temporal maps of pressure along isolated segments of intestine. These PMaps have been combined with DMaps to detail the relationships between changes in diameter and changes in intraluminal pressure, again represented as 2-dimensional maps (DPMs) (Costa *et al.* 2013b). The application of simplified principles of muscle mechanics allows the dynamic state of the intestinal smooth muscle to be determined at each point along DPMs (i.e. whether muscle is actively contracting or relaxing, or passively shortening or lengthening, during motility patterns (Fig. 2; Costa *et al.* 2013b). All of this has added to an accurate measurement and display of the repertoire of colonic motility patterns and an understanding of how some of the patterns are generated.

Mechanisms of propulsion of colonic contents

One simple insight from this methodology was the demonstration that the propulsion of a bolus in the isolated rabbit colon was due to an area of active muscle contraction occurring at the oral end of the bolus and an active relaxation at the anal end (Dinning *et al.* 2014b).

These processes could be blocked by tetrodotoxin (TTX), suggesting that the active contractions and relaxations require the activity of excitatory and inhibitory enteric motor neurons, respectively. These results are a more detailed investigation of the polarized enteric pathways demonstrated by Bayliss & Starling (1899) that were shown to be responsible for propulsion of luminal contents. The existence of polarized enteric neural pathways capable of mediating ascending excitation and anal inhibition of the circular muscle has been demonstrated convincingly in several species, using localized mechanical stimuli (Crema *et al.* 1970; Costa & Furness, 1976; Grider, 1989; Spencer & Smith, 2001) (Fig. 1A).

'The Neuromechanical Loop hypothesis'

In the isolated colon of experimental animals, inserting natural or artificial faecal pellets into the oral end of the preparation evokes propulsive circular muscle contractions, after a short delay (Costa & Furness, 1976; Kadowaki *et al.* 1996; D'Antona *et al.* 2001; Spencer *et al.* 2011; Sia *et al.* 2013). This is driven by a contraction oral to the pellet by activation of polarized enteric neural pathways as described above.

Content-dependent neural propulsion initiated by liquid or pellet distension has been reported in guinea-pig, dog, cat, sheep and rat, and have been variously called 'peristaltic contractions', 'peristaltic waves', 'giant contractions' or 'giant migrating contractions', 'rhythmic propulsive motor complexes', 'long distance contractions', 'antegrade propagating long distance contractions', or 'mass peristaltic events' (Crema *et al.* 1970; Sarna *et al.* 1988; Bedrich & Ehrlein, 2001; D'Antona *et al.* 2001; Gonzalez & Sarna, 2001; Hipper & Ehrlein, 2001; Lentle *et al.* 2008; Dinning *et al.* 2012a; Chen *et al.* 2013; Costa *et al.* 2013a). Thus the propulsive motor patterns generated by the neuromechanical loop could be described generically as 'neural peristalsis' to distinguish them from migrating motor complexes or even possible myogenic peristalsis.

Importantly, in the colon of guinea-pigs, fluid infusion generates repeated peristaltic propulsive contractions that propagate at much higher speeds than solid pellets (Costa *et al.* 2015). Thus, the consistency of the contents influences the rate of progression of the circular muscle contraction along the colon. This simple observation indicates that a peristaltic contraction is not a simple reflex (which would happen in a stereotyped, invariant fashion). Rather, it is a more complex mechanism that adapts to the luminal content. Bayliss & Starling (1899) proposed that polarized reflexes are the basis of propagating contractions. A bolus activates ascending excitation and descending inhibition which cause movement of the bolus aborally. From its new location, it activates another bout of polarized reflexes, causing further propulsion. We

have extended this proposal into the ‘neuromechanical loop’ hypothesis (Costa *et al.* 2013*b*; Dinning *et al.* 2014*b*). This hypothesis suggests that distension by luminal material activates polarized reflexes (as Bayliss and Starling proposed). However, the physical consistency of the material influences how it is redistributed by the contractions and relaxations of smooth muscle in the gut wall. This, in turn, affects the pattern of distension along the gut and thus modifies the subsequent re-activation of polarized enteric neural pathways. In this way, polarized reflex pathways and mechanical factors, including distributed distension, form a dynamic functional loop, which adapts gut motility to deal effectively with a wide range of contents (ranging from fluid to solid pellets of various dimensions).

How does the ‘neuromechanical loop’ work?

The ‘neuromechanical loop’ hypothesis predicts that the consistency, shape and size of the luminal contents should influence the speed of propulsion. This relationship has been experimentally demonstrated in the isolated guinea-pig colon (Costa *et al.* 2015). Using boluses of various lengths and diameters, a clear relationship between surface area and speed of propulsion was demonstrated (Costa *et al.* 2015; Fig. 3). This relationship probably reflects the number of enteric mechano-sensitive neurons activated by the bolus. The larger the surface area, the more sensory neurons are simultaneously activated and the greater activation of polarized ascending excitatory and descending inhibitory pathways. This leads

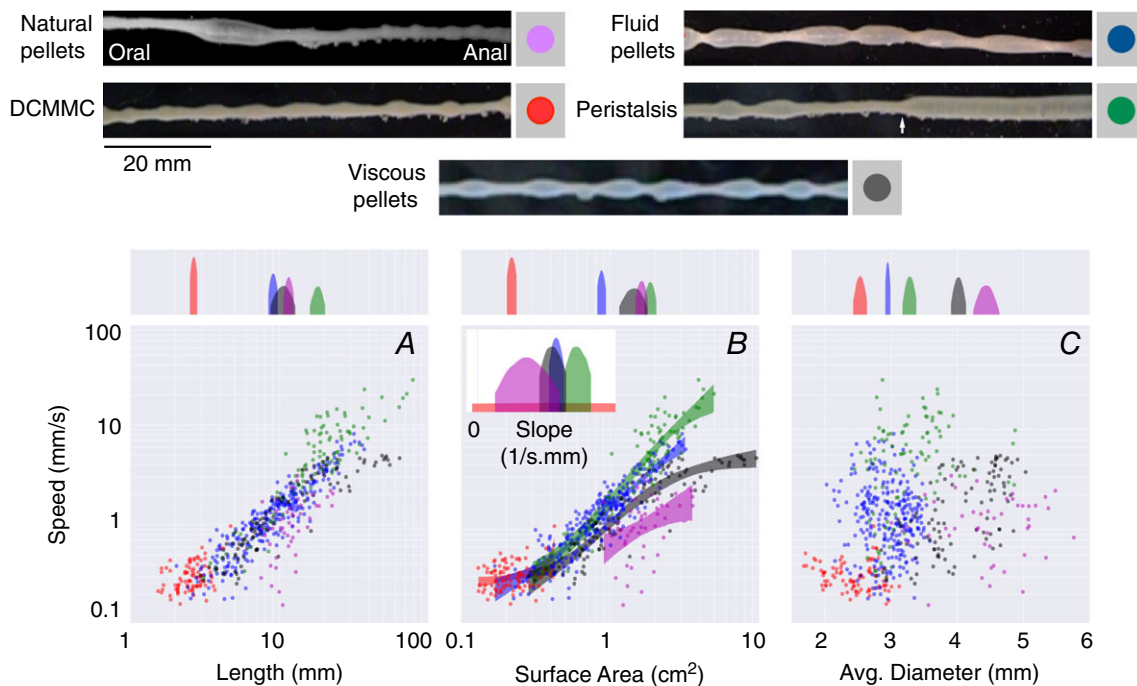


Figure 2. Propulsion of luminal contents is modified by differences in the consistency of luminal contents

In the upper half of the figure, five different images are shown where boluses of different sizes and shapes (pellets) are present in the distal colon. Natural pellets are represented in purple, distal colonic migrating motor complexes (DCMMCs) in red, fluid pellets in blue, viscous pellets in grey, and peristaltic contractions in green. It was noted that peristaltic contractions often started approximately half-way along the preparation (white arrow). A–C shows the relationships between speed of propulsion and the bolus size; speed correlates positively with bolus length (A); speed in relation to bolus surface area (B); speed in relation to average bolus diameter (C). In the upper quadrant of each graph (A–C) the probability density is represented on a log scale. The distributions of means are shaded within their 95% highest density interval (HDI, see text) and the gaps between shaded 95% HDIs represent significant differences between the various measures. Note that for the average diameter (C) there is a clear separation between all of the shaded 95% HDIs, indicating that the diameters of different boluses are significantly different from one another. However, a poor correlation exists between the speed of propulsion and average diameter. For surface areas (B) from about 1 cm², the speeds of all bolus types (except DCMMCs) differ significantly, as shown by the gap between their shaded 95% HDIs (see centre square), with natural, viscous, liquid and peristaltic liquid boluses moving at increasing speeds. The inset in B depicts the distributions of the steepest slope of the sigmoid curve for each bolus type. A shows that a significant overlap exists between the length of the natural, viscous and fluid pellets. DCMMCs (shown in red) are shorter than all other bolus types and peristaltic contractions (shown in green) are significantly longer than all other bolus types. Figure reproduced from Costa *et al.* (2015), with permission.

to a consequential increase in the amplitude of oral contraction and anal relaxation, causing a high speed of propulsion. According to the neuromechanical loop hypothesis, increasing the fluidity of contents should increase the speed of propulsion. Indeed natural solid pellets are propelled at a slower speed than viscous material or liquid (Fig. 3). The speed of propulsion is constrained by influences that impede movement of the content. Increasing the diameter of a faecal-shaped pellet beyond a limit, or increasing the load against which propulsion operates, slows the velocity at which contractions propagate (Costa *et al.* 2015). These observations

support the existence of a neuromechanical loop which ensures that simple enteric neural circuitry can efficiently propel content with a wide range of physical properties. It may also explain some of the variability of colonic motor patterns studied under varying conditions in different laboratories.

Neurally dependent cyclic motor activity in the colon

There is considerable evidence for a neurally generated cyclical motor pattern (colonic migrating motor complex; CMMC), which is less dependent on content than

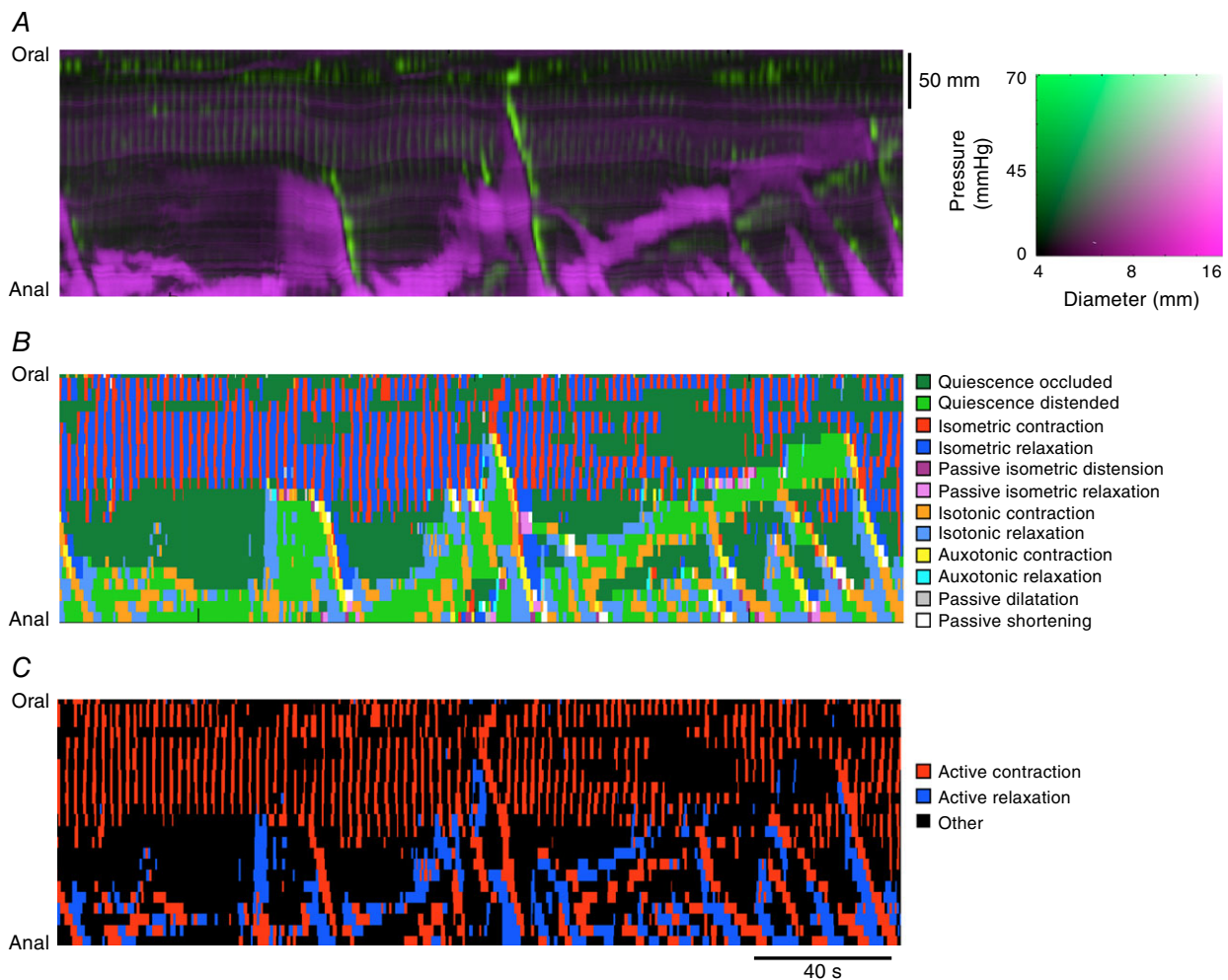


Figure 3. Spatio-temporal map of the steady states of linear and quiescent orbits

This was constructed from the composite DPMs (shown in A) and the extracted orbits that were formed from the independent variables (diameter and pressure) (see B). In B each of the 12 possible mechanical states are mapped in different colours. The map portrays the periods of quiescence either when the intestine remains passively dilated (light green) or passively occluded (dark green). Red, orange and yellow areas represent active contractions and mark the propagating area of contraction during neural peristalsis. Active relaxation (aqua and light blue) precedes both in time and space the propagating contraction. Myogenic activity in this particular example consists of isometric relaxations and contractions. The last map (C) represents a simplification of the full composite map of states by clustering all areas undergoing active contraction (red) and active relaxation (blue). All other passive states are in black. The spatio-temporal nature of active contractions and relaxations during neurogenic activity and myogenic activity is made very distinct. Figure reproduced from Costa *et al.* (2013b).

neural peristalsis. CMMCs occur in the colon of many experimental animals. They have been reported *in vivo* in rabbits and sheep (Ehrlein *et al.* 1982; Bedrich & Ehrlein, 2001), dogs (Sarna, 1986), and *in vitro* in guinea-pigs (Costa & Furness, 1976; D'Antona *et al.* 2001; Costa *et al.* 2015), rabbits (Lentle *et al.* 2008; Dinning *et al.* 2012a) and mice, where the most detailed studies on the CMMC have been performed (Fida *et al.* 1997; Brierley *et al.* 2001; Powell & Bywater, 2001; Spencer & Bywater, 2002; Roberts *et al.* 2008; Spencer *et al.* 2013). These complexes can migrate either orally, aborally, or appear simultaneously along regions of colon (Sarna *et al.* 1984), suggesting that their direction of propagation is not hardwired into the enteric nervous system. They do not appear to be lumen-occlusive and in the rabbit and guinea-pig proximal colon, where they have been correlated with the actual profile of the colonic wall, they result in indentations that begin to separate soft faecal material into pellets (Lentle *et al.* 2008; Dinning *et al.* 2012a; Costa *et al.* 2015).

Recent studies have challenged the idea that CMMCs occur entirely independently of content. In many studies of CMMCs, tension or length transducers were attached to the gut wall to record the complexes; the resting tension applied by the transducers may constitute an effective stimulus to drive the initiation of CMMCs. Without transducers, CMMCs actually occur infrequently and propagate over shorter distances, with slower propagation velocities (see Fig. 4) (Barnes *et al.* 2014). Certainly, distension can activate premature CMMCs (Zagorodnyuk & Spencer, 2011). This remains a fertile area of research that requires a combination of recording at both cellular and organ level to identify the mechanisms of initiation of this cyclical enteric neural activity.

Neural mechanisms underlying generation of peristalsis and CMMCs

Considerable work has been carried out analysing the site of initiation of content-dependent neural peristalsis and of CMMCs. By removing layers of the gut wall of preparations of guinea-pig (Spencer *et al.* 2011) and mouse large intestine, *in vitro*, (Keating & Spencer, 2010) it has been shown that the neural circuits responsible for both neural peristalsis and CMMCs lie in the myenteric plexus and/or muscularis externa (Keating & Spencer, 2010; Spencer *et al.* 2011). This suggests that the submucosal plexus and mucosa (including enterochromaffin (EC) cells) are not essential for either the initiation or propagation of these neurogenic motor patterns. Distension of the bowel increases the frequency of CMMCs; this response is also preserved after removal of the submucosa and mucosa (Zagorodnyuk & Spencer, 2011). Similar findings have been reported in the guinea-pig colon where the frequency of peristaltic waves increased substantially in response to maintained local distension, after removal of

mucosa and submucosal plexus (Spencer *et al.* 2011). This suggests that all of the mechanosensory elements and the neural circuits controlling CMMCs and neural peristaltic contractions must reside in the myenteric plexus and/or muscularis externa, at least in small laboratory animals. Interestingly, removal of the circular muscle layer, but not longitudinal muscle layer, has a major inhibitory effect on stretch-activated polarized neural reflex pathways in the colon (Spencer *et al.* 2006) (Fig. 1B). This is why sensory elements in the circular muscle/or connectivity between the circular muscle and myenteric plexus was determined to be critically important in activating stretch-induced polarized reflexes (Fig. 1B). In larger animals, such as dogs and pigs (Sanders & Smith, 1986; Furness *et al.* 1990; Timmermans *et al.* 1994; Hens *et al.* 2002) some motor neurons have cell bodies in the submucosal ganglia, so motility circuitry may involve the submucosal plexus in these species.

Of course given that there are nerve endings of intrinsic sensory neurons in the mucosa and that reflex responses can be elicited by mechanical and chemical stimulation of the mucosa, it is likely that neural input from the mucosa modulates the circuits underlying the neuromechanical loop.

It is likely that most of the propulsion achieved by colonic motility is mediated by the combination of CMMCs and activation of the neuromechanical loop by contents working in concert leading to propulsive movements. Both neural activities involve mechanical stimuli. While maintained distension generates cyclical enteric neural activity even in the absence of moving contents, the neuromechanical loop provides a self-sustained propulsive mechanism. The flexibility in the speed of propulsion conferred by the neuromechanical loop may allow these patterns to propel content of a wide range of consistencies very effectively. In addition, in some conditions, extrinsic excitatory neural inputs may be required for making the enteric circuits more sensitive to content distension.

The roles of chemical factors resulting from the diet and microbiota in the modulation of these highly propulsive patterns have yet to be identified.

Role of myogenic mechanisms in colonic motility

In all species studied to date, colonic smooth muscle has rhythmic 'myogenic' motor activity, often with several frequency components. These are generated by networks of pacemaker ICCs (Sanders *et al.* 2014). We will not review here in full the role of myogenic activity, but a short summary is needed. Three main frequencies have been described in the colon of most mammalian species studied.

An intermediate frequency, generated by pacemaker cells in the deeper part of the circular muscle, drives 'slow

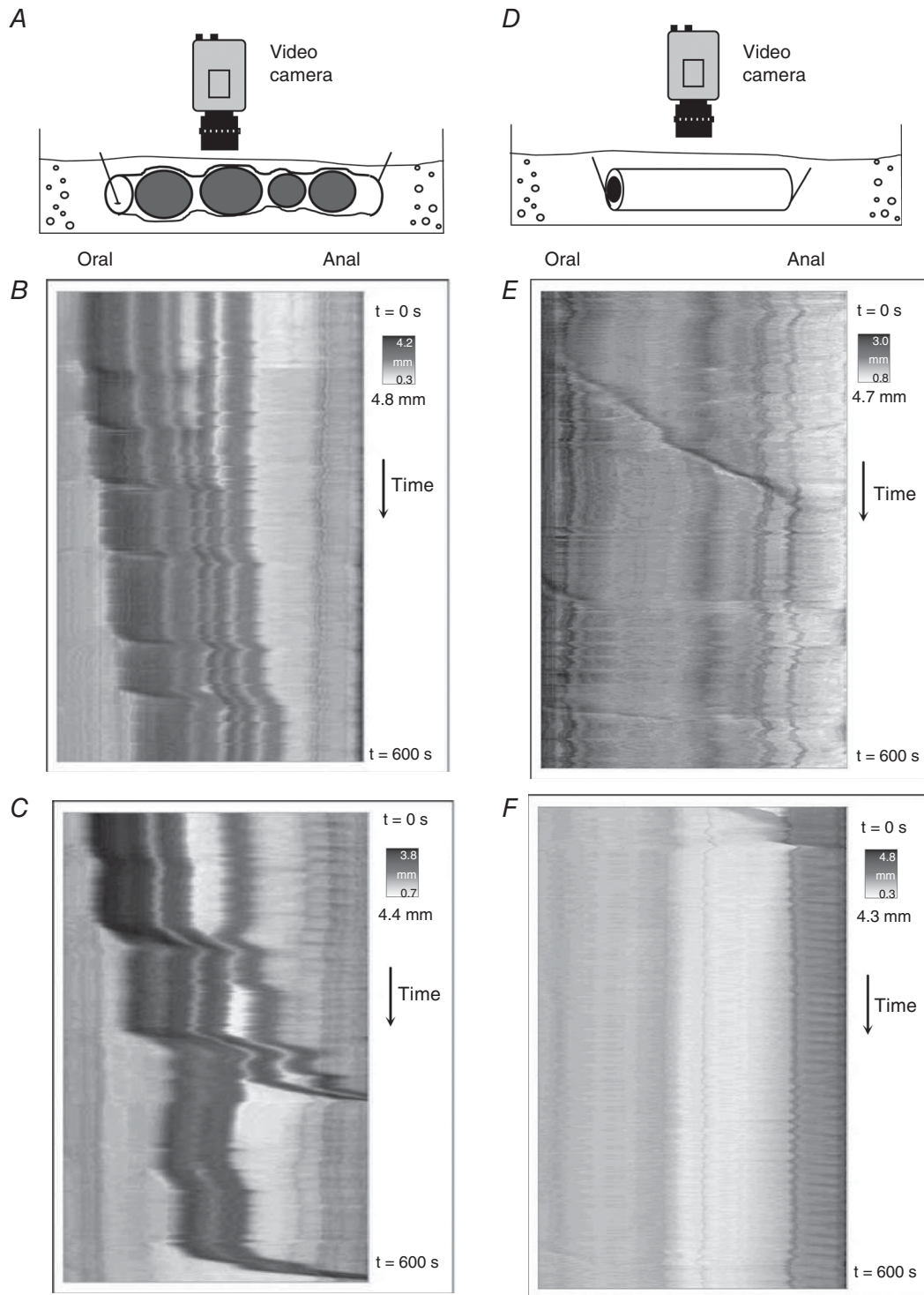


Figure 4. The frequency of colonic migrating motor complexes (CMMCs) is significantly increased by the presence of faecal contents

A, schematic diagram showing an isolated whole mouse colon containing multiple faecal pellets. B and C show spatio-temporal maps from the whole colon of different mice *in vivo*. CMMCs occur frequently and propagate over significant lengths of colon. D and E, when the colon has expelled all contents, the same segments of colon shown in B and C, respectively, rarely generate CMMCs. When CMMCs do occur, their velocity of propagation is significantly slower. This shows that the presence of multiple pellets in the lumen enhances the velocity and frequency of CMMCs. Figure reproduced from Barnes *et al.* (2014).

waves' in the circular muscle. The likely source of these intermediate slow waves is the "... ICC at the level of the submucosal border, as demonstrated in the dog colon" (Smith *et al.* 1987*b*). These often activate shallow contractions described as 'ripples' in isolated preparations of colon of experimental animals (Fig. 5) (D'Antona *et al.* 2001; Huizinga *et al.* 2011; Dinning *et al.* 2012*a*; Costa *et al.* 2013*a*). Ripples occur at frequencies which correspond to the intrinsic frequency of slow waves in the colon of each species. For example, in the rabbit colon, under conditions where neural activity is abolished, spatio-temporal maps revealed ripples occurring at just under 10 min^{-1} and propagating short distances orally and aborally (Dinning *et al.* 2012*a*).

A second faster set of oscillations is generated by pacemaker cells located near the myenteric plexus. Myogenic activity at over 30 min^{-1} occurs during distension of rabbit and rat colon (Lentle *et al.* 2008; Dinning *et al.* 2012*a*; Costa *et al.* 2013*a*). This probably reflects activity driven by myenteric ICCs in the colon – the so-called 'myenteric potential oscillations' or 'MPOs' described in dog colon (Smith *et al.* 1987*a*). These appear to play an important role in longitudinal muscle activity but may sum with intermediate frequency slow waves (Smith *et al.* 1987*a*; Sabourin *et al.* 1990).

A third myogenic activity sometimes appears after blocking neural activity. It consists of slow phasic contractions, usually occurring at intervals of 1 min or more. This type of tetrodotoxin-resistant activity has been recorded in rat colon (Pluja *et al.* 2001; Huizinga *et al.* 2011; Mane *et al.* 2015), rabbit colon (Dinning *et al.* 2012*a*), dog colon (Sabourin *et al.* 1990) and in the guinea-pig colonic flexure (Fujimoto *et al.* 2010).

In general, myogenic activity alone is not sufficient for significant propulsion of content and it is clear that a

functioning enteric nervous system is essential for survival (Ro *et al.* 2006; Roberts *et al.* 2008). Interactions between the three myogenic rhythmic activities and inputs from enteric motor neurons play important roles in normal colonic motility. Distension by luminal content activates neural pathways for peristalsis or CMMCs which sum with slow-wave-driven ripples; this activity is readily apparent in spatio-temporal maps of rabbit colon (Dinning *et al.* 2012*a*). Excitatory motor neuron input to a region of muscle increases the amplitude of ripples in that same region, so that they form clusters of contractions, which can fuse into larger summated contractions (Ehrlein *et al.* 1982; Karaus & Sarna, 1987; Sabourin *et al.* 1990; Lentle *et al.* 2008; Dinning *et al.* 2012*a*) although excitatory and inhibitory inputs can also alter their frequency and duration resulting in more variable rhythmic activity (Sanders & Smith, 1986).

Relation between colonic motor patterns in human and experimental animals

Studies on the cellular bases of intestinal motility indicate that humans share with other mammalian species similar fundamental mechanisms. It is therefore valuable to establish the correspondence of colonic motor patterns in humans and experimental animals. This is not a simple process; the conditions used to record human colonic motility are very different from the experimental arrangements for animal studies *in vivo* and *in vitro*.

Solely because of their amplitude, the most striking colonic motor patterns in humans are the 'mass movements' first described in 1909 (Holzknechtg, 1909). These are associated with powerful contractions of circular muscle which propagate down the colon; similar events have been observed in animals *in vivo*, including sheep (Bedrich & Ehrlein, 2001), pigs (Hipper & Ehrlein, 2001),

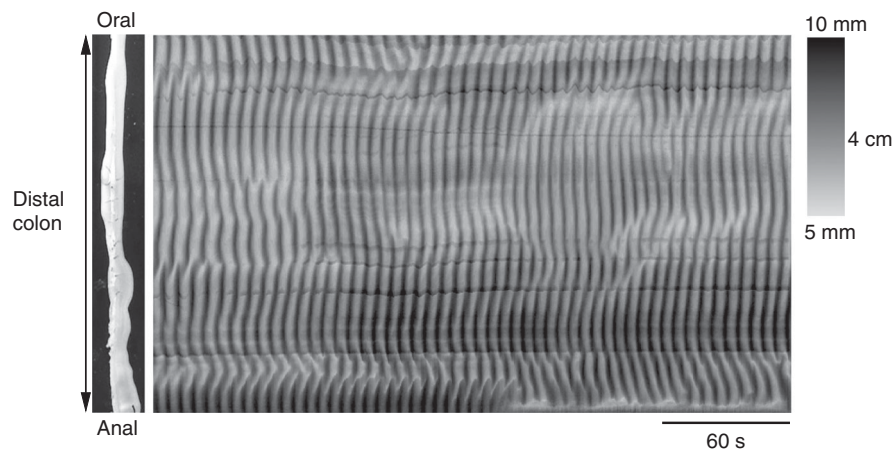


Figure 5. Myogenic 'ripples' recorded from isolated rabbit distal colon

In the presence of hexamethonium and tetrodotoxin, random chaotic ripple contractions can be visualized, with initiation sites that vary and an irregular direction of propagation. Figure reproduced from Dinning *et al.* (2012*a*).

dogs (Sarna *et al.* 1988) and rabbits (Ehrlein *et al.* 1982, 1983). Luminal infusion of a laxative induces large, single pressure waves that propagate considerable distances along the human colon (Hardcastle & Mann, 1968; Torsoli *et al.* 1971). These motor patterns were called 'true peristalsis' or 'colonic peristalsis', and were associated with 'mass movements' (Torsoli *et al.* 1971). These peristaltic motor patterns were neurally mediated because their initiation by bisacodyl could be blocked by prior application of the local anaesthetic lidocaine (lignocaine) (Hardcastle & Mann, 1968).

Narducci *et al.* recorded similar motor patterns occurring spontaneously and coined the name 'high amplitude propagating contractions' (HAPCs) to describe them (Narducci *et al.* 1987) (Fig. 6A). These high amplitude events were infrequent, occurring between 6 and 20 times per 24 hours in normal human subjects, but were often prevalent after morning waking (Bampton *et al.* 2001). They often appeared shortly after a high calorie meal (Bassotti & Gaburri, 1988; Bassotti *et al.* 1989, 1990) and were often followed by defaecation (Kamm *et al.* 1992; Bampton *et al.* 2000). These large propagating contractile events stand out in manometric recordings of colonic motility and have received more attention than any other pattern.

HAPCs are strikingly similar to the neurogenic peristaltic contractions evoked by fluid or pellet distension of the colon of experimental animals, *in vitro* and described above. Large peristaltic contractions in the human colon, as in experimental animals, are usually initiated by luminal distension, and probably modulated by mucosal stimuli. In conscious human patients, HAPCs are almost absent in the empty 'prepared' bowel (Dinning *et al.* 2014a) suggesting that they require distension to be initiated. Further, they are likely to require extrinsic autonomic input because they can be triggered by a meal (see above) or by stress. This suggests an involvement of vagal and sacral parasympathetic pathways to the bowel (Bharucha, 2012). The distinctive nature of the HAPCs/peristaltic contractions is supported by discriminant and multivariate analysis applied to pressure waves (duration, gradient and amplitude) from high-resolution recordings from human colon. This showed that these neurally mediated, high amplitude propagating peristaltic sequences formed a category that was clearly distinguishable from all other propagating motor patterns in the human colon (Dinning *et al.* 2014a).

As described above, activation of enteric ascending excitatory and descending inhibitory neural pathways, linked in neuromechanical loops, can explain this powerful pattern of highly propulsive motility in the human colon. Consistent with this, polarized reflexes can be activated by local luminal distension in isolated specimens of human colon (Spencer *et al.* 2012).

Colonic migrating motor complexes in humans

In humans CMMCs have been recorded less often, but there are a few reports suggesting they can occur (Adler *et al.* 1941; Cook *et al.* 2000; Hagger *et al.* 2002). Hagger *et al.* (2002) showed repetitive small amplitude contractile complexes in ambulant studies of normal subjects. Furthermore, a recent study of human colon *in vitro* reported motor complexes that travelled in both directions, measured with force transducers (Spencer *et al.* 2012). The paucity of recordings of CMMCs in human colon may be because the associated contractions are of small amplitude, or because widely spaced sensors used in traditional manometry were unable to detect them. Some evidence of motor complexes is apparent in high resolution colonic recordings where sensors are spaced at 1 cm intervals (Fig. 7).

Myogenic colonic activity in humans

As in experimental animals, in humans extensive nets of pacemaker cells generate at least three independent frequencies of myogenic activity. Cyclic contractions at an intermediate frequency of 2–8 min⁻¹ (Narducci *et al.* 1987; Rao & Welcher, 1996; Dinning *et al.* 2014a) are frequently recorded in human colon or rectum. These are probably due to the myogenic slow waves which have a similar frequency in human colonic tissue *in vitro* (Rae *et al.* 1998; Auli *et al.* 2008) and correspond to the intermediate slow wave-generated ripples in experimental animals.

An early study in the human descending and sigmoid colons distinguished several types of pressure waves (Adler *et al.* 1941) which usually occurred asynchronously in adjacent balloon sensors. It was concluded that many colonic contractions lack close coordination along the colon (Adler *et al.* 1941). Segmental, non-propagating, uncoordinated motor activity was believed to be the major pattern of activity between HAPCs in the healthy human colon. This description was widely accepted for the next 70 years (Narducci *et al.* 1987; Soffer *et al.* 1989; Bampton *et al.* 2001; Rao *et al.* 2001). However, in the rectum, several reports identified regular, rhythmic pressure waves (Kumar *et al.* 1989; Rao *et al.* 2001), with similar frequency (2–8 min⁻¹) to those described by Adler (Adler *et al.* 1941). They proposed that this activity was stimulated by the arrival of stool or gas, and might act to retard the flow of colonic contents into the rectum (Rao & Welcher, 1996). Other studies showed that pressure waves with a similar frequency could be occasionally recorded in other regions of the colon (Kerlin *et al.* 1983; Soffer *et al.* 1989; Bampton *et al.* 2001). Hagger *et al.* (2002) provided evidence that the events can migrate either orally or aborally (Rao & Welcher, 1996).

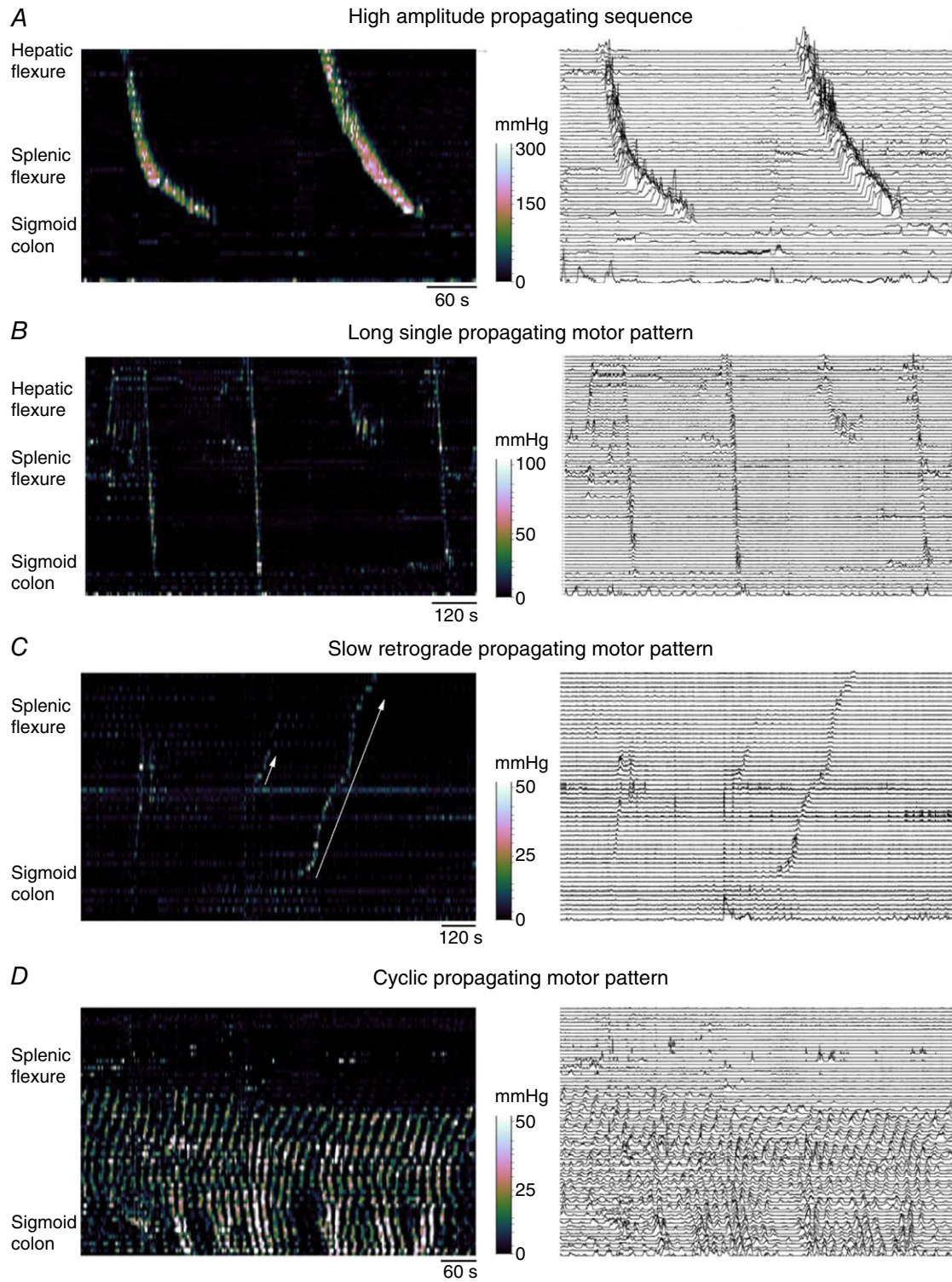


Figure 6. Colonic manometry recordings made using high resolution fibre optic technology taken from healthy adult human colon

The left-hand images are displayed as spatio-temporal colour plots and on the right is the same image displayed as a conventional line plot. *A* shows the well-described high amplitude propagating sequences. In *B*, three long single propagating motor patterns can be seen (black arrows). These motor patterns rapidly propagate across the transverse and descending colon and the component pressure waves have a lower amplitude than the pressure wave shown in *A*. In *C*, an example of a slowly propagating retrograde motor pattern is shown (solid white arrow).

In recent years, high resolution catheters have greatly improved the ability to detect small amplitude propagating motor patterns in the healthy adult colon. It has become apparent that earlier studies using balloons and other widely spaced sensors mis-categorized much of the contractile activity of the human colon (Dinning *et al.* 2013) (Fig. 7). Indeed when colonic motor patterns are recorded with sensors spaced at ≥ 7 cm intervals, approximately half of all propagating motor patterns are either missed or mislabelled compared to recordings with sensor spacing of 1 cm (Dinning *et al.* 2013).

High resolution recordings have now shown that these intermediate frequency cyclic contractions ($2\text{--}6\text{ min}^{-1}$) are the most common motor pattern in the human colon. These ripples propagate antegradely or retrogradely, or

sometimes occur synchronously over several centimetres of bowel (Dinning *et al.* 2013, 2014a), just as in other species (see above). As noted previously, these short-extent, small amplitude contractions occur in all regions of the colon but are most often evident in the distal colon, sigmoid and rectum (Fig. 6D). The predominant retrograde direction of these complexes in the sigmoid colon and rectum supports the suggestion that they resist anally directed flow and assist in the maintenance of continence and control of defaecation (Dinning *et al.* 2014a).

Ingestion of a meal (Dinning *et al.* 2014a) or electrical stimulation of sacral nerves (Dinning *et al.* 2012b; Patton *et al.* 2013) rapidly increases the occurrence and amplitude of this cyclic activity, suggesting that extrinsic nerves can modulate it.

These originate in the sigmoid colon and over several minutes propagate into the transverse colon. These motor patterns appear during an unstimulated period of recording (i.e. before a meal). Preceding the slow retrograde motor patterns is a short single motor pattern (short white arrow). In *D*, the cyclic propagating motor pattern is shown. This is the most common postprandial propagating motor pattern. It is largely confined to the distal regions of the colon and propagates predominantly in a retrograde direction, although in this instance both antegrade and retrograde propagation can be seen. The cyclic propagating motor patterns occur at the colonic slow wave frequency of $2\text{--}4\text{ cycles min}^{-1}$. Figure constructed from data published in Dinning *et al.* (2014a).

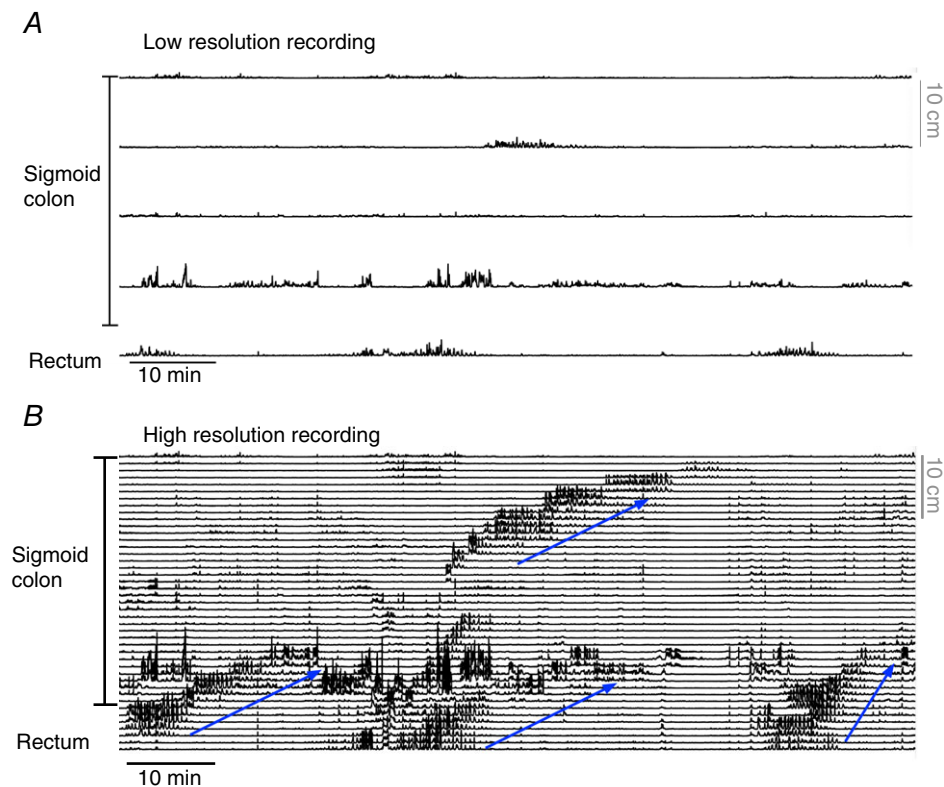


Figure 7. Colonic motor patterns recorded with a fibre optic manometry catheter in the sigmoid colon and rectum

A shows the low-resolution recording (10 cm spaced sensors) used for most colonic manometry studies. In B, the complete data set is shown. With the high resolution recording (1 cm spaced sensors) four propagating motor patterns can be seen (blue arrows indicate the actual direction of propagation). These propagating contractions may represent the colonic motor complex. Figure constructed from data published in Dinning *et al.* (2013).

Other colonic motility patterns in humans

Three other patterns of colonic motility have been distinguished in human colon by high resolution manometry:

(1) **Short single motor pattern.** As their name suggests, these are isolated patterns that propagate antegradely or retrogradely and occur in the proximal or distal colon. Their rate of propagation overlapped with cyclic myogenic contractions, as did their amplitude and duration (Fig. 6C).

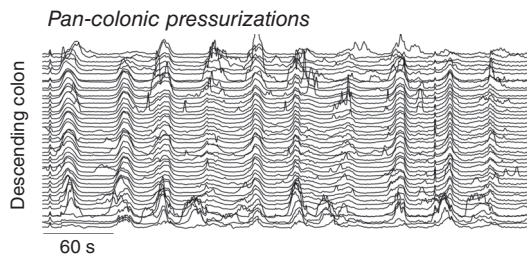


Figure 8. Examples of pan-colonic pressurizations recorded from descending colon of a patient with slow transit constipation, using a fibre optic manometry catheter
Figure constructed from data published in Dinning *et al.* (2015).

(2) **Long single motor patterns.** These consist of a single pressure event which propagates along the colon at rate slightly higher than cyclic myogenic contractions ($1.8 \pm 1.2 \text{ cm s}^{-1}$). They had similar durations to the myogenic cyclic contractions, but propagated over much longer distances. These occurred more than 1 min apart when occurring repetitively, and generally originated proximal to the mid-descending colon (Fig. 6B).

(3) **Retrograde slow propagating motor patterns.** These were recorded in only 2 out of 10 subjects, but were a distinctive pattern, travelling slowly at less than 0.5 cm s^{-1} over distances exceeding 40 cm, starting in the sigmoid colon and extending into the transverse colon (Fig. 6C).

Discriminant analysis suggested that all three of these patterns were more similar to cyclic myogenic contractions than they were to HAPCs/peristaltic contractions in terms of amplitude and duration (Dinning *et al.* 2014a). This raises the possibility that the time course of the contractions may be influenced by myogenic mechanisms. However, the distinctive velocities, directions, extents and intermittency of these contractions suggest involvement of neural coordination. Exactly how neural activity and

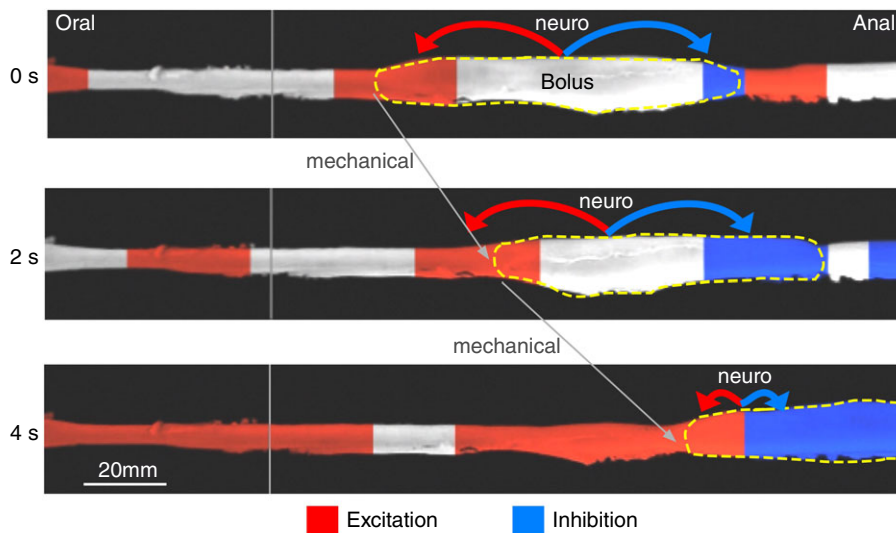


Figure 9. Schematic interpretation of the neuro-mechanical loop mechanisms for propulsion of intestinal content initiated and sustained by a bolus

Three separate images have been taken of a bolus (yellow dashed line) moving along the rabbit distal colon at 2 s intervals. The inferred state of enteric motor neuron activity was determined from the relationships that exist between changes in diameter (video image) and the corresponding changes in intraluminal pressure (manometry). These relationships allow for the calculation of the mechanical state of the muscle (Costa *et al.* 2013b; Dinning *et al.* 2014b). The red regions indicate activation of excitatory motor neurons and the blue regions indicate the activation of enteric inhibitory motor neurons. The liquid bolus is propelled by oral excitation and anal inhibition, as proposed by Bayliss & Starling (1899), and predicted by the neuro-mechanical loop mechanisms. The bolus distends the gut and activates the polarized ascending excitatory reflex pathways resulting in oral active contraction of the muscle (red) and anal active relaxation (blue). This results in a mechanical event of propulsion of the bolus in the anal direction distending a new area of intestine, then initiating neural activity of polarized enteric pathways, resulting in further propulsion of the bolus, which becomes self-sustained.

myogenic activity interact to drive these patterns is currently unclear.

In the human colon, as in other species, there are two other frequencies of myogenic activity. A faster pacemaker system with higher frequency than slow waves has been demonstrated both electrophysiologically (Rae *et al.* 1998) and mechanically (Carbone *et al.* 2013). A third type of slower myogenic activity has also been observed in human colon preparations, when neural activity is blocked. It consists of contractions of longer duration and larger amplitude (Mane *et al.* 2015). Isolated specimens of human colon *in vitro* show slow spontaneous contractions, at intervals of 0.5–5 min, called ‘slow phasic contractions’ (Carbone *et al.* 2013). They could be initiated prematurely by electrical activation of enteric neural pathways, which then re-set their rhythmicity. After tetrodotoxin similar slow phasic contractions persisted, confirming that also in the human colon, as in other species, all three myogenic frequencies exist. These slow phasic contractions continued after the submucosa and mucosa had been removed, suggesting that, as in other species they were initiated by myenteric or intramuscular pacemakers. In fact, this rhythm appears to be seen most commonly in colonic preparations that lack submucosal ICCs, suggesting that it may normally be suppressed when the gut wall is intact.

The existence of the slower myogenic activity *in vivo* in an intact colon has not been clearly demonstrated. However, in recent high-resolution manometry recordings a ‘pan-colonic pressurization’ has been described (Corsetti *et al.* 2015). This motor pattern consists of a pressure increase of 15 ± 3 mmHg amplitude and 24 ± 4 s duration, which occurs simultaneously in all colonic sensors (Corsetti *et al.* 2015). These motor patterns are recorded in both healthy controls and patients with constipation and can occur in isolation or in a rhythmic pattern ranging from 3 min^{-1} to $\sim 1 \text{ min}^{-1}$ (Fig. 8). The physiological role of this motor pattern in the human colon remains unknown, but it has been associated with relaxation of the internal anal sphincter (Corsetti *et al.* 2015).

Complex interaction between neural inputs and myogenic mechanisms

The relationship between enteric neural circuitry and ICCs is complex. There is considerable evidence that neurotransmission from excitatory and nitroergic inhibitory enteric motor neurons to gut smooth muscle is mediated via ICCs (Sanders *et al.* 2010), although this has been disputed (Goyal & Chaudhury, 2010). More recent evidence, based on inducible knockdown of kit expression, has confirmed that ICCs play a critical role in motor neurotransmission, including in the colon (Klein *et al.* 2013). ICCs also play an important role in the stretch

sensitivity of smooth muscle excitability (Won *et al.* 2005), another potent contributor to smooth muscle excitability (Bulbring, 1955). Enteric neurons are also capable of phase shifting or altering the frequency of myogenic activity (Smith *et al.* 1989; Beckett *et al.* 2003; Bayguinov *et al.* 2010).

Important challenges for future studies

The relation between enteric neural inputs and the slow phasic myogenic contractions remains one important issue to be resolved. In fact, the role of the myogenic slow phasic contractions is an area of uncertainty. Certainly, they can mimic some of the propulsive, content-dependent contractions, but generally they show considerably less spatial coordination. As these slow large phasic myogenic contractions appear only after blocking neural activity it is possible that they are normally suppressed, presumably by enteric inhibitory circuits, and that they may emerge either in experimental conditions, or in colonic dysfunction. The contributions of the fast myogenic events is also uncertain. While they play a role in patterning longitudinal muscle contractions, their role in shaping circular muscle activity and interactions with motor neuronal input remain uncertain. The muscularis mucosa is the Cinderella of the muscle layers with little or no investigations addressing the role of this muscle layer in motility. Furthermore, how the enteric nervous system coordinates its activity to give rise to short and long-extent contractions is another area lacking a good understanding (Fig. 9). Working out the details of the interactions between enteric neural pathway and local myogenic mechanisms promises to be a difficult but fruitful target for future study. The other major challenge for the future remains to determine how these patterns are modified in gastrointestinal disease and which mechanisms can be selectively targeted.

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Additional information

Competing interests

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