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Circumferential and functional re-entry of *in-vivo* slow wave activity in the porcine small intestine

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Abstract

Background—Slow waves modulate the pattern of small intestine contractions. However, the large-scale spatial organization of intestinal slow wave pacesetting remains uncertain because most previous studies have had limited resolution. This study applied high-resolution (HR) mapping to evaluate intestinal pacesetting mechanisms and propagation patterns *in-vivo*.

Methods—HR serosal mapping was performed in anesthetized pigs using flexible arrays (256 electrodes; 32×8; 4 mm spacing), applied along the jejunum. Slow wave propagation patterns, frequencies, and velocities were calculated. Slow wave initiation sources were identified and analyzed by animation and isochronal activation mapping.

Key Results—Analysis comprised 32 recordings from nine pigs (mean duration 5.1±3.9 min). Slow wave propagation was analyzed, and a total of 26 sources of slow wave initiation were observed and classified as focal pacemakers (31%), sites of functional re-entry (23%) and circumferential re-entry (35%), or indeterminate sources (11%). The mean frequencies of circumferential and functional re-entry were similar (17.0±0.3 vs 17.2±0.4 cycle min⁻¹; p=0.5), and greater than that of focal pacemakers (12.7±0.8 cycle min⁻¹; p<0.001). Velocity was anisotropic (12.9±0.7 mm s⁻¹ circumferential vs 9.0±0.7 mm s⁻¹ longitudinal; p<0.05), contributing to the onset and maintenance of re-entry.

Conclusions & Inferences—This study has shown multiple patterns of slow wave initiation in the jejunum of anesthetized pigs. These results constitute the first description and analysis of circumferential re-entry in the gastrointestinal tract and functional re-entry in the *in-vivo* small intestine. Re-entry can control the direction, pattern, and frequency of slow wave propagation, and its occurrence and functional significance merit further investigation.

Keywords

Pacemaker; High-resolution mapping; Electrophysiology; Frequency gradient; Interstitial cells of Cajal

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Introduction

Slow waves generated by the interstitial cells of Cajal modulate the pattern of gastrointestinal contractions (1). The initiation and organization of normal gastric slow waves is well documented and understood (2),(3),(4). However, slow wave organization in the small intestine remains much less certain, and particularly, the organization of small intestine slow wave pacesetting remains a matter of some debate.

Previous studies have revealed that an initial pacemaker site exists in the proximal duodenum, followed by a declining frequency gradient along the intestine in the form of a series of frequency plateaus (5),(6),(7). Investigations into the mechanism of these frequency plateaus have presented differing outcomes. *In-vivo* studies have suggested that the frequency gradient and plateaus can result from a hierarchy of peripheral pacemakers ‘competing’ along the intestinal tract (8),(9),(10), whereas a feline *in-vitro* study instead showed that they could result from successive propagation blocks to the antegrade wavefront (11). Recently, another *in-vitro* study found that functional re-entrant circuits can occur in the healthy and diabetic rat small intestine (12), which might also control slow wave frequency. However, re-entry has not yet been examined in the *in-vivo* small intestine.

Historically, the technical complexity of recording and analyzing slow wave activity over large areas of the intestine proved a barrier to defining slow wave propagation in spatial detail (8). Classic studies primarily focused on analyzing slow wave frequency using small numbers of electrodes in a linear configuration spaced along the intestine (6),(7),(9), preventing a spatial analysis of propagation dynamics, pacemaker behaviors, and wavefront interactions. However, spatial analysis is necessary to accurately resolve, quantify, and classify pacemaking mechanisms (13),(14). More recently, high-resolution (HR) platforms for mapping GI slow waves have been introduced, whereby simultaneous recordings are taken from dense arrays of electrodes, to define activation sequences in fine spatiotemporal detail (15). New techniques have recently been developed to map curved anatomical surfaces in HR and to efficiently analyze the results, now presenting the opportunity to study intestinal slow wave pacesetting dynamics in more comprehensive spatial detail (16),(17), (18).

The aim of this study was to apply these new HR mapping techniques to record slow wave activity around the intestinal circumference, in a large animal model, and to use these data to better define the mechanisms governing the organization and patterns of intestinal slow wave pacesetting *in-vivo*. We hypothesized that spatiotemporal analysis of slow wave activation over the intestinal circumference could reveal a combination of mechanisms to influence small intestine slow wave frequency *in-vivo*, such as focal pacemakers, conduction blocks, and possibly re-entry.

Materials and Methods

Animal preparation

Ethical approval was obtained from the University of Auckland Animal Ethics Committee (R698). All experiments were performed *in-vivo* on white cross-breed weaner pigs (n=9; 33.1 ± 2.6 kg). These animals and their preparation methods were similar to those used in a previous study defining porcine gastric slow wave activity (4). In summary, the animals were fasted overnight and then subjected to general anesthesia, induced with Zoletil (Tiletamine HCl 50 mg mL⁻¹ and Zolazepam HCl 50 mg mL⁻¹) and maintained with isoflurane (2.5–5% with an oxygen flow of 400 mL within a closed circuit anesthetic system). Vital signs were continually monitored and temperature was maintained in the normal physiological range (38.5–39.5 °C) by external heating. A midline laparotomy was

performed to expose the small intestine. At the conclusion of the experiments, the animals were euthanized with a bolus injection of 50 mL of a saturated solution of magnesium sulfate while they were still under anesthesia.

Recording Methods

Serosal HR slow wave mapping was performed using validated flexible printed-circuit-board (PCB) multi-electrode arrays (16),(18). Arrays of 256 electrodes were arranged in a 32×8 configuration with an inter-electrode spacing of 4 mm, covering approximately 35 cm². The PCB arrays were contained in gauze-padded silicone cradles that were sized to match the circumference of the porcine jejunum, with the cradled arrays placed such that the electrodes conformed around most of the intestinal circumference, while exerting only gentle pressure on the serosa (18).

Recordings were conducted at periodic intervals along the proximal jejunum, with sequential recordings then obtained across a segment of the jejunum that displayed a frequency change. All of these intestinal manipulations were accomplished with the minimum necessary visceral handling. During recordings, the intestine was replaced into the abdomen, the wound edges were approximated, and the residual opening packed with warm, saline-soaked gauze to prevent tissue cooling and drying.

Unipolar recordings were acquired at a sampling frequency of 512 Hz using the ActiveTwo System (Biosemi, Amsterdam, Netherlands), with reference electrodes placed on the lower left abdomen and the left hindquarter thigh.

Signal Processing and Analysis

The data analysis pipeline was performed as summarized in Figure 1. These methods were implemented in the Gastrointestinal Electrical Mapping Suite (GEMS) software v1.5 (19). All data were first filtered with a Gaussian moving median filter (20 second moving window) to remove baseline drift and a Savitzky-Golay filter ('low-pass', window 1.7 s, polynomial order 9) to remove high frequency noise (20). Slow wave activation times were then detected using the falling-edge variable-threshold (FEVT) algorithm (17), with parameters tuned for porcine small intestine slow wave detection (18). Automatically-detected slow wave events were manually reviewed to correct false-positive and false-negative marks.

Animations of slow wave activation were generated for all recorded data sequences (19) and propagation patterns were quantified and classified. Based on this classification, recordings that showed sites of wavefront initiation or re-entrant activities were focused on for comprehensive analysis. An automated grouping algorithm ('REGROUPS'), initially designed for gastric recordings, was applied to cluster individual slow waves into groups representing the same cycle of activity (21), although subsequent manual correction was often necessary, particularly around pacemaker and re-entrant sites. Isochronal mapping of activation times was then performed to visualize and quantify the propagation of successive wavefronts, using automated methods (21). Areas of conduction block were manually indicated as thick black lines, and inaccuracies in the automated maps were manually corrected (22).

Slow wave frequencies were determined by calculating the cycle-to-cycle intervals of successive events across an array of electrodes over the duration of interest. Velocities were calculated using a finite difference approach with a Gaussian smoothing filter (23), and circumferential and longitudinal propagation patterns were analyzed and compared.

Statistical Methods

Slow wave frequencies and velocities were averaged and compared using Student's t-test or one-way ANOVA with Tukey's post-test, as appropriate. Means $\pm$ SEMs are reported unless otherwise specified.

Results

Analysis comprised a total of 32 recordings from nine pigs, of mean duration 5.1 ± 3.9 min (total duration 163.1 min; approximately 450,000 individual slow wave events).

Propagation Direction, Velocity, and Initiation

Slow wave activity was observed to propagate in the antegrade, retrograde, and circumferential directions, with wavefront collisions and multiple propagation patterns sometimes being observed in the duration of a single recording. Antegrade propagation was the most prevalent pattern, present in 30/32 recordings, with 11 of those recordings characterized exclusively by antegrade propagation (Figure 2A). Retrograde propagation was observed in 21/32 recordings, with only two recordings characterized exclusively by retrograde propagation (Figure 2B). Circumferential propagation was observed in 15/32 recordings, always accompanying a source of wavefront initiation and, thus, accompanied by antegrade and retrograde propagation. The velocity of propagation in the circumferential direction (12.9 ± 0.7 mm s⁻¹) was higher than that of propagation in the longitudinal direction (9.0 ± 0.7 mm s⁻¹); ($p < 0.05$).

Spatiotemporal HR analyses revealed a total of 26 incidences of wavefront initiation occurring in 15 recordings from 6 animals. These were classified into three distinct mechanisms of wavefront initiation (refer to Table 1 for a glossary of terms): focal pacemakers ($n = 8/26$; illustrated in Figure 3), functional re-entry ($n = 6/26$; illustrated in Figure 4), and circumferential re-entry ($n = 9/26$; illustrated in Figure 5). In three instances, the source of wavefront initiation could not be definitively classified because of insufficient data coverage at those sites. Wavefronts emanated from all of these sources, and due to the more rapid circumferential velocity, these wavefronts ultimately developed into complete rings of activation propagating longitudinally along the organ in the antegrade and retrograde directions. Collisions between antegrade and retrograde propagating wavefronts were frequently observed.

Animations corresponding to Figures 2-5 are also provided as supplementary material.

Focal Pacemakers

In 8/26 sources of wavefront initiation, slow wave activity was observed to originate from a focal pacemaker and to propagate in all directions from that location (observed in 4 animals). Antegrade and retrograde wavefronts were established, together with circumferential propagation that traveled around the intestine in opposite directions, colliding and terminating at a site opposite to the focal pacemaker. An example is shown in Figure 3 (and Supplementary Animation 3). The location of focal pacemakers occasionally migrated by a few centimeters over the duration of the pacemaker before other competing slow wave activity overtook and entrained the tissue where the focal pacemaker had been located.

Re-entrant Activities

The majority of sources of wavefront initiation were re-entrant foci (15/26), where slow wave activity propagated over a 'loop' of tissue, re-activating (i.e., 're-entering') that loop

over multiple successive cycles. Two distinct types of slow wave re-entry were observed, as described below.

Functional Re-entry—Functional re-entry was identified by repeated slow wave activation occurring in either a clockwise or anticlockwise loop around a line of functional block (24)(25), and was seen to be the source of wavefront initiation in six instances (6/15 re-entries; 6/26 sources of wavefront initiation; 2 animals). In all of these instances, wavefronts were observed to propagate through the area where re-entry had occurred, either prior to, or after, the actual re-entrant activity, which indicated that these re-entrant circuits were best explained by functional mechanisms rather than by structural defects or anatomical mechanisms (24)(25). In one instance, ‘figure-of-eight’ re-entry (26), as defined in Table 1, was observed (Figure 4A and Supplementary Animation 4A). An additional example of functional re-entry from a different animal is shown in Figure 4B (and Supplementary Animation 4B).

Circumferential Re-entry—Circumferential re-entry was characterized by a single wavefront establishing a continuous and repeated loop around the intestinal circumference, and was observed in nine instances (9/15 re-entries; 9/26 sources of wavefront initiation; 4 animals). Two examples of circumferential re-entry from different animals are shown in Figure 5 (and Supplemental Animation 5A and Supplemental Animation 5B). In some instances, functional blocks were also present adjacent to the area of circumferential re-entry, with subsequent propagation activating the tissue behind the block in a further non re-entrant loop, as shown in Figure 5B.

Re-entry frequency and duration—The mean frequencies of functional and circumferential re-entry were similar (17.2 ± 0.4 vs 17.0 ± 0.3 cycle min^{-1} ; $p=0.5$), and both were higher than the frequency of the focal pacemakers (12.7 ± 0.8 cycle min^{-1}) ($p<0.001$). Wavefronts arising from re-entrant circuits propagated antegrade and retrograde from the site of re-entry at the same frequency as that re-entry, thereby establishing a high-frequency ‘plateau’ along adjacent sections of intestinal tissue (Figures 4 and 5, and corresponding animations, supplementary material).

The duration of the three observed slow wave initiation mechanisms was analyzed. There was no significant difference in the average recorded duration of focal pacemakers (median 41 s; range 20-200 s), functional re-entry (median 41.5 s; range 25-150 s), or circumferential re-entry (median 80 s; range 37-200 s) ($p=0.14$). In some cases, focal pacemakers and circumferential re-entry were present at the beginning of the recording (2 focal; 3 circumferential) or persisted to the termination of the recording period (1 focal; 3 circumferential), meaning that the duration values were an underestimate for those recording periods.

Physical Principles of Re-entry—Re-entry has physical implications for the frequency, velocity, and wavefront spacing (refer to glossary in Table 1) of the resultant slow wave entrainment (27). The physical principles of the observed re-entrant events were formulated in terms of mathematical equations in Figure 6, which mirror those previously established in cardiac re-entry (27). In order to maintain re-entry, the period of re-entry must be greater than the combined activation and refractory periods, such that there is an excitable gap of tissue between cycles of re-entry (28), as described in Table 1. The frequency, and thereby also the period, of circumferential re-entry is governed by the relationship between intestinal circumference and circumferential velocity. For any pacemaker site, the spacing of resultant longitudinally-propagating wavefronts is governed by the slow wave frequency and longitudinal velocity. For circumferential re-entry, this wavefront spacing can be further

formulated in terms of longitudinal velocity, circumferential velocity, and intestinal circumference (Figure 6D).

To further validate that the data presented above was indicative of circumferential re-entry, the above principles were applied to the experimental data recorded in this study by inserting the average frequency ($f = 17.0 \text{ cycles min}^{-1}$), circumferential velocity ($V_C = 12.9 \text{ mm s}^{-1}$), and longitudinal velocity ($V_L = 9.0 \text{ mm s}^{-1}$) into the formulae presented in Figure 6. The result was a predicted average intestinal circumference of $\approx 46 \text{ mm}$ and an average longitudinal wavefront spacing resulting from a site of circumferential re-entry of $\approx 32 \text{ mm}$ (as illustrated in Supplemental Animation 5A and Supplemental Animation 5B). This estimate agrees closely with intestinal circumference data from porcine controls in another study (median: 43 mm; range: 33-55 mm) (29).

Discussion

In this study, HR electrical mapping was performed around the circumference of the porcine jejunum to examine the range of slow wave propagation patterns and wavefront initiation mechanisms occurring in the *in-vivo* small intestine. In addition to observing focal pacemakers, re-entrant slow wave propagation was observed and quantified, including both functional re-entry and a novel form of circumferential re-entry. These re-entrant propagation patterns determine the direction, frequency, and period of slow wave propagation along the jejunum, and operated at a higher frequency than focal pacemakers. This study serves as the first description of circumferential slow wave re-entry in the gastrointestinal tract, and the discovery of functional re-entry in the *in-vivo* small intestine complements the recent descriptions of functional re-entry in the gastric antrum and corpus (13),(30) and isolated *in-vitro* small intestine (12).

This study demonstrated how organ-level mechanisms may influence the pattern of intestinal slow wave propagation. Previously, it has generally been assumed that *in-vivo* intestinal slow wave frequencies and propagation patterns are governed solely by the intrinsic frequencies of ICC, influenced by the variable coupling between adjacent segments (5). The enteric nervous system and intrinsic agents, such as prostaglandins, are also known to influence slow wave frequencies (31),(32). Our study showed that *in-vivo* intestinal slow wave frequency can also be governed by re-entrant foci, in which frequencies of ICC are entrained according to relationships of velocity, wavelength, and organ geometry, as seen in Figure 6. It is likely that re-entry, particularly circumferential re-entry, has been missed in previous studies due to insufficient spatial resolution, limited flexibility or coverage of HR mapping arrays, or preparation methods of *in-vitro* tissue (e.g., opening the sample along the longitudinal axis, which eliminates the circumferential re-entrant pathway). The flexible PCB electrode arrays that wrapped around the intestinal circumference in this study were vital to our ability to record circumferential re-entry. Circumferential coverage was not complete in experimental cases, due to the necessity for a small gap at the location of mesenteric attachment (approximately 5-30%, depending on subject variability). However, the slow wave activation times, distances, velocities, and lack of any circumferential wavefront collisions (Figure 5 and Supplemental Animation 5A and Supplemental Animation 5B) were indicative that circumferential re-entry was the most favorable explanation for these sources of wavefront initiation, as quantified above.

'Plateaus' of stable slow wave frequency have been shown to occur along the intestine (6), (7), caused by either intermittent conduction blocks *in-vitro* (11), or a hierarchy of 'peripheral' pacemakers *in-vivo* (9),(10). Our study further supports the concept of peripheral pacemakers as the dominant *in-vivo* pattern, albeit with the added complexity of re-entry also causing frequency plateaus. Discrepant *in-vivo* and *in-vitro* findings may be

due to the fact that preparation of tissues for *in-vitro* studies may elevate ICC frequencies and / or impair frequency gradients, for example, as a consequence of prostaglandin release (32). Suzuki *et al.* previously demonstrated that ‘waxing and waning’ (or ‘spindling’), where the amplitude of slow waves cyclically increases and decreases, thought to be a consequence of adjacent frequencies operating slightly out of phase, was greatly diminished in the isolated *in-vitro* intestine, compared to *in-vivo* preparations (8), suggesting a loss of competing peripheral pacemakers *in-vitro*.

The anisotropy of small intestine conduction reported in this study (velocity 40% faster circumferentially than longitudinally) accords with a previous study of anisotropy in the feline small intestine (velocity 30% faster circumferentially) (33). This velocity anisotropy is important for the initiation and maintenance of circumferential re-entry because it enhances the likelihood of circumferentially-directed activation to re-enter a circuit of tissue before a competing longitudinal wavefront arrives at the excitable gap at that location. The cause of this velocity anisotropy requires further investigation. Conduction through ICC-MP (myenteric plexus ICC) is considered to be isotropic based on small animal studies (34),(35), and the anisotropy demonstrated here could be a result of the coupling and differing conductivities between ICC-MP and other classes of ICC and / or smooth muscle layers (36).

Lammers *et al.* recently demonstrated the occurrence of functional re-entry in the rat small intestine *in-vitro* (12). In that study, re-entry was observed in 15% (10/66) of preparations studied, in both control and streptozocin-induced diabetic rats. Unlike in our porcine study, however, it was found that in rats the re-entrant frequency operated within a similar frequency range to the intrinsic intestinal frequency, likely reflecting differences in conduction velocity and refractory periods between these species. Our study serves as the first description of the figure-of-eight re-entry pattern in the intestine, which is well known in the heart, and was recently observed in the canine antrum (13),(26).

In cardiology, re-entry has been widely studied and occurs exclusively as a dysrhythmic pattern, including in ventricular fibrillation (25). Re-entry in the gastrointestinal tract is a relatively new avenue of research, with its implications only beginning to be investigated. Gastric re-entry is considered dysrhythmic and is associated with tachygastria (13),(30), though it has not yet been demonstrated in disease states or to be associated with significant organ dysfunction. Lammers *et al.* also considered intestinal functional re-entry to be dysrhythmic in their *in-vitro* rat study because although the slow wave frequency was unchanged, re-entry was accompanied by increased retrograde propagation (12). In this study, re-entry was observed to occur in the healthy porcine small intestine. However, the experimental manipulations in this study, although limited, may have played a role in promoting re-entrant slow wave activity as a dysrhythmic pattern (30). It is also possible that re-entry will occur in the normal small intestine in health, given the inherent anisotropy of the tissue. To help resolve this question further, it would be useful to perform circumferential mapping in the healthy human small intestine. Importantly, the methods used in this study are suitable for human research (18). Furthermore, a recovery study with chronic recordings may help to determine whether re-entry is associated only with acute surgery, or also occurs in the recovered fed state and disease models.

As was detailed in Figure 6, and previously in the cardiac field (27),(28),(37), the physical properties of re-entry are characterized by a complex relationship between the size of the re-entrant circuit, refractory period, and slow wave velocity, which is further affected by anisotropic conductivity (38). In functional re-entry, the leading edge directly follows the refractory tail of the previous cycle, and the circuit size is thereby kept to a minimum, defined by the velocity and refractory period. By contrast, the size of the circuit in

circumferential re-entry is anatomically determined by the intestinal circumference, and is larger than that in functional re-entry. It was therefore an interesting result that the frequencies of functional and circumferential re-entry were found to be similar, despite their differing mechanisms. This result could be explained by the anisotropic conduction. Although circumferential re-entry has a larger circuit, the entire circuit is in the direction of maximum velocity, whereas functional re-entry has a smaller circuit but also a slower velocity because a portion of the circuit will always be in the longitudinal direction.

The anisotropic conductivity should also serve to stabilize circumferential re-entry because rapid circumferential activation reduces the size of the excitable gap, reducing the likelihood for competing wavefronts to invade this gap and terminate reentry. A similar stabilizing effect of anisotropic conductivity on anatomical re-entrant excitation has been described in cardiac arrhythmias (28),(38).

This study investigated re-entry in the jejunum, but functional re-entry has previously been shown to also occur in the duodenum and ileum of the *in-vitro* rat (12). It is currently unknown whether circumferential re-entry could also occur in the *in-vivo* duodenum and ileum of large animals. However, the slow wave velocity has been shown to be high in the duodenum of dogs (in the order of 10 cm s^{-1}) (10), such that the very long resultant wavelength makes duodenal reentry unlikely. The decreased velocity of slow waves (in the order of 1 cm s^{-1} in dogs) in the ileum means that re-entry may be more likely in that part of the tract (10),(12). Further experimental studies are now needed to confirm or refute the above theoretical considerations.

The effects on intestinal motility of re-entry and its accompanying high-frequency retrograde propagation require further research, but useful comparisons can be drawn with studies where pacing above intrinsic frequencies was applied to the distal limb of an isolated jejunal loop to similarly induce retrograde slow wave activity (39),(40). This pacing approach was explored as a potential therapy for short gut syndrome (39). These, and other, studies have shown that the intestinal transit, particularly of liquids, is substantially slowed with retrograde slow wave propagation, increasing proximal volume and absorption (39), (40). Similar effects could perhaps be expected from the relatively high-frequency retrograde propagation resulting from re-entry. If, as discussed above, re-entry is a dysrhythmic pattern in the intestine, then it could potentially be relevant to the pathophysiology of post-operative ileus and other motility disorders, such as intestinal ischemia, where dynamic conduction blocks and tachyarrhythmias have been shown to occur, but with uncertain mechanisms (41),(42). It is also possible that this type of re-entry may be associated with dysrhythmias observed in other parts of the gut, such as the stomach, where the high degree of anisotropy (circumferential velocity 2.5 times faster than longitudinal in human) promotes circumferentially-directed propagation during dysrhythmia (14).

Recent debate has arisen as to whether extracellular electrical recordings truly reflect slow wave potentials or movement artifact (43). Based on the morphological and biophysical characteristics of the recordings reported here, which are similar to those in other intestinal extracellular studies (6),(10), it is these authors' view that the recorded events were strongly indicative of true bioelectrical potentials rather than contractions (44).

In summary, this study serves as the first description of circumferential re-entry in the GI tract, and the first example of functional re-entry in the *in-vivo* intestine. The occurrence of slow wave re-entry and its effect on intestinal function merits further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TRA, GOG, AJP, & LKC designed the study; TRA, GOG, PD, & NP collected the experimental data; TRA, GOG, PD, & NP analyzed the data; TRA & GOG wrote the draft; AJP, IPB, and LKC supervised the research; all authors except AJP revised and approved the manuscript.

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Figure 1.

Pipeline of the data analysis process from raw data through visualization. Sample electrograms are shown for each step, and each step is described in the ‘Signal Processing and Analysis’ section of the methods. Identified slow wave activation times are marked by red dots. Three forms of visualization are shown. The isochronal activation map shows the propagation of Wave 4 from the electrogram above (see Figure 2 for a complete description of the activation maps). The velocity field map displays the magnitude as a color gradient and the direction at each electrode as an arrow. A single frame of the propagation animation shows multiple waves in the mapped area at one time, represented in different colors and corresponding to Wave 4 and Wave 5 in the electrogram above.

Figure 2.

Antegrade and retrograde slow wave propagation. An 8×13 electrode section of the array is shown in these examples. The electrode array was wrapped around the circumference of the intestine such that the top and bottom of each map correspond to nearly-adjacent tissue at the mesenteric border. Electrograms are shown on the left, and isochronal activation maps for three successive slow wave cycles (*a*, *b*, and *c*) are shown on the right. The displayed electrograms correspond to the channels labeled on maps A(*a*) and B(*a*). Each activation map shows a single wavefront, with each color band indicating the area of slow wave propagation per 0.5 s in A and 1.0 s in B, progressing from red (early) to blue (late). Each black dot represents an electrode, and white dots outlined in red represent electrodes where activity was interpolated. **A.** Consistent antegrade propagation. Slow wave activity originated oral to the array and propagated antegrade across the array. **B.** Consistent retrograde propagation. Slow wave activity originated aboral to the array and propagated retrograde across the array. (See also: Supplemental Animation 2A and Supplemental Animation 2B of the same data).

Figure 3.

Focal pacemaker. The figure panel is arranged as described in Figure 2. Each map depicts a single wavefront and each color band represents 0.25 s of propagation time. An 8×11 electrode section of the array is shown. Slow wave activity was observed to originate at a point near the mesentery, and spread in all directions. The circumferentially-directed propagation traveled around the intestinal circumference (from top and bottom of map), colliding at a point approximately opposite the site of activation (near the middle of the map; also observed in converging waveforms in the electrograms). Focal pacemakers generated wavefronts traveling longitudinally along the intestine in both the antegrade and retrograde directions (see also: Supplemental Animation 3 of the same data).

Figure 4.

Functional re-entry. The figure panels are arranged as described in Figure 2, with each map depicting a single re-entrant cycle and each color band representing the area of propagation per 0.5 s. Blank areas represent locations where data was not reliably recorded. An 8×10 electrode section of the array is shown in these examples. The top electrode in the electrogram ('electrode 1') is repeated at the bottom of the electrogram to demonstrate the continuous propagation. **A.** In this example, slow wave activity formed a 'figure-of-eight' re-entrant circuit where a single wavefront broke into two wavelets (wave fragments that separate from the main wavefront) that propagated in clockwise and anti-clockwise directions around two distinct lines of functional block. These wavelets repeatedly re-excited the same loops of tissue, reactivating after subsequent cycles (at point marked 'R' in maps *a*, *b*, and *c*) and forming a re-entrant circuit. In this example, the anti-clockwise propagation loop was slightly out-paced by the clockwise loop, causing the clockwise loop to become dominant and the anti-clockwise loop to collide and terminate at a functional block in maps *b* and *c*. **B.** An episode of functional re-entry from a second animal, occurring in an anti-clockwise direction. The loop of activation again traveled around a line of functional activation block and re-excited the same tissue circuit. In both *A* and *B*, wavefronts were seen to propagate antegrade and retrograde from the sites of functional re-entry, thereafter assuming 'rings' of activation moving longitudinally. (see also: Supplemental Animation 4A and Supplemental Animation 4B).

Figure 5.

Circumferential re-entry. The figure panels are arranged as described in Figure 2, with each map depicting a single wavefront and each color band representing the area of propagation per 0.5 s. An 8×14 electrode section of the array is shown. **A.** Slow wave activity was observed to propagate from the bottom to top of the maps and re-enter at the bottom, illustrating a continuous loop wavefront that propagated circumferentially around the intestine (i.e., the wavefront at the top of map *a* propagated continuously onto bottom of map *b*, and so on). **B.** A further example from a second animal again illustrates a continuous loop of slow wave activity that propagated around the intestinal circumference, in this case in the opposite direction as in A. An additional conduction block was present in this case at the top of the map, represented by the thick black line. In A and B, ring wavefronts were observed to travel longitudinally antegrade and retrograde along the intestine from the site of circumferential re-entry (see also: Supplemental Animation 5A and Supplemental Animation 5B).

Figure 6.

Mathematical properties of re-entry. Slow wave propagation is represented with arrows, where red represents the wavefront and white represents the excitable gap (see Table 1 for definition of terminology). **A.** Schematic of a focal pacemaker, shown on a cross-section of the intestine. Activity originates from a point represented with the asterisk, travels in both directions around the circumference of the intestine, and collides on the opposite side. **B.** Schematic of circumferential re-entry, shown on a cross-section of the intestine. A wavefront propagates continuously in a loop around the circumference of the intestine, re-exciting the same circuit of tissue over successive cycles. **C.** Schematic of functional re-entry, shown on the serosa of the intestine. Activity propagates in a loop around a functional block, represented by a thick black line. The functional block is created by the refractory period such that the leading edge of one cycle follows the refractory tail of the previous cycle. In both circumferential and functional re-entry, an excitable gap must be present between the leading edge of one cycle and the refractory period of the previous cycle to avoid a collision between the two, resulting in termination of the re-entry. **D.** Equations outlining the physical properties governing re-entrant slow wave activity.

Table 1

Glossary of terms used to describe intestinal slow wave propagation and re-entry. Several cardiac electrophysiology terms (25),(28) have been adapted to describe similar phenomena in the intestine.

Terminology	Description
Leading Edge	Depolarized / excited tissue at the front of a propagating slow wave wavefront.
Refractory Tail	Depolarized tissue behind the leading edge of a slow wave that is unable to be excited.
Wavefront Spacing	The distance between two successive wavefronts.
Anisotropic Conduction	A propagation profile that is directionally non-uniform. In the case of gastric and intestinal slow waves, anisotropic conduction manifests as circumferential propagation being faster than longitudinal propagation (36).
Conduction Block	Abnormal termination of a propagating wavefront, for example when refractory tissue or a structural defect is encountered.
Functional Block	A specific type of conduction block resulting from dynamic interactions between the leading edge and refractory tissue rather than by anatomical means.
Focal Pacemaker	Slow wave/s arising from a point-source and propagating in all directions, colliding in the opposite aspect of the intestine circumferentially, and forming rings of activation propagating longitudinally.
Re-entry	A slow wave propagation pattern where slow wave activity propagates in a circuit around a defined obstacle, forming a loop pattern of activation and re-activating that circuit over successive cycles.
Excitable Gap	A section of excitable tissue between the refractory tail of one cycle of re-entry and the leading edge of the following cycle. The presence of an excitable gap is necessary to maintain re-entry, as re-entry will terminate if there is a collision between the leading edge and refractory tail. The size of the excitable gap is governed by the size of the re-entrant circuit, the slow wave velocity, and the refractory period.
Anatomical Re-entry	A type of re-entry where a structural feature forms the obstacle around which slow wave activity establishes a re-entrant loop (27).
Circumferential Re-entry	A specific type of anatomical re-entry where the intestinal lumen forms the anatomical obstacle around which slow wave activation establishes a re-entrant loop.
Functional Re-entry	A type of re-entry where a functional conduction block forms the obstacle around which slow wave activation establishes a re-entrant loop of propagation (24)(25).
Figure-of-Eight Re-entry	A variant of functional re-entry where a common wavefront divides into clockwise and anticlockwise wavelets that propagate around separate functional blocks before rejoining, forming a re-entrant circuit in a figure-of-eight pattern (26).