CYCLIC MOTOR PATTERNS AND HAUSTRAL ACTIVITY IN THE HUMAN COLON

CHARACTERIZATION OF CYCLIC MOTOR PATTERNS AND HAUSTRAL ACTIVITIES IN THE HUMAN COLON BY HIGH-RESOLUTION MANOMETRY

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Abstract

Colonic manometry tests and measures strength and coordination of colonic muscles contractions. This tool was used to understand the rhythmic colonic motor patterns and their contribution to motility in healthy subjects and patients with constipation. Rhythmic activity in the gut is mediated by pacemaker cells, Interstitial cells of Cajal (ICC). We present a detailed characterization of ICC-mediated rhythmic activity that (1) occurs in the small pouches making up the colon (haustra) and (2) is greater than 5cm along the length of the colon (cyclic motor patterns-CMP).CMP possess high-frequency activity (7-15cpm), in addition to activity observed in the low-frequency range (2-6cpm). Activity in the haustra, or haustral activity, is comprised of 2 boundaries with activity within these bounds (intra-haustral activity); the overexpression in patients serves to retard flow of colonic content. Sphincter of O'Beirne is the last haustral boundary at the rectosigmoid junction; its persistent presence was characterized in a patient with refractory constipation.

Abstract

This thesis focuses on the characterization of rhythmic activity in the colon of healthy subjects and patients diagnosed with refractory constipation; this activity is mediated by pacemaker cells in the gastrointestinal system, the Interstitial cells of Cajal (ICC). The myogenic activity described are the cyclic motor patterns (CMP) and haustral activity; characterization of these motor patterns in healthy subjects provided control values for the subsequent comparison in patients. Frequency analysis of CMP revealed a novel high-frequency activity (7-15cpm) unrelated to the breathing artefact. Three categories of cyclic motor patterns were observed: (1) CMP following mass peristaltic events (HAPW); (2) those that occur in isolation of other colonic motor patterns (HAPW) in the colon; and (3) low-frequency (2-6cpm), prominently retrograde rhythmic activity in the rectum. CMP were scarcely present in majority of the patients; however, elevated retrograde CMP in the distal colon and rectum in some patients plays a role in retarding flow of colonic content. A detailed characterization of haustral activity (comprised of 2 boundaries and the activity within a haustrum) is reported for the first time using high-resolution colonic manometry. Furthermore, we find that over expression of haustral boundary activity in patients serves as a disproportionate hindrance in colonic transit. An in-depth methodology is developed for the identification and subsequent analysis of haustral activity and CMP; this provides transparency in the data acquisition and analysis. Lastly, a sphincter at the rectosigmoid junction, sphincter of O'Beirne is presented in a patient case report. The persistent presence and paradoxical contractions of this sphincter served to impede flow colonic content, an important factor contributing to the pathophysiology of severe refractory constipation.

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Abbreviations

HRCM: high-resolution colonic manometry

HAPW: high-amplitude propagating pressure wave

SPW: simultaneous pressure wave

HAPW-SPW: a proximal HAPW followed by SPW at the splenic flexure

CMP: cyclic motor pattern

HAPW-CMP: cyclic motor pattern that follows an HAPW

HRV: heart rate variability

ENS: enteric nervous system

CNS: central nervous system

ICC: Interstitial cells of Cajal

PBD: proximal balloon distention

DBD/RBD: distal/rectal balloon distention

List of Publications and Conferences

Publications

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Chen, J., Pervez, M., Hanman, A., Shokrollahi, M., Parsons, S., Ratcliffe, E., & Huizinga, J. D. (2018). Different types of simultaneous pressure waves in the human colon: Biomarkers for gas transit and colon function assessment. *Neurogastroenterology and Motility, 30*, 1st ser. doi:30-S1:10.1111/nmo.13422

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Conferences

February 2018 – Canadian Digestive Diseases Week (poster)
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March 2020 – Farncombe Family Digestive Health Research Institute: Research-In-Progress (oral)

Chapter 1 : General Introduction

Chronic constipation

Chronic constipation is a prevalent functional gastrointestinal disorder, present in anywhere from 2-27% of the general population[30, 31, 50]. It significantly impacts the quality of life with a significant reduction in psychological health[, 50]. The costs associated with medical care, in particular outpatient and emergency room visits, are high for patients diagnosed with constipation since childhood as per the results of a longitudinal study conducted in 2011[39]. Costs associated with chronic constipation were estimated to have exceeded 230 million dollars in the United States[30]. While some doctors may define constipation based on the frequency of bowel movements, patients report bothersome symptoms to describe constipation; these symptoms include infrequent bowel movements, straining, bloating, report of incomplete evacuation following bowel movement, abdominal discomfort and hard/lumpy stool[39]. Some studies suggest that only 25% of those afflicted with chronic constipation seek medical treatment; many of those who do seek treatment also report dissatisfaction of the treatment outcome[45, 46].

Most treatment options, such as lifestyle changes (exercise, and increased fiber and fluid intake), and osmotic and stimulant laxatives, work to manage symptom(s), but do not target the underlying pathology[30, 39]. Nevertheless, many patients diagnosed with constipation find relief with the aforementioned therapeutic measures, but not all[29]. When all therapeutic approaches have been exhausted, and careful clinical re-evaluations have been conducted, patients may be diagnosed with refractory constipation. These patients require detailed diagnostic evaluation to better understand the underlying pathophysiology for a more tailored treatment option[29]. On the extreme end of the treatment spectrum, irreversible surgical options such as subtotal colectomy are exercised that have mixed outcomes on the quality of life of these patients[28, 48].

Colonic manometry has been recommended for the identification of colonic motor dysfunction in children and adults diagnosed with treatment-refractory constipation prior to considering surgical intervention as per a consensus statement[36]. Increased colonic activity is observed after waking up and after meal intakes. High amplitude propagating pressure waves (HAPWs) are generated in response to meal and bisacodyl. These are all considered normal colonic responses as observed in colonic manometry (as per a consensus report in 2008[32]). Conversely, the absence of HAPW over 24h[42] and/or following bisacodyl administration[47], and lack of increase in retrograde pressure waves following meal intakes[40, 41, 44, 49] have been touted as indicators of severe colonic motor dysfunction as per colonic manometry findings (Consensus report 2008[32]).

Low-resolution manometry allowed partial identification of HAPWs due to its pronounced pressure and its progression over a long segment of the colon; this motor pattern served as a marker for healthy colonic motility despite its infrequent appearance. With the advent of high-resolution colonic motility, new markers that were previously unidentified or invisible due to low resolution, became visible; and their contribution to colonic motility became more apparent. Simultaneous pressure waves (SPW), which are pan-colonic pressurizations previously attributed to artefactual pressure increases, now present their association with gas expulsion and concomitant presence with anal sphincter relaxation[34]. Motor patterns of shorter length and/or lower amplitudes such as cyclic motor patterns and haustral activity are also seen with the higher resolution; this enables for their detailed characterization and understanding of their potential functions/contributions to colonic motility. Furthermore, colonic manometry provides insight into the neural input involved in colonic motility. An HAPW generated in the proximal colon in response to rectal bisacodyl infusion (as observed during colonic manometry) provides evidence for (1) spinal and vagal innervation, and (2) the communication between autonomic nerves and the colon[33, 43].

The colon

The colon is an important organ responsible for the absorption of water and electrolytes, and fermentation of nutrients. The colon stores fecal contents, with initial storage in the caecum, ascending and transverse colon[1, 2]. This content is slowly processed while being propelled in the anal direction with concomitant formation of stool followed by defecation when socially acceptable[3, 4, 5]. Colonic motility is regulated through the collaboration of and communication between multiple control systems[6]. Colonic motor patterns require the collaboration and communication between extrinsic and enteric nervous systems, the gastrointestinal pacemaker network of interstitial cells of Cajal (ICC), smooth muscle cells, endocrine or paracrine factors, secretory mucosal cells and the luminal environment (gut flora, mechanical and chemical composition of intestinal content)[4, 6.] Sensory and motor neurons that are intrinsic and extrinsic to the smooth muscle mediate the response to stimuli that ultimately leads to colonic contractions[7, 8, 9]. Normal variability in heart rate (HRV) is due to autonomic neural regulation of the heart and circulatory system. A balance between the sympathetic and parasympathetic input controls heart rate, and thus, variability in heart rate provides information about the functioning of the nervous system [54]. In the lab, away to understand the role of the extrinsic autonomic nervous system in the generation of colonic motor patterns is through the simultaneous assessment of the changes in heart rate variability (HRV). As suggested by a recent publication by the lab[10], the HAPW observed through high-resolution colonic manometry correspond to changes in parameters measuring parasympathetic and sympathetic activity; colonic motor patterns are associated with an increase in parasympathetic activity and decrease in sympathetic activity.

Enteric nervous system

The enteric nervous system (ENS) is the intrinsic nervous system of the gastrointestinal system capable of local and autonomous function [11]. It contains an extensive neural circuit that can detect physiological conditions and integrate this information to control gut motility, local blood flow to the gut, and regulate the exchange of fluids between the gut and its lumen. The ENS obtains information about the state of the gastrointestinal

tract via afferent sensory neurons; some of this information is relayed to the central nervous system (CNS). In turn, the CNS exercises control over gut motility relayed through the ENS[11, 12, 13]. Enteric nerves have innervations directly into the smooth muscle cells and in ICC networks[14]. There is close association and proximity between the ICC and the ENS as evidenced from ultrastructural studies, which suggests that one of the major functions of the enteric nervous system is to have neural control over pacemaker activity[15]; the interaction of the ICC and ENS system is integral for normal motor patterns to occur[5, 11].

Interstitial cells of Cajal

Electrical oscillations in smooth muscle are present throughout the digestive tract; these electrical oscillations are driven by pacemaker cells identified as interstitial cells of Cajal (ICC)[4, 5, 6]. The electrical oscillations consist of rapid upstrokes, a long plateau and then repolarization. The networks of ICC are present at the interface between the submucosa and the circular muscle layer (ICC-SMP (submuscular plexus)) intra-muscular (ICC-IM) and inter-muscular (ICC-MP (myenteric plexus); between circular and longitudinal muscle layers) layers of the gastrointestinal tract[16, 17]. The networks commonly focused on in the human colon related to motility are ICC-SMP and ICC-MP because their pacemaker activity is implicated in the generation of myogenic-mediated rhythmic muscle motor patterns[13, 18]. These networks are in close association with (1) smooth muscle cells, and neural plexi, demonstrating the role of ICC in neurotransmission[13] and (2) the ENS[19] where the ICC orchestrate propagating contractions in concert with the ENS[5]. The omnipresent electrical oscillations generated by the ICC-SMP are termed slow waves and serve as the primary pacemaker of the colon; these correspond with frequencies in the range of 3-9 cpm in the human colon[20] ; this low-frequency activity is implicated in non-propulsive contractions[5]. ICC-MP generate high-frequency propulsive contractions through stimulus-dependent electrical oscillations; these are secondary pacemaker cells[5, 13, 51]. The electrical coupling of the ICC and smooth muscle cells allows for slow waves to actively propagate into the muscle layers[6] through gap junction

proteins[14, 21, 22] or through the proximity of ICC and smooth muscle cells [23]. Slow waves induce alternating periods of high and low-excitability; high-excitability relates to the plateau phase of the slow wave[17]. When a threshold amplitude is achieved for activation of L-type calcium channels, action potentials are generated superimposed on the slow waves. Slow waves are either maintained continuously (omnipresent) or are induced by neural activation[11, 24]. Beyond pace-making activity, ICC also play a role in sensing distention in the gastrointestinal tract[25].

Colonic smooth muscle contractions

Smooth muscle contractions, and thus colonic motility, are the ultimate result of the collaboration of the multiple control systems noted above. Influx of calcium into the smooth muscle cells from voltage-sensitive calcium channels and/or intracellular calcium stores result in the depolarization of the gut smooth muscle cells, thereby generating action potentials and gut contractile activity[26, 51]. The rhythmic depolarization of the underlying ICC network brings the smooth muscle membrane potential periodically closer to the threshold for calcium influx, however further stimulation is required for contractile activity to occur. Distention (stretch of smooth muscle)[27], excitatory neuro-transmission and inhibition of inhibitory neurotransmission[6] will further depolarize the smooth muscle to bring the slow wave plateau over the action potential threshold to generate contractile activity at the slow wave frequency. The amplitude of the contractile activity is determined by the extent of depolarization of the smooth muscle past the threshold for calcium channel activation; the greater the depolarization above the threshold, the greater the contraction[51]. The stomach and small intestines have distinct ICC-mediated frequency gradients, with high frequencies in the proximal regions. This gradient dictates the direction of contractile propagation from region of high frequency to region to low frequency resulting in anal-directed propagation[17, 24]. The colon does not appear to have a distinct frequency gradient and the labile nature of the frequency gradient explains the mixed propagations seen in pressure waves[52]. High-frequency ICC electrical oscillations are often not reflected in the rhythmicity of the muscular

contractions; contractile activity may occur at a much lower frequency[11, 24]; ICC-MP may have a frequency of 17cpm but the contractile activity may be 1cpm[53]. Contractions at 17 cpm may summate since not enough time is present to allow for relaxation. This happens because the ICC-generated slow wave propagates into the smooth muscle creating a region of depolarization bound by time and space; the smooth muscle action potentials for the generation of contractions cease to occur beyond this region[51]. The plateau of the slow waves may be superimposed by action potentials which signifies smooth muscle contractions, however not every plateau of depolarization corresponds with an action potential peak if the depolarization is not sufficient enough to result in a calcium influx. While colonic motility is orchestrated by the interaction of both neurogenic and myogenic processes, some motor patterns and colonic features have an underlying mechanism dominated by neurogenic or myogenic activity[4, 6]. When considering ICC-mediated rhythmic activity, these will be deemed as myogenic motor patterns despite having some dependency on enteric nervous system-mediated neural excitation[5, 13].

With this thesis, I have contributed to the optimization and analysis of human high-resolution colonic manometry (HRCM), ultimately to diagnose patients with colonic motor dysfunction associated with functional gastrointestinal disorders. To achieve this, I have assisted during the manometry recording through thorough notetaking, identified and analyzed normal motor patterns and motor complexes, and obtained an understanding of their function and contribution towards colonic motility. Quantitative analysis of HRCM in healthy volunteers allowed for the development of 'normal' or 'control' values which were utilized for the assessment of colonic motor patterns in patients. Furthermore, our discussions around HRCM protocols have led to the optimization to evoke the motor patterns to be evaluated. Once interventional impact on colonic activity is assessed, this insight will serve as a clear and tailored diagnostic tool in the evaluation of the underlying pathophysiology.

The overall objective of my work was to characterize specific prominent motor patterns, namely the cyclic motor patterns and haustral activity in both healthy subjects and patients, and to understand the contribution of these motor patterns to the pathophysiology in those afflicted with constipation. I also contributed to the identification of a functional sphincter, the sphincter of O'Beirne, and conducted the data analysis pertaining to this sphincter in a case report.

The specific goals of my thesis work were as follows:

1. Assessment of colonic motor function through High-Resolution Colonic Manometry (HRCM) and the development of identification and analysis methods – Motor pattern acquisition (**Chapter 2- Results 2.1**)

   This chapter signifies the first step prior to any data analysis and requires effective and accurate data acquisition in our clinical setting. This clinical setting requires maintaining a balance in conducting the study objectively while exercising empathy and flexibility when working closely with research participants and patients. Furthermore, accurately obtaining data requires close monitoring and reporting of research participant-induced artefacts in the manometry recording such as body movements, and episodes of urination, coughing, and talking. Chapter 2 of the thesis highlights the steps taken to make the process of the manometry study session efficient and the methods through which breathing artefacts were definitively identified. **Appendix 1A, B, C** include checklists/manuals developed outlining the numerous tasks pertaining to HRCM data acquisition.

2. Assessment of colonic motor function through High-Resolution Colonic Manometry (HRCM) and the development of identification and analysis methods – The cyclic motor pattern and haustral activity (**Chapter 2- Results 2.2-2.3**)

This chapter is also tasked with the development of methods to identify and analyze (qualitative and quantitative analysis) the diverse colonic motor patterns with a focus on cyclic motor patterns (CMP), and haustral activity (comprised of haustral boundary activity and intra-haustral activity). While cyclic motor patterns have been reported on in the past, the careful identification, classification, and analysis of haustral activity has not been reported on with the advent of HRCM. This chapter serves as a guide for future analysis on both cyclic motor patterns and haustral activity and serves to provide transparency in the methods of data acquisition and pertinent analysis. **Appendix 2** provides a guide for haustral activity selection and data input into excel file. Sample excel files containing categories for data sorting are provided for cyclic motor patterns (**appendix 3**) and haustral activity (**appendix 4**).

3. Cyclic motor patterns in the human colon (**Chapter 3**)

   Chapter 3 serves to provide a detailed analysis of the cyclic motor pattern, pressure waves spanning greater than 5cm along the length of the colon; these were characterized in healthy controls. Here, we focus on the frequency distribution of this motor pattern to elucidate information about the underlying networks of ICC. The frequency distribution reveals 2 distinct frequency ranges: low-frequency range which is 2-6 cpm centered at 3 cpm; and high-frequency range which are frequencies greater than 6cpm centered around 11-13 cpm. The low frequency range has been researched extensively in the past, but this chapter highlights a never before published high-frequency pressure wave range, typically attributed to the respiratory artefact.

4. Characterization of haustral activity in healthy controls and the assessment in patients using high-resolution colonic manometry (**Chapter 4**)

   This chapter focuses on the detailed characterization of haustral activity which

includes both isolated haustral boundary contractions and intra-haustral activity in the colon. A characterization of this type has not been done before using HRCM. Frequency distribution of haustral activity is analyzed to gain insight on the underlying ICC networks and the potential link to cyclic motor patterns. Cyclic motor patterns were identified on the tenet of rhythmicity being a distinct feature but required to be greater than 5cm in colonic length. This definition was used to exclude haustral activity- the focus of this chapter. Once healthy subjects were analyzed using a strict set of parameters that I developed, the control values were used for the comparison of haustral activity in patients. Furthermore, the control parameters obtained from chapter 3 on cyclic motor patterns were used to draw comparisons to adult patients. Individualized analysis of haustral activity reveals the potential role of haustral activity in their pathophysiology.

5. On the sphincter of O'Beirne and autonomous dyssynergia in chronic constipation (**Chapter 5**)

   This study is a case report for a patient with severe refractory constipation. HRCM was utilized to assess the preserved and potentially impaired motor function of the colon. In doing so, we observed a high-pressure zone at the recto-sigmoid junction that was consistently present, the sphincter of O'Beirne. This high-pressure zone of 1-2cm was associated with rhythmic contractions of 2-3cpm and was immediately distal to a persistent low-frequency retrograde cyclic motor pattern and several active haustra. I analyzed the sphincter of O'Beirne for its amplitude and interaction with high-amplitude propagating pressure waves (HAPW), simultaneous pressure waves (SPW) anal sphincter activity, and the cyclic motor pattern bordering the recto-sigmoid junction. This case study reports of autonomous dyssynergia where a propagating motor pattern (e.g. HAPW) is followed by a maintained and/or paradoxical contractions of the sphincter of O'Beirne and the anal sphincter instead

of a relaxation as observed in controls; a feature attributed to the pathophysiology of chronic constipation.

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Chapter 2 : Assessment of colonic motor function through High-Resolution Colonic Manometry (HRCM) and the development of identification and analysis methods.

Introduction

The pathophysiology of constipation is heterogenous. Constipation may result from primary disorders in the colonic and/or anorectal function, or it may be secondary to other conditions such as metabolic disorders, and colorectal issues[11] Abnormal motor activity in the colon is correlated to several colonic disorders such as chronic constipation[7]. Although symptom-based assessments are important, it alone is insufficient to direct therapy. When change of diet and laxative medication prove to be insufficient, and secondary causes of constipation are discarded, a thorough work-up is performed to determine the cause of primary chronic constipation assessed to be colonic inertia, outlet obstruction syndrome or functional disorders[14]. As of yet, the intricate movements within the human colon are not well understood and lack a community wide consensus. Despite this knowledge, ill- advised surgical resections are being prescribed to resolve colonic motility disorders without weighing the consequences[8]. Overall, the non-specificity of constipation lends to the importance of understanding the underlying pathophysiology and striving towards optimal management. One such tool that may contribute to the understanding of the underlying pathophysiology of constipation is manometry.

Colonic manometry is the only measure of colonic motility that can measure pressure/force from multiple regions within the colon in real time[3]. In traditional manometry techniques, there was constant compromise between getting rich details over shorter spans of the colon, or losing spatial resolution by increasing the inter-sensor distance (between 10 and 20cm) over the colon to accommodate its large size (~120cm)[11]. Both methods resulted, ultimately, in incomplete and mislabeled data pertaining to colonic motor patterns/complexes[15, 16,18] when using low-resolution manometry. With the advent of high-resolution colonic manometry (HRCM) as used in

our lab, the water- perfused catheter allows for a dramatic increase in sensors (84) with an inter-sensor spacing of only 1cm, provide detailed pressure profiles of the colo-anal region, while retaining flexibility for intubation. Furthermore, topographical colon maps are generated by the interpolation of the pressure between the sensors; this allows for ease of reading the maps and motor pattern detection compared to the traditional line traces of pressure[17]. This technique provides clearer information on the propagation direction, frequency, duration, and length of the motor patterns. With the small interval between the sensors, HRCM resolves the problem of missing or incomplete data. Using HRCM, we can analyze various colonic motor patterns/complexes in healthy volunteers and patients; investigating various parameters to better understand their function, and how their presence or lack of is potentially linked to pathophysiology.

Challenges in interpreting HRCM

The subjects were in the supine position for the duration of the study except during meal intake period where the subject is in Fowler's position at 45 degrees. Only during the short period of meal intake is the pressure in the manometry tracing elevated and contains artefacts, and thus analysis during meal intake in omitted and recommences following completion of meal. Subject-induced artefacts (various movements) were noted in the recording with a placement of a marker with a corresponding comment marking the time of artefact; these artefacts were excluded from analysis. There is 3-4L of water introduced at a low infusion rate in the colon through the water perfusion system, however a significant elevation of pressure is not observed in the prepared colon from the baseline period to the period following bisacodyl administration. The lack of increase in pressure is mitigated by the colon's absorptive capabilities (up to 5-6 L of fluid[29] with slow infusion of fluids and approximately 2L per day[30, 31]) and the passive outflow of fluid via the drainage tube inserted in the subject's rectum. While a rapid infusion of water into the colon may result in increased fecal weight, however slow infusion, as found in this study, will not impact the stool frequency or weight[29].The introduction of water may trigger the osmolality sensors in the colon resulting in

potential additional colonic motor patterns. Water perfusion manometry requires a prepared colon in a non-ambulatory setting. Given the non-physiological nature of a prepared colon, the colonic activity reported may deviate from colonic motility under normal, physiological circumstances. However, the objective of the study is to document colonic response to different stimuli, thus the data produced is valid.

Colonic manometry provides detailed information regarding the types of motor patterns and quantitative features of colonic motility (e.g. amplitude, frequency, durations). The limited sensors in low-resolution manometry (6-8 sensors with intersensory distance of 5 -15cm) assessed amplitude based on limited sensors[20], often obtaining the maximum recorded amplitude from a single sensor for the evaluation of HAPWs. Moreover, low-resolution traditionally resulted in an inaccurate determination of HAPW velocity as propagation distance were rough approximations. Detailed characterization of high-amplitude propagating pressure waves (HAPW) which included features such as point of initiation and termination, propagation distance, velocity and amplitude was conducted in healthy controls, only possible with the data provided by the high-resolution[19]. High-resolution manometry allows for both accurate determination of (1) pressure amplitude recorded from multiple external transducers via a perfused silicone catheter, and (2) propagation velocity through the accurate determination of initiation and termination point and the distance of propagation. Simultaneous pressure waves (SPW) is a colonic motor pattern previously ignored due to the uncertainty of it signifying abdominal pressure changes[10]. Through the visualization of the entire colon including the anal sphincter by HRCM, led to the finding that SPWs are a prominent motor pattern that propagates into the anal canal, it is associated with anal sphincter relaxation and gas expulsion in healthy subjects[4].

When I started to research the cyclic motor pattern, it became apparent that our assessment was fundamentally different from reports on this pattern in the literature[5]. This forced me to develop a comprehensive quantitative analysis which led to the

discovery of a never published cyclic motor pattern at a prominent frequency of 12cpm; this frequency is beyond the 2-6cpm activity reported in the literature[9, 22, 23].

Since my work is the first to give a detailed quantitative assessment of haustral activity, I needed to understand the features of haustral activity observed through colonic manometry. This insight was provided by the qualitative analysis of haustral activity by Chen et al.[24] which laid the foundation for the identification and analysis of haustral activity. Thus, the objective of this chapter is to outline steps pertaining to the optimization of HRCM with respect to protocol development and execution. This is followed by devising an effective and efficient method to analyze colonic motor patterns. This chapter outlines the specific methodology developed to (a) identify and (b) qualitatively and quantitatively analyze cyclic motor patterns (CMP) and haustral activity (haustral boundaries and intra-haustral activity).

General Methods

High Resolution Colonic Manometry (84 sensor water-perfused catheter) studies are conducted on adult volunteers, and patients from both adult and pediatric populations. We recruited about 2 volunteers per month that were screened using detailed criteria by Dr. Chen, supervising the study. The subjects were adequately prepared for the study by (a) discontinuing the use of drugs influencing gastrointestinal motility for 48 to 96h prior to the procedure, (b) utilizing a bowel cleaning prep (4L of PEG (70 g/L) over the course of 24 hrs) and (c) undergoing an overnight fast. On the day of the study, the catheter is inserted with minimal sedation (fentanyl i.v. 50–100 mcg and midazolam i.v. 2 – 5 mg) with the assistance of a colonoscope.

Manometry is utilized under different conditions following a 90-minute baseline period which includes balloon distensions (proximal, and distal/or rectal balloon distensions), standardized 1000-calorie meal (500 g of organic vanilla yoghurt fortified with organic milk fat (Mapleton Organics, Moorefield, ON, Canada), providing 800 – 1000 kcal

(based on the volume consumed)), administration of prucalopride (oral or intra-luminal administrations) and bisacodyl (proximal or rectal administration). Bisacodyl is a laxative and a powerful stimulus of enteric and extrinsic sensory nerves for the stimulation myoelectrical and motor activity in the colon and stimulation of intestinal secretions[27]. Prucalopride, a serotonin ($5HT_4$) receptor agonist possesses enterokinetic properties resulting in increased stool frequency and loosens stool consistency via increased colonic motility[26].

This project entails (1) taking part in performing the HRCM followed by (2) subsequent analysis of the recordings. Prior to the HRCM, the prepping and calibration of the equipment (Medical Measurement System (MMS ®; Laborie, Toronto, Canada) is executed. During the HRCM, biopsies are obtained from five segments of the colon, and notes pertaining to the interventions and the volunteer/patient are taken and recorded. These notes, which include reports of patient/volunteer reporting symptoms and artefactual events, are of critical importance.

Without diligent note input into the HRCM recording, several true colonic events may falsely be considered artefactual; notes on symptoms also provide insight into correlations with colonic events. Lastly, the HRCM session is concluded with the sterilization of the catheter and the related equipment.

Signal processing of Medical Measurement System (MMS)

The sampling frequency of the MMS which records the manometry data is 10 per second (600 cpm). This sampling frequency is sufficient in assessing colonic activity in the frequency range of interest (1-20 cpm), thus aliasing error is not a concern in the acquisition of motor patterns recorded and characterized here. The manometry data was not filtered for any artefacts or frequencies when analysis was conducted; rather the original data from the MMS was analyzed on an ImageJ platform for amplitude (mmHg), length (cm), duration (sec and min) and frequency (cycles per minute (cpm)) assessment.

General data analysis

Following the successful acquisition of the data, the next portion of the project requires extensive analysis. Here, qualitative analysis is conducted, whereby all instances of colonic activity and specific motor patterns are investigated. These motor patterns are analyzed using programs developed by Sean Parsons using Image J ® (National Institutes of Health, Bethesda, Maryland, USA) and Matlab ® (Mathworks Natick, MA, USA) to acquire quantitative information such as amplitude, duration, length, velocities, frequencies and location. In addition, special attention is paid to colonic events that have rhythmicity as a constant feature, thus those suspected to be governed by ICC networks. Frequencies are both manually determined on the Medical Measurement System (MMS) which contains all the unfiltered manometry recordings, and the inverse of the intervals between successive pressure wave is calculated through programs developed by Sean Parsons on Image J for verification of manual estimation. The frequencies and propagation direction of such motor patterns are recorded to gain insight into the electrophysiological nature of the underlying ICC network. This data is collected in a spreadsheet format and compiled to create a scientific/academic report for each volunteer/patient for the subsequent creation of a clinical report and for later referencing .

Results

The results are separated into 3 sections that pertain to (1) data acquisition and reporting for high-resolution colon manometry study; (2) the criteria for the identification and subsequent analysis of cyclic motor patterns and (3) haustral activity.

Results 2.1. Motor pattern acquisition

1. Documenting observations

During the HRCM, detailed notes are taken to ensure appropriate analysis of the HRCM recordings. These detailed notes include noting incidences of volunteer and patient body

movements, laughter, deep breathing, and talking, all of which often produce an artefact that is very characteristic of colonic motor patterns such as SPWs. Detailed comments need to be taken to avoid mislabeling an artefact as a motor pattern or attributing a true colonic event to an artefact.

Further notes are taken to document subject- reported symptoms such as pain levels during an intervention, nausea, bloating, cramping, periods of urination, vomiting, and gas or liquid expulsion. Often these reported symptoms are associated with colonic motor patterns. One such occurrence is exemplified in **figure 2-1** where the subject reports of abdominal rumbling and gas sensations, which coincides with a low-amplitude SPW originating in the proximal colon. These reports help us to better correlate symptoms with parallel activities of colon which in turn provide insight into the function of various motor patterns. This point is particularly evident in the recent publication by the lab (Chen et al., 2018) focusing on the Simultaneous Pressure Wave (SPW) as a colonic motility biomarker with its close association with gas expulsion.

Comments are also taken during the colonoscopy procedure during catheter placement. While the scope is maneuvered into the proximal colon, notes reported by Dr. Chen on potential locations of the functional sphincters and regions with other distinct features (e.g. polyps, short rectum, redundant sigmoid) are recorded. These notes are essential as they describe the anatomy of the colon and help to better understand/correlate the contributions/impact of these features on colonic functions.

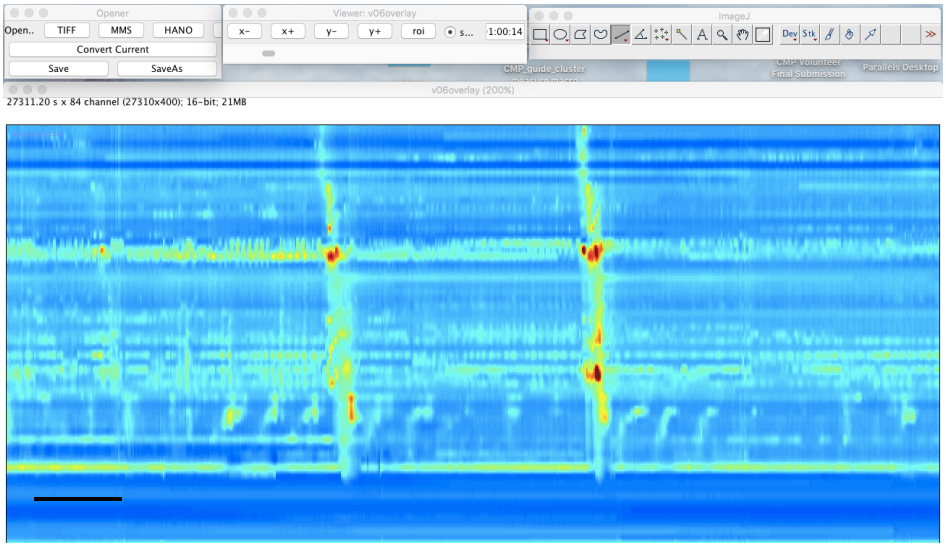


Figure 2-1: Correlation of subject-reported symptoms and motor patterns.

2-D plot of 2 simultaneous pressure waves with anal sphincter relaxations that are followed by subject-reported gas expulsion during baseline period. X-axis is time passed in mins; y-axis is distance in cm from the most proximal sensor (at 0cm). The color map corresponds to the pressure in mmHg. Black horizontal line at the bottom left of the figure corresponds to a scale of 1 min.

Breathing artefact

For further optimization of the HRCM, we set out to definitively identify what was and was not a breathing artefact, and not to eliminate all activity that was in the range of the breathing frequency. Breathing frequency is the low-amplitude, simultaneous rhythmic pressure waves that are present in the background of the colon and rectum visible in the manometry; these are caused by the rhythmic oscillations in the abdominal pressure while breathing that are sensed by the catheter (**fig. 2-2**). In many studies, several low-amplitude, simultaneously propagating rhythmic motor patterns that fall in a large frequency range (8cpm to 15cpm) are dismissed as a breathing artefact[5, 11, 24]. We identified pressure waves that fell within this broad range of frequencies. To address this problem, we began asking the volunteer/patient to do a few exercises. These included holding their breath and doing a few deep breathing exercises. The subject would hold their breath most commonly during baseline when the low-amplitude background rhythmic activity is present and very evident (**fig. 2-2)**. The hypothesis was that if in the

duration of holding their breath the low-amplitude background activity ceases, then that activity was indeed the breathing artefact, while the persistence of the activity would suggest otherwise. However, often when the subject held their breath, it would result in an increased abdominal pressure which exceeded that of the low amplitude background activity. Hence, the low visibility of that activity did not conclusively indicate whether the absence of the activity while the subject held their breath corresponded to their respiratory artefact. Modification to this technique were made whereby the subject was asked to stop their breath momentarily without taking a deep breath in to avoid an increase in abdominal pressure. In addition, we often see rhythmic motor patterns possessing a frequency of 12cpm which was identical to or close to the breathing frequency of the subject. Thus, we requested the subject to hold their breath or stop breathing momentarily during instances of the cyclic motor pattern (at frequencies in the range of 11 to 13 cpm) to record its absence or presence. However, rhythmic motor patterns with this frequency would often present itself during periods of high stimuli and while experiencing some discomfort, such as during proximal balloon distention where it was not practical to ask the subject to hold their breath. Thus, to manually verify the low-amplitude simultaneous pressures in the background as the respiratory artefact, the oscillation of the subject's abdomen was closely followed for its similarity to the feature observed in manometry with respect to the oscillations over a period of a minute (i.e. frequency).

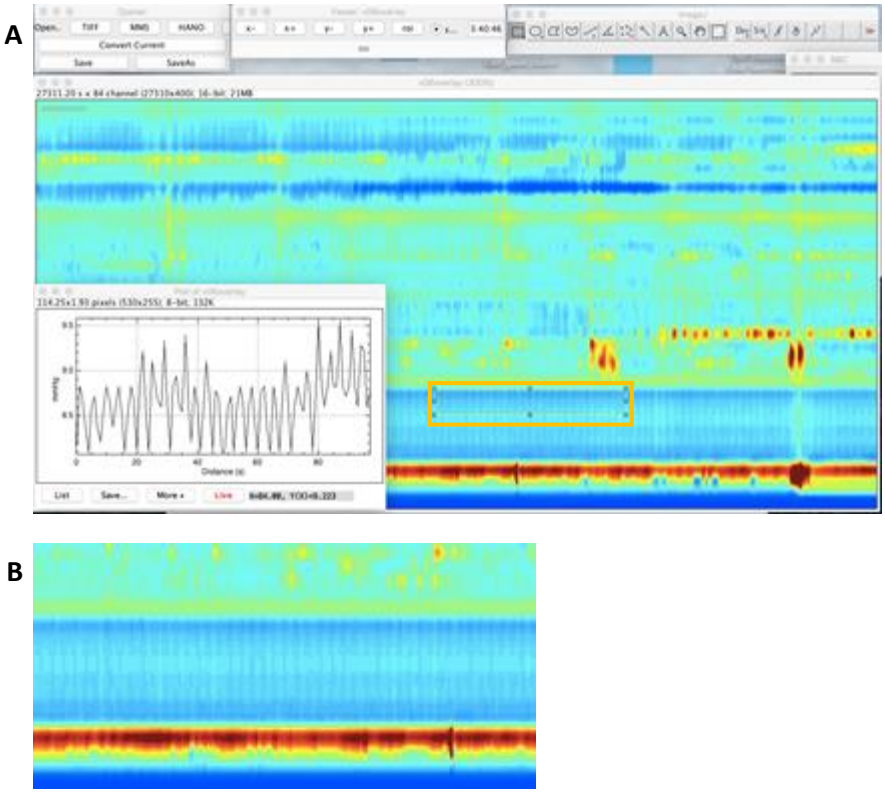


Figure 2-2 : Identification of breathing artefact in the manometry recording.

The breathing frequency is identified in the rectum as low-amplitude rhythmic pressure waves in the background of the recording.

(A) Selected region (in yellow) captures the breathing frequency in the rectum; the frequency is manually estimated using the plot profile tool (bottom left window in A; x-axis is the duration in seconds; y-axis is the amplitude in mmHg). Note the low amplitude of the breathing artefact; this rhythmicity is due to oscillations in abdominal pressure during breathing.

(B) Close-up of the breathing frequency in the rectum. This is in stark contrast to the activity occurring in the distal colon possessing higher amplitude.

In order to correctly identify the identity of low-amplitude rhythmic activity in the background (commonly dismissed as the respiratory artefact) and record all variations of subject breathing, we utilized the impedance portion of the simultaneously conducted cardiography during HRCM. In most subjects, the electrocardiogram and cardiac impedance were recorded to analyze heart rate variability and impedance. In this study, the only data we used from this analysis is the breathing frequency obtained from the

impedance measurements. Impedance was recorded by affixing four electrodes in a standard tetrapolar electrode configuration, where 2 electrodes supplied the constant current source and 2 electrodes sensed the changes in the transfer impedance assessed as a voltage change. The respiratory frequency was determined by measuring the changing impedance of the thoracic cavity where an increase and decrease in impedance corresponded to the inhalation and exhalation[28]. Signals were recorded using a MindWare impedance cardio GSC monitor and Heart Rate Variability (HRV) analyzed by MindWare HRV 3.1 and IMP 3.1 software (MindWare Technologies LTD., Gahanna, OH).

HRCM Preparation

One of the key problems encountered with HRCM with respect to the preparation pertains to setting up the equipment. HRCM possesses 84 sensors that are comprised of fragile parts which are prone to damage. With each HRCM session, it requires the calibration of all 84 channels. The calibration is a tedious and meticulous task that requires careful adjustments to ensure proper calibration of equipment, and to minimize damage and costs associated with the replacement of parts. A protocol was developed outlining steps to be taken to calibrate and prepare the HRCM equipment (see **appendix 1 A**).

2. Data Analysis

Quantification of motor patterns allows for direct comparison amongst volunteers and serves as a comparison to patient data. All data pertaining to the motor patterns are recorded in a spreadsheet format with parameters that include frequencies, length, duration, amplitudes, propagation direction and velocities, intervention it was found during and its association with symptoms the volunteer/patient reported (see **appendix 3, 4**). Regular methods to optimize data collection and analysis are practiced whether that is by adjusting the HRCM protocol (see **appendix 1 B**) or the means through which analysis is conducted (e.g. programs such as macros for ImageJ for the measurement of

individual pressure waves in an episode of CMP, developed by Sean Parsons). Time is spent in the planning and modifications of the data acquisition and analysis to best cater to the objectives of a project. Novel methods of identification and analysis were devised to characterize cyclic motor patterns and haustral activity reported below.

Results 2.2. Cyclic motor patterns identification and analysis

Identification on the Medical Measurement System

The Medical Measurement System (MMS) is the data acquisition system used to record the manometry sessions for volunteers and patients. This tool is used to analyze the colonic manometry recordings and identify colonic motor patterns and activity. The key feature of the cyclic motor pattern is a series of pressure waves of variable propagation directions, often a mix of antegrade, retrograde and simultaneous propagation occurring in a persistent and rhythmic fashion and spanning over 5cm in the colon (refer to **chapter 3**). Rhythmicity is manually assessed as the number of pressure waves in an episode occurring over a duration of 60 seconds. Such instances are noted by recording the initiation and termination time points, alongside their sensor location.

Comments stored in the MMS software and the x-ray obtained during the manometry recording are utilized to construct the anatomy of the colon. The x-ray helps to identify the approximate location of the first sensor through the metal endoclip attached on the catheter. The X-ray also marks the location of the proximal balloon (and distal balloon, when applicable); the balloons are slightly inflated prior to the x-ray for clearer appearance of the balloon in the x-ray image. Lastly, the distinct high-pressure band present in the distal end of the manometry recording marks the sensors corresponding to the location of the anal sphincter. Dr. Chen informs of any distinct colonic features such as a short rectum or redundant sigmoid colon. Together, these identifiers help in estimating the anatomy of the colon to better assign locations of the cyclic motor patterns

in the colon.

Cyclic motor patterns are assessed for their presence in proximity to other motor patterns such as HAPW, HAPW-SPW and SPWs. Instances where the cyclic motor pattern is within 2 min of the initiation of said motor patterns is categorized as being in proximity of the motor pattern. The episodes, defined as continuous instances of rhythmic pressure waves spanning greater than 5cm along the length of colon, are organized based on the period in the study they occur during (e.g. baseline and other study stimuli). All quantitative and qualitative data pertaining to the cyclic motor pattern analysis is recorded in an excel file with all data pertaining to the identity of the patient and volunteer anonymized.

Quantitative analysis using ImageJ

Cyclic motor patterns are exported into a program developed by Dr. Sean Parsons using Image J ® (National Institutes of Health, Bethesda, Maryland, USA). A guide developed by Parsons (Analysis of Colonic Manometry and ECG Data, Parsons, 2017) was utilized to format the manometry recording to be further analyzed. Using the cyclic motor pattern initiation and termination time points recorded in the excel file, these episodes are identified and selected using the rectangle select tool on image J, and a plot profile is generated (**fig. 2-3**). Using the plot profile tool, a threshold amplitude (mmHg) is manually estimated where the maximum number of distinct pressure wave peaks can be incorporated for each episode of cyclic motor pattern. This amplitude threshold value is inputted into a code developed by Parsons titled ClusterSegment_Macro for every manual selection of the cyclic motor pattern episode which subsequently generates a region of interest (ROI) in the ROI Manager. Once all cyclic motor patterns have been selected and inputted into the ROI Manager, the manometry recording will have all the pressure waves pertaining to the cyclic motor pattern episode selected (**fig. 2-4**). Next, another macro developed by Parsons titled ClusterMeasure_Macro is used to measure parameters such as the interval (measured in seconds) between successive pressure waves

in an episode (later used to run automated frequency analysis), the length in cm of each pressure wave, and the mean and maximum amplitudes of the selected pressure waves. These measurements are obtained by selecting the events in the ROI Manager and then running the ClusterMeasure macro (**fig. 2-5**). The output (Results tab) from the results is copied and pasted into the excel file with the corresponding cyclic motor pattern episode.

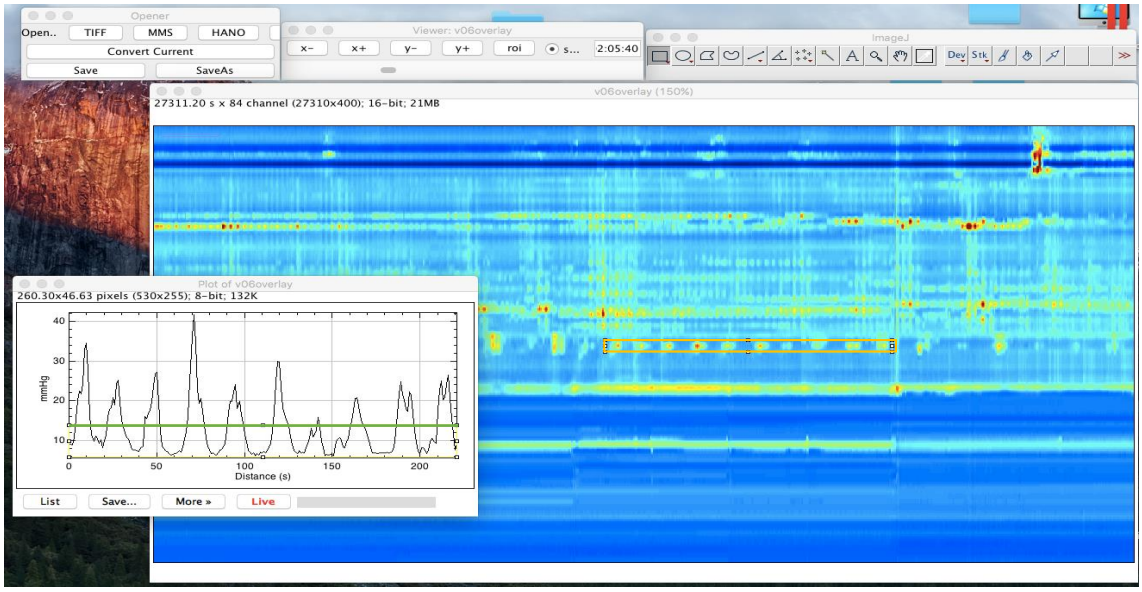


Figure 2-3: Cyclic motor pattern amplitude threshold Plot profile

Bottom left: plot profile with x-axis is duration in seconds and y-axis is amplitude in mmHg, of the cyclic motor pattern (yellow rectangle in the image). The plot profile is generated to (a) confirm the rhythmicity and (b) manually assign a threshold amplitude (green line) which includes the maximum number of pressure waves.

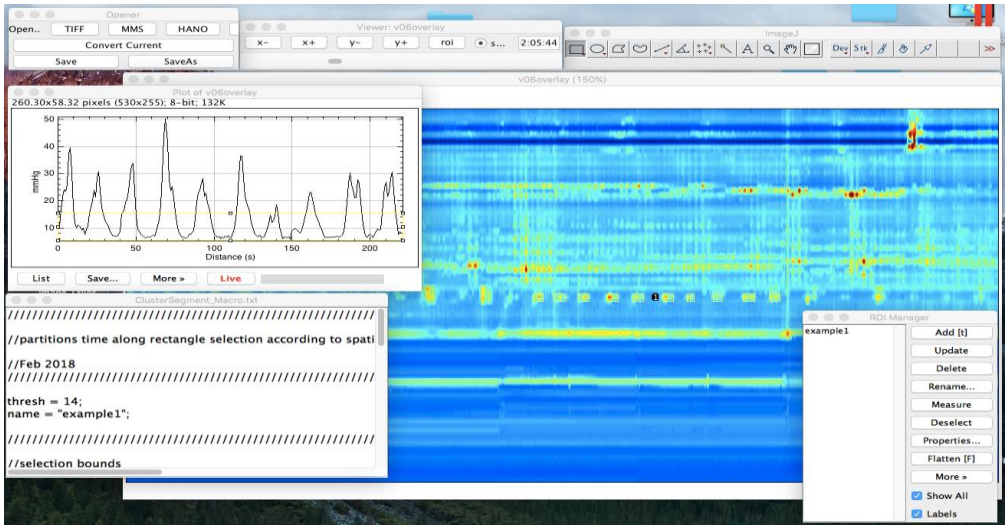


Figure 2-4: ImageJ cluster_segment macro.

The threshold determined manually from the plot profile is specified for the cluster_segment macro (thresh) alongside a name of the cyclic motor pattern event. Note that following the execution of the macro, the manometry recording image contains yellow outlined boxes highlight the individual pressure waves in the CMP that fall above the assigned threshold.

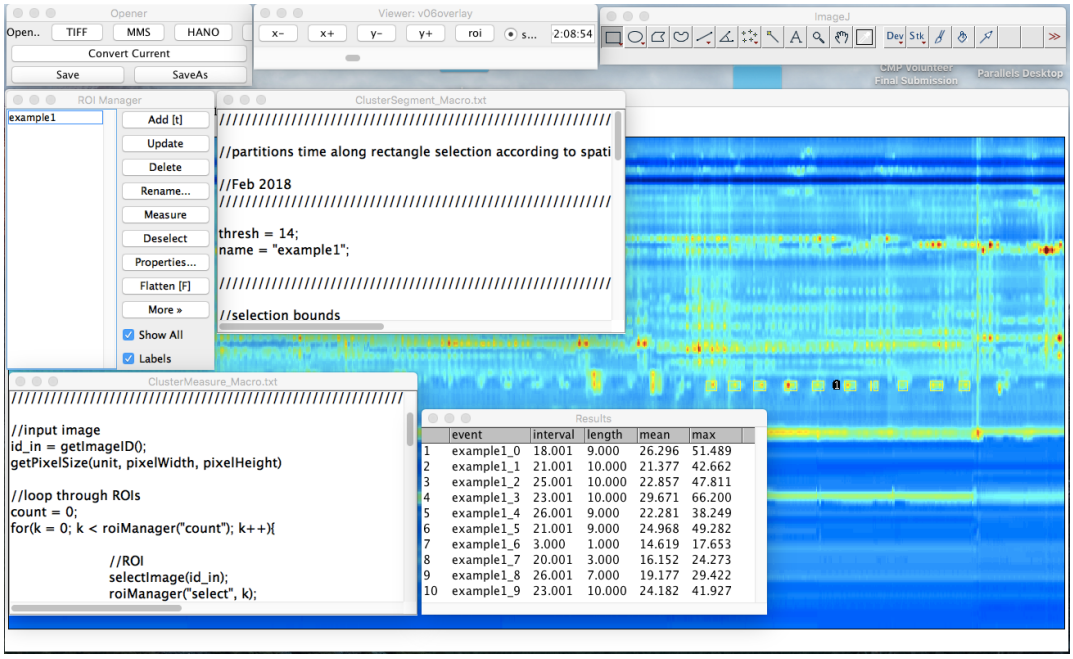


Figure 2-5: ImageJ cluster_measure macro output.

Screenshot of the results output (bottom; center) following the application of the macro. This macro yields length (cm), mean and max amplitude (mmHg) measures for each pressure wave in a cyclic motor pattern event. In addition, interval between every adjacent pressure wave is reported (sec).

The volunteer/patient image file is saved as a ".tiff" file format. The ROI Manager and the pixel size of the file is noted. The saved recording file will save the selected cyclic motor pattern pressure waves as overlays for later referencing.

Propagation velocity assessment using ImageJ

For each episode of cyclic motor pattern, 1 to 2 pressure waves with distinct propagation direction (antegrade and retrograde) are selected and the velocity is calculated. This calculation is done by selecting the line tool on the ImageJ toolbar and dragging the tool alongside a pressure wave with distinct propagation (**fig. 2-6**) from the point at which the pressure wave commences to where it ends; this is inputted into the ROI Manager. Once in the ROI Manager, the pressure wave is selected followed by clicking on the "measure" button. The velocity is estimated by dividing the height (length along the colon; cm) of

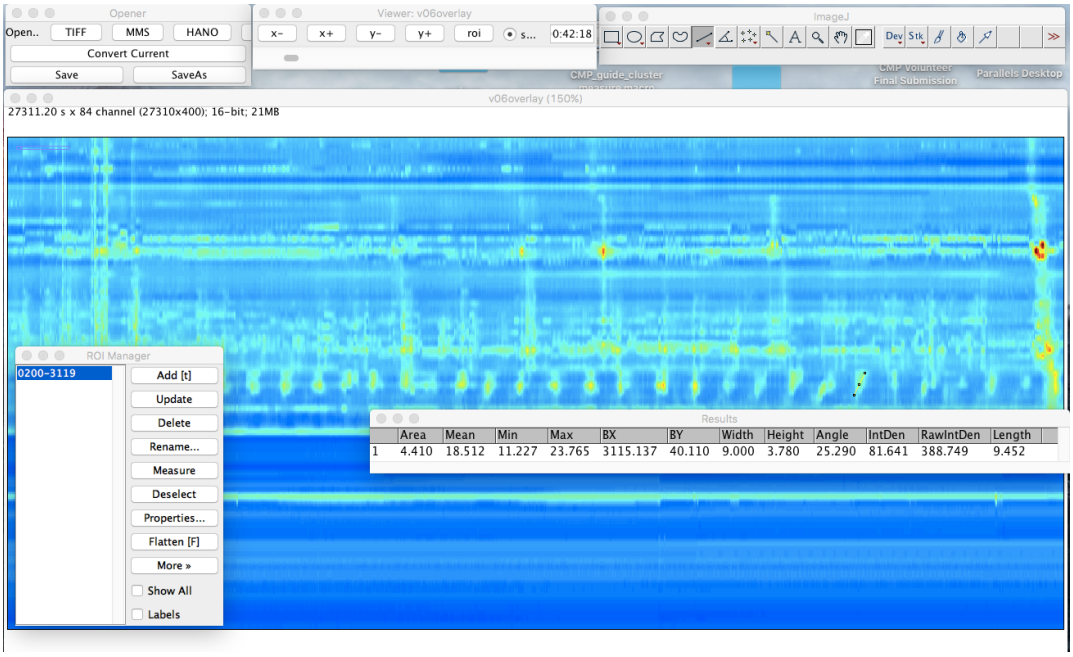


Figure 2-6: ImageJ measurement output for velocity calculations.

Screenshot of the results table in ImageJ. This data is selected and transferred into the data collection excel file for cyclic motor pattern for subsequent analysis.

the pressure wave by the width (duration), thus producing the value for velocity in cm/s. It is important to note that when calculating the height, extra caution is taken to note if a balloon in the catheter is present; this is assessment is done by adding the "height" to the "BY" value (point of origin along the length axis) to see whether a balloon was present (refer to Fig. 3-3 (pg. 73) for information on catheter types). If the sensor values fall in the range of the balloon sensor, then an additional 10cm (corresponding to the length of the balloon) are added to the height and then divided by the width to obtain the final velocity value (velocity = (Height + 10)/ Width).

Cyclic motor pattern analysis using Matlab

Matlab ® (Mathworks Natick, MA, USA) was used for further data analysis and image development for illustration purposes. Using Matlab, 3-D, 2-D and line plots are generated which allows for the viewing and illustration of specific features of the cyclic motor pattern episodes. Line plots are used for specific cyclic motor pattern episodes to

calculate the number of antegrade, retrograde and simultaneous propagating pressure waves; this data is recorded into the excel file. **Figure 2-7** illustrates a 3-D and a line plot; the latter is used to calculate propagation direction of each pressure wave in a cyclic motor pattern episode. Since propagation velocity can be accurately described only up to 7cm/s, any propagation of pressure waves greater than this value is deemed simultaneous [45]; a pressure wave is deemed to have simultaneous propagation when it is distinctively not antegrade and retrograde in propagation .

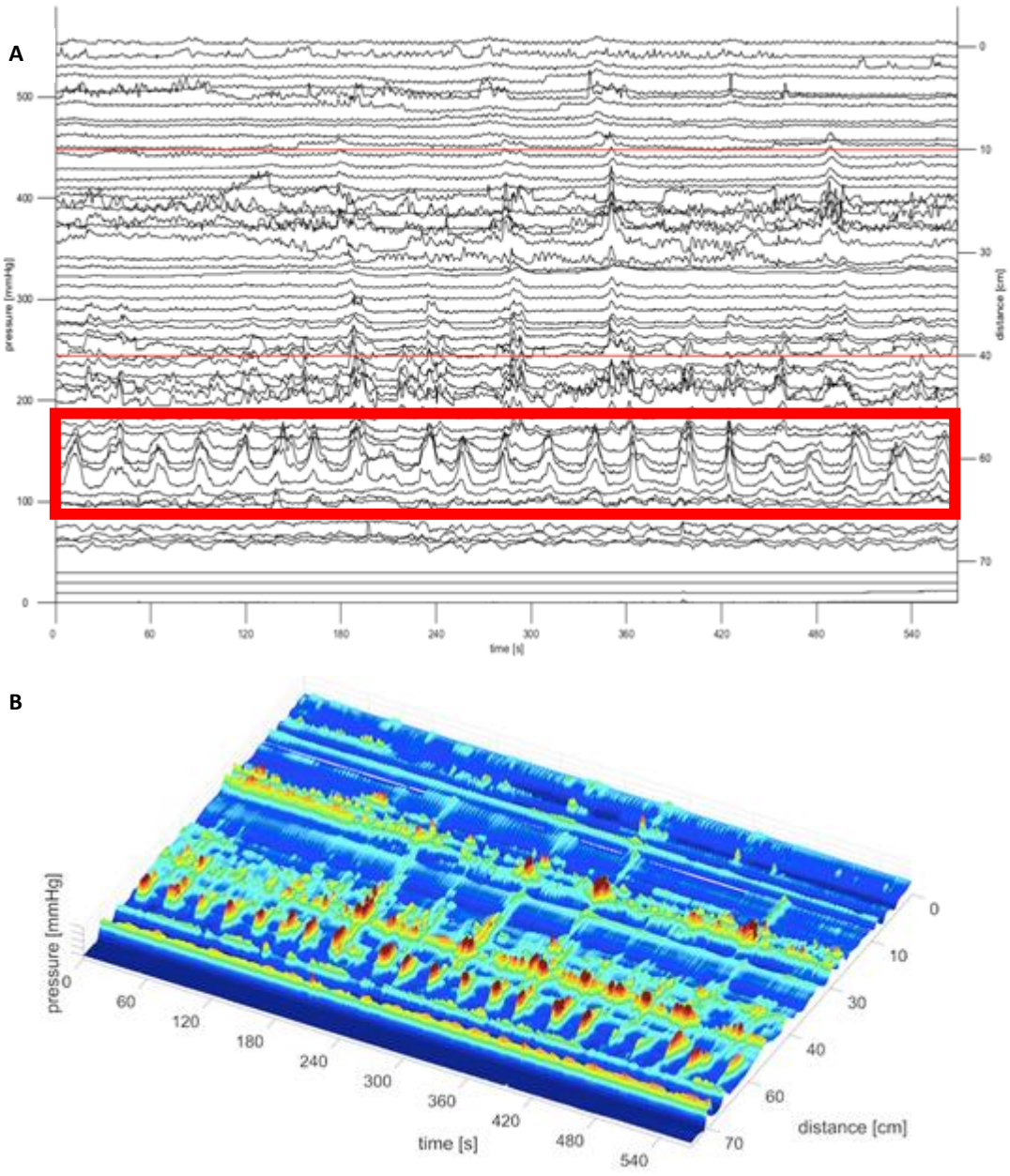

A
pressure [mmHg]
distance [cm]
time [s]
B
pressure [mmHg]
time [s]
distance [cm]

Figure 2-7. Quantification of propagation. Line- and 3-D plot of cyclic motor pattern in the rectum with distinct propagation direction.

(A) Line plot illustrating cyclic motor pattern in the rectum outlined in red. Each individual pressure wave is assessed for its propagation direction. Each horizontal line in the plot represents the pressure from a single sensor. X- axis is time passed in seconds. Left y-axis is the pressure in mmHg and right y-axis represents the distance in the colon in cm (red line represents placement of balloons).

(B) 3-D plot of (A). Color maps (2-D and 3-D) average the amplitude between successive sensor to generate continuous pressure waves. X- axis is time passed in seconds. Y-axis represents the distance in the colon in cm. Z-axis is the pressure in mmHg.

Results 2.3. Haustral activity identification and analysis

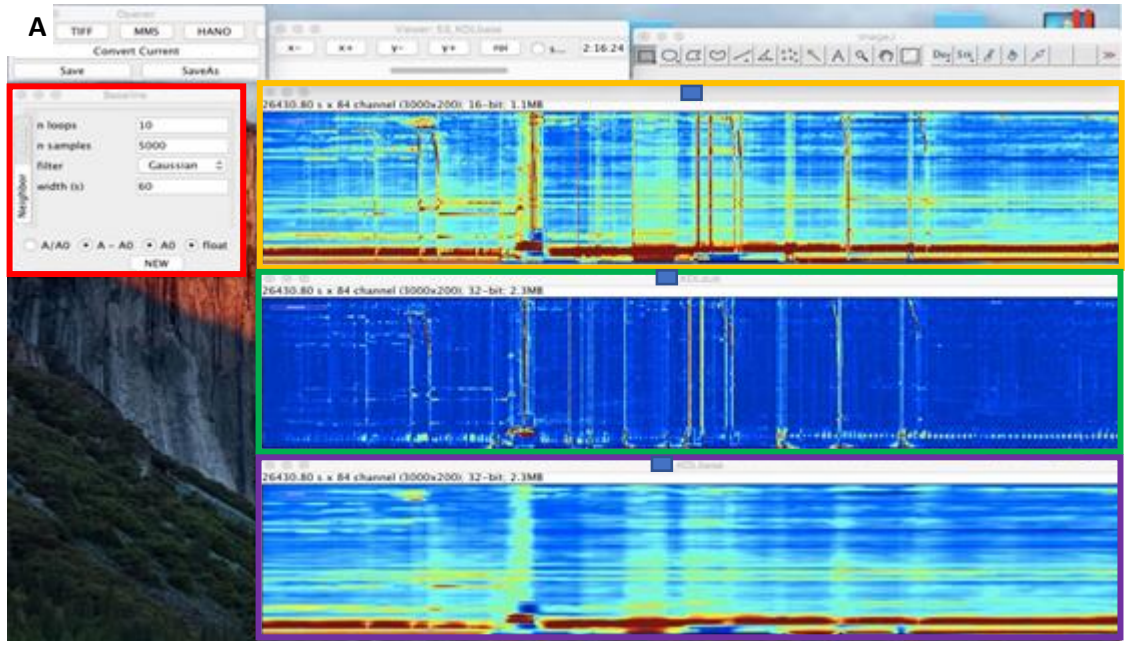
Recording formatting on ImageJ prior to identification of haustral activity

Similar to CMP, episode identification and categorization was accomplished using the MMS tool with subsequent quantification completed using ImageJ. Alternatively, identification and the associated analysis can also be done using the ImageJ platform alone, based on features of haustral activity described in **chapter 4 results**. The steps below highlight the image formatting necessary for haustral activity identification and quantification on imageJ. Prior to any haustral activity analysis on ImageJ, background amplitude adjustment is made to the manometry recording (refer to **chapter 4 methods**). After importing the tiff file (manometry recording saved as a .tiff format), use the "convert current" feature on the Opener window of ImageJ. If the tiff file contains regions where the water was being refilled for the catheter's water perfusion system (artefactual data), then go on Plugins>Event Series>Corrections>Refill; select the region of water refill with the rectangle tool. Click on "interpolation fill", which converts the water refill region into an area with the average amplitudes of periods immediately before and after. Next, go on Plugins>Event Series>Corrections>Baseline; this will prompt a window called "Baseline" as outlined in **figure 2-8a** (red outlined box). Input the values into the window as listed in **figure 2-8**, followed by pressing "NEW"**.** This

will generate 2 additional images: (1) "*image name*_KOLsub" (where image name corresponds to the tiff file name given (green-outlined window in **fig. 2-8a)**) which is the image with the background amplitude removed; and (2) "*image name*_KOLbase" (purple-outlined window in **fig. 2-8a**) which is the recording of the background amplitude only. The "KOLsub" window is utilized for haustral activity identification and reports the amplitude generated by haustral activity without the influence of any ambient background pressures.

Identification of haustral activity

Haustral activity is defined as activity restricted to 4-5cm with or without boundary-like features; these could be rhythmic or arrhythmic. Haustral activity is prominently observed in the vicinity of HAPW, SPW and HAPW-SPW motor patterns (**fig. 2-9**), displaying activation or termination at the motor patterns, thus initial identification of active haustra may be made here.


A
TIFF
MMS
HANO
Convert Current
Save
SaveAs
n loops
10
n samples
5000
filter
Gaussian
width (s)
60
NEW
26430.80 s x 84 channel (3000x200): 16-bit: 1.1MB
26430.80 s x 84 channel (3000x200): 32-bit: 2.3MB
26430.80 s x 84 channel (3000x200): 32-bit: 2.3MB

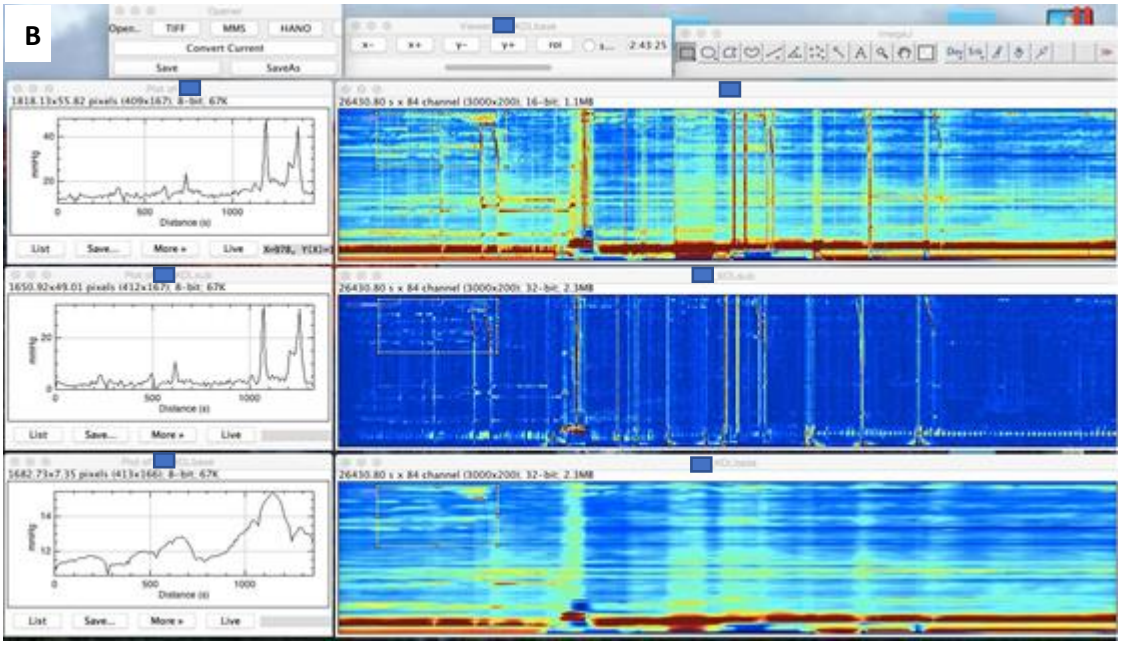

B
Open...
TIFF
MMS
HANO
Convert Current
Save
SaveAs
1818.13x55.82 pixels (409x167): 8-bit: 67K
1650.92x49.01 pixels (412x167): 8-bit: 67K
1682.73x7.35 pixels (413x166): 8-bit: 67K
Distance (s)
List
Save...
More »
Live
26430.80 s x 84 channel (3000x200): 16-bit: 1.1MB
26430.80 s x 84 channel (3000x200): 32-bit: 2.3MB
26430.80 s x 84 channel (3000x200): 32-bit: 2.3MB

Figure 2-8: Background amplitude adjustment.

Steps to remove background amplitude are provided.

A) Red-outlined box is the 'baseline' window with the specific limits outlined where width(s) is the interval between which background amplitude is removed; 'A-Ao', 'Ao' and 'float', where Ao is background amplitude and A is the original tiff image. Orange-outlined window is the original manometry recording in .tiff format; green-outlined window is the image without the background amplitude; purple-outlined window is the background amplitude removed.

B) The 3 image windows in (A) with corresponding plot profiles (y-axis is the amplitude in mmHg; and x-axis is duration in seconds). The decline in amplitude from the plot profile is seen in the middle plot profile when compared to the original plot profile

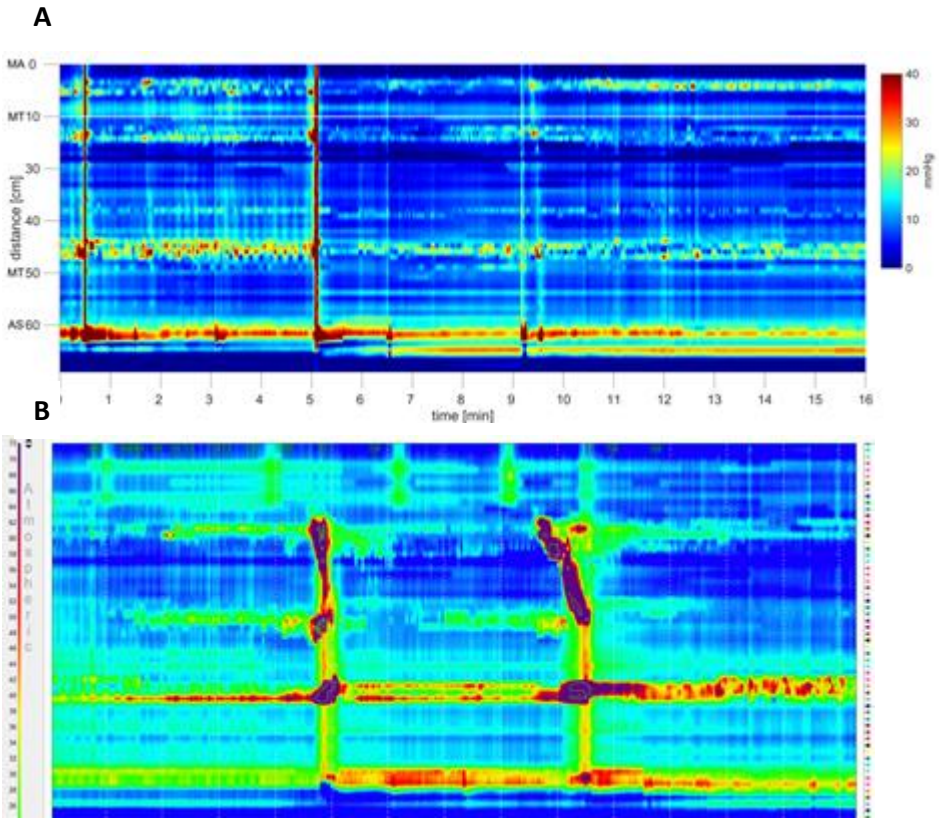


Figure 2-9: Haustral activity and other motor patterns.

2-D plot illustrating the appearance of haustral activity in proximity of SPWs and HAPW-SPW for initial identification. X axis is the duration passed in minutes; y-axis is the distance in the colon where 0cm corresponds to the most proximal sensor. The color bar represents the pressure in mmHg. The abbreviations MA (mid-ascending), MT (mod-transvers), PS (proximal sigmoid) and AS (anal sphincter) are colonic locations.

A) 2 SPWs (0.5- and 5-mins point) between which haustral activity is spotted (at distance along the length of the colon corresponding to 5, 23 and 45cm). Following initial identification, haustral activity can be followed along these colonic locations.

B) 2 HAPW-SPWs between which haustral activity is observed.

The sensors corresponding to identified haustral activity are highlighted and closely followed through the manometry recording for their presence. The sensors corresponding to the potential active haustra are also monitored for any activity (e.g. continuous pressure band or erratic activity) prior to catheter insertion or following the extubation; these are indicative of sensor error rather than haustral activity.
Haustral activity that is not in proximity of motor patterns presents (not within 2 min from the initiation of the motor pattern) the features defined below.

(a) Activity confined in a region 3-5cm in length.
(b) Rhythmicity of the haustral boundary, occupying 1 sensor (but sometimes 2 sensors), and may possess a distinct frequency of typically 2-6cpm (but may present higher frequencies (>7cpm)).
(c) Rhythmicity of activity inside the haustrum, termed intra-haustral activity, typically 3-5cm in length, with frequencies in prominently the low-frequency range (2-6cpm), but sometimes in the high-frequency range (>7cpm). This rhythmic pattern may display distinct antegrade or retrograde propagation, mixed propagation, simultaneous propagation and/or display a segmentation pattern.
(d) Presence of two haustral boundaries corresponding to a single haustrum, typically 4-5 cm apart.
(e) A combination of (b) and (c).

For further details on haustral activity features, refer to **Chapter 4 results** section.

Selection and categorization on ImageJ

Using the background amplitude adjusted "KOLsub" tiff files, the size of the file is assigned through the ImageJ window>Image>Adjust>Size. Deselect "constrain aspect

ratio", the height in pixels is set to 400, and the width (pixels) is assigned to be the same as the number of seconds of the manometry recording rounded to the whole integer; the size in seconds is reported on the image window (**fig. 2-10**; red line). The pixel size for each subject are recoded for future referencing.

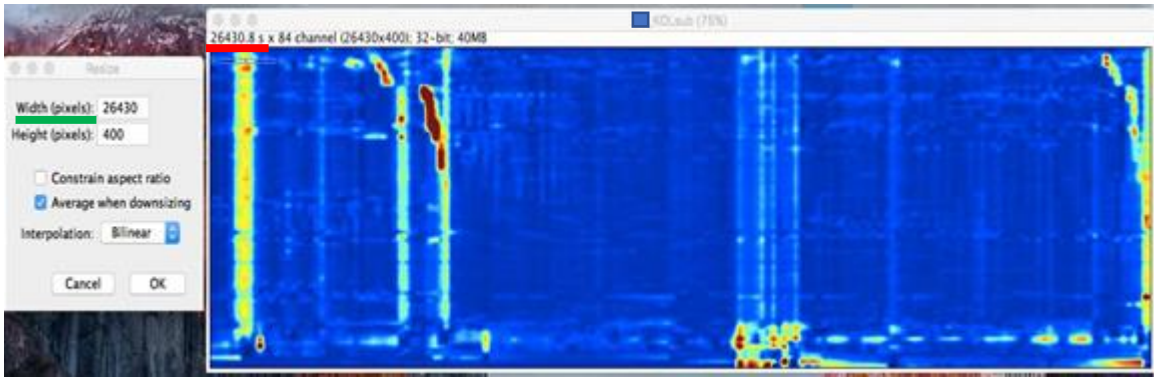


Figure 2-10: Resizing manometry recording.

Resize window with parameters of width and height (pixels); new width (green line) matches the size of the original recording rounded to whole number (red line).

The episodes of haustral activity are selected using the rectangle select tool in ImageJ. It is important to ensure regions of haustral activity that overlap other artefacts (e.g. body movement) and other motor pattern (e.g. HAPW, HAPW-SPW and SPW) are avoided to mitigate their influence on haustral characterization (e.g. the amplitude of HAPW would greatly increase the average amplitude of haustral activity). Furthermore, efforts should be made to closely select the haustral activity to ensure that the length and amplitude outputs from ImageJ accurately reflect those of the haustral activity. Selections should also be made keeping different types of activities (intra-haustral and isolated haustral boundary activity, segmentation and propagation intra-haustral activity, rhythmic and arrhythmic activity) separate to allow for ease of categorization. Once a region has been selected, use the keyboard shortcut, letter T, to input the selection into the ROI Manager (regions of interest). Once all ROIs have been selected, follow the methods of cyclic motor pattern measurements output outlined in **chapter 2.2** (**fig. 2-6**). To obtain specific measurements, on the ImageJ tab select Analyze>Set Measurements; select the

following: Area, Min & max gray value, Mean gray value, Bounding rectangle, Integrated density. Record the measurement output from ImageJ into an excel file (see **appendix 4**) which is integral for the extrapolation of other pertinent data explained below. Save the ROIs in the ROI Manager by selecting More>Save; these ROIs will be saved as an overlay on the tiff file for later reference.

Discussion

This chapter reports the measures taken for data collection on the day of the HRCM study and details a comprehensive methodology on the identification and analysis of cyclic motor patterns and haustral activity.

An important aspect in the optimization of HRCM is trying to (a) validate that the background, low-amplitude simultaneous activity is a true representative of the breathing artefact, and (b) confirm that a commonly filtered frequency of 12cpm is a true colonic frequency, before any analysis can take place. We employed several measures in our unfiltered data (such as those described earlier) to correctly identify rhythmic pressure wave activity seen in frequency range of 10-18 cpm as the breathing frequency from true colonic frequencies. In doing so, we uncovered rhythmic colonic events that share this frequency range. Once true colonic events with high frequencies were conclusively identified, this allowed us to further understand the underlying pacemakers in the colon and the intrinsic frequencies of their slow waves which modulate the smooth muscle contractions and in turn the rhythmic frequencies observed in the HRCM recording (detailed in **chapter 3**).

In the identification of each episode of cyclic motor pattern, data was obtained for each pressure wave with a rhythmicity. The selection of each pressure wave was automated based on a manual threshold assigned using an amplitude plot profile for each occurrence of the cyclic motor pattern. In assigning the threshold, the objective was to capture the maximum number of pressure waves in a cyclic motor pattern episode by assigning a low

enough amplitude as the threshold. Due to the labile nature of colonic frequencies and lack of frequency gradient[25], episodes of the cyclic motor pattern may demonstrate short-lived appearances of pressure waves of contrasting frequencies, that is, pressure waves may demonstrate an overall frequency of 12cpm based on a manual frequency assessment, but the plot profile may reveal 2 pressure waves with a small interval between them (thus high-frequency). Since it is difficult to capture all intervals, and thereby all frequency variations between pressure waves in a cyclic motor pattern episode, an amplitude threshold was assigned to omit the limited presence of such pressure waves. As a result, the average amplitudes reported may be slightly elevated given the threshold assignment.

Haustral activity is composed of haustral boundary and intra-haustral activity. A haustral boundary is identified based on its rhythmic appearance, however once it is identified as part of an active haustrum, the haustral boundary may also appear as transient pressure activity with no distinct rhythmicity and/or as a tonic pressure band for short durations (10-15 seconds); this activity is categorized together as arrhythmic for ease of classification. In a localized region of 3-5cpm, the most typical type of intra-haustral activity is segmentation and propulsive-type activity, however there are infrequent instances of isolated pressure increases along 1-2 sensors which were omitted from the analysis instead of taking the risk of misclassifying those.

The high-resolution with 1cm spacing between successive sensors is adequate for the identification and classification of HAPWs, SPWs, CMPs, anal sphincter activity, however it sometimes proves to be insufficient for the analysis of intra-haustral activity[25]. Intra-haustral activity spans 3-5cm and thus 3-5 sensors are relied on for its classification as propulsive or segmentation. In majority of instances, it is easy to distinguish segmentation from propulsive activity or determine the direction of propagation, but in some cases using the pressure interpolation in the color maps may result in misclassification. To mitigate this possibility, instances where there is uncertainty in the

type of intra-haustral activity, the haustral activity episode is viewed using a line plot which illustrates the data per sensor rather than an interpolation. Line plots help with determining the direction of the propagation, but the limited number of sensors in a single active haustrum (5cm) proves to be low-resolution.

A macro was developed for the identification of cyclic motor pattern pressure waves to provide information on the frequencies. This macro was not used for the frequency assessment of intra-haustral activity due to the overall low amplitude of haustral activity (2-15mmHg). Since segmentation may also show rhythmicity (refer to **chapter 4**), assigning a threshold amplitude proved difficult. Frequencies were manually estimated; and based on the low and high frequency ranges determined in **chapter 3**, haustral activity was also classified as presenting low or high frequencies (see **appendix 4**).

The identification and further classification of both cyclic motor patterns and haustral activity were designed in a way so that the data could be used to pursue other hypotheses. For instance, the haustral activity is also categorized as being active in the proximity of motor patterns such as HAPWs and SPWs. While **chapter 4** has not included the analysis pertaining to the potential interaction of haustral activity and other motor patterns extensively, the data is recorded in such a way to have the foundation ready for exploring this and other questions further.

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Chapter 3 : Cyclic motor patterns in the human colon.

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Abstract

Background

High-Resolution Colonic Manometry gives an unprecedented window into motor patterns of the human colon. Our objective was to characterize motor activities throughout the entire colon that possessed persistent rhythmicity and spanning at least 5 cm.

Methods

High-resolution colonic manometry using an 84-channel water-perfused catheter was performed in 19 healthy volunteers. Rhythmic activity was assessed during baseline, proximal balloon distention, meal and bisacodyl administration.

Key Results

Throughout the entire colon, a cyclic motor pattern occurred either in isolation or following a High-Amplitude Propagating Pressure Wave (HAPW), consisting of clusters of pressure waves at a frequency centered on 11-13 cycles/min, unrelated to breathing. The cluster duration was 1 – 6 min; the pressure waves traveled for 8-27 cm, lasting 5-8 sec. The clusters itself could be rhythmic at 0.5-2 cpm. The propagation direction of the individual pressure waves was mixed with >50% occurring simultaneous. This high-frequency cyclic motor pattern co-existed with the well-known low-frequency cyclic motor pattern centered on 3-4 cpm. In the rectum, the low-frequency cyclic motor pattern dominated, propagating predominantly in retrograde direction. Proximal balloon distention, a meal and bisacodyl administration induced HAPWs followed by cyclic motor patterns.

Conclusions and Inferences

Within cyclic motor patterns, retrograde propagating, low-frequency pressure waves dominate in the rectum, likely keeping the rectum empty; and mixed propagation, high-frequency pressure waves dominate in the colon, likely promoting absorption and storage, hence contributing to continence. Propagation and frequency characteristics are likely determined by network properties of the interstitial cells of Cajal.

Keywords: cyclic motor pattern, colonic motility, high-resolution colonic manometry, rectal pressure waves, interstitial cells of Cajal.

Key Points:

High-Resolution Colonic Manometry allows exploration of motor patterns of the human colon as pressure waves. We characterized all motor patterns with persistent intrinsic rhythmicity, using 84 sensors, 1 cm apart, throughout the entire colon.

A prominent high-frequency cyclic motor pattern, centered around 11-13 cpm, was characterized that was unrelated to breathing and co-existed with the well-known cyclic motor pattern centered around 3-4 cpm. The high-frequency cyclic motor pattern occurred prominently in the wake of 44% of the high amplitude propagating pressure waves (HAPWs).

Introduction

Colonic manometry is the method of choice to diagnose potential colonic motor disorders and the procedure is recommended by consensus guidelines [1,2]. Detailed analysis of colonic motility patterns is now possible using high-resolution manometry. Studies in patients have proposed biomarkers of disease identified by high-resolution manometry [3,4]. However, detailed analysis of normal motor patterns in healthy subjects is still in its infancy and claims for biomarkers of disease deserve scrutiny as more features of normal motor activity become known. In pediatric colonic manometry, the primary goal is to

confirm or exclude colonic inertia which is assessed by the colon's ability to generate High-Amplitude Propagating Pressure Waves (abbreviated as HAPWs or HAPCs or HAPSs), either spontaneously, in response to a meal or in response to bisacodyl. Based on the HAPW appearance, guidelines have been developed to distinguish normal from abnormal activity [1,2]. Although very useful, in particular when colonic inertia is excluded, focusing only on HAPWs assesses only a very small part of colonic motor activity. It is now clear from studies using high-resolution colonic manometry (HRCM) that other motor patterns deserve to be incorporated in the assessment of colon motility such as "simultaneous pressure waves" [5–7] and the "cyclic motor pattern" [3,4,8] or "periodic motor activity" [9]. Dinning et al. proposed that the absence of an increase in the cyclic motor pattern after a meal was a feature of slow transit constipation [4] and Lindley and coworkers proposed that the occurrence of a cyclic motor pattern in between bisacodyl-induced HAPWs was a biomarker of constipation [3]. Hence cyclic motor patterns deserve extensive investigation. Dinning and co-workers defined the cyclic motor pattern as repetitive propagating pressure events with a frequency of 2–6 per min and proposed that the pressure wave frequency and propagation characteristics were orchestrated by interstitial cells of Cajal (ICC)[4]. Consistently, myogenic activity in the human colon has most commonly been attributed to pacemaker-generated activity ranging from 2-8 cpm, often very evident in the human rectum [10,11 12 13]. Interestingly, direct measurement of slow wave activity in the human colon also showed prominent frequencies around 12 cpm [14–16].

A major reason for exploration of rhythmic motor patterns that are likely associated with ICC pacemaker activity [13,17,18 19] is the consistent finding that ICC are markedly reduced in patients with severe constipation who underwent surgery [20 3 21]. It is not known if loss of ICC is a primary cause of constipation but loss of ICC causes dysmotility [22,23]. Hence, an understanding of ICC-directed motor patterns in healthy volunteers and patients may shed light on the consequences of loss of ICC. This may contribute to our understanding of the pathophysiology of severe constipation. Research into plasticity of ICC may lead to future opportunities for restoration of function [24].

Although all motor patterns of the human colon can occur in a rhythmic manner, the objective of the present study was to explore all propagating motor patterns over at least 5 cm in the colon and at least 3 cm in the rectum that have rhythmicity as a defining and constant feature.

Methods

Study Subjects

Nineteen healthy subjects with an average age of 36.4 $\pm$ 14.0 years (range: 19-60 years; 7 females) were recruited as volunteers through local advertising. In 17 of these volunteers, the characteristics of the simultaneous pressure waves have been analyzed and published [6], but the cyclic motor patterns were not analyzed previously nor reported on. All participants gave written informed consent and all procedures were approved by the Hamilton Integrated Research Ethics Board (HiREB). Exclusion criteria included: abdominal surgery, hepatic, kidney or cardiac diseases, connective tissue disorders, central nervous system disorders, thyroid diseases, prostate diseases, or malignancies. All subjects had normal stool consistency and normal bowel frequency; between 1 every 3 days and 3 per day. None of the subjects experienced defecation difficulty, nor did they take any medication that might influence bowel movements.

High-Resolution Colonic Manometry (HRCM)

HRCM was performed on a custom-made platform (Medical Measurement Systems (MMS); Laborie, Toronto, Canada). One of two 84-sensor water-perfused catheter were used (diameter: 8.0mm; Mui Scientific, Mississauga Canada) that included either two 10-cm long balloons between sensors 10 and 11 (proximal balloon) and 40 and 41 (distal balloon), or a single balloon between sensors 10 and 11 only. No sensors were present in these 10 cm segments. In seven volunteers, a separate rectal balloon was used in place of the distal balloon. The catheter is calibrated the morning of the colonoscopy with 0mmHg pressure corresponding to the height of the hospital bed used for the duration of the manometry session. The bed is raised during the colonoscopy and lowered thereafter.

Upon insertion of the catheter in colon, the pressure is mildly elevated relative to the pressure at the initial calibration position. The catheter was inserted with minimal sedation (fentanyl i.v. 50–100 mcg and midazolam i.v. 2 – 5 mg) with the assistance of a colonoscope after a bowel cleaning procedure using an inert osmotic laxative (PEG-Lyte, Pendopharm, Montreal, QC, Canada), but no use of stimulant laxatives such as bisacodyl. For the bowel cleaning procedure, 3 L of PEG (70 g/L) was taken between 4 and 6 pm the day before the procedure, with more water consumed if needed to have all solids removed. The next morning, 1 L was taken at 4 am. The tip of the catheter was clipped to the mucosa via a fish line, a few cm distal to the cecum. The catheter was made of 100% silicon; after use, an extensive approved cleaning procedure was executed followed by sterilization. A disposable dual lumen stomach tube (3.3 mm x 91 cm; Salem Sump^{TM}, Covidien Ilc, USA) was placed in the rectum for passive liquid drainage introduced by the water perfusion system. In 18 of the 19 volunteers, the drainage tube was present for the entire duration of the study. During the recording, the total diameter of the tubing (accounting the water-perfused catheter and the drainage tube) in the participant was 11 mm. This diameter is comparable to the 3D high-definition anorectal manometry (HDAM) catheters (diameter: 10.8 mm) [25].

Protocol

A minimum of thirty minutes after the scope was withdrawn, a 90 minutes recording of baseline activity was started (Table 3-3). The response to a 5 min balloon distension at the proximal colon and/or the rectum was investigated. The balloon was inflated until first sensation was reported; this was followed by incremental increases in balloon volume by 60 mL until the maximum tolerated volume was achieved which was between 250-400 ml air. In each of these periods, the volume was sustained for a short period (between 2-3 min). The extent of the balloon inflation was determined by the subject's level of discomfort in response to the distension. Inflation was stopped when the discomfort reached 6-7 on a 10-point scale, but such that the subject could manage the balloon distention for 5 min. After the 5 min distention, the balloon was deflated.

Analysis of the response to balloon distention was performed on the 5 min period of sustained distention, as well as a 15 min period after deflation. After the balloon distension, a meal was given (500 g of organic vanilla yoghurt fortified with organic milk fat to 40% fat (Mapleton Organics, Moorefield, ON, Canada), providing 800 – 1000 kcal (based on the volume consumed). Its effect was observed for 90 min. Lastly, the effects of 10 mg of bisacodyl (Dulcolax; Boehringer Ingelheim, Sanofi Canada, Quebec) in the proximal colon via the catheter or per rectum (Table 3-3) were observed for 30 min; the bisacodyl suspension was made in saline by crushing 2 x 5 mg tablets, with a pestle and mortar for 5 min.

Water perfusion

The water perfusion rate of the manometry machine was 0.1 ml/min via each sensor, resulting in a maximum total of 0.5 L per hour if all pressure sensors were inside the colon; the pressure was calibrated to 1000 mBar. Each manometry study would last 6-8 hours, resulting in 3-4 L of water being delivered into the colon. The drainage tube diverted 1.2 ± 0.7 L water (N=18). In addition, water was expelled by the subject through colonic motor activities, in particular HAPWs and SPWs, and water absorption will have taken place. Whether inflow of water for the duration of the 6-8-hour manometry session was affecting intraluminal pressure, that might induce motor patterns, was assessed through the Image J-automated comparison of intraluminal pressures made during the baseline period and just before bisacodyl administration as outlined in "statistical analysis" below. Across 19 volunteers, the mean difference in pressure between a period in baseline and a period just prior to bisacodyl administration was 1.2 ± 2.0 mmHg ($p=0.056$), hence no statistically or physiologically significant difference. The subjects were informed about water "leakage" due to propulsive motor patterns, and absorptive pads were provided to give maximal comfort. The discomfort level due to water leakage was different for each subject; from no discomfort at all to some embarrassment with some leakage episodes associated with motor patterns. Comparable results of solid-state

and water-perfused catheters have been reported for the detection of HAPWs [26], and cyclic motor patterns [27].

Visual identification analysis of motor patterns

Data were not filtered prior to analysis. All data were acquired using the software developed by Medical Measurement Systems (MMS ®; Laborie, Toronto, Canada), and analyzed using programs developed by Parsons using Image J ® (National Institutes of Health, Bethesda, Maryland, USA) and Matlab ® (Mathworks Natick, MA, USA). With an acquisition rate of 10/s in high-resolution manometry, the data were used to calculate the amplitude (mmHg), interval between successive pressure waves in a cyclic motor pattern occurrence (sec), propagation velocity (cm/s) and direction (antegrade, retrograde, simultaneous), number of pressure waves within a cyclic motor pattern occurrence, duration (sec, min), and length (cm) in Image J. The direction was assessed according to the upstroke of the pressure event. Since the data did not undergo automated filtering, the intraluminal pressures reported (e.g. in the case of cyclic motor pattern amplitude assessment), were relative to the atmospheric pressure outside the colon. Image J was also used to conduct FFT analysis to confirm the manual frequency assessment. FFT spectra, with decibel scaling, were calculated for each pressure channel over the time period indicated in the figure legends. Thus pressure (channel, time) maps were converted into spectral-power (channel, frequency) maps. Lastly, Matlab was used to generate the 2D, 3D, and line plot images.

Motor patterns in the human colon

HAPWs: High Amplitude Propagating Pressure Waves. HAPWs are defined as transient increases in pressure of > 50 mmHg (average of all measured points within the area of the HAPW) that propagate, almost always in anal direction [28]. They are also referred to in the literature as high amplitude propagating contractions or sequences. HAPWs can occur rhythmically at ~ 1 cpm but it is not a defining characteristic.

SPWs: Simultaneous Pressure Waves. SPWs occur in isolation or following an HAPW; they can occur in a rhythmic fashion at ~ 2 cpm, but it is not a defining characteristic [29] [6,7,28].

CMPs: Cyclic Motor Patterns. The cyclic motor pattern is defined as a cluster of pressure waves; the pressure waves occur with persistent rhythmicity and propagate over a distance of at least 5 cm. The present study developed a comprehensive analysis of cyclic motor patterns with pressure wave frequencies ranging from 0.3-20 cpm. This includes the periodic rectal motor activity at ~ 3 cpm as described by Rao et al. [5] and the cyclic motor pattern extensively reported on by Dinning and co-workers, defined as "repetitive propagating events with a frequency range of between 2 and 6 cycles/min" [8]. Since this motor pattern is always centered around 3 cpm [9 30], we refer to this motor pattern as the "low-frequency cyclic motor pattern". New in the present study is the discovery of a cyclic motor pattern that centers around 11-13 cpm, ranging from 7-20 cpm, we refer to this motor pattern as the "high-frequency cyclic motor pattern".

Synchronized haustral pressure waves. These occur in a very defined space of 3-5 cm bordered by haustral boundaries [28]. These activities were not considered in the present study; their relationship with cyclic motor patterns deserves future studies.

Statistical analysis

The present study was designed to record the baseline colonic motor activity, which was followed by sessions with different stimuli. It is a descriptive study to document all rhythmic activity in the colon and rectum of healthy volunteers. The stimuli were given consecutively, therefore, data following a stimulus might have been influenced by the remaining activity of the previous stimulus, except the first stimulation (proximal balloon distention (PBD)). Frequency, number of pressure waves, amplitude, propagation velocity and direction, length and duration were determined for each motor pattern and recording period; the data are given as mean ± SD. N represents the number of subjects

and n indicates the number of motor patterns. Each parameter noted above was compared as a function of recording period (baseline, proximal balloon distention, meal and bisacodyl administration) and/or as a function of frequency group (high- vs low-frequency). Duration and number of pressure waves were analyzed and reported per subject or reported overall across all volunteers (the latter mode applied in the reporting of average frequency, amplitude, and length (cm) of cyclic motor patterns). Propagation direction of cyclic motor patterns was compared based on overall presence in each recording period or as a function of colonic location in which the motor pattern was present. Significance of the above parameters was determined by one-way ANOVA with the Bonferroni's correction procedure using Prism 8 software (GraphPad USA). The Kruskal-Wallis test was conducted for small sample datasets pertaining to the calculation of cyclic motor pattern duration per volunteer with a follow-up of Dunn's multiple comparison test. Wilcoxon signed-rank test was conducted to assess whether there was a difference in the number of pressures waves before and after a meal per volunteer as a function of high- and low-frequency cyclic motor patterns. Pearson's correlation coefficient test was conducted to assess a potential relationship between the amplitudes of the HAPWs and the amplitudes of the pressure waves in the associated cyclic motor pattern. $P<0.05$ was considered significant.

Potential changes in the intraluminal pressure during the manometry sessions were assessed. In the unfiltered manometry recording, 5-min segments were obtained where artefacts impacting colonic pressure were absent (e.g. coughing, lying on side, bed raised, periods of urination) during baseline and just prior to bisacodyl administration. A segment immediately prior to bisacodyl administration was used given the number of motor patterns present in response to bisacodyl. These segments were analyzed using Image J where baseline corrections were made as outlined previously [31], a Gaussian filter was used with width of the Gaussian defined as 60 sec. Upon determining the average pressure $\pm$ SD of the 5-min intervals during baseline and immediately prior to bisacodyl administration in each volunteer, a non-parametric Wilcoxon signed-rank test was

conducted, given the limited sample size, to assess whether there is a difference in intraluminal pressures in the duration of each recording. $P<0.05$ was considered significant.

The analysis of overall cyclic motor pattern frequency and interval data was carried out with Python in a Jupyter Notebook. Scikit-learn was used for Gaussian kernel density estimation (sklearn.neignbors.KernelDensity) and Gaussian mixture estimation (sklearn.mixture.GaussianMixture). Gaussian kernel density estimation fits the data with a large number (as many as are required to reach fitting tolerance) of Gaussian functions of the same, fixed scale (standard deviation): we used a scale of 1 cpm. Gaussian mixture estimation fits the data with a fixed number of Gaussians (we used 2) with differing scale.

Determining the position of the sensors

The position of a balloon in all figures is identified by a white line which represents a gap of 10 cm where no data were recorded. The length and velocity of the pressure waves indicated in the figures is accurately reported, taking the balloon position into account. The tip of the catheter was clipped near the ileocecal valve in 15 of 19 volunteers (Table 3-3). Recording of the anal sphincters pressure made it easy to identify rectal motor activity. The figures mark the location of the first sensor, the position of the balloon(s) and the anal sphincter which were verified by the x-rays taken. A small amount of air was injected into the balloon(s) to facilitate recognition by X-ray. Radiopaque markers were present at channel 1 and at both sides of the balloon(s).

Impedance cardiography

In all subjects, the electrocardiogram and cardiac impedance was recorded to analyze heart rate variability and impedance. In this study, the only data used from this analysis was the breathing frequency that can be extracted from the impedance measurements. Impedance was recorded by affixing four electrodes in a standard tetrapolar electrode

configuration for impedance recording, where 2 electrodes supplied the constant current source and 2 electrodes sensed the changes in the transfer impedance. The electrocardiogram was obtained by a standard electrode configuration. Signals were recorded using a MindWare impedance cardio GSC monitor and Heart Rate Variability (HRV) analyzed by MindWare HRV 3.1 and IMP 3.1 software (MindWare Technologies LTD., Gahanna, OH).

Results

The cyclic motor patterns

Cyclic motor patterns were defined as clusters of cyclic pressure waves with a distinct pressure wave frequency ranging from 0.3 to 20 cpm (Figures 3-1,2,3,4). Incidental clusters of cyclic SPWs or cyclic HAPWs were not classified within the cyclic motor pattern category. Frequency analysis of the cyclic motor patterns showed that there are two groups of cyclic motor patterns, a "low-frequency" and a "high-frequency" pattern (Figure 3-5, Table 3-1). This was calculated by measuring the average frequency of the pressure waves within each manually identified cyclic motor pattern (Figure 3-5A), and by interval analysis of all pressure waves within all cyclic motor patterns (Figure 3-5B). Both methods showed the same probability of a motor pattern belonging to the high-frequency or low-frequency group (Figure 3-5C). Using the average frequency analysis, the average frequency of the low- and high-frequency cyclic motor pattern were 3.8 cpm and 12.2 cpm, respectively. The interval analysis showed an average frequency of 3.4 cpm and 11.2 cpm, respectively.

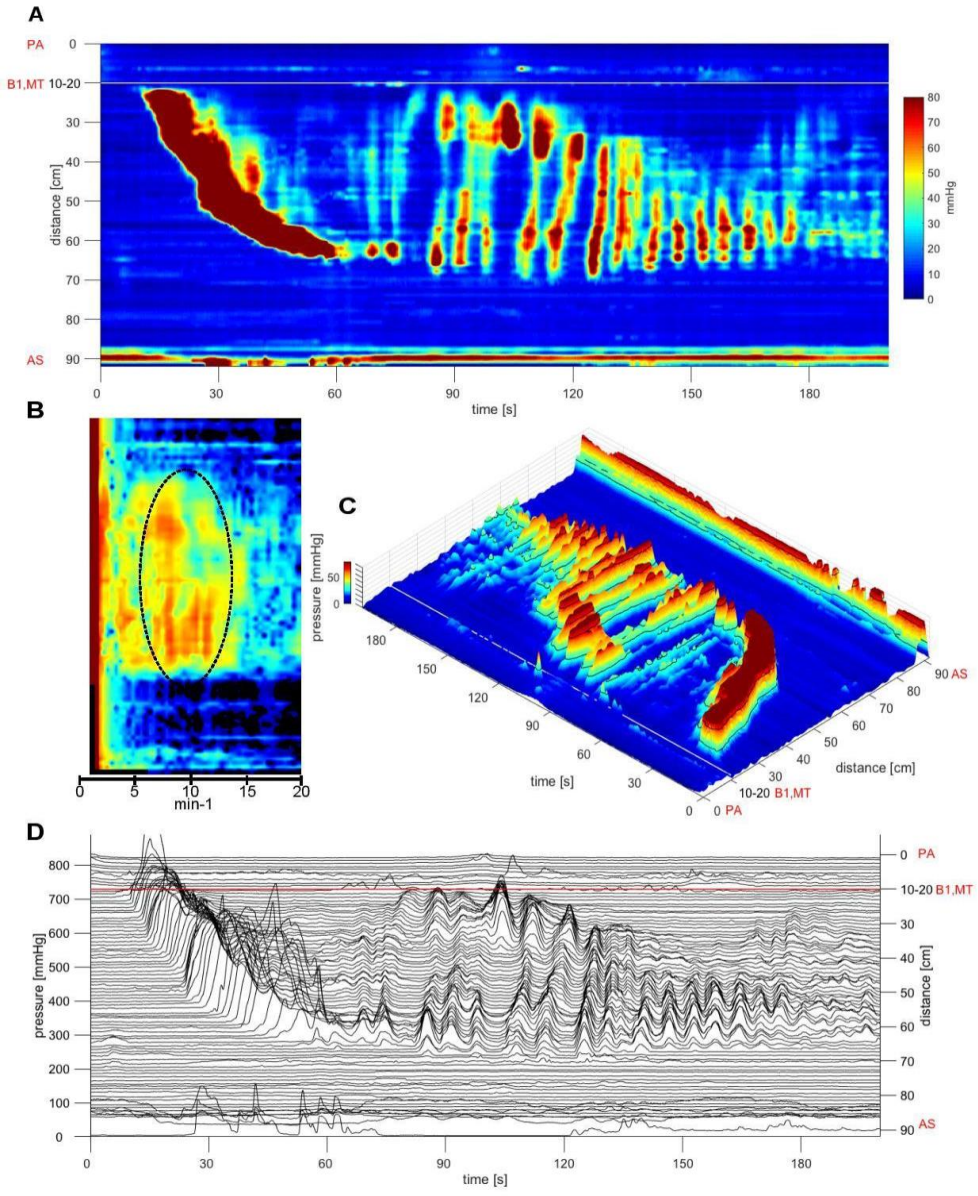


Figure 3-1: The high-frequency cyclic motor pattern occurring post HAPW (1).

(A), (C) and (D). A cyclic motor pattern that follows an HAPW seen in the 2-D, 3-D, and line plot respectively during proximal balloon distention (PBD) with the most proximal sensor (0 cm) in the proximal ascending colon (PA), balloon 1 (B1) in the mid-transverse colon (MT) and anal sphincter (AS) approximately 90cm from the first sensor. White line and (red line in (D)) represent position of the 10 cm balloon. The HAPW average amplitude is 174.0 mmHg which was cut off in the plots to allow for the visualization of lower-amplitude events.

(B) FFT spectrum with a broad peak from 6 to 13 cpm circled with a dotted line. X-axis represents the frequency from 0 to 20 cpm. Y-axis represents the sensors where the vertical scale bar in the spectrum equates to 20 sensors. The FFT analysis was run on the same 140 s duration of the cyclic motor pattern in (A), (C) and (D) following the HAPW. The pressure waves express simultaneous, antegrade and strong retrograde propagation. This contrasts with the breathing frequency, which is also commonly expressed at 11-13 cpm but shows exclusively simultaneous events. Breathing frequency noted in this subject was 15 cpm.

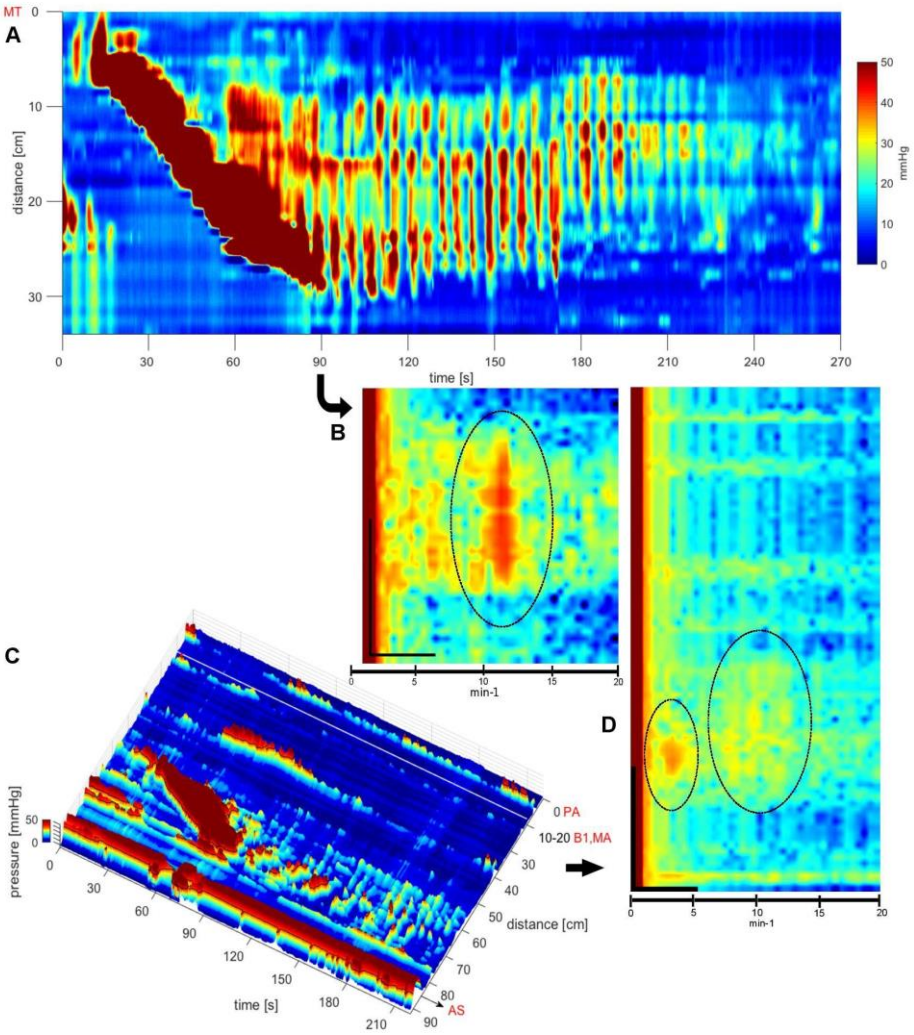


Figure 3-2: The high-frequency cyclic motor pattern occurring post HAPW (2).

(A) 2-D plot of a cyclic motor pattern following HAPW during proximal balloon distention (PBD) with the first sensor in the mid-transverse (MT) colon and no balloons.

(B) FFT spectrum of (A) with a peak between 11 and 12 cpm circled with a dotted line. X-axis represents the frequency from 0 to 20 cpm. Y-axis represents the sensors where the vertical scale bar in the spectrum equates to 20 sensors. The FFT analysis was run on the same 180 s duration of the cyclic motor pattern in (A) following the HAPW. The HAPW average amplitude was 160.2 mmHg which was cut off in the plots to allow for the visualization of lower-amplitude events.

(C) 3-D plot of a cyclic motor pattern following an HAPW in the distal sigmoid colon during baseline, showing interaction of two distinct frequencies estimated at approximately 3 and 12 cpm. The first sensor is in the proximal ascending colon (PA), with balloon (B1-white line) in the mid-ascending (MA) colon and the anal sphincter (AS) located approximately 95cm from the first sensor.

(D) FFT spectrum of (C) with 2 peaks circled with dotted lines at frequencies of 3.2 cpm and another broad peak from 8-13 cpm. X-axis represents the frequency from 0 to 20 cpm. Y-axis represents the sensors where the vertical scale bar in the spectrum equates to 20 sensors. The FFT analysis was run on the same 130 s duration of the cyclic motor pattern in (C) following the HAPW. The HAPW average amplitude was 174.7 mmHg which was cut off in the plots to allow for the visualization of lower-amplitude events.

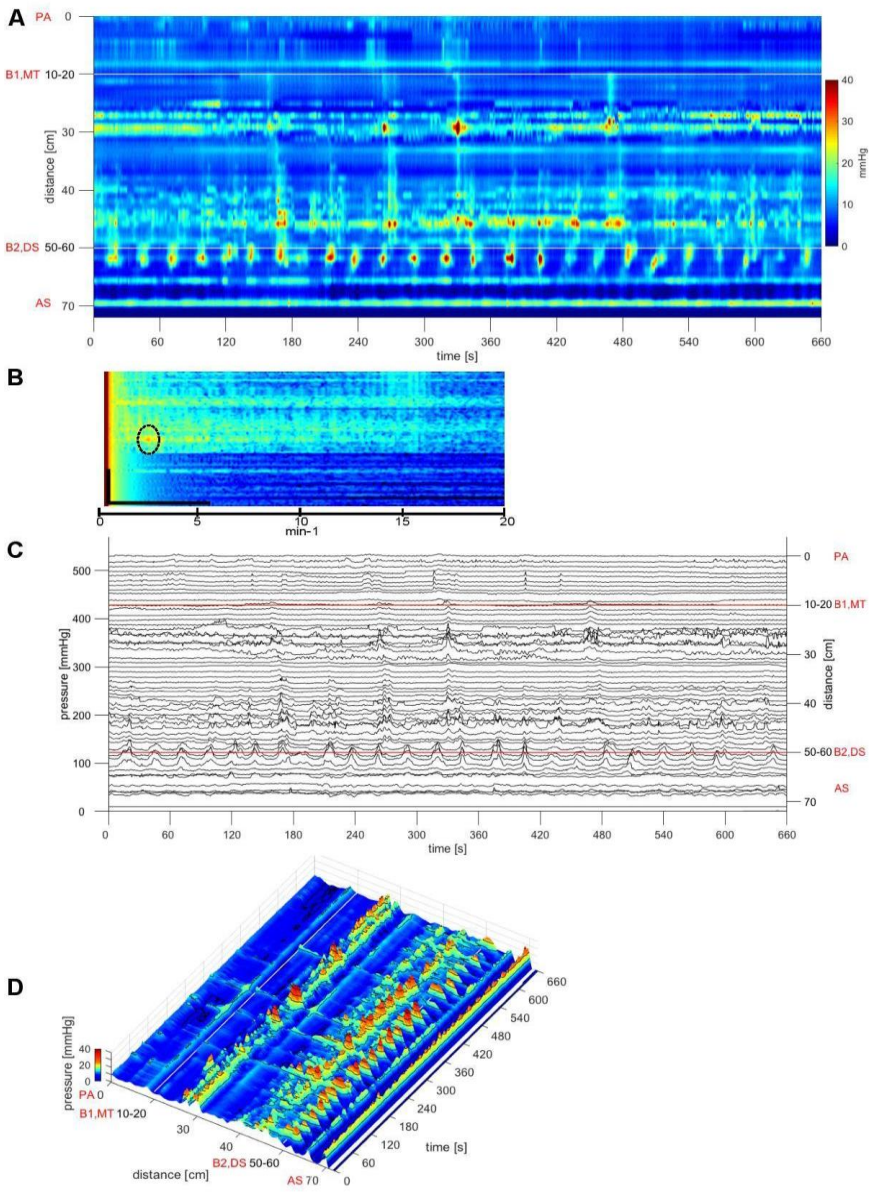


Figure 3-3: The low-frequency (3 cpm) cyclic motor pattern in the rectum..

(A) 2-D rectal cyclic motor pattern, primarily retrograde activity at 2.2 cycles/min during baseline with an average length of 4 cm, duration of 660 s. Two white lines represent 10 cm sections of the balloons (B1 and B2).

(B) FFT spectrum with a peak at 2.2 cycles per min (cpm) circled with a dotted line. X-axis represents the frequency from 0 to 20 cpm. Y-axis represents the sensors where the vertical scale bar in the spectrum equates to 20 sensors. The FFT analysis was run on the same 660 s duration of the cyclic motor pattern in (A).

(C) Line plot of the rectal cyclic motor pattern as seen in (A) operating primarily in the retrograde direction (seen between 60 and 70 cm on the right y-axis). Two red lines represent the balloons (B1 and B2).

(D) 3-D plot of (A): rhythmic activity during baseline in the distal sigmoid colon and rectum. Note lack of rhythmic activity preceding the sigmoid colon. Two white lines represent position of 10 cm balloon (B1 and B2). In (A), (C), and (D) the most proximal sensor is located in the proximal ascending colon (PA), balloon 1 (B1) and balloon 2 (B2) are located in the mid-transverse (MT) and distal sigmoid colon (DS), respectively, and the anal sphincter (AS) is located 70 cm from the location of the first sensor.

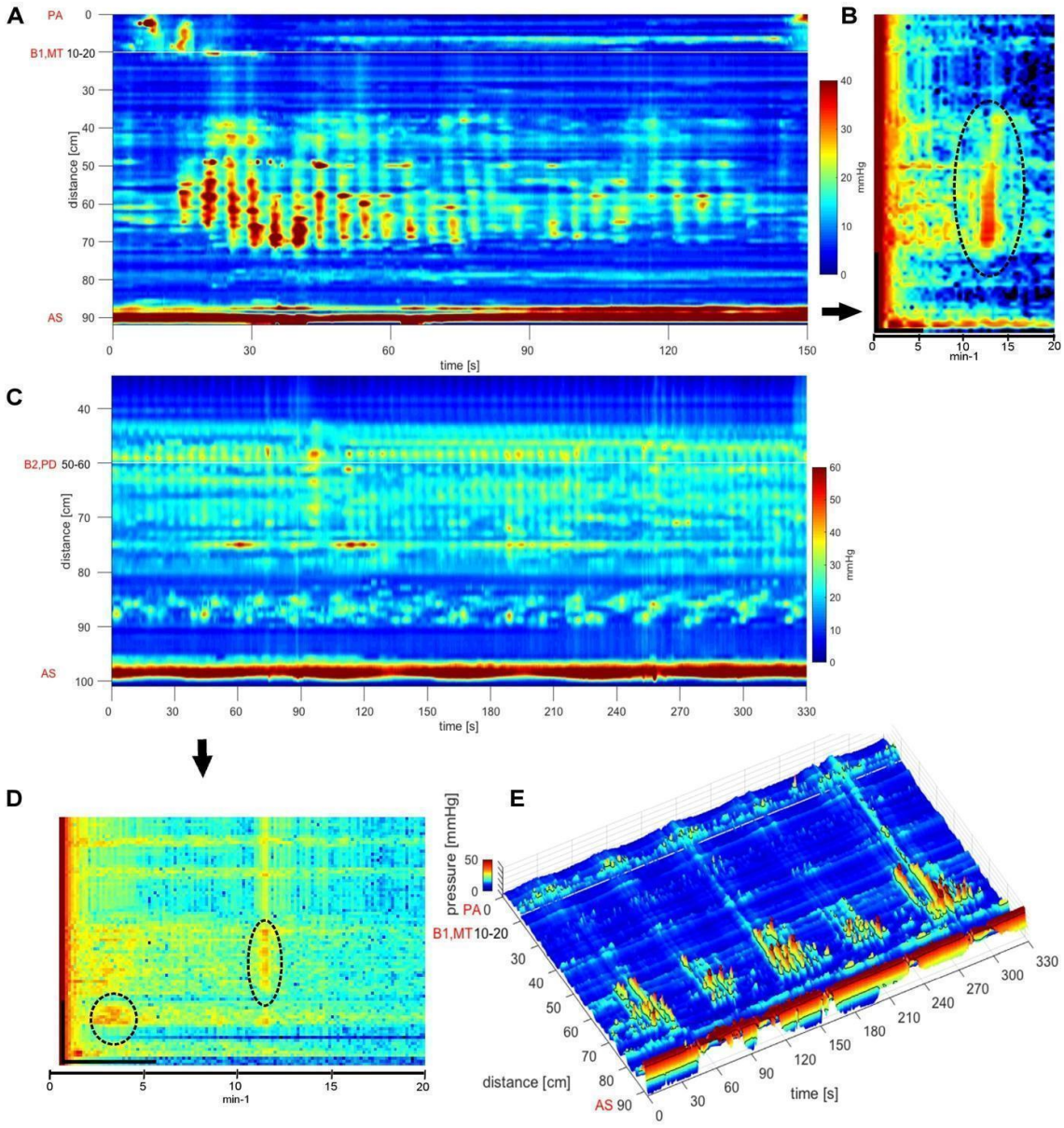


Figure 3-4: The high-frequency colonic cyclic motor pattern.

(A) 2-D plot of colonic cyclic motor pattern in the descending and sigmoid colon during proximal balloon distention (PBD); the first sensor is in the proximal ascending colon (PA), and the balloon (B1) is located in the mid-transverse (MT) colon with the anal sphincter (AS) located approximately 90cm from the most proximal sensor. White line represents positioning of 10 cm balloon.

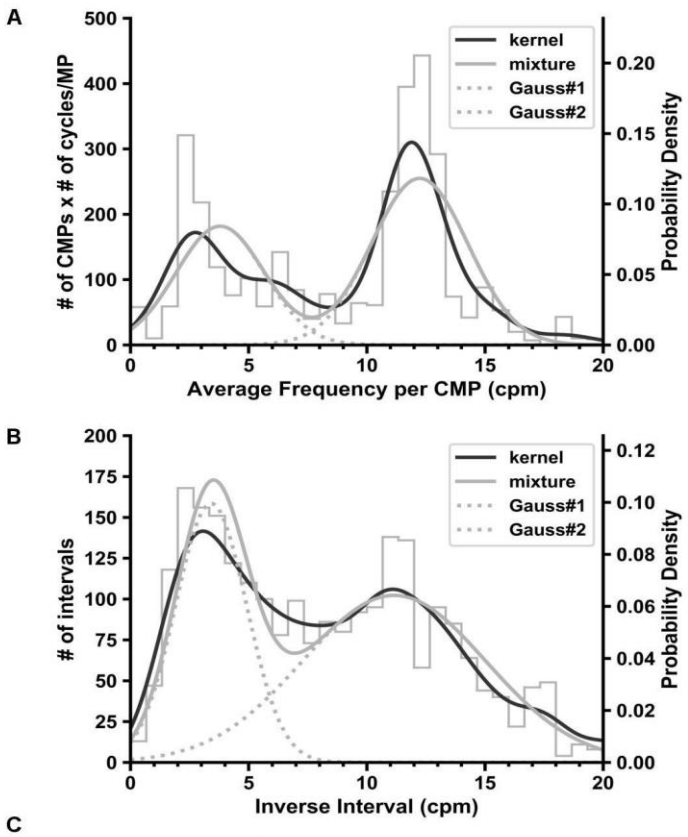
(B) FFT spectrum of (A) with peak centered at 12.7 cpm corresponding to the colonic cyclic motor pattern. X-axis represents the frequency from 0 to 20 cpm. Y-axis represents

the sensors where the vertical scale bar in the spectrum equates to 20 sensors. The FFT analysis was run on the same 130 s duration of the cyclic motor pattern in (A).

(C) 2-D plot of a high-frequency colonic-CMP occurring in the distal colon, during baseline, with a concomitant presence of the low-frequency rectal-CMP and 1 cpm anal sphincter oscillations. The first sensor is in the mid ascending colon; the balloons, B1 and B2, are located in the distal ascending and proximal descending colon (PD), respectively, with the anal sphincter (AS) located approximately 97cm from the proximal most sensor. This figure does not illustrate the location of the first sensor and the proximal balloon location as they were omitted to allow for the clear visualization of the shown motor patterns.

(D) FFT spectrum of (C) with a low-frequency peak centered at 2.7 cpm corresponding to the cyclic motor pattern in the rectum, and a high-frequency peak at 11.1 cpm representative of the high-frequency colonic cyclic motor pattern. X-axis represents the frequency from 0 to 20 cpm. Y-axis represents the sensors where the vertical scale bar in the spectrum equates to 20 sensors. The FFT analysis was run on the same 330 s duration of the cyclic motor pattern in (C).

(E) 3-D plot of the rhythmicity of colonic-CMP clusters appearing at 1 cpm, originating in the proximal colon. The proximal most sensor is in the proximal ascending colon (PA); the balloon is present in the mid-transverse colon (MT) and the anal sphincter (AS) is located approximately 85 cm from the first sensor. The pressure waves within a single cluster of the colonic-CMP in the sigmoid colon displayed high-frequency (10-12 cpm). White line represents positioning of 10 cm balloon (B1).



Gaussian mixture estimation of all cyclic motor pattern frequency data.

Data	Gaussian components		
	Mean (cpm)	S.D. (cpm)	Area under curve (probability)
Average Frequency per CMP	3.79	1.87	0.39
	12.24	2.04	0.61
Inverse Interval	3.41	1.50	0.38
	11.15	3.88	0.62

Figure 3-5: Analysis of cyclic motor pattern frequency by average frequencies and intervals.

(A) All cyclic motor patterns were identified, the average frequency of each individual cyclic motor pattern occurrence calculated and the number of pressure waves corresponding to the average frequency noted. The histogram (stepped grey line) shows the number of CMPs with a particular average frequency, multiplied by (i.e. weighted by) the number of pressure waves present in the occurrence (left y axis). If the histogram is scaled such that its total area is equal to one, the relevant height scale is the probability density (right y axis) which indicates the probability of a CMP having the particular frequency. The histogram was modeled (fitted) by a sum of Gaussian distributions. In Gaussian kernel density estimation (dark smooth line) the histogram is fitted with as many Gaussians as are required for a good fit, but they all have the same width (standard deviation; here set to 1.0 cpm). In Gaussian mixture estimation (light

smooth lines) the histogram is fitted with a fixed number of Gaussians (here 2; dotted lines) of any width.

(B) The interval between every pressure wave in every CMP is measured and is converted into a frequency (1/interval). The histogram (stepped grey line) shows the number of intervals with a particular frequency (left y axis). As in (A) the right y axis shows the corresponding probability density scale and the data is fitted with a Gaussian kernel density estimate (1.0 cpm S.D.) and a Gaussian mixture estimate of two components.

(C) reports the parameters of the two Gaussians (dotted lines) in (A) and (B).

Taking the low-frequency pattern as a frequency range of 2-6 cpm [8,13] and the high-frequency range as 7 cpm or higher, the average amplitude of the high-frequency pressure waves, 22.2 ± 8.7 mmHg, is significantly higher compared to the low-frequency pressure waves at 16.5 ± 6.4 mmHg (Figure 3-6F, $p<0.0001$). Overall, their direction of propagation, retrograde or antegrade, was not significantly different, with simultaneous propagation dominating (Figure 3-6A).

The total number of pressure waves within the cyclic motor patterns per subject were not significantly different before and after the meal. The total number of pressure wave corresponding to low-frequency cyclic motor patterns in the 90 min before the meal was 33.2 ± 33.8 and 20.5 ± 18.1 in the 90 min after the meal ($p=0.34$). Focusing only on low frequency retrograde pressure waves, we observed 19.5 ± 30.4 pressure waves per 2hr per subject during baseline and 7.2 ± 14.9 retrograde pressure waves per 2hr per subject after a meal ($p=0.86$). The total number of pressure wave corresponding to high-frequency cyclic motor patterns in the 90 min before was 30.7 ± 45.4 and 29.0 ± 44.4 in the 90 min after the meal ($p=0.78$).

Three areas were identified where the occurrence of the cyclic motor pattern was prominent (Table 3-2). 1. A cyclic motor pattern often followed an HAPW which was dominated by high-frequency pressure waves with frequencies in the range of 7-20 cpm (Figure 3-1, 2). 2. A cyclic motor pattern was prominent in the rectum (rectal CMP), dominated by low-frequency pressure waves with an average frequency of 3.3 ± 2.3 cpm (range 2-6 cpm). This cyclic motor pattern could be restricted to the rectum but was often congruent with a 3 cpm cyclic motor pattern in the sigmoid (Figure 3-3). 3. A cyclic

motor pattern occurred throughout the colon (colonic CMP), but primarily in the transverse, descending, sigmoid colon, not associated with an HAPW; its pressure wave frequency was a mix of low- and high-frequency cyclic motor patterns, with a range of 0.3 to 16.3 cpm (Figure 3-4; Table 3-2).

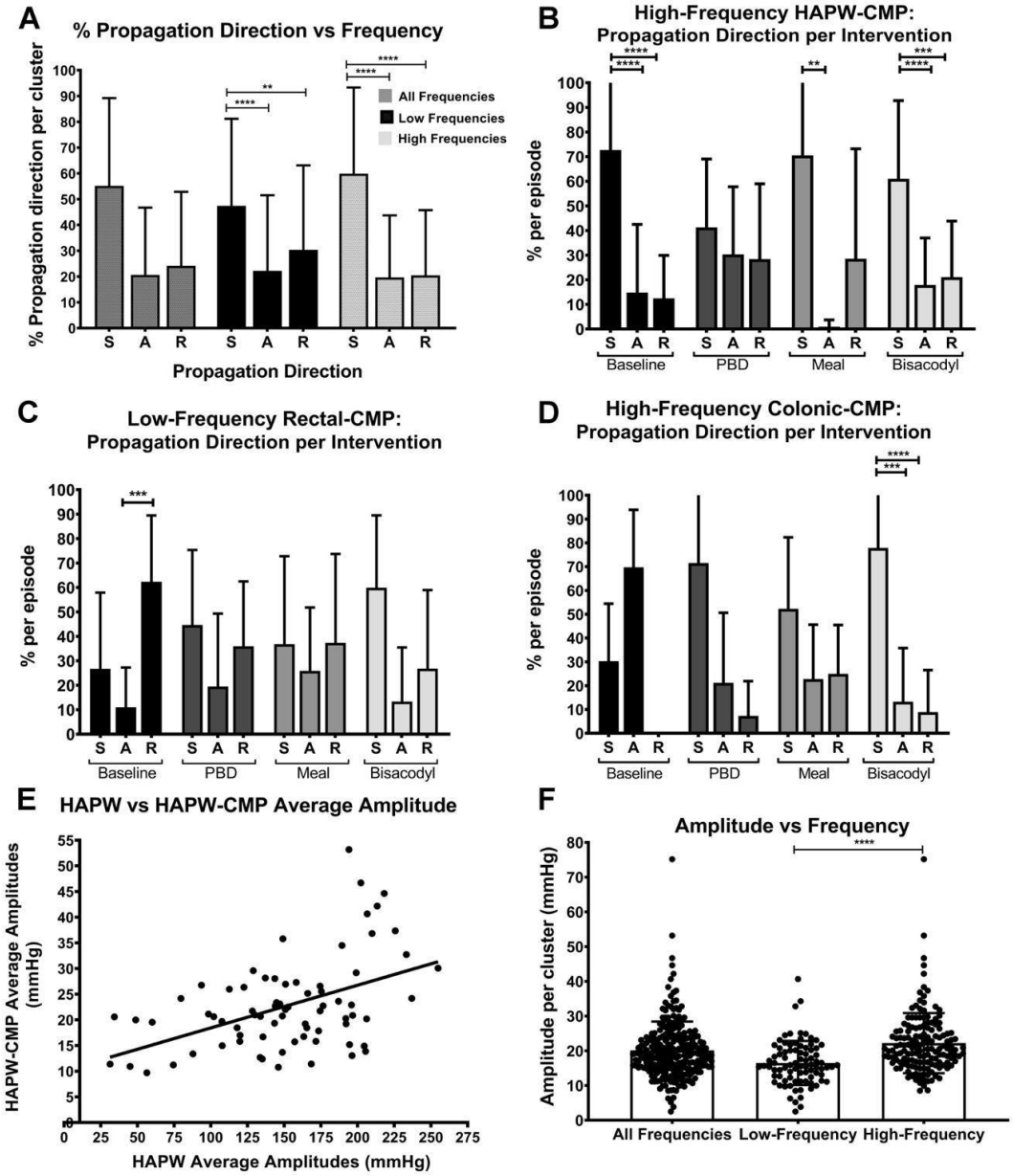


Figure 3-6: Features of the cyclic motor patterns.

(A) Propagation direction (simultaneous (S), antegrade (A) and retrograde (R)) reported as a function of high- and low-frequency groups, and as a combination of all frequencies. Note within each frequency group, simultaneous propagation is most prominent while no statistically significant difference exists between the two frequency groups nor between antegrade and retrograde propagation within the frequency groups.

(B) Propagation direction reported as percent of pressure waves per cluster of high-frequency cyclic motor patterns following HAPWs.

(C) Propagation direction reported as percent of pressure waves per cluster of rectal CMP.

(D) Propagation direction reported as percent of pressure waves per cluster of high-frequency colonic cyclic motor patterns.

(E) Amplitude correlation of all HAPWs and their corresponding cyclic motor patterns (Pearson's correlation coefficient = 0.4662; p<0.0001).

*(F) Average amplitude of pressure waves per cluster of all CMPs and those categorized as the high- and low-frequency cyclic motor patterns. The y-axis reports the average pressure (mmHg) of the pressure waves within the clusters. Each point represents a cluster of the CMP's. **** = p<0.0001, *** = p<0.001,** = p<0.01, * = p<0.05.*

The high-frequency cyclic motor pattern dominates following the HAPW.

From the 243 HAPWs observed, 43.6% were followed by a cyclic motor pattern (n=106; N=17; Figures 3-1, 2). 82.4 % of the frequencies of the cyclic motor patterns were between 7 and 20 cpm (12.8 ± 3.3 cpm); 17.6% of the frequencies were between 2 and 6 cpm, as assessed by the percent durations of cyclic motor patterns. The average amplitude of the high-frequency cyclic motor pattern was 22.7 ± 8.9 mmHg associated with HAPWs of an average amplitude of 140.0 ± 50.7 mmHg. There was a strong correlation between the amplitude of the HAPW and the amplitude of the pressure waves within the cyclic motor pattern following it (p<0.0001; Figure 3-6E). This significance was very evident in the bisacodyl-induced HAPW and the cyclic motor pattern following it (p=0.003). The propagation direction of all pressure waves following HAPWs were as follows: 58.9% occurred simultaneously, 24.2% propagated in oral direction and 16.9% in anal direction. There was no statistical difference between the number of oral and anal propagating pressure waves (Figure 3-6B). Simultaneous propagation was the most common direction in the transverse, descending and sigmoid colon (70.8%, 54.5%, 51.1%, respectively) in any occurrence of the cyclic motor pattern. There was no difference in the percentage of anterograde and retrograde propagation directions within a location, and no statistical difference in the appearance of a particular direction in any location of the colon.

The average length and duration of the cyclic motor patterns that followed the HAPWs were 15.8 ± 8.1 cm and 76.2 ± 53.3 seconds, respectively (Table 3-2). When comparing different locations along the colon, the average frequencies did not differ significantly; transverse 11.9 ± 3.3 cpm (n=51), descending 11.3 ± 4.3 cpm (n=19) and sigmoid 11.1 ± 5.1 cpm (n=42).

The individual pressure waves within the cyclic motor pattern were almost always occurring without interruption but occasionally a clear segmentation pattern was observed, that is, the wave consisted of a pressure-relaxation cycle (Figure 3-2C), resulting in a checkered pattern of isolated pressure transient, identical in appearance to the classic Cannon-type segmentation in the small intestine [32]. This occurred when the low- and high-frequency cyclic motor patterns appeared in the same section of the colon. The pattern was distinctly seen in the distal colon amidst propagating and simultaneous pressure waves.

Distinguishing the high-frequency cyclic motor pattern from the breathing frequency

In most subjects, breathing was identifiable as background simultaneous pressurizations at the breathing frequency established by manually counting breaths per minute, and through the impedance measurements which were obtained simultaneously with the manometry assessment. In 10 subjects with a cyclic motor pattern pressure wave frequency of 12.0 ± 1.2 cpm, the breathing frequency ranged from 15-18 cpm. Figure 3-7 shows that a subject with a breathing frequency of 15 cpm identified by background and impedance had a concomitant pressure wave frequency of 12 cpm. Another distinct feature of the high-frequency cyclic motor pattern is that it shows direction of propagation, that is, any cyclic motor pattern consists of a mixture of simultaneous, retrograde and antegrade propagating pressure waves, whereas the breathing frequency artefact always showed exclusively simultaneous pressure changes. Figure 3-1 shows a typical motor pattern with distinct antegrade or retrograde propagation, in stark contrast to the simultaneous pressurizations as the hallmark of the breathing artefact.

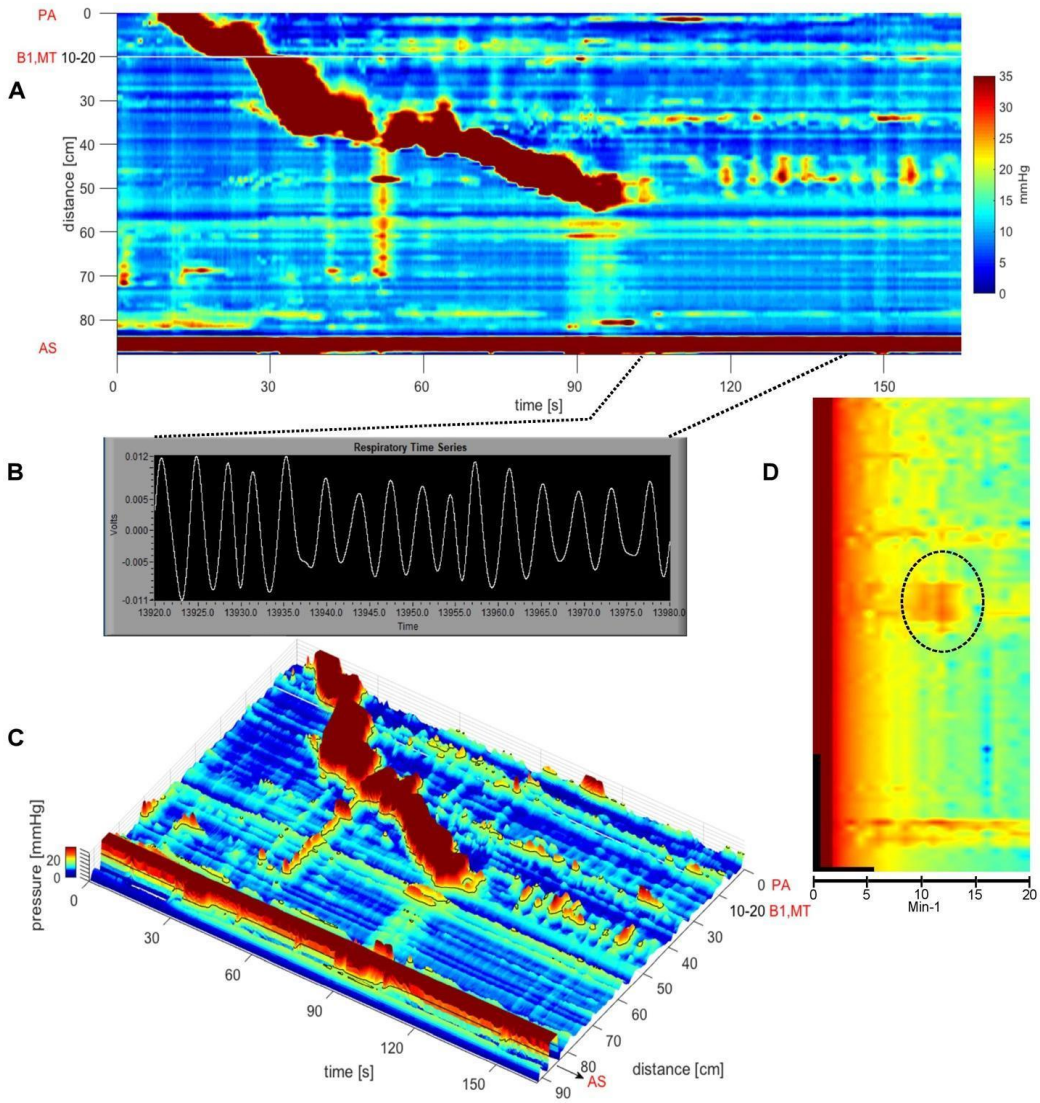


Figure 3-7: The high-frequency cyclic motor pattern is distinct from breathing artefacts.

(A) 2-D plot: HAPW-CMP during the meal intervention with the most proximal sensor in the proximal ascending colon (PA), a balloon (B1) at the mid-transverse colon (MT) and the anal sphincter (AS) approximately 85cm from the proximal sensor. The white line represents positioning of 10 cm balloon.

(B) Respiratory Time Series: y-axis reports the change in voltage with a maximal change of 23 mV; x-axis reports the time of the recording in seconds which is used to time match with the segment of the cyclic motor pattern in the HRCM recording in (A) seen by the 2 dotted lines. The impedance recording directly corresponds to the low-amplitude

background frequency seen between 60 and 80cm (left y axis in (A)), thereby providing evidence that the breathing is 16 cpm.

(C) 3-D plot of HAPW-CMP in (A). The true HAPW average amplitude was 110.0 mmHg which was cut-off in the plot to allow for the visualization of lower-amplitude events.

(D) FFT spectrum of (A) and (C) with peak centered at 11.8cpm corresponding to the cyclic motor pattern following HAPW. X-axis represents the frequency from 0 to 20 cpm. Y-axis represents the sensors where the vertical scale bar in the spectrum equates to 20 sensors. The FFT analysis was run on the same 60 s duration of the cyclic motor pattern in (A) and (C) following the HAPW.

The low-frequency cyclic motor pattern in the rectum

The rectal cyclic motor pattern (Figure 3-3) was present in 14 subjects, occurring a total of 37 times, most prominent during baseline (N=8, n=15), and meal periods (N=6, n=11), although it was also present during balloon distention (N=5, n=7) and bisacodyl (N=3, n=4). The overall amplitude of the cyclic motor pattern in the rectum was 18.0$\pm$5.6 mmHg (Table 3-2). The rectal cyclic motor pattern presented a combination of simultaneous, antegrade and retrograde propagation. The most prominent propagation direction was retrograde representing ~50.2% of total propagation overall, followed by simultaneous direction at 32.8% and antegrade at 16.9%. The overall propagation velocities for retrograde and antegrade direction were found to be 0.82$\pm$0.77 cm/s and 1.10$\pm$1.10 cm/s respectively, not significantly different. Only during baseline was the retrograde direction more prominent compared to the antegrade activity (Figure 3-6C; P<0.001). The average length and duration of the rectal cyclic motor pattern were 5.7$\pm$2.5 cm and 6.0 $\pm$ 4.9 min, respectively (Table 3-2).

The colonic cyclic motor pattern not associated with HAPWs

The colonic cyclic motor pattern, not associated with HAPWs was observed in 18 subjects (n=107; Figure 3-4), most prominently found during baseline (N=16, n=47) and meal periods (N=12, n=29). Three quarters of this activity originated in the descending or sigmoid colon whereas 25% originated more proximally. The colonic motor pattern episodes would also occur simultaneously with rhythmic activity in the rectum (Figure 3-

4C). The propagation direction of the pressure waves across all the colonic cyclic motor patterns was predominantly simultaneous (47%), followed by retrograde (26%) and antegrade (27%) pressure waves. When different interventions and different sites of origin were compared, there were no significant differences between antegrade and retrograde directionality of the pressure waves (Figure 3-6D, Table 3-2). The mean propagation velocity was 6.6 ± 5.8 cm/s and 5.2 ± 6.7 cm/s for antegrade and retrograde propagation, respectively, not significantly different. The length (along the colon) and duration (of the cyclic motor pattern cluster) were both highly variable at 20.1 ± 13.5 cm and 5.0 ± 10.0 min, respectively (Table 3-2). The colonic cyclic motor pattern itself also occurred in a rhythmic fashion (N=9, n=17) at 1.4 ± 0.5 cpm with a range of 0.5-2.3 cpm (Figure 3-4E). Most pressure waves associated with colonic cyclic motor patterns that were not associated with HAPWs belonged to the high-frequency category. The proportion of total pressure waves associated with low- and high-frequency cyclic motor patterns were 37.2% and 62.8%, respectively.

Comparison between the cyclic motor patterns in the rectum, colon and following the HAPWs

Taking all pressure wave frequencies into account, we compared the cyclic motor pattern characteristics in the rectum, colon and following HAPWs (Table 3-2). The pressure waves following the HAPW possessed much higher amplitudes than those within the colonic cyclic motor pattern ($p=0.0002$) and within the rectum ($p=0.0088$.) The largest increase in amplitude across all cyclic motor patterns was from baseline to bisacodyl, specifically in cyclic motor patterns following the HAPW ($p=0.0264$) and within the colonic cyclic motor pattern ($p<0.0001$), while there were no interventional effects on rectal cyclic motor pattern amplitudes. The cyclic motor patterns in the colon and following the HAPW had the greatest proportion of simultaneous propagation. In addition, both the retrograde and antegrade proportions of the HAPW and colonic cyclic motor patterns were significantly less than the retrograde proportions found in rectal cyclic motor pattern ($p<0.05$ overall). The durations and lengths of the cyclic motor

pattern differed (Table 3-2). The colonic cyclic motor pattern was on average present for the longest duration when compared to the durations of the rectal cyclic motor pattern (p=0.002) and the cyclic motor patterns following the HAPW (p=0.0006). The rectal cyclic motor pattern was the shortest motor pattern in terms of length when compared to colonic cyclic motor pattern (p<0.0001) and the cyclic motor patterns following the HAPW (p<0.0001).

TABLE 3-1 The Cyclic Motor Patterns of the human colon			
		Low-Frequency CMP (2-6cpm)	**High-Frequency CMP (7-20cpm)**
Frequency (cpm)		3.6 ± 1.7	12.4 ± 1.8
Number of Pressure Waves/ subject	**Mean ± SD**	12.2 ± 7.5	14.0 ± 14.1
	Min – Max	3 – 35	3 – 107
	25th – 75th percentile	6 – 15.75	6 – 17
Pressure Wave Amplitude (mmHg)	**Mean ± SD**	16.5 ± 6.4 ****	22.2 ± 8.7
	Min – Max	2.5 – 40.7	8.5 – 75.2
	25th – 75th percentile	12.4 – 20.5	16.7 – 26.0
Pressure Wave Propagation Direction (%)	**Percent Direction per CMP episode (mean ± SD)**	Simultaneous: 47.4 ± 33.8% Anterograde: 22.3 ± 29.3% Retrograde: 30.4 ± 32.8%	Simultaneous: 59.9 ± 33.4% Anterograde: 19.7 ± 24.1% Retrograde: 20.5 ± 25.3%
CMP Duration /subject	**Mean ± SD**	296.3 ± 355.3 sec	99.8 ± 218.5 sec
	Min – Max	20 sec – 47 min	14 sec – 38 min
	25th – 75th percentile	90 – 390 sec	33 sec – 105 sec

Table 3-1 The cyclic motor patterns of the human colon.

*(*) represents the comparison between cyclic motor patterns belonging to the 3cpm and 12cpm frequency ranges. **** = p<0.0001, *** = p<0.001,** = p<0.01, * = p<0.05. The values for the 25th and 75th percentiles were included to report where majority*

(50%) of the data was present without the impact of potential outliers that may be present in the min and max values stated in the table above.

TABLE 3-2 Comparison of Cyclic Motor Patterns in the Colon and Rectum				
		HAPW-CMP	**Colonic-CMP**	**Rectal-CMP**
Pressure Wave Frequency (cpm)	Mean ± SD	11.6 ± 4.2 ****	8.1 ± 4.4 # / °°°°	3.3 ± 2.3 ♦♦♦♦
	Min – Max	3.3 – 20	0.3 – 16.3	0.6 – 12
	25th – 75th percentile	9.1 – 13.7	3.9 – 12.0	2.3 – 3.3
Pressure Wave Amplitude (mmHg)	Mean ± SD	22.7 ± 8.9 ***	18.2 ± 6.4	18.0 ± 5.6 ♦♦
	Min – Max	9.7 – 78.2	3.4 – 38.3	2.5 – 32.8
	25th – 75th percentile	18.8 – 26.4	14.5 – 21.7	15.0 – 21.7
Pressure Wave Propagation Direction	Percent Direction per CMP episode (mean ± SD)	Simultaneous: 47.1 ± 31.7%	Simultaneous: 58.6 ± 33.3%	Simultaneous : 36.7 ± 32.8%
		Anterograde: 26.7 ± 29.3%	Anterograde: 16.5 ± 22.3%	Anterograde: 17.2 ± 22.8%
		Retrograde: 26.2 ± 29.8%	Retrograde: 24.9 ± 28.3%	Retrograde: 46.1 ± 32.4%
Pressure Wave Length (cm)	Mean ± SD	15.8 ± 8.1 *	20.1 ± 13.5 °°°°	5.7 ± 2.5♦♦♦♦
	Min – Max	4 – 45	3 – 68	3 – 13
	25th – 75th percentile	10 – 21	8 – 27	4 – 7
CMP Duration	Mean ± SD	1.3 ± 0.9 min ****	5.0 ± 10.0 min	6.0 ± 5.0 min ♦♦
	Min – Max	14 sec – 4.3 min	15 sec – 74 min	40 sec – 24 min
	25th – 75th percentile	35 – 105 sec	47 sec – 5.5 min	2.5 – 8.0 min

Table 3-2: Comparison of the cyclic motor patterns in the colon and rectum without separation in high- and low-frequency categories

*All * represent comparisons between HAPW- and Colonic-CMP; All □ represent comparisons between Colonic- and Rectal-CMP. All ♦ represent comparisons between HAPW- and Rectal-CMP. Four symbols = p<0.0001. Three symbols = p<0.001. Two symbols = p<0.01. One symbol = p<0.05. The values for the 25th and 75th percentiles were included to report where majority (50%) of the data was present without the impact of potential outliers that may be present in the min and max values stated in the table above. # = This value reflects a combination of low-frequency and high-frequency activity.*

TABLE 3-3 Protocol per Subject (Supplementary Table 3-1)													
		Sensor 1 placement					Balloon placement				Bisacodyl administration		
Volunteer	**Catheter Type**	Ascending	Transverse	Descending	Sigmoid	Clip Dislocation	Ascending	Transverse	Descending	Sigmoid	Proximal Infusion	Distal Infusion	Rectal Infusion
01	A	✓m					✓d		✓p			✓	
02	A	✓m						✓m		✓p	✓	✓	
03	A	✓m				✓		✓m	✓d				✓
04	A	✓p					✓d		✓p			✓	
05	A	✓p						✓m		✓d		✓	
06	A		✓p						✓p	✓d	-	-	-
07	A	✓p					✓d		✓d				✓
08	C		✓m				-	-	-	-			✓
09	A	✓m						✓m		✓m	✓		✓
10	A	✓p					✓d		✓d				✓
11	A	✓p						✓m	✓d				✓
12	A	✓p						✓p		✓p	✓		✓
13	A	✓p						✓p		✓m	✓		
14	B	✓p						✓p					✓
15	B	✓p					✓m						✓
16	B	✓p						✓m					✓
17	B			✓p						✓p			✓
18	B		✓d						✓d				✓
19	B	✓m					✓d						✓

Table 3-3: Protocol per healthy subject.(Supplementary Table 3-1)

p, m, and d refers to proximal, mid- and distal sites in specific regions of the colon, respectively. - refers to an aspect of the protocol not performed due to experiment feasibility. The catheters used in the study evolved across successive subjects with respect to the number of balloons incorporated in the design of the catheter. A represents the catheter with a proximal balloon (between sensors 10 and 11) and a distal balloon (between sensors 40 and 41). B refers to catheter with only a proximal balloon between sensor 10 and 11. C refers to catheter with no balloons.

Discussion

The present study shows that in addition to the low-frequency cyclic motor pattern, defined previously as clusters of pressure waves between 2 and 6 cpm [8,9], the colon exhibits a prominent high-frequency cyclic motor pattern that is often seen in the wake of a high-amplitude propagating pressure wave (HAPW) but is also seen throughout the transverse, descending and sigmoid colon as an isolated motor pattern. The high-frequency cyclic motor pattern centers around 11-13 cpm. It is rarely reported on in human colonic manometry studies but noted in early research. Code [33] reported in 1952 type 1 waves in the human colon, described as simple monophasic waves that had the highest rhythmic frequency characteristic for the colon at 13 cpm, in addition to waves at 3-8 cpm. Kerlin, Zinsmeister and Phillips [34] reported colonic frequencies which they could not distinguish from respiratory artefacts. Due to this uncertainty, in many studies, rhythmic activity that was deemed to be potentially caused by respiration, in the frequency range of 8-12 cpm was not analyzed [35–37 37]. In other studies, frequencies between 16 and 20 cpm were deemed potential respiratory movements and removed from the analysis [4,13 30,38]. Other studies defined the cyclic motor pattern by its frequency range from 2-6 cpm and hence did not analyze data > 6 cpm [30]. We did not filter our data nor did we exclude any frequency domain. We did carefully identify artefacts due to body movements and removed those from the analysis. We show that pressure waves within the high-frequency cyclic motor patterns are not caused by breathing since the pressure waves caused by breathing, which were always visible in the background, had a distinctly different frequency as determined by impedance measurements and manual assessments,

and was usually 15-16 cpm. Furthermore, the individual pressure waves within the high-frequency cyclic motor patterns always showed a percentage of antegrade or retrograde propagation, which was never seen in breathing artefacts.

The occurrence of the high-frequency cyclic motor pattern with rhythmicity between 9 and 15 cpm is consistent with human colon electrophysiological studies that showed a prominent presence of slow waves within this frequency range. Sarna et al. recorded with serosal electrodes during surgery and found a frequency of 11.4 cpm to be extensively present (see figure 2 in [14]). Bueno et al. found a prominent presence of cyclic electrical activity between 10 and 12 cpm [15]. Couturier et al. found the most prominent frequency between 8.4 and 10.6 cpm [39], similar to Schang and Devroede, their average was 10 cpm [40]. Consistently, in vitro studies of the human colon circular muscle showed prominently a slow wave activity in a similar frequency range [16 41,42]. Hence, the pressure wave pattern will be caused by rhythmic circular muscle contractions generated by short bursts of smooth muscle action potentials superimposed on slow wave activity in the 9-15 cpm range. The slow waves are generated by one of the dense networks of interstitial cells of Cajal [16,43,44]. Human and animal studies have shown that, in contrast to the stomach and small intestine, the colon pacemaker activity is labile and does not show a persistent frequency gradient [45]. If the frequency is "noisy", that is, if it fluctuates around, for example, 12 cpm, both at the proximal and distal end of the cyclic motor pattern activity, propagation direction will be "chaotic" and can switch direction quickly and can occur simultaneous; in a system of coupled oscillators, the propagation is not determined by sequential depolarization of consecutive cells but rather by the synchronization of cyclic electrical activities with or without phase lags [46 47 48 49]. The high-frequency cyclic motor pattern consists of rather randomly alternating retrograde and antegrade propagating, or simultaneous pressure waves. The electrical activity underlying simultaneous pressure waves can be synchronized slow wave activity without phase lag, or slow wave activity at a very high apparent velocity. In the rabbit colon, where we recorded contractile activity and pressure waves simultaneously, simultaneous pressure waves were often associated with clusters of contractions that propagated at high velocity [50]. Mixed direction of

propagation is likely associated with net non-propulsive activity promoting absorption and storage [51,52].

The present study uses a water-perfused catheter over a period of 6-8 hours. A consideration is whether or not the water perfusion would lead to colonic distention that might introduce motor activity. We observed no significant increase in baseline pressure. Furthermore, the high-frequency cyclic motor pattern was often seen during baseline with features no different from patterns observed later in the recording. The colon has a large capacity to absorb water and water was expelled by the many propulsive motor patterns and some water was drained out via a rectal catheter. Importantly, a frequency spectrum of cyclic activity in the human colon obtained using a fibre optics catheter also showed peaks at ~3 and ~11 cpm, see figure 2 in [53]. In general, comparison between studies that use water-perfused or solid-state catheters shows no significant differences [27].

Many human manometry studies have shown an increase in motility in response to a meal, in particular an increase in HAPWs [54 55 34 56 11 5 13,37,57]. Dinning et al. reported that retrograde propagating cyclic motor patterns at 2-6 cpm occurred on average 5.6 times per 2hr during baseline, which changed after a meal to 34.7 ± 19.8 per 2hr [8,13 38]. This impressive post-prandial increase in the low-frequency cyclic motor pattern became a benchmark for normal colonic response parameters [58], which was then implemented in a later study [4] assessing colonic motor abnormalities in slow-transit constipation. It was concluded that in patients with STC, on average, the occurrence of the low-frequency cyclic motor pattern did not show a difference before and after a meal although in 5 out of 14 patients, the meal initiated the cyclic motor pattern when no pattern was present during baseline (table 1 in [4]); hence the absence of a meal response does not appear to be a consistent feature in patients with constipation. Relatedly, Bassotti et al [59] reported an *increase* in regular rhythmic activity with a dominant frequency of 3 cpm from 10 to 34 % in patients with slow transit constipation after a meal compared to the period preceding the meal. The present study shows that although some subjects showed an increase in the

low-frequency cyclic motor pattern and some showed an increase in the high-frequency cyclic motor pattern, on average there was no significant difference before and after meal intake. This means that the absence of a meal response in a patient with constipation should probably not be seen as an abnormal feature and does not necessarily indicate pathophysiology.

In the present study, the rectal cyclic motor pattern was predominantly retrograde and around 3 cpm. Rao et al. [12], comparing activity at 7 cm (rectum) to 14 cm (sigmoid) from the anus, found that the *clusters* of rectal cyclic motor activity to be 14% retrograde and 47% simultaneous, whereas at night time it was 16% simultaneous and 36% retrograde. This analysis is different from the present study and other studies using high resolution manometry where the characteristics of the individual pressure waves can be analyzed which is not possible with low resolution manometry. In the present study, the presence of the rectal cyclic motor pattern was quite variable (it occurred in 56% of 21 healthy volunteers) and did not, on average, increase with a meal [12]. It was proposed to be an intrinsic braking mechanism preventing untimely flow of colonic contents into the rectum, particularly during sleep [12]. Patients with slow transit constipation showed a 50% increase in this rectal cyclic motor pattern and the hypothesis was put forward that an increase in the rectal cyclic motor activity may contribute to the pathogenesis of slow transit constipation [9]. The rectosigmoid brake hypothesis was supported by studies from Lin et al [8] pointing to the prominence of retrograde propagation in the sigmoid and rectum. This motor pattern was very strong and persistent in the distal colon in patients after surgery involving right hemicolectomy [30] suggesting that it may serve to restore normal bowel function [8]. Interestingly, the pattern shows all the characteristics of a network of pacemaker cells underlying it (see figure 3 in [30]), such as a frequency gradient, patterns of Cannon-type segmentation and dislocations (the sudden disappearance of a pressure wave), as reported on recently, based on comparisons between recorded slow wave activity and a mathematical model of coupled oscillators [49,60].

Giorgio et al. [3] came to the conclusion that simultaneous pressurizations found between bisacodyl-induced HAPWs was a biomarker of neuromuscular pathology in children with constipation since it was not observed in children who were deemed not to have constipation based on HRCM features and subsequent assessments. In the present study in adults from 19 years of age and older, 4.5% of the HAPWs during all interventions across all healthy volunteers were followed by absolute quiescence (and 5.4% post bisacodyl), whereas 43.6% of HAPWs were followed by a cyclic motor pattern (and 36.6% post bisacodyl). Because of the prominence of post HAPW cyclic motor patterns in healthy subjects it appears that this cyclic motor pattern is not a unique feature of constipation in adults. The cyclic motor pattern that follows the HAPWs is primarily high frequency, which may explain why Lin et al did not find a correlation between HAPWs and cyclic motor patterns as they analyzed only the 3 cpm cyclic motor pattern [8].

The high-frequency oscillator and low-frequency oscillator are likely coming from two different ICC networks, since we saw these cyclic motor patterns operate in the same spatial and temporal region in the distal sigmoid colon overlapping with one another (Figure 3-2C). Interestingly, where these two distinct frequencies overlap, there is sometimes evidence of the Cannon-type segmentation pattern similar to the small intestine where we showed that this segmentation pattern is caused by phase-amplitude coupling of two oscillators [32].

In summary, the human colon has two prominent cyclic motor patterns, clusters of pressure waves centering on 3-4 and 11-13 cpm. Within almost all clusters, pressure waves show a mix of antegrade, retrograde and simultaneous propagation, likely to move content in alternating directions. We proved the high-frequency pattern to be unrelated to breathing. This pattern should be regarded as a prominent feature of normal colon motility likely associated with segmentation (absorption and storage) and maintenance of continence.

Contributions and Acknowledgements

HRCM was developed by J-HC and JDH. All HRCM studies were executed by J-HC. All analysis for the present study was executed by MP. MP made significant contributions to data acquisition and data interpretation and wrote the first extensive draft of the paper. SPP made significant contributions to data acquisition, data analysis and interpretation. ER obtained funding and was involved in aspects of data interpretation. The identification and characterization of the motor patterns described here was the result of extensive discussions between all authors. All authors discussed and revised the manuscript and approved the final version.

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Chapter 4 : Characterization of haustral activity in healthy subjects and patients

Introduction

The colon is comprised of haustra, sac-like structures spanning the length of the colon. Graphical representations of the colon always illustrate regular indentations; the out-pouching between adjacent indentations marks the haustrum. The thickening of the outer longitudinal muscle layer results in the formation of thickened bands, the taeniae[1, 2]. Colons with 3 taeniae coli, as seen in humans, rabbits and guinea pigs, form a triangular opening of the haustrum when the circular muscles of the muscular layer contract[2, 3]. The last major sub-structure of the colon inner wall are haustral mucosal folds which are thin elongated structures that protrude from the flat colon wall into the lumen of the colon[4]. In studies analyzing haustration of the colon from cadavers, it is suggested that some haustra may be fixed anatomical fixtures, while others are transient inter-taeniae circular muscle contractions; muscular haustral boundaries cause the distinctive appearance of the triangular opening of the haustrum at the haustral boundary[5] seen during colonoscopies and video-endoscopies.

The indentations observed on the exterior of the colon are not consistently parallel to the folds observed in the inside of the colon. This is because the folds are mucosal. There is much confusion regarding the formation of haustra and what constitutes the boundaries. Haustra may be the result of circumferential circular muscle contractions which form the indentations observed both inside and outside[6]; the circular muscle contractions make up the boundaries of the haustrum. Conversely, the boundaries may be haustral folds- these haustral folds could be (a) whole colon wall folds, thus the indentation is visible in the inside and outside; or (b) a passive fold which is visible in the inside only because of the absence of the muscle contractions; or (c) there could be mucosal folds which are only visible indentations in the inside[7]. Another opinion suggests that the relative shortness of three taeniae coli, that run the length of the colon, “cause the outpouchings of the bowel wall”. Taeniae coli are $1/6^{th}$ shorter than the length of the colon and thus the short length

causes constriction of the colonic smooth muscle, thereby resulting in the formation of haustra[1, 8, 9]. To summarize, haustral boundaries are defined in the literature by mucosal or muscles folds (passive folds due to taenia contractions), or by circular muscle contractions[10]. Fortunately, our study using high-resolution colonic manometry and spacing between sensors of 1cm, allowed for the assessment of active contractions visible from the rhythmic appearances of pressure waves. Our definition of haustral boundaries is "circumferential circular muscle contractions bounding haustra" evidenced by the definitive identification in the manometry recording with evidence of intra-haustral activity and/or haustral boundary activity at regular intervals (intervals of 4-5cm).

Haustral activity has been described as local activity that is not associated with mass movements, but mostly consists of segmentation[11]. Several decades later, high-resolution colonic manometry allows for a more in-depth analysis of colonic features. Chen et al.[10] describes components of haustral activity: haustral boundaries and the intra-haustral activity. Haustral boundaries are pressure transients spaced 3-4cm, but with little activity captured by the sensors in between the boundaries. These boundaries may present rhythmicity which was frequently 3cpm. Intra-haustral activity was demonstrated by activity in 2-5 adjacent sensors; when this activity would produce a complex pressure pattern, segmentation activity is observed. Intra-haustral activity could be synchronized in 4-5 sensors with distinct propagation and a rhythmicity of 3cpm. In the 3-taeniated rabbit colon, haustral boundary activity was also observed as rhythmic circumferential ring contractions, however propagating typically in the anal direction[12].

This study comprises the identification and description of haustral activity in healthy subjects and patients. The first objective of this study is to characterize haustral activity in healthy subjects using features observed in high-resolution colonic manometry. Since the entire colon is comprised of haustra, all colonic activity is haustral activity, but here we focus on single haustra. Hence, by our definition, haustral activity is activity in a confined haustrum of approximately 4-5cm and includes activity of the haustral boundaries and the

intra-haustral activity. The cyclic motor pattern project focused on all rhythmic activity (with the exclusion of HAPW and SPWs that may occur rhythmically) beyond 5 cm of length, which, consequently excluded all single haustral activity that may possess rhythmicity. In this chapter, I also explore the potential links between haustral activity and cyclic motor patterns. The second part of this chapter focuses on patients to (a) utilize the characteristics defined in the first part of the study to identify haustral activity, (b) compare haustral activity to the values derived in controls to increase our understanding of the role of haustral activity in identifying the pathophysiology of constipation on a case-by-case basis. Lastly, cyclic motor patterns will be analyzed in the adult patients followed by comparison to the control values derived in chapter 3.

Methods

Methods utilized here are as per those outlined in Chapter 3.

Statistical analysis

The present study was designed to record the baseline colonic motor activity, which was followed by sessions with different stimuli. It is a descriptive study to document all haustral activity in the colon of healthy volunteers and patients. The stimuli were given consecutively, therefore, data following a stimulus might have been influenced by the remaining activity of the previous stimulus, except the first stimulation (proximal balloon distention (PBD)). Normality of data is determined using Shapiro-Wilk test. Frequency, amplitude, number of haustra, length and duration were determined for each instance of haustral activity and recording period; the data are given as mean ± SD with the 95% confidence intervals provided. N represents the number of subjects and n corresponds to the number of haustral activity occurrences.

Haustral activity was assessed as a whole, and as its components (intra-haustral and haustral boundary activity) individually. Haustral activity and its components were reported as a function of different colonic locations (proximal and distal colon), and its

appearance during baseline and following different stimuli (proximal and distal/rectal balloon distention, meal and bisacodyl administration). The unit used to quantify haustral activity (except for amplitude (mmHg)) was duration in minutes; duration was also utilized to report proportions (e.g. proportion of haustral activity that displayed distinct frequencies). Significance of the above parameters was determined by one-way ANOVA with the Bonferroni's correction procedure using Prism 8 software (GraphPad USA). The Kruskal-Wallis test was conducted for non-normally distributed datasets. Paired t- tests and Wilcoxon signed rank tests were used for comparison of paired data (e.g. amplitude and duration of haustral activity during baseline vs. meal periods). $P<0.05$ was considered significant. Haustral activity and cyclic motor patterns in patients was compared to the 95% confidence intervals determined from healthy subjects.

Graphical representation of data

Violin plots were used for the visualization of the variability in amplitudes and durations of haustral activity for healthy controls. Violin plot is a valuable tool in visualizing both the distribution and the probability density, especially useful in portraying multimodal distributions. Individual data points from each healthy subject are reported in the plots. Graphical representation of data for patients is in a line graph format with control 95% confidence intervals reported for reference; individual patient values that fall above or below control values are categorized as "higher-" and "lower-than control" activity or "above-" and "below-average activity". In the results text, the control 95% confidence intervals are reported in parentheses.

Baseline pressure adjustment

Background pressure may be generated by (a) the difference in height between the original height at calibration and the final height at which the catheter was placed in the subject's colon; and (b) the ambient pressure in the colon. Due to these reasons, the manometry recordings do not demonstrate a true 0mmHg reference. The manometry recordings have been filtered according to the methods described in **chapter 2.3**. The

background pressure has been removed from the manometry recordings, thus amplitudes reported in the text below represent the pressure generated by haustral activity, specifically those generated by haustral boundary and intra-haustral activity.

Reporting methods for haustral activity durations

The durations of haustral activity were reported in 3 ways:

(1) Total duration of all episodes of an active haustrum.

The duration of activity was totaled for each active haustrum. Next, the average duration of activity across all haustra was determined to yield the average duration of activity observed in an active haustrum. For this chapter, this method of reporting the duration will be referred to as duration of activity per active haustrum.

(2) Total duration and number of active haustra.

The total duration of haustral activity across all active haustra is tallied and reported. This value is reported alongside the number of active haustra.

(3) Total duration per 1hr period.

This measure was utilized for reporting duration of haustral activity during different periods in the study (baseline and the successive stimuli). Due to the differences in the length of each period (baseline and meal last 90 min vs. balloon distention which last 20 min), the duration of haustral activity determined in (2) was reported for a 1hr study session period.

Estimation of the number of haustra

To estimate the number of haustra in each subject, first, the length of the colon in the recording was determined using the number of sensors present in the colon and the inter-sensor distance based on the catheter type used (appendix 2). The length of the colon was measured from the proximal-most sensor in the catheter to the sensor that corresponded with the recto-sigmoid junction. The total number of haustra were determined by dividing the length of the colon by the average length of a haustrum determined from the analysis

in this chapter. Next, a ratio of the active haustra (those identified as isolated haustral boundary or intra-haustral activity) to the estimated number of haustra was derived to provide a numerical value for the proportion of active haustra; this ratio allows for comparison between subjects.

Identification and classification of haustral activity

Haustral activity is comprised of haustral boundary activity and intra-haustral activity. The analysis of haustral activity using manometry poses some challenges in determining whether intra-haustral activity also has the corresponding haustral boundaries. In comparison, isolated haustral boundary activity without intra-haustral activity is easier to identify. Thus, data below is reported with respect to haustral boundary activity in isolation as seen in **figure 4-1**, and intra-haustral activity, which may or may not include haustral boundary activity (**Fig. 4-2**). Isolated haustral boundary and intra-haustral activity are further categorized based on its rhythmic and arrhythmic appearance (**Fig. 4-1,2**). Isolated haustral activity span 1-2cm and are first identified based on rhythmicity and/or its association with intra-haustral activity. Intra-haustral activity, which spans 3-4cm, is further classified as demonstrating propulsive and/or segmentation-type behavior (**Fig. 4-2,3**); propulsive pressure waves are the synchronization of 3-4 sensors with distinct propagation direction whereas segmentation appears like a checkerboard-type activity with scattered pressure peaks within 3-4cm. Rhythmicity of haustral activity is assessed and reported as belonging to high (>6cpm) vs. low frequency range (2-6cpm) as defined in chapter 3. Frequencies were recorded with specific values and, due to some haustral activity episodes demonstrating multiple indiscernible frequencies, range of frequencies (e.g. 4-5cpm) observed were noted; thus, specific values for average frequencies were not reported in the results below. Once active haustra are identified, the sensors corresponding with the haustral activity are recorded. Haustral activity continued along the same sensors for the duration of the study, and any activity present in the sensors was categorized as isolated haustral boundary activity or intra-haustral activity. **Table 4-1** reports an in-depth analysis for healthy volunteers collectively, and **Table 4-2**

presents the data for each of the 7 adult patients. **Table 4-3** reports the data pertaining to the cyclic motor patterns in patients. The control values determined from the healthy subjects are used to draw comparisons with each adult patient with respect to haustral activity and cyclic motor patterns.

Results

Features of haustral boundary activity

Isolated haustral boundary activity is pressure observed over 1-2 sensors (1-2cm; n=1059). These may show rhythmicity with frequencies in the low- (2-6cpm) and high- (>6cpm) ranges. Haustral boundary may switch from rhythmic to arrhythmic activity with short durations of sustained pressure as seen in **figure 4-1A**. Single haustral boundary (**fig. 4-2C**), and multiple haustral boundaries (**fig. 4-1C**) corresponding to adjacent haustra, can occur at both low- and high-frequency ranges. A single haustrum is distinctly visible by the upper and lower boundary rhythmic activity 4-5cm apart without the presence of intra-haustral activity (**fig. 4-1B)**. Haustral boundaries are a result of circular muscle contractions that form a triangular-shaped opening of the haustrum (**fig. 4-1E**); complete contractions of circular muscle results in occlusion of the haustrum (**fig. 4-1D**).

Features of intra-haustral activity

Intra-haustral activity is activity bound by 2 haustral boundaries spanning 4.6 ± 1.1 cm (n=1343) along the length of the colon (**fig. 4-2A, fig. 4-2C**). This activity is further classified as segmentation activity when it is seen as a complex pressure pattern inside the haustrum with a distinctive checkerboard pattern (**fig. 4-3A-B);** mixed peaks of pressure waves confined to a region 4-5cm in length. Intra-haustral activity may demonstrate propulsive activity with rhythmicity in the low-frequency (**fig. 4-3C**) and high-frequency (**fig. 4-7B**) ranges. Propulsive intra-haustral activity presents antegrade (**fig. 4-14B**), retrograde (**fig. 4-14B, fig. 4-3C**) simultaneous (**fig. 4-14B**) and mixed propagation (**fig. 4-2C**).

Video colonoscopy

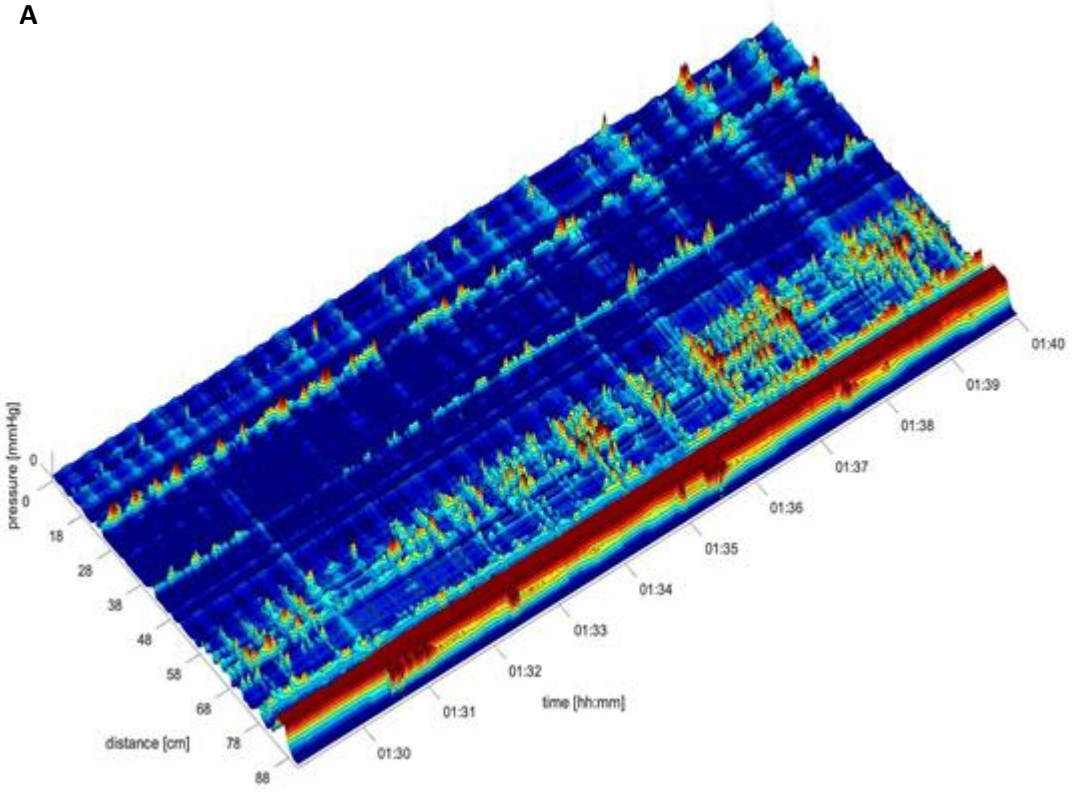
Using video colonoscopy to view the inside of a haustrum reveals that the boundaries of the haustrum are bound by circular muscle (**fig. 4-3D**-1st from the left). The walls of the haustrum as viewed from the inside may be flat (**fig. 4-3D**-2nd from the left) and may show haustral folds (**fig. 4-3D**-3rd from the left; ridge-like appearance) where previously the colonic walls were flat. The height of the haustral folds may be as high as 1cm (**fig. 4-3D**- 4th from the left).

Changes in types of haustral activity within a single haustrum

There is variability over time in the types of haustral activity that occur within a single haustrum. Switch between segmentation and propulsive intra-haustral activity is seen in **figure 4-3A** where segmentation activity transitions to mixed propagation intra-haustral activity. A haustrum with intra-haustral activity may transition to isolated haustral boundary activity with distinct rhythmicity (**fig. 4-1B** and **4-3A**). Intra-haustral activity may or may not include haustral boundary activity because the synchronization of activity across the entire haustrum makes it hard to definitively spot haustral boundaries. In some instances, a single haustral boundary and the adjacent intra-haustral activity may be apparent (intra-haustral activity with lower boundary (**fig. 4-2B)** and upper haustral boundary (**fig. 4-1B**)).

Once active haustra were identified based on the presence of isolated haustral boundary activity and/or intra-haustral activity, they almost always continued at the same sensors for the duration of the study (Table 3-3 reports of catheter shift in 1 subject). We rarely identified haustral boundary propagation; if there was some propagation, it was not possible to ascertain if it was active propagation. Thus, once a haustrum was identified, the activity could be described for the duration of the study despite that in some instances the boundaries became "lost" due to loss of pressure.

A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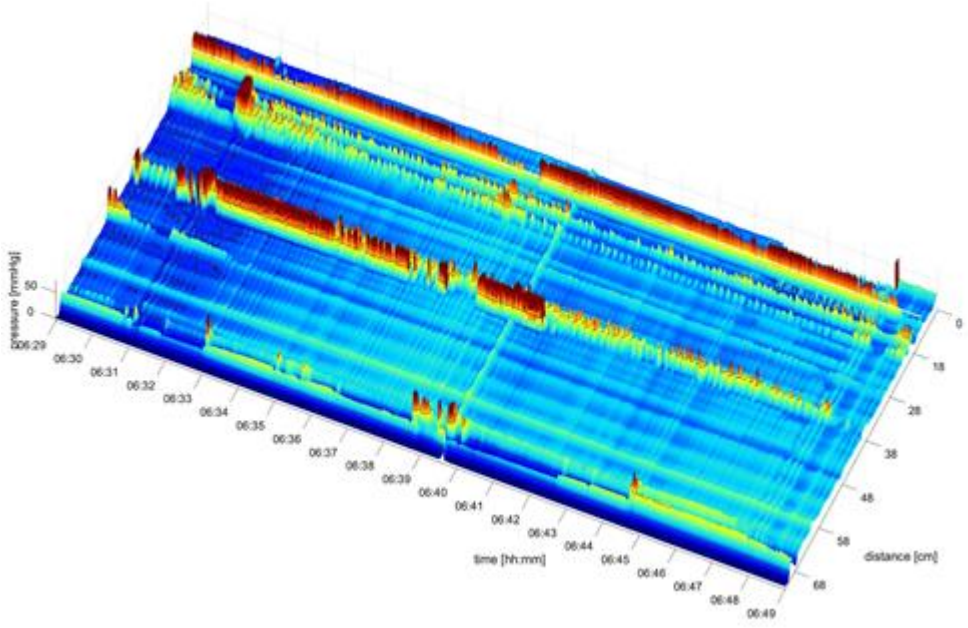
B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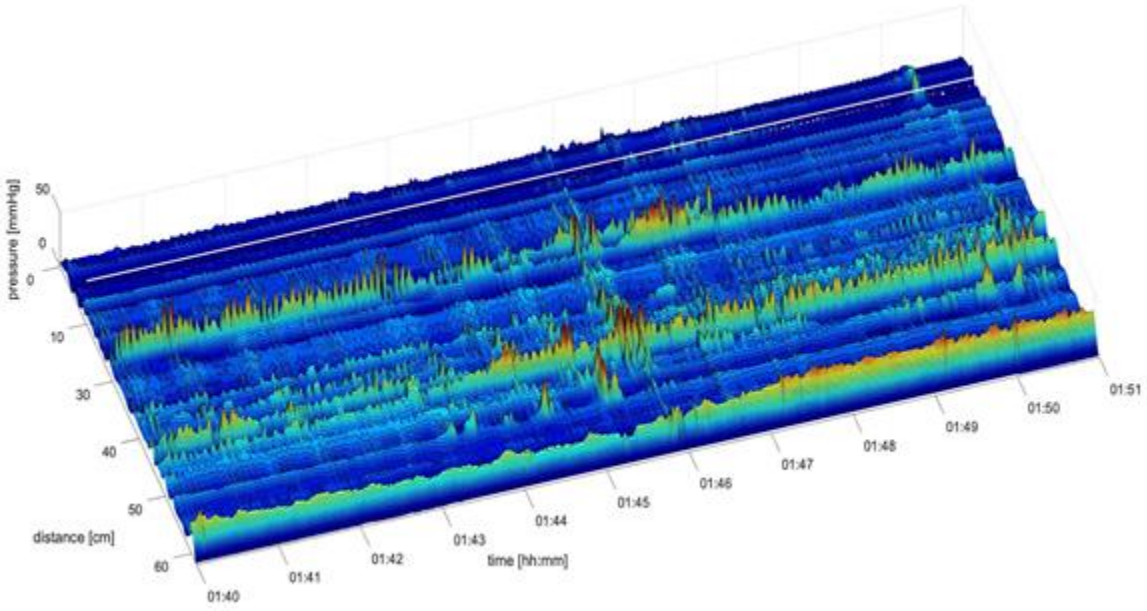
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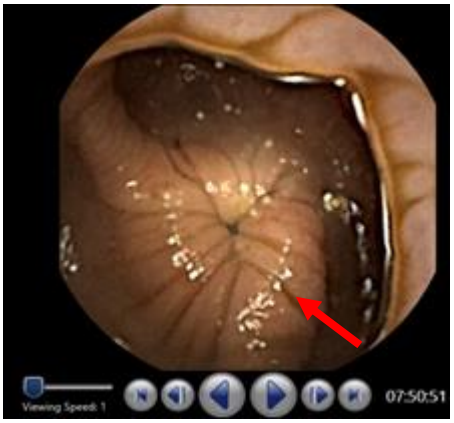

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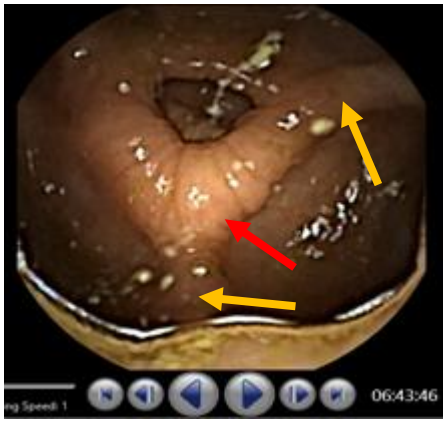


Figure 4-1: Isolated haustral boundary contractions

A-D 3-D plots of isolated haustral boundary contractions. D and E illustrate images obtained through video colonoscopy procedure.

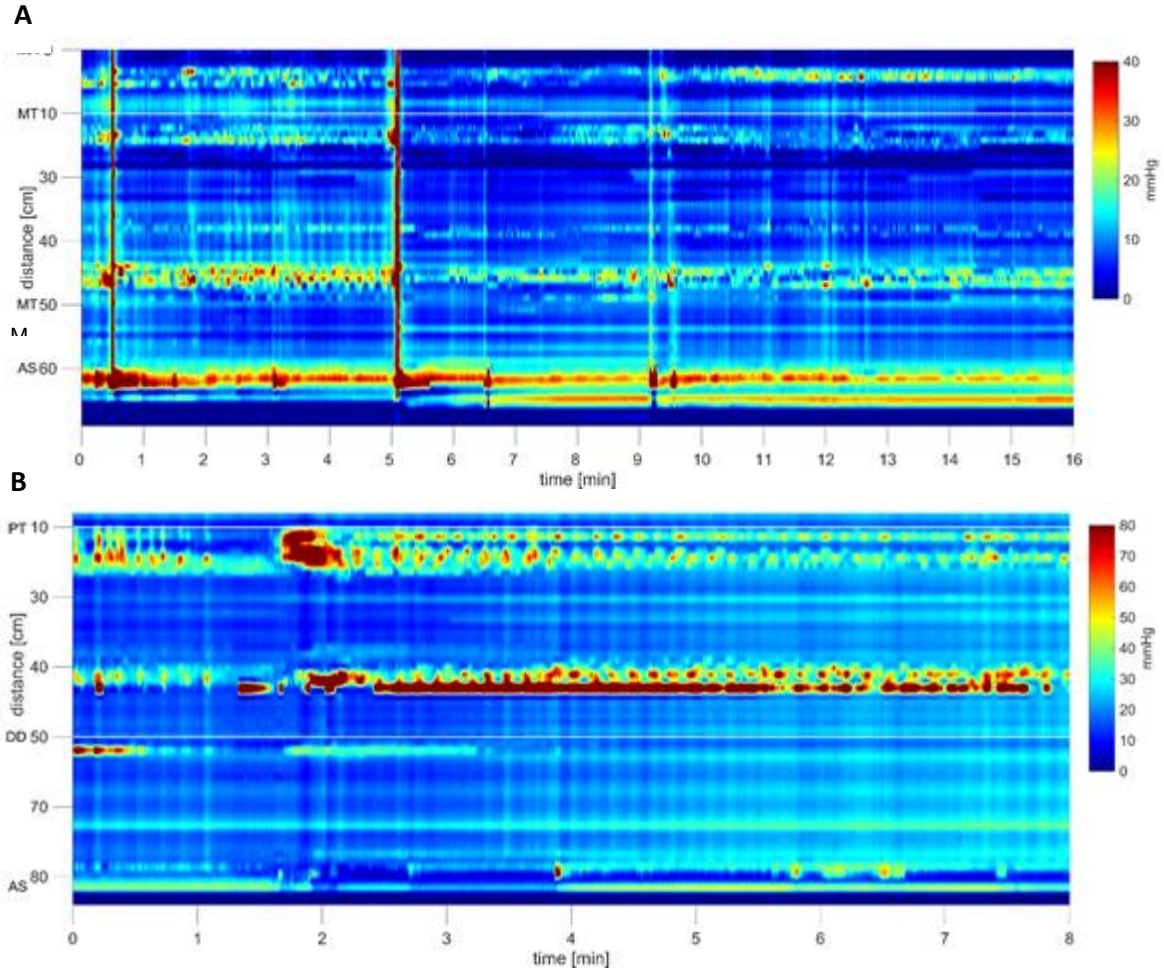
(A) At 22cm is an isolated HB contracting rhythmically at 3cpm.

(B) Commencing at 6:40, 2 distinct rhythmic haustral boundary (upper and lower boundary) contractions at 5-6cpm appear at 16 and 21cm representative of the 2 circular muscle contractions bounding a single haustrum.

(C) The distal colon (36cm-50cm), 4-5 distinct haustral boundaries are present. These boundaries are operating at a high frequency of 9cpm (higher amplitude haustral boundary (45cm, 1:47)). Y-axis represents the distance where 0cm corresponds to the most proximal sensor location. X-Axis is the time in hours and minutes; z-axis represent the pressure in mmHg.

(D) Circular muscle contraction forming the haustral boundary (red arrow) resulting in complete occlusion of the haustrum (at least from one side).

(E) Interaction of the circular muscle (red arrow) and the 2 of the 3 taeniae (yellow arrow) forming a triangular opening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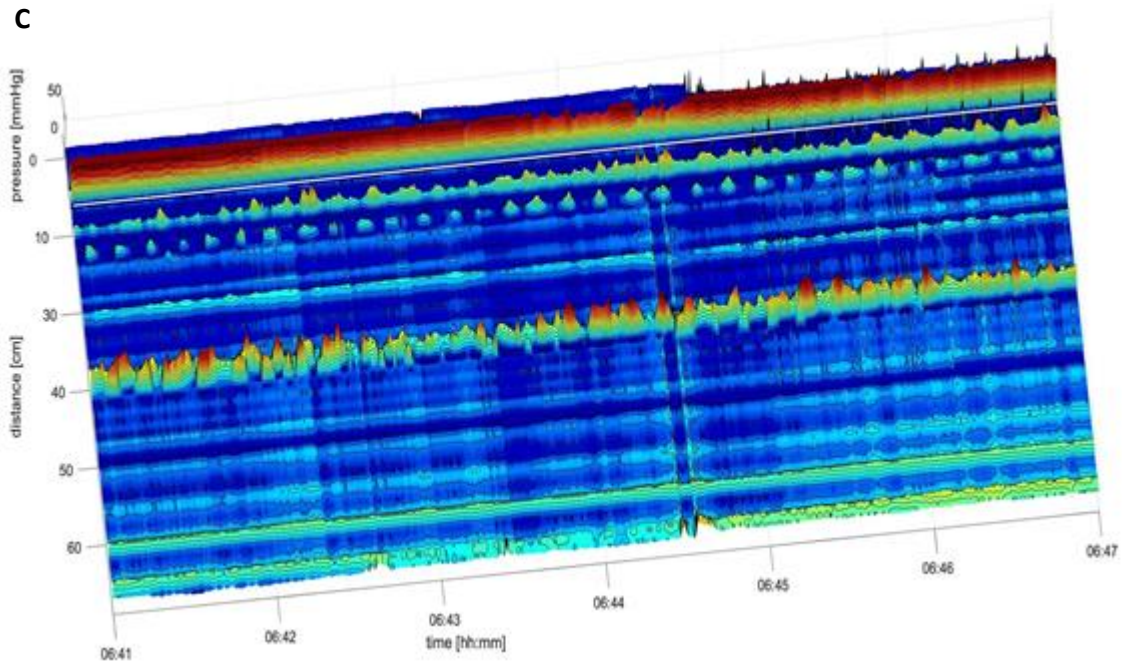


Figure 4-2: Intra-haustral activity

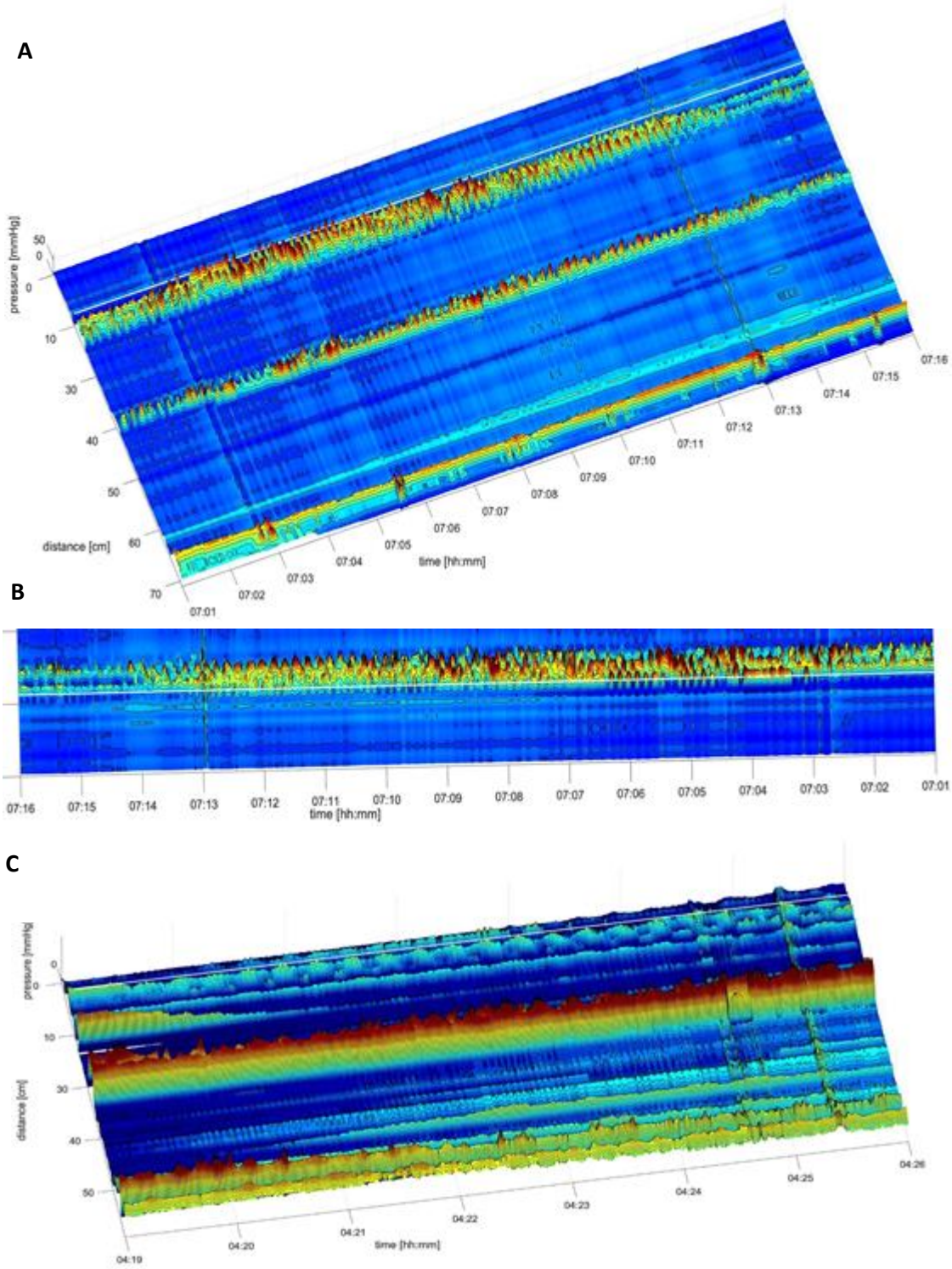
A-B 2-D plots, and C 3-D plot of intra-haustral activity.

(A) 2 simultaneous pressure waves (SPWs) between which at 44-48cm is rhythmic intra-haustral segmentation activity at ~3cpm (3-5min) with some earlier instances of arrhythmic segmentation (1-3min). At 10 to 14 min, 2 haustral boundaries, appearing in an arrhythmic fashion, mark the upper and lower boundaries of the intra-haustral activity described earlier. At 2-4 min around the 5cm region, arrhythmic segmentation is apparent.

(B) Commencing at 3.3 min, an upper boundary and intra-haustral activity are present. Between 4-5 min and around 25cm, retrograde propagation at 6cpm is visible, whereas earlier mixed propagation was present. For (A) and (B) x-axis is the time in minutes; y-axis represents the distance where 0cm corresponds to the most proximal sensor location and pressure (in mmHg) is represented using a color bar. The letters along the y axis represent colonic locations: mid-ascending (MA), proximal transverse (PT), mid-transverse (MT), distal descending (DD), mid-sigmoid (MS), anal sphincter (AS).

(C) At 40cm (3cm in length), intra-haustral activity is present displaying mixed propagation operating at 4cpm. In the proximal colon (23cm), isolated haustral boundary operating at 4cpm is present.

Y-axis represents the distance where 0cm corresponds to the most proximal sensor location; x-axis is the time in hours and minutes; z-axis represent the pressure in mmHg.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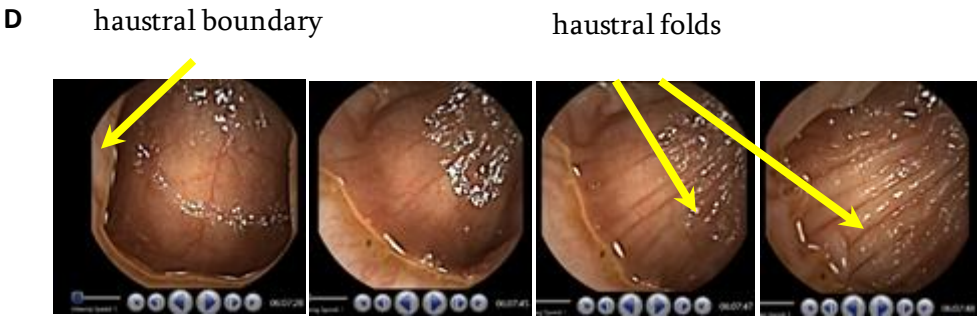


Figure 4-3: Segmentation and propulsive intra-haustral activity

A-C 3-D plots of different types of the intra-haustral activity.

(A) At ~10cm, there is segmentation-type intra-haustral activity that initially is arrhythmic, but from 7:10- 7:14 has a distinct rhythmicity of 4-6cpm. This activity later resolves into the upper and lower haustral boundaries. Y-axis represents the distance where 0cm corresponds to the most proximal sensor location; x-axis is the time in hours and minutes; z-axis represent the pressure in mmHg.

(B) A close-up of the activity described in figure 3A, however the orientation is reversed (top-to-bottom is distal to proximal locations in the colon; and right-to-left corresponds to increasing time). This zoomed version illustrates the checkerboard, non-propulsive nature of segmentation with both an arrhythmic and rhythmic component.

(C) Distinct propulsive intra-haustral activity around 5cm from the most proximal sensor. At 04:22:00 to 04:24:30, antegrade propagation is most distinct.

(D) video colonoscopy with evidence of haustral boundary (1st panel from left) and haustral folds inside the haustrum (3rd and 4th panel from left).

Part 1: Characterization of haustral activity in healthy subjects

All 21 healthy subjects presented haustral activity in their colonic manometry recording (n=2402). However, every intervention did not demonstrate evidence of haustral activity (**table 4-1**).

Amplitude of haustral activity

The average amplitude of haustral activity, when assessed collectively, is 8.7 ± 3.6 mmHg (N=21). Amplitudes of isolated haustral boundary activity were similar to those of intra-haustral activity (p=0.71, **table 4-1**). A bimodal distribution in haustral activity amplitudes is observed (**fig. 4-4**) corresponding to mean amplitudes of 5.3mmHg and 11.2mmHg; the bimodal distribution is evident in the amplitudes of isolated haustral boundary activity, intra-haustral activity, haustral activity in the distal colon, and haustral activity observed during baseline (p<0.0001). The average amplitude of haustral activity was highest following the administration of bisacodyl (11.5 ± 6.2 mmHg), significantly different from the baseline period by 4.3 mmHg (p=0.03). There were no significant differences in the amplitudes of haustral activity in other interventions when compared to baseline, and between amplitudes of haustra present in the proximal versus distal colon.

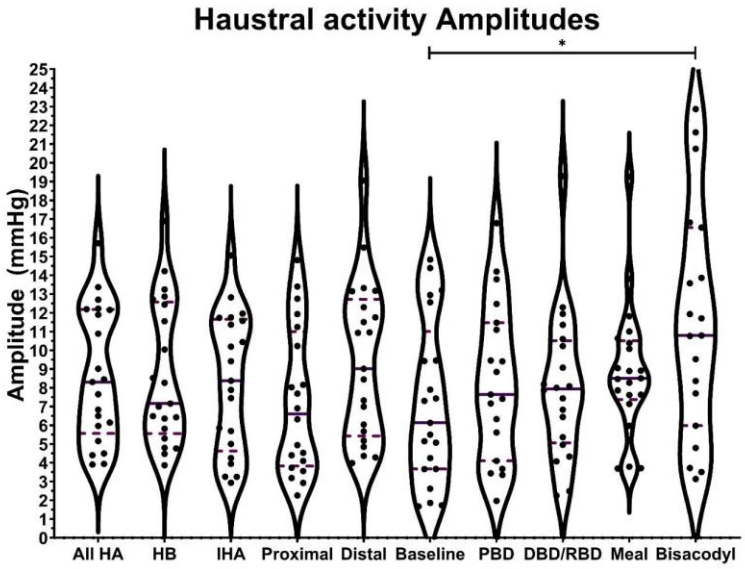


Figure 4-4: Violin plot of the amplitudes of haustral activity

The violin plot combines the box plot and kernel density plot to illustrate the distribution and probability density of the haustral activity amplitudes. The dark dots represent the individual datapoints from the healthy volunteers; the horizontal dark purple solid line and wide dotted lines (2) are the median and 25th (bottom line) and 75th (top line) percentiles line, respectively. The dark black line forming the perimeter of the plot depicts the probability density corresponding to each amplitude value. The y-axis corresponds to the amplitude of haustral activity in mmHg, whereas the x-axis displays the various parameters by which haustral activity was stratified (haustral activity type, location of haustra and interventional analysis). Overall, haustral activity amplitudes show bimodal

*distribution centering at 5.3 and 11.2 mmHg. * represents a p value of 0.03 marking the significant difference between the amplitudes of haustral activity during bisacodyl when compared to baseline period. HA is haustral activity, HB is haustral boundary, IHA is intra-haustral activity, PBD is proximal balloon distension, and DBD/RBD distal/rectal balloon distention.*

Haustral activity amplitudes during baseline and following bisacodyl administration

In a recently published study conducted in the same cohort of healthy subjects (Milkova, Parsons, Ratcliffe, Huizinga, & Chen, 2020), 11.4% of HAPWs occurred during baseline (90min), and 20% of the 290 total HAPWs occurred bisacodyl periods (20min). Given the large proportion of HAPWs following bisacodyl, the percent duration of haustral activity in proximity to HAPW during was bisacodyl 45.1%, whereas during baseline it was only 19.7%. The amplitude of HAPWs during baseline (89.1 ± 4.9 mmHg) was significantly different from those occurring following bisacodyl (104.1 ± 5.2 mmHg; p=0.03); this increase in amplitude is also reflected in the haustral activity that occurs following bisacodyl administration when compared to baseline (p=0.03). Furthermore, amplitudes of haustral activity in the vicinity of HAPW (within 2 mins from the onset of the HAPW as seen in manometry) are greater than the amplitudes of haustral activity occurring in isolation during baseline (p=0.03) and following bisacodyl (p=0.04) with a mean difference of 2mmHg.

Duration of haustral activity

On average, in 21 healthy subjects, 5.5 ± 2.1 haustra (range 2 to 10) exhibited activity out of an estimated total of 12.9 ± 3.8 (range 8 to 21) monitored by the catheter (**table 4-1**). Following these haustra for the duration of the study, the average duration of activity per active haustra was 81.9 ± 45.0 min, ranging from 11.2 to 167.2 min out of a total of 274.0 ± 49.2 min study duration. A healthy subject exhibited on average 114.0 ± 52.0 episodes of continuous haustral activity (a "haustral activity episode") lasting on average 3.5 ± 3.4 min (range 8 sec to 35.3 min). The duration of haustral activity in the proximal and distal colon were indiscernible, with durations in proximal colon ranging from 3.0 to 174.3 min and those in the distal colon from 10.1 to 208.6 min (**table 4-1**, **fig. 4-5**); 2-3 haustra in

the proximal colon and 2-4 haustra in the distal colon were identified (95% confidence interval).

Duration of haustral activity during baseline and other stimuli

The total average duration of haustral activity spanning baseline and all interventions including all haustral activity episodes was 402.6 ± 211.6 min (n=21) ranging from 56.2 to 850.9 min (total study duration of 274.0 ± 49.2 min). The total duration of haustral activity per 1hr period was variable with no intervention distinctively encompassing increased haustral activity relative to baseline, presenting a minimum of 1.9min to 330.5 min per 60 min period (**fig. 4-6**). The number of active haustra during baseline was 3.9 ± 1.8 (range 2-8 haustra), during meal 4.3 ± 2.1 (range 1-10 haustra), during bisacodyl 4.1 ± 1.6 (2-7 haustra) (**table 4-1**). The periods of balloon distention saw a reduction in active haustra to 3.2 ± 2.0 (range 1-9); 15 of the 21 healthy subjects showed a decline in the number of active haustra exhibited from baseline to a balloon distention. The number of active haustra as a percentage of haustra covered by the catheter was 47.2 ± 17.0%.

Duration of episodes of active haustral boundaries and intra-haustral activity

The average duration of active haustral boundaries was 34.2 ± 23.6 min per boundary, while the duration of episodes of intra-haustral activity per haustrum was 50.2 ± 29.75 min across 5.0 ± 2.0 (range 2-9) haustra. The total duration of haustral boundary activity and intra-haustral activity was 151.3 ± 86.7 min and 251.3 ± 169.3 min; 13 subjects showed a greater proportion of intra-haustral activity, 5 subjects showed a greater proportion of haustral boundary activity, and 3 subjects showed similar proportions of both haustral boundary and intra-haustral activity. No difference was seen comparing the total duration of haustral boundary and intra-haustral activity in the proximal and distal

colon, and when comparing durations between baseline and other stimuli.

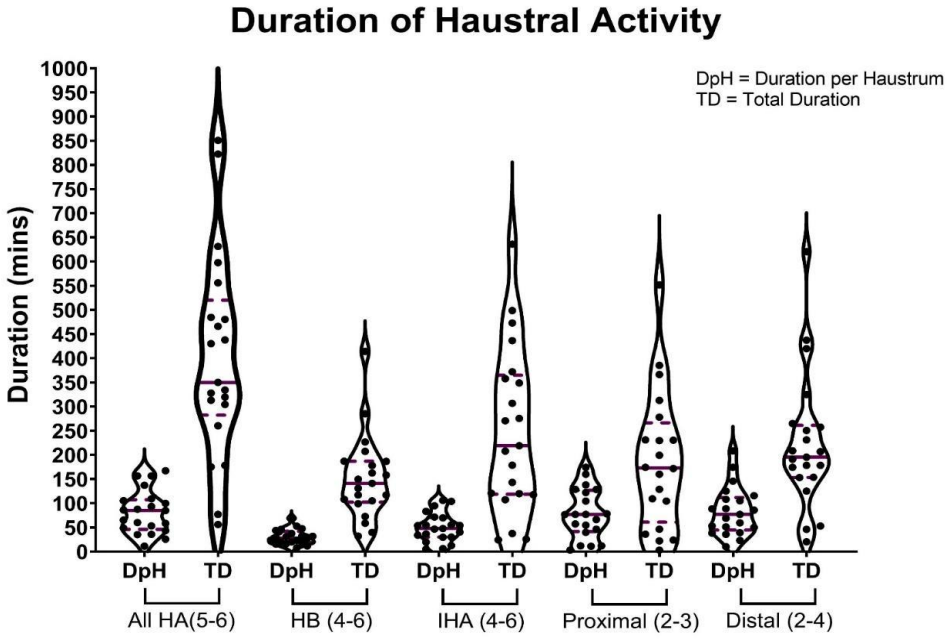


Figure 4-5: Violin plot of the durations of haustral activity

The violin plot combines the box plot and kernel density plot to illustrate the distribution and probability density of the haustral activity durations. The dark dots represent the individual datapoints from the healthy volunteers; the horizontal solid line and wide dotted lines (2) are the median and 25th (bottom line) and 75th (top line) percentiles line, respectively. The solidline forming the perimeter of the plot depicts the probability density corresponding to each duration value. The y-axis corresponds to the duration of haustral activity in min, whereas the x-axis displays the various parameters by which haustral activity was stratified (haustral activity type and location of haustra).This plot encompasses the 2 modes of duration analysis which includes the duration of haustral activity per active haustrum (DpH) and the total duration (TD) which takes all haustra into account. The y-axis corresponds to the durations in min of haustral activity, whereas the x-axis displays the various parameters by which haustral activity was stratified (haustral activity type, and location of haustra). The abbreviations HA, HB, IHA correspond to haustral activity, haustral boundary, and intra-haustral activity, respectively. In brackets next to the abbreviations are the number of haustra (95% confidence interval).

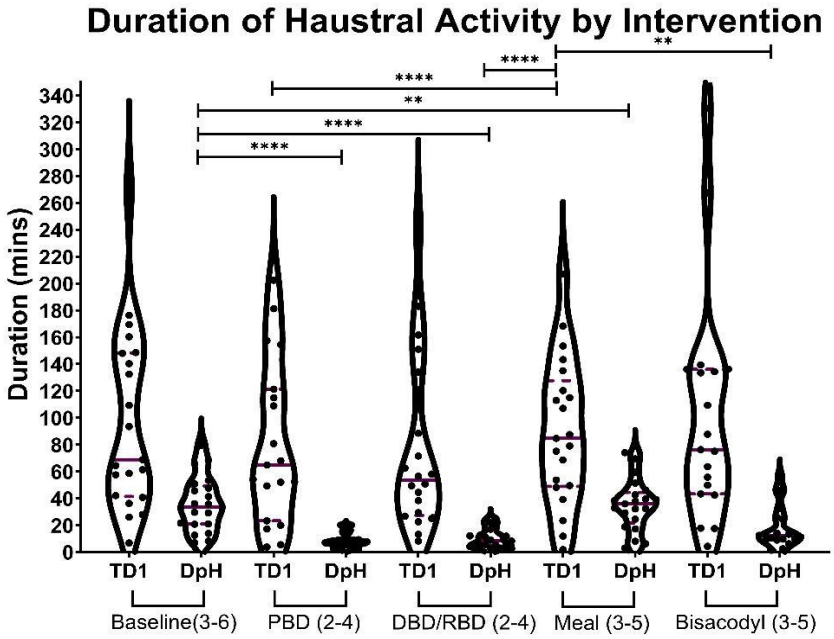


Figure 4-6: Durations of haustral activity per intervention.

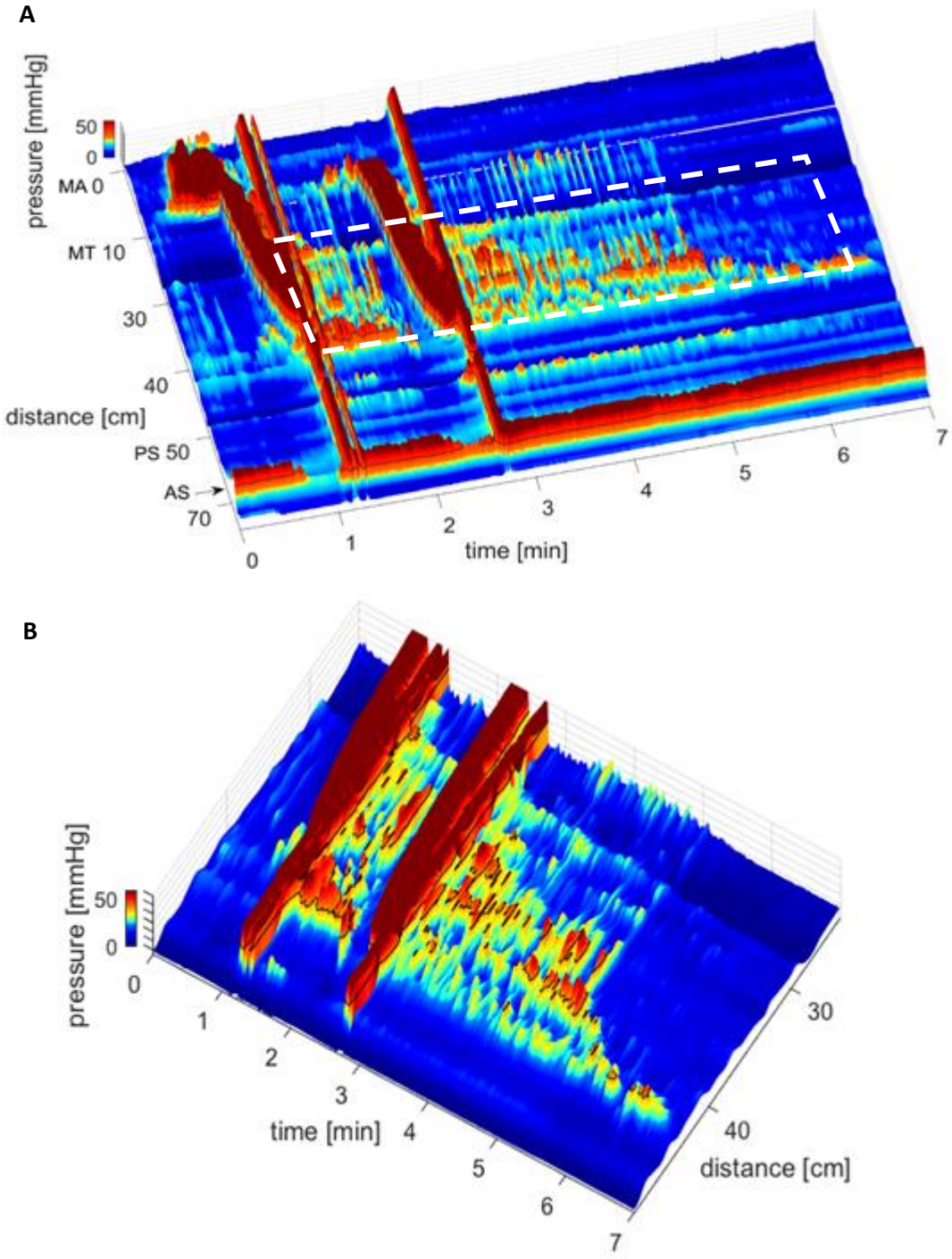
*Violin plot combines the box plot and kernel density plot to illustrate the distribution and probability density of the haustral activity durations. The dark dots represent the individual datapoints from the healthy volunteers; the horizontal dark purple solid line and wide dotted lines (2) are the median and 25th (bottom line) and 75th (top line) percentiles line, respectively. The dark black line forming the perimeter of the plot depicts the probability density corresponding to each duration value. This plot encompasses the 2 modes of duration analysis which includes the haustral activity duration per active haustrum (DpH) and the total duration with the intervention duration scaled to 1hr (TD1). In brackets below the interventions are the number of haustra (95% confidence interval). DpH Baseline vs DpH bisacodyl p=0.0042 (**); DpH Meal vs DpH bisacodyl p=0.007 (**). **** p<0.0001.*

Rhythmic haustral activity

On average, 36.6 ± 12.6% of all haustral activity showed rhythmicity with 79.9 ± 20.1% in the low-frequency range (2-6cpm), the remainder demonstrated frequencies in the high-frequency range (>7 cpm). 70.1 ± 16.8 % of rhythmic haustral activity was intra-haustral activity, and 29.9 ± 16.8 % was haustral boundary activity ($p<0.0001$).

Haustral Activity and cyclic motor patterns in healthy subjects

High amplitude propagating pressure waves are often followed by a cyclic motor pattern as reported in chapter 3, and here we report that this cyclic motor pattern is often followed by active haustra. It appears that the strong neural activity in the generation of HAPW diminishes in strength over time resulting in the development of a cyclic motor pattern followed by haustral activity. When a high frequency cyclic motor pattern occurs at 12 cpm, this develops into haustra with the same intra-haustral pressure wave frequency, suggesting that the cyclic motor pattern was synchronized haustral activity (**fig. 4-7D**). When the cyclic motor pattern exhibits de-synchronized activity, that is the pressure waves within the cyclic motor pattern show mixed retrograde and antegrade as well as simultaneous pressure waves, the developing haustra show segmentation activity (**fig. 4-7A, B**). When the cyclic motor pattern exhibits primarily simultaneous pressure wave activity at 9 cpm, the ensuing haustra show a similar motor pattern frequency (**fig. 4-7C**). We also see that haustral activity is followed by a cyclic motor pattern, hence adjacent active haustra synchronize, following an HAPW (**fig. 4-7C**). Taken together, the synchronization of adjacent active haustra, with sufficient stimulation results in the cyclic motor pattern. The de-escalation in stimuli from the HAPW to CMP to the high frequency haustra suggests that haustral activity is the base component of the CMP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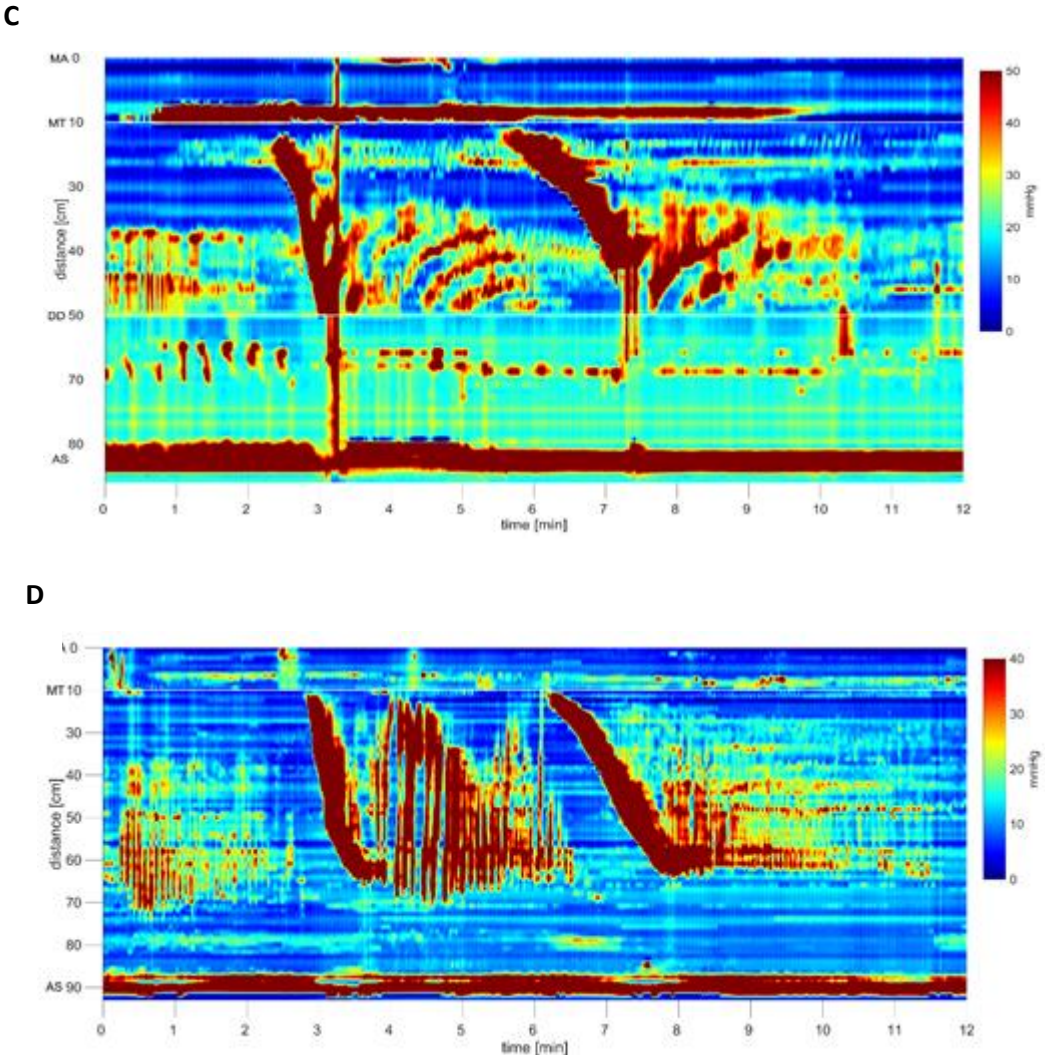

Figure 4-7: Haustral activity and cyclic motor patterns

A-B 3-D plots, and C-D 2-D plots of haustral activity in close association with cyclic motor patterns (CMP) following HAPWs (HAPW-CMP).

(A) 2 consecutive HAPW-CMP, the distal portion of which presents as a haustrum as the interval from the termination of HAPW increases, possessing mix-propagation/segmentation activity of high-frequency.

(B) Close-up of (A) to view the segmentation-type intra-haustral activity appearing as the CMP resolves and shows evidence of lack of synchronization.

(C) Retrograde CMP following an HAPW resolving into component high-frequency intra-haustral activity. With the increase in interval from termination of HAPW, CMP resolves and haustral activity appears.

(D) A period during proximal balloon distention with increasing colonic stimulation (increasing balloon distention). With initial stimulation, CMP appear with component haustra apparent. Increasing stimulation generates HAPW and synchronized haustra producing mixed propagation and high-frequency CMP. With deflation, HAPW is followed by lower-amplitude CMP which, with increasing time quickly resolves into component haustra seen at the 1-minute point. Y-axis represents the distance where 0cm corresponds to the most proximal sensor location. X-Axis is the time in minutes (hours and minutes for A-B). Z-axis (for A-B) represents the pressure in mmHg; pressure (mmHg) is represented using a color bar which is present in C-D. In A- D, the letters along the y axis represent colonic locations: proximal ascending (PA), mid-ascending (MA), mid-transverse (MT), distal descending (DD), proximal sigmoid (PS), anal sphincter (AS).

Propulsive and segmentation patterns in intra-haustral activity

Intra-haustral activity exhibited a segmentation pattern 87.5 ± 13.4 % of the time and 18.9 ± 13.9% ($p<0.0001$) of the activity was propulsive. Some episodes of intra-haustral activity (n=5) encompassed both forms of activity thus there was some overlap. Of the segmentation-type intra-haustral activity, 41.5 ± 15.5 % demonstrated low-frequency rhythmicity; compared to 58.5 ± 15.5% arrhythmic segmentation activity ($p > 0.001$). Propulsive intra-haustral activity was categorized as displaying majority antegrade, majority retrograde, mixed, and simultaneous pressure waves. The vast majority of propulsive intra-haustral activity was simultaneous ($p<0.0001$, **Table 4-1**), while the other propagation types were infrequently present.

Part 2: Haustral activity in adult patients and comparison to healthy controls

All adult patients exhibited active haustra (N=7), however 3 adult patients did not have recordings from the proximal colon, thus comparison made to healthy subjects will be those pertaining to the distal colon.

Amplitude of haustral activity

The amplitudes of haustral activity, isolated haustral boundary activity and intra-haustral activity in adult patients (N=7) were not significantly different from controls (**table 4-2**, p=0.90) with an average amplitude of 8.4 ± 2.6 mmHg. Of these patients, 1 demonstrated amplitudes within normal range, 2 with lower than normal amplitudes and 3 had higher than normal amplitudes (**fig. 4-8**). The meal period had the highest average amplitude of 12.4 ± 4.6 (7.4-10.4) mmHg. Of the 7 adult patients, 5 show amplitudes of haustral activity higher than control values during the meal at 14.1 ± 4.2 mmHg (compared to 8.9 ± 3.52 mmHg control value; p=0.003) with a mean difference of 5.2 ± 1.8 mmHg.

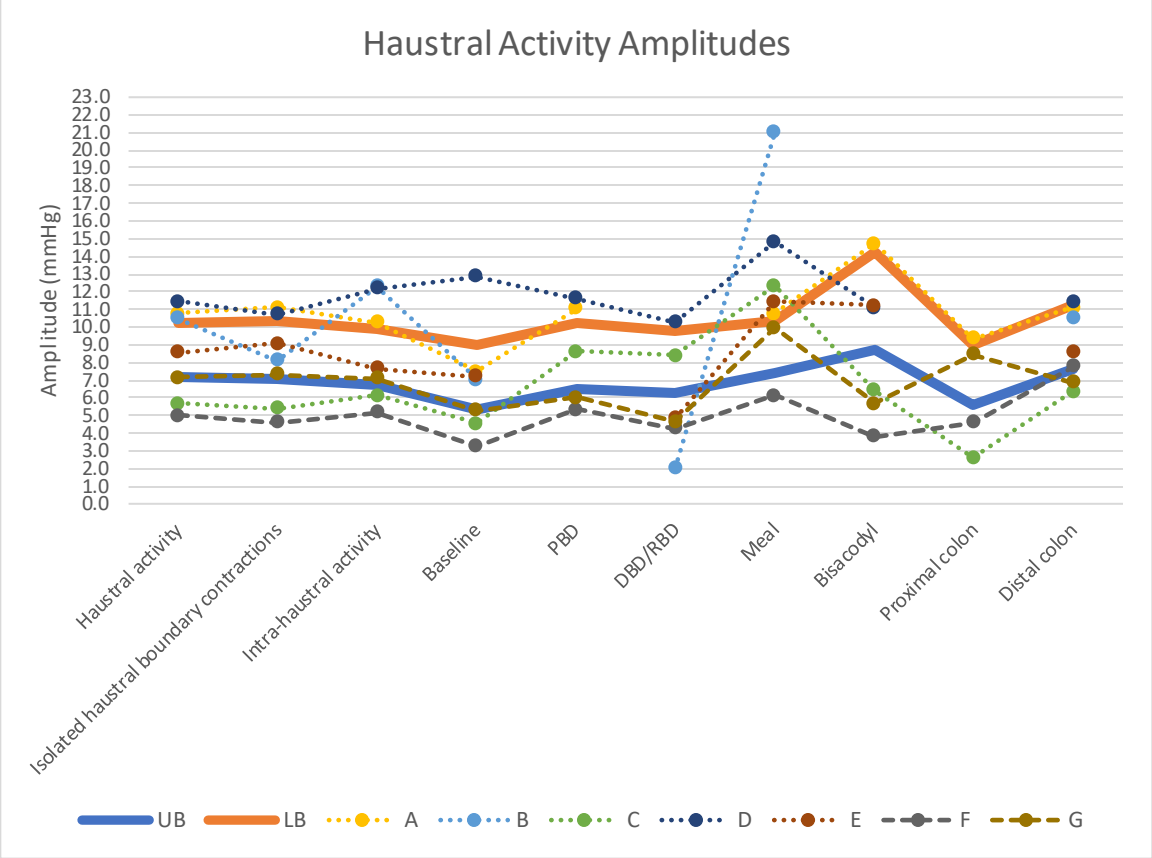

Figure 4-8: Haustral activity amplitude

The control values are represented by the thick lines, the lower and upper bounds of the 95% confidence interval depicted by the blue and red line, respectively. The wider dotted lines (green and purple) represent the two high responders (F-G), whereas the narrow-dotted lines correspond to the weak responders (A-E; N=5). The y-axis is the amplitude reported in mmHg. The x-axis reports haustral activity overall, the types of haustral activity, the different sessions in the study, and the locations where the haustral were active generalized to the proximal and distal colon. Due to distal catheter placement, 3 of the 7 adult patients only presented distal haustra represented by missing data. Missing data points during the interventions is suggestive of lack of active haustra or an incomplete intervention (N=3).

Categorization of adult patients: Strong, weak and non-responders

The analysis pertaining to duration showed a stark contrast when analyzing individual subjects. For this section, the terms "strong responders", "weak responders" and "non-responders" will be used to group patients, based on the nomenclature defined in Milkova

(2020). The amplitudes of HAPW and the autonomic response (measures of parasympathetic and sympathetic tone assessed from heart rate variability) in strong responders are within the intervention-based normal range determined from the control values; these consists of subjects F and G. Weak responders are characterized by HAPW generation in response to 1-2 stimuli, and have HAPW amplitudes lower than the normal range; weak responders include subjects C and D. Non-responders do not generate any HAPW within normal parameters and consist of subjects A, B and E.

Haustral activity duration analysis of Strong Responders versus Weak and Non-Responders

The weak responders and non-responders (N=5; subjects A-E) had an average duration of 30.5 ± 13.1 min of haustral activity per active haustrum (62.7-101.1 min), whereas the strong responders' (subjects F and G) duration of haustral activity per active haustrum was within the control range (**table 4-2**, **fig. 4- 9**). The total duration of haustral activity (**table 4-2**,**fig. 4- 10**) was elevated for the strong responders who had a duration of 980.3 and 639.5 min (312.1-493.1min) across 12 and 10 active haustra, respectively; the number of active haustra were double of controls (5-6). The number of active haustra during baseline was 5.1 ± 2.7 (range 1-9 haustra), during a meal 4.7 ± 3.7 (range 2-10 haustra), during bisacodyl 4.3 ± 2.3 (1-7 haustra) (**table 4-2**). The periods of balloon distention saw a reduction in active haustra to 3.3 ± 2.9 (range 1-9); 6 of the 7 adult patients showed a decline in the number of active haustra exhibited from baseline to a balloon distention. The number of active haustra as a percentage of haustra covered by the catheter was 46.6 ± 25.0% (range 23.1 - 85.7%). Strong responders produced higher-than-control proportion of haustra; in these patients, 71.4% and 85.7% of the estimated number of haustra were active at some point during the manometry recording. Conversely, with the exception of one other adult patient with marginally above-control proportions of active haustra, 4 adult patients collectively presented an average of 28.0 ± 6.39% of haustra that were active, well below the 95% confidence interval determined from controls (40.0-54.5%).

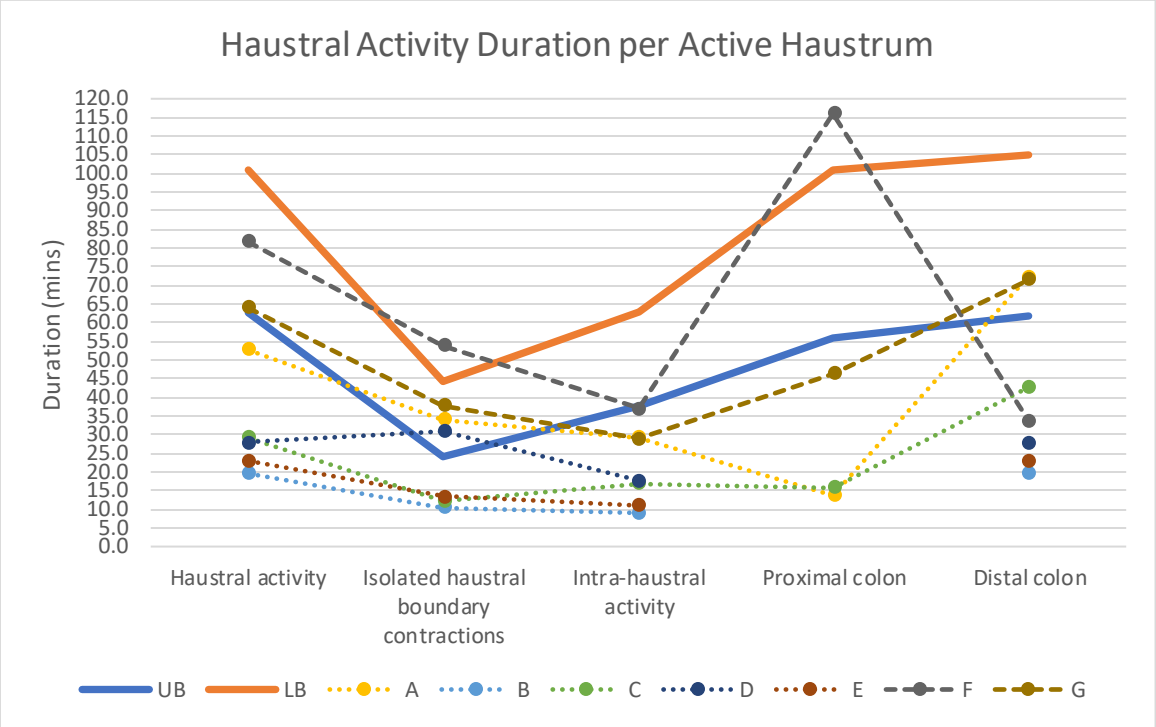

Figure 4-9: Haustral activity duration per active haustrum

The control values are represented by the thick lines, the lower and upper bounds of the 95% confidence interval depicted by the blue and red line, respectively. The wider dotted lines (green and purple) represent the two high responders (F-G), whereas the narrow-dotted lines correspond to the weak responders (A-E; N=5). The y-axis is the duration reported in min. The x-axis reports haustral activity overall, the types of haustral activity, and the locations where the haustra were active generalized to the proximal and distal colon. Due to distal catheter placement, 3 of the 7 adult patients only presented distal haustra

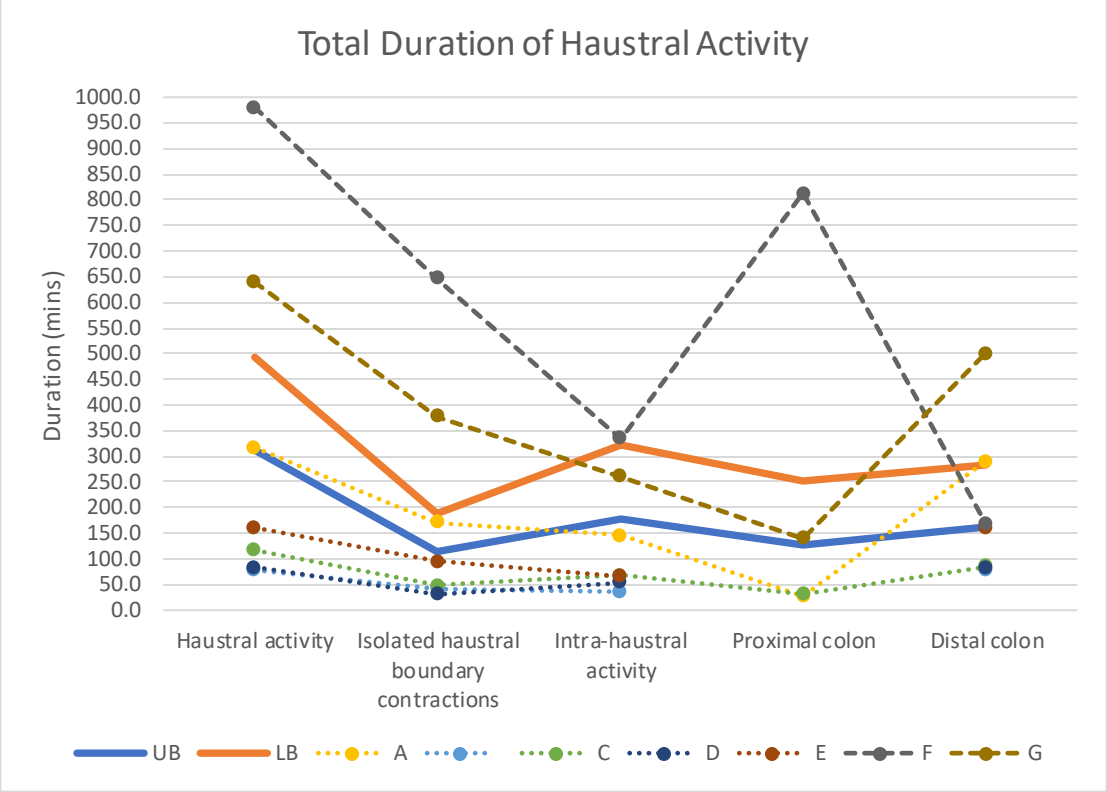

Figure 4-10: Total haustral activity

The control values are represented by the thick lines, the lower and upper bounds of the 95% confidence interval depicted by the blue and red line, respectively. The wider dotted lines (green and purple) represent the two high responders (F-G), whereas the narrow-dotted lines correspond to the weak responders (A-E; N=5). The y-axis is the duration reported in min. The x-axis reports haustral activity overall, the types of haustral activity, and the locations where the haustra were active generalized to the proximal and distal colon. Due to distal catheter placement, 3 of the 7 adult patients only presented distal haustra

Haustral durations based on colonic location

Weak and non- responders displayed consistently below-average duration of haustral activity per active haustrum and the total duration overall (**fig. 4- 9, 10**). Three patients with distal catheter placement (weak responders) and 1 other weak responder showed low duration of haustral activity (**table 4-2**) in the distal colon when compared to control (61.8-105.0 min of activity per active haustrum and 163.9-282.7 min of activity per 1hr). Subject F showed more active haustra in the proximal colon (7/12 active haustra; **fig. 4-**

12C) whose increased haustral activity was reflected in the total duration in the proximal colon of 811.8 min (126.1-250.4 min), whereas subject G had more active haustra in the distal colon (7/10 active haustra; **fig 12A-B**) with a total haustral activity duration of 500.1min colon (163.9-282.7min). Both strong responders demonstrated an elevated proportion of active haustra (**table 4-2**) in the proximal (2-3) and distal (2-4) colon.

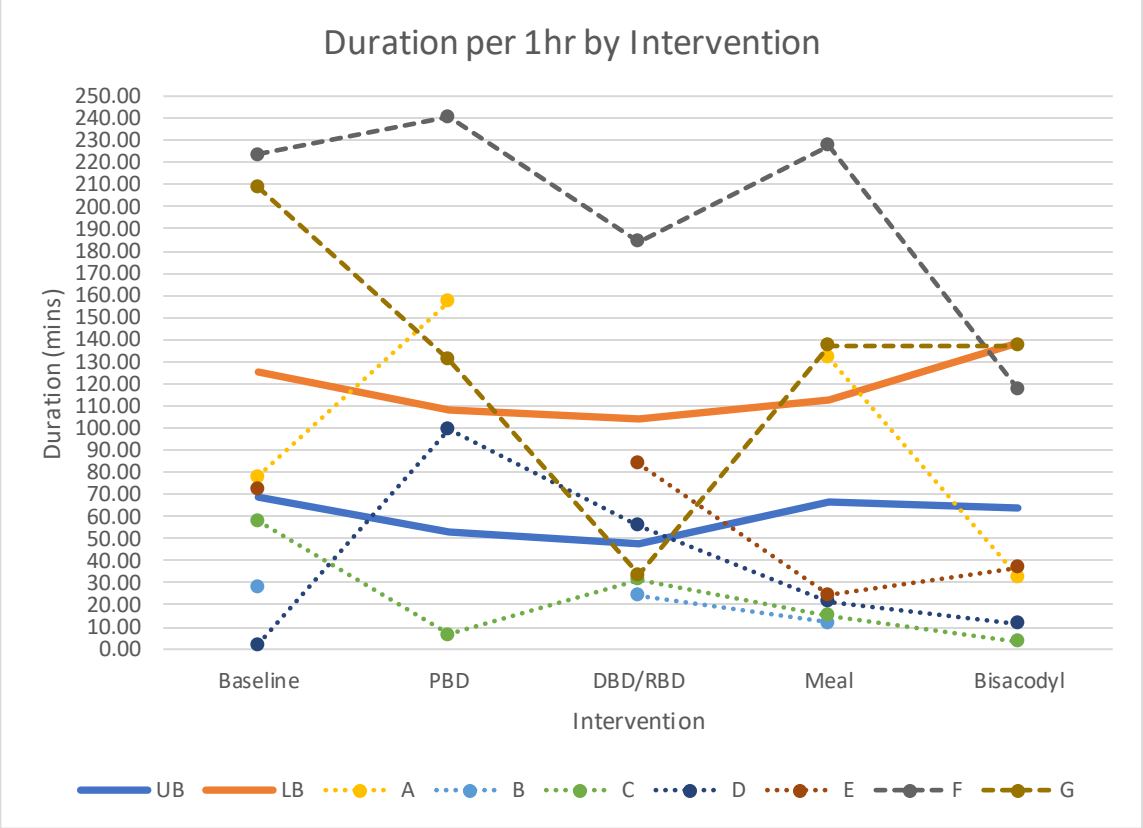

Figure 4-11: Haustral activity duration per 1hr as a function of interventions

The control values are represented by the thick lines, the lower and upper bounds of the 95% confidence interval depicted by the blue and red line, respectively. The wider dotted lines (green and purple) represent the two high responders (F-G), whereas the narrow-dotted lines correspond to the weak responders (A-E; N=5). The y-axis is the duration reported in min. The x-axis reports the different sessions in the study. Missing data points during the interventions is suggestive of lack of active haustra or an incomplete intervention (N=3).

Haustral boundary and intra-haustral activity

Weak responders demonstrated below-average durations with respect to haustral boundary activity and intra-haustral activity. Strong responders elicited above-average isolated haustral boundary activity, 646.3 min (subject F) and 378.5 min (subject G), when total duration is considered (114.2-188.4 min). The ratio of the total durations of isolated haustral boundary activity to intra-haustral activity revealed that subject A and B (weak responders) showed equal proportions of activity; subject C and D showed a 40-70% increase in intra-haustral activity (non- responders); and subject E (weak responder), F and G (strong responders) elicited 45-90% increase in isolated haustral boundary activity.

Rhythmic haustral activity

From the total duration of rhythmic haustral activity, 54.1% corresponded to haustral boundary activity, whereas 45.9% was intra-haustral activity (**fig. 4- 12**); furthermore, 6 adult patients demonstrated elevated rhythmic isolated haustral boundary activity (**table 4-2**; range 43.5- 66.2%) when compared to healthy controls (22.8-37.1%). Of all the rhythmic activity in the adult patients, 96.1% contains frequencies in the low-frequency range of 2-6cpm and centralized at 3cpm; this proportion of activity is much higher in patients than in controls (71.3- 88.5%), elevated in 5 patients.

Intra-haustral Activity – Propulsion and Segmentation

Collectively, patients have below-average segmentation-type intra-haustral activity of 78.5% and just within-range propulsive activity at 24.6%. Despite the lower-than-normal proportions, the proportion of segmentation-type intra-haustral activity is significantly higher than the proportions of propulsive activity ($p=0.0002$) with a mean difference of $53.9 \pm 9.76\%$. Six patients demonstrated propulsive intra-haustral activity, and all elicited high proportions of mixed propagation; while 3 patients also had distinct instances of antegrade and retrograde propagation intra-haustral activity, that were otherwise absent in

controls.

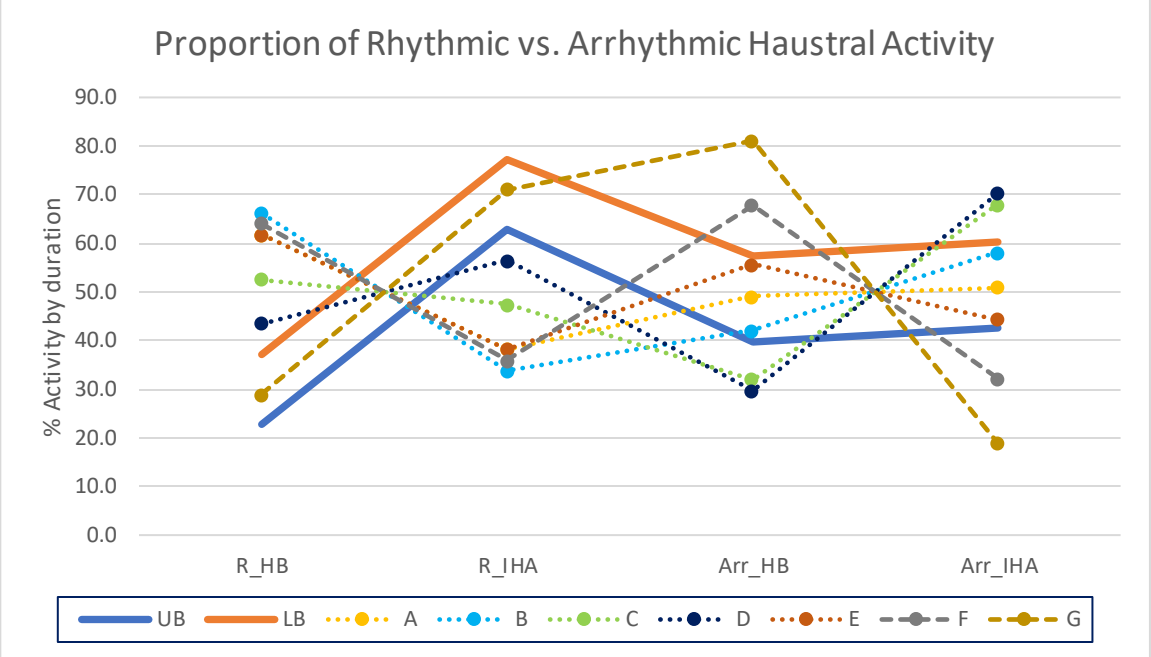

Figure 4-12: Proportion of rhythmic and arrhythmic isolated haustral boundary activity and intra-haustral activity

The control values are represented by the thick lines, the lower and upper bounds of the 95% confidence interval depicted by the blue and red line. The wider dotted lines (green and purple) represent the two high responders (F-G), whereas the narrow-dotted lines correspond to the weak responders (A-E; N=5). The y-axis is the duration reported as the percent proportion of rhythmic and arrhythmic activity as assessed by duration. The abbreviations along the x-axis : rhythmic (R), arr (arrhythmic), haustral boundary (HB) and intra-haustral activity (IH)

Part 3: Cyclic motor patterns in adult patients and comparison to healthy controls

The presence of cyclic motor patterns was heterogeneous amongst adult patients where 4 of 7 adult patients had no occurrences of cyclic motor patterns based on the criteria presented in chapter 3; this is in contrast to the controls who all demonstrated cyclic motor patterns. Of these 4 patients, 3 were non-responders and 1 was a weak responder . Of those that showed evidence of cyclic motor patterns (**table 4-3**), 2 were those categorized as strong responders; the strong responders overall showed increased presence of cyclic motor patterns evidenced by the number of low-frequency pressure waves when compared to the weak responder (subject C**, table 4-3**).

Characteristics of cyclic motor patterns in patients

The average length of the cyclic motor patterns in the colon of all 3 patients were on average 9.2± 2.1cm less than the lengths in controls ($p<0.0001$); and in the rectum, the length of the cyclic motor patterns were on average 3.6± 0.7cm less than those found in controls ($p<0.0001$). The frequencies of the cyclic motor patterns in the colon were also lower than those found in controls ($p<0.0001$) with a mean difference of 5.7± 0.8cpm; the average frequency observed in the colon were in the low-frequency range at 4.0± 3.1cpm. Furthermore, the high-frequency cyclic motor patterns were minimally observed in adult patients. Two patients presented cyclic motor patterns following a HAPW , a single patient had cyclic motor patterns in the rectum (rectal-CMP), and all had isolated occurrences of cyclic motor patterns in the colon (**table 4-3**).

Variations in cyclic motor patterns

The 3 patients with cyclic motor patterns displayed much variation in the frequency range and types of cyclic motor patterns. Subject C showed 7 occurrences of cyclic motor

patterns presenting both low and high-frequency. The cyclic motor patterns in both the distal descending/sigmoid colon and the proximal colon presented elevated simultaneous propagating pressure waves (**table 4-3**) with an average length of 10.4 ± 4.4cm. Furthermore, an increase in number of pressure waves from 18 pressure waves during baseline to 30 pressure waves following meal.

Subject F presented all 3 types of cyclic motor patterns (**figure 4-13**) with an increased expression of the cyclic motor pattern in the rectum (n=17 out of 30 occurrences) and elevated low-frequency cyclic motor patterns (**table 4-3**). The amplitude of the rectal-CMP is higher than those observed in controls with the proportion of retrograde propagating pressure waves in a cyclic motor pattern occurrence also prominent overall (**table 4-3**). Subject F presented elevated cyclic motor patterns during baseline (116 pressure waves), whereas meal intake elicited 16 rhythmic pressure waves.

Subject G had an increased presence of haustral activity in the distal sigmoid colon; this location coincided with the presence of 9 occurrences of cyclic motor patterns that bordered the sphincter of O'Beirne. The cyclic motor patterns were an average length of 7.0 ± 1.3 cm and all were colonic-CMP. There was minimal to no cyclic motor patterns during baseline and meal periods. This patient demonstrated elevated retrograde propagation with exclusive low-frequency cyclic motor patterns (2.1 ± 0.3 cpm); the number of pressure waves and duration of this motor pattern were within normal range (**table 4-3**).

Adjacent active haustra

All cyclic motor patterns in the 3 patients were present in locations containing active adjacent intra-haustral activity or haustral boundary activity at some point during the

duration of the study. The cyclic motor patterns were comprised of the synchronization of adjacent 1.5-2 previously active intra-haustral activity (**figure 4-14**) and/or synchronization of intra-haustral activity with the rhythmic sphincter of O'Beirne activity (**Figure 4-15**). In 3 of the 4 adult patients without cyclic motor pattern activity, there were no active adjacent haustra with intra-haustral activity and/or haustral boundary activity.

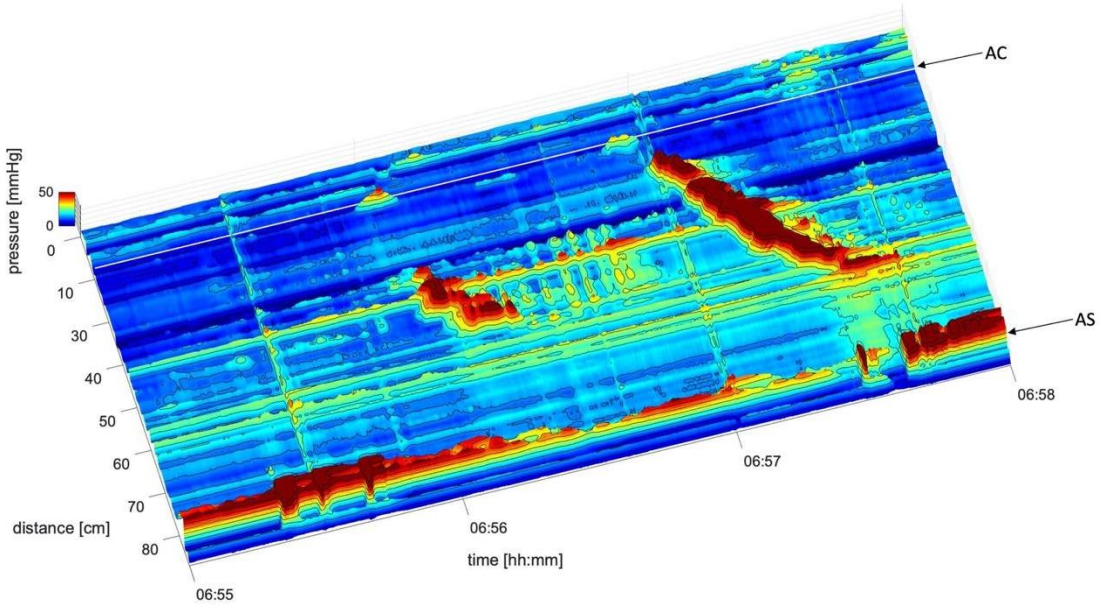

Figure 4-13: Cyclic motor pattern following HAPW

3D figure of cyclic motor pattern following bisacodyl-induced HAPW. The HAPW amplitudes are higher than reported but capped to allow for the visualization of the cyclic motor pattern. The proximal balloon is represented by the white line and is located in the ascending colon (AC). The anal sphincter (AS) is 80cm from the proximal-most sensor.

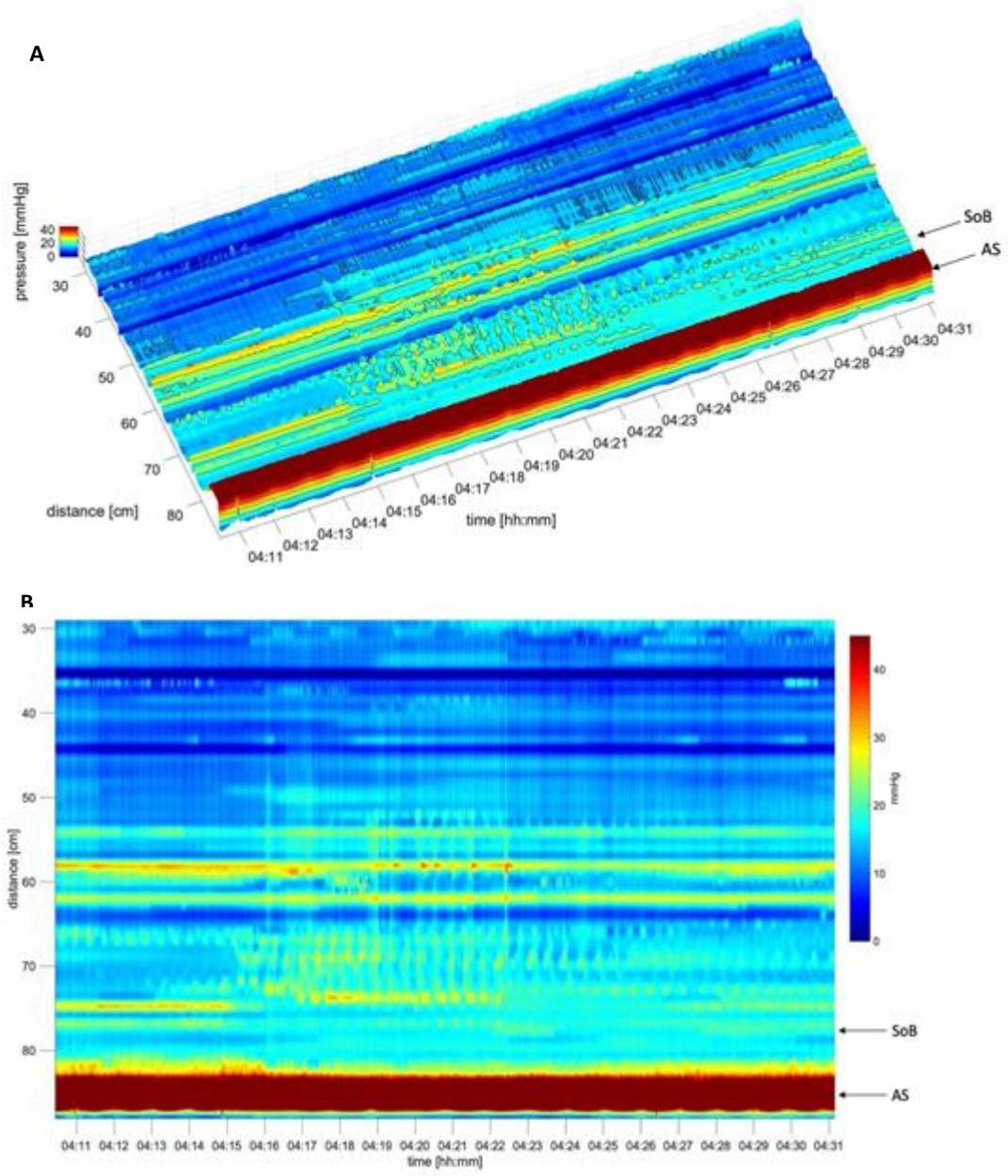

Figure 4-14: Cyclic motor pattern in the distal sigmoid colon

(A) 3-D figure of cyclic motor pattern occurring just proximal to the sphincter of O'Beirne (SoB). Mixed propagation of the cyclic motor pattern is visible with a frequency of 2 cpm. This motor patter is preceded by a rhythmic boundary operating at approximately 2cpm and the cyclic motor pattern is followed by intra-haustral activity at the same frequency with an adjacent haustral boundary activity distal to it.

(B) 2-D figure of (A). Mixed propagation of the cyclic motor pattern and the following intra-haustral activity is visible. AS marks the anal sphincter.

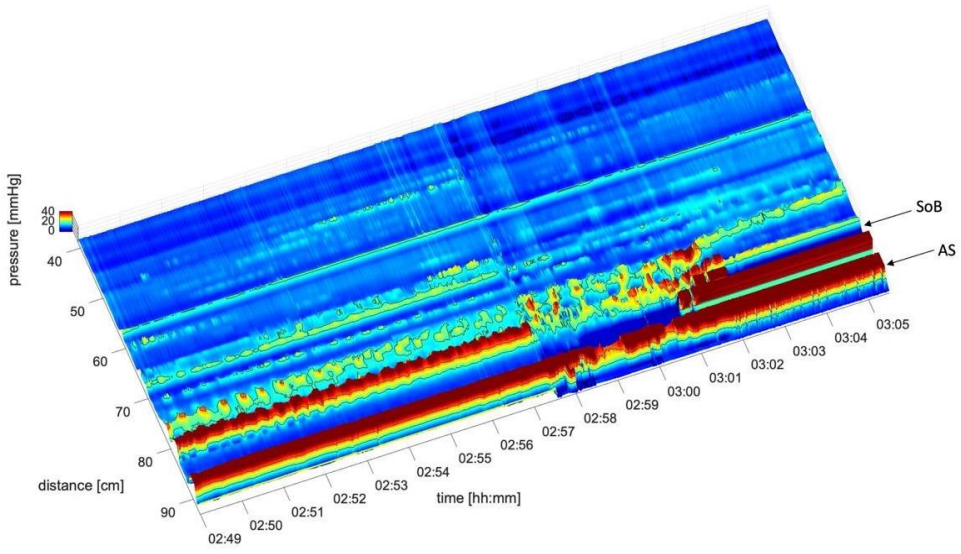

Figure 4-15: Cyclic motor pattern proximal to the sphincter of O'Beirne

3-D figure of distal intra-haustral activity occurring prior to the rhythmic sphincter of O'Beirne (SoB) both around the frequency of 2-2.5cpm. Rhythmic pressure waves of the intra-haustral activity extend to the sphincter of O'Beirne merging to produce mixed propagation rhythmic cyclic motor pattern (7 pressure waves; approximately 1.8cpm). The cyclic motor pattern is followed by the rhythmic haustral boundary activity and the tonic sphincter of O'Beirne.

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Control Values									
Parameters						Amplitude (95% CI)			
1. Amplitude (mmHg)	Sig.	Mean		Std	N	Lower bound	Upper bound	Min	Max
Haustral activity		8.7	±	3.6	21	7.1	10.2	3.9	15.7
Isolated haustral boundary activity	0.71	8.7	±	3.8	21	7.1	10.4	3.9	16.9
Intra-haustral activity		8.3	±	3.7	21	6.7	9.9	2.9	15.1
2. Amplitude (mmHg) by intervention									
Baseline		7.2	±	4.3	21	5.3	9.0	1.7	14.8
PBD		8.3	±	4.2	19	6.5	10.2	2.0	16.8
DBD/RBD		8.1	±	4.0	20	6.3	9.8	2.3	19.3
Meal		8.9	±	3.5	21	7.4	10.4	3.7	19.3
Bisacodyl	*	11.5	±	6.2	19	8.7	14.2	3.1	22.9
3. Amplitude (mmHg) by location									
Proximal colon	0.09	7.3	±	3.9	20	5.5	9.0	2.3	14.8
Distal colon		9.4	±	4.2	21	7.6	11.2	4.0	19.1

	Average haustral activity duration per active haustrum				Duration per active Haustrum (95% CI)					Average Total Duration				Total duration (95% CI)				Number of haustra (95% CI)	
4.Duration (mins)	Sig.	Mean	±	Std	Lower bound	Upper bound	Min	Max	N	Sig.	Mean	±	Std	Lower bound	Upper bound	Min	Max	Lower bound	Upper bound
Haustral activity		81.9	±	45.0	62.7	101.1	11.2	167.2	21		402.6	±	211.6	312.1	493.1	56.2	850.9	5	6
5. Duration (mins) by location																			
Proximal	0.75	78.3	±	51.2	55.9	100.8	3.0	174.3	20	>0.99	188.2	±	141.8	126.1	250.4	3.0	551.8	2	3
Distal		83.4	±	50.5	61.8	105.0	10.1	208.6	21		223.3	±	138.9	163.9	282.7	20.2	620.5	2	4
6. Duration (mins) of isolated HB contractions and IH activity																			
HB	0.06	34.2	±	23.6	24.1	44.2	8.0	113.5	21	0.4	151.3	±	86.7	114.2	188.4	32.1	414.3	4	6
IHA		50.2	±	29.8	37.5	62.9	5.2	106.0	21		251.3	±	169.3	178.9	323.7	24.1	635.9	4	6
7. Duration (mins) of isolated HB contractions and IH activity by location																			
Proximal HB	0.09	38.9	±	29.7	25.58	52.3	3.0	122.9	19	0.09	69.9	±	44.7	49.9	90.0	3.0	159.4	1	3
Proximal IHA		48.9	±	34.9	33.21	64.6	3.8	116.7	19		125.9	±	119	72.3	179.4	7.6	466.9	2	3
Distal HB		30.4	±	24.4	19.96	40.8	3.1	104	21		85.9	±	75.2	53.7	118.0	3.1	341.1	2	4
Distal IHA		56.7	±	46.1	37.02	76.5	5.3	181.4	21		137.4	±	95.3	96.7	178.2	15.8	321.9	2	3

Parameters	N	Sig.	Total Duration/1hr			Duration/hr (95% CI)				Number of haustra (95% CI)	
			Mean		Std	Lower bound	Upper bound	Min	Max	Lower bound	Upper bound
8. Duration (mins) by intervention											
Baseline	21	0.73	97.1	±	66.3	68.8	125.5	6.8	269.7	3	5
PBD	19		80.7	±	60.7	53.4	107.9	3.8	202.5	2	4
DBD/RBD	20		75.9	±	64.4	47.7	104.1	8.3	242.0	2	4
Meal	21		89.8	±	53.7	66.9	112.8	1.9	207.1	3	5
Bisacodyl	19		101.1	±	83.0	63.8	138.4	4.3	330.5	3	5
9. Duration (mins) of isolated HB contractions and IH activity by intervention											
Baseline HB	21	0.38	32.6	±	22.4	23.0	42.1	2.4	93.7	2	4
Baseline IHA	21		64.6	±	58.8	39.4	89.7	3.8	219.1	2	4
PBD HB	16		41.5	±	33.2	25.2	57.8	3.8	123.4	2	3
PBD IHA	16		54.3	±	39.5	34.9	73.7	5.5	135.9	2	4
DBD/RBD HB	19		38.7	±	46.7	17.7	59.7	1.3	212.6	2	3
DBD/RBD IHA	18		43.5	±	38.5	25.7	61.2	4.0	133.7	2	4
Meal HB	20		35.6	±	22.8	25.6	45.6	5.0	97.4	3	4
Meal IHA	21		55.9	±	38.8	39.4	72.5	1.9	130.8	3	4
Bisacodyl HB	18		38.1	±	31.0	23.8	52.4	3.4	138.9	3	4
Bisacodyl IHA	19		65.0	±	62.4	37.0	93.1	0.8	256.2	3	4

Parameters							(95% CI)			
10. Rhythmicity (% values determined from the duration)		Sig.	Mean		Std	N	Lower bound	Upper bound	Min	Max
% of rhythmic haustral activity	HB		30.0	±	16.8	21.0	22.8	37.1	0.0	62.1
	IHA	####	70.1	±	16.8	21.0	62.9	77.2	37.9	100.0
% of arrhythmic haustral activity	HB	0.99	48.5	±	20.3	21.0	39.9	57.2	17.0	90.9
	IHA		51.5	±	20.3	21.0	42.8	60.1	9.1	83.0
11. Propulsive vs. Segmentation (% values determined from the duration)										
% propulsive IHA			18.9	±	13.9	20.0	12.8	25.0	1.0	46.0
% segmentation IHA		%%%%	87.5	±	13.4	21.0	81.8	93.2	52.6	100.0
% rhythmic segmentation			41.5	±	15.5	21.0	34.9	48.1	17.7	65.7
% arrhythmic segmentation		++++	58.5	±	15.5	21.0	51.9	65.1	34.3	82.3
12. Propulsive intra-haustral activity (% values determined from the duration)										
% Majority antegrade			4.2	±	7.1	21.0	1.1	7.2	0.0	21.5
% Majority retrograde			4.7	±	11.3	21.0	-0.2	9.6	0.0	37.2
% Mixed			8.5	±	18.7	21.0	0.5	16.5	0.0	74.4
% Simultaneous		^^^^	78.0	±	32.0	21.0	64.4	91.7	0.0	100.0
13. Low- vs. High-frequencies										
% rhythmic HA – Low-frequency			79.9	±	20.1	21.0	71.3	88.5	21.6	100.0
% rhythmic HA – High-frequency			24.8	±	19.5	17.0	15.5	34.1	2.3	78.4
14. Number of Haustra										
% Active overall			47.2	±	17.0	21.0	40.0	54.5		

Table 4-1: Haustral activity in healthy subjects

This table includes the data pertaining to various parameters studied. HB and IHA (IH) refers to haustral boundary and intra-haustral activity, respectively. For interventions, PBD and DBD/RBD correspond to balloon distention, those in the proximal and distal/rectal colon, respectively. 3 different duration measures are reported: average duration of haustral activity per active haustrum, total duration (for haustral activity overall, haustral activity in the proximal and distal colon and the two types of haustral activity) and duration per 1 hr study period (for interventional analysis). In the significance column, 1 symbol p<0.05, 2 symbols p<0.01, 3 symbols p<0.001 and 4 symbols p<0.0001.

** p = 0.03; Amplitude of Bisacodyl > Baseline*

 # p < 0.0001; Proportion of rhythmic IHA > isolated HB contractions

% p<0001; Proportion of segmentation IHA> propulsive IHA

+ p<0.001; Proportion of arrhythmic > rhythmic segmentation

^ p<0.0001; Proportion of simultaneous propagation > mixed, majority antegrade and majority retrograde

Patient Values		**A**	**B**	**C**	**D**	**E**	**F**	**G**
Parameters								
1. Amplitude (mmHg):	Sig.							
Haustral activity		10.75	10.48	5.68	11.43	8.56	4.99	7.17
Isolated haustral boundary activity	0.90	11.12	8.13	5.38	10.70	9.05	4.59	7.31
Intra-haustral activity		10.27	12.27	6.15	12.20	7.63	5.17	7.11
2. Amplitude (mmHg) by intervention:								
Baseline		7.51	7.01	4.56	12.85	7.20	3.25	5.31
PBD		11.07		8.60	11.60		5.33	5.99
DBD/RBD			1.98	8.39	10.26	4.81	4.23	4.66
Meal	*	10.77	21.00	12.33	14.82	11.45	6.13	9.96
Bisacodyl		14.74		6.42	11.12	11.23	3.82	5.70
3. Amplitude (mmHg) by location:								
Proximal colon	0.12	9.36		2.61			4.59	8.43
Distal colon		11.11	10.48	6.37	11.43	8.56	7.74	6.85

	A		B		C		D		E		F		G	
4. Duration (mins):														
Haustral activity	52.9	317.3	19.6	78.2	29.2	116.8	28.0	84.1	23.0	160.7	81.7	980.3	64.0	639.5
5. Duration (mins) by location:														
Proximal	13.7	27.4			15.7	31.4					116.0	811.8	46.5	139.5
Distal	72.5	289.9	19.6	78.2	42.7	85.4	28.0	84.1	23.0	160.7	33.7	168.5	71.4	500.1
6. Duration (mins) of isolated HB contractions and IH activity:														
Haustral Boundary (HB)	34.1	170.7	10.6	42.2	12.2	48.9	30.9	30.9	13.5	94.8	53.9	646.3	37.9	378.5
Intra-Haustral Activity (IHA)	29.3	146.6	9.0	36.0	17.0	67.9	17.7	53.2	11.0	65.9	37.1	334.0	29.0	261.0
7. Duration (mins) of isolated HB contractions and IH activity by location:														
Proximal HB	6.1	12.1			12.4	24.8					72.1	504.8	33.0	99.0
Proximal IHA	7.6	15.2			3.3	6.6					51.2	307.0	20.2	40.4
Distal HB	52.9	158.5	10.6	42.2	12.1	24.1	30.9	30.9	13.5	94.8	28.3	141.5	39.9	279.5
Distal IHA	43.8	131.4	9.0	36.0	30.6	61.3	17.7	53.2	11.0	65.9	9.0	27.0	31.5	220.6

	A	B	C	D	E	F	G
8. Duration (mins) by intervention:							
Baseline	77.99	28.27	57.93	2.28	72.16	223.44	208.81
PBD	157.12		6.84	99.42		240.66	130.93
DBD/RBD		24.18	31.48	55.76	84	184.19	33.75
Meal	131.68	12.28	15.47	21.71	24.61	227.65	137.41
Bisacodyl	32.9		3.34	11.62	37.04	117.7	137.47
9. Duration (mins) of isolated HB contractions and IH activity by intervention:							
Baseline HB	46.62	16.4	20.14		54.6	147.89	115
Baseline IHA	31.37	11.86	37.8	2.28	17.56	75.55	93.81
PBD HB	52.27			38.32		149.61	74.24
PBD IHA	104.85		6.84	61.1		91.04	56.69
DBD/RBD HB		7.43	26.49	32.33	36.93	99.83	33.75
DBD/RBD IHA		16.75	4.99	23.43	47.06	84.36	
Meal HB	46.3	6.71	8.59	3.27	10.47	153.39	87.19
Meal IHA	85.38	5.58	6.88	18.44	14.14	74.26	50.22
Bisacodyl HB	27.53		0.79	3.15	20.11	88.32	83.41
Bisacodyl IHA	5.37		2.55	8.47	16.93	29.37	54.06

Control Values			**A**	**B**	**C**	**D**	**E**	**F**	**G**
10. Rhythmicity (% values determined from the duration):		Sig.							
% of rhythmic haustral activity	HB	0.28	61.79	66.18	52.55	43.54	61.81	64.11	28.89
	IHA		38.21	33.82	47.45	56.46	38.19	35.89	71.11
% of arrhythmic haustral activity	HB		48.98	41.98	31.93	29.73	55.57	67.78	81.11
	IHA		51.02	58.02	68.07	70.27	44.43	32.22	18.89
11. Propulsive vs. Segmentation (% values determined from the duration):									
% propulsive IHA			9.89		19.15	18.96	30.26	12.76	56.62
% segmentation IHA		%%%	52.30	77.03	99.12	95.68	70.02	92.15	62.87
% rhythmic segmentation		0.06	52.14	37.16	38.71	42.71	36.65	49.29	59.63
% arrhythmic segmentation			47.86	62.84	61.29	57.29	63.35	50.71	40.37
12. Propulsive intra-hasutral actviity (% values determined from the duration):									
% Majority antegrade			20.34					18.69	5.39
% Majority retrograde			48.74					20.17	34.46
% Mixed			30.92		71.41	77.19	32.02	25.41	52.90
% Simultaneous					28.59	22.81	47.41	35.73	7.25
13. Low- vs. High-frequencies:									
% rhythmic HA – Low-frequency			88.50	100.00	83.95	100.00	101.72	99.45	98.83
% rhythmic HA – High-frequency			11.50	0.00	16.05	0.00	0.00	0.55	1.17
14. Number of Haustra:									
% Active overall			28.57	57.14	23.53	23.08	36.84	85.71	71.43

Table 4-2: Haustral activity in adult patients

This table includes the data pertaining to various parameters studied and compares values pertaining to each parameter to the control 95% confidence interval determined in Table 4-1. Values below the control 95% confidence interval is colored blue; values above the control 95% confidence interval are colored red. HB and IHA (IH) refer to haustral boundary and intra-haustral activity, respectively. For interventions, PBD and DBD/RBD correspond to balloon distention, those in the proximal

and distal/rectal colon, respectively. For parameters 4 through 7, each patient column contains 2 sub columns; these sub columns represent (in order) (1) average duration of haustral activity per active haustrum, (2) total . For parameters 8 and 9, duration per 1 hr study period is reported for interventional analysis. In the significance column, 1 symbol $p<0.05$, 2 symbols $p<0.01$, 3 symbols $p<0.001$ and 4 symbols $p<0.0001$.

** $p = 0.03$; Amplitude of Meal > DBD/RBD*

% $p=0002$; Proportion of segmentation IHA> propulsive IH

Table 4-3 Cyclic Motor Patterns in Adult Patients						
Parameters			**Normal Range (95% CI)**	**Patients w/ cyclic motor patterns**		
				C	**F**	**G**
Types of cyclic motor patterns (n= # of occurrences)	HAPW-CMP		NA	2	5	0
	Colonic-CMP		NA	5	8	9
	Rectal-CMP		NA	0	17	0
Frequency (cpm)	HAPW-CMP		10.7-12.1	6.8±2.3	5.8±3.9	NA
	Colonic-CMP		7.3-8.9	6.5±3.5	2.5±0.4	2.1±0.3
	Rectal-CMP		2.5-4.1	NA	2.5±0.3	NA
Amplitude (mmHg)	HAPW-CMP		20.7-24.4	6.6±1.1	19.4±5.0	NA
	Colonic-CMP		16.9-19.4	6.8±1.2	17.2±4.8	17.3±3.8
	Rectal-CMP		16.2-19.9	NA	24.7±4.4	NA
Pressure wave propagation per CMP occurrence (%)	HAPW-CMP	Simultaneous	51.6-65.6	96.7±4.7	50.0±54.8	NA
		Anterograde	11.8-21.1	0	20.0±40.0	NA
		Retrograde	19.1-30.9	3.3±4.7	30.0±46.9	NA
	Colonic-CMP	Simultaneous	41.0-53.2	85.0±33.5	45.8±47.8	20.0±32.8
		Anterograde	21.0-32.3	5.0±11.2	18.8±37.2	14.4±32.7
		Retrograde	20.5-32.0	10.0±22.4	35.4±49.1	61.4±42.1
	Rectal-CMP	Simultaneous	25.7-47.7	NA	32.8±47.0	NA
		Anterograde	9.7-24.9	NA	22.0±27.4	NA
		Retrograde	32.3-36.9	NA	45.2±38.2	NA
Length (cm)	HAPW-CMP		14.1-17.4	6	10.2±7.1	NA
	Colonic-CMP		17.4-22.7	12.2±3.8	8.9±3.9	6.6±1.2
	Rectal-CMP		4.9-6.5	NA	2.1±0.9	NA
	HAPW-CMP		1.1-1.5	2.9±1.1	0.9±0.5	NA

Duration per CMP episode (min)	Colonic-CMP	3.1-10.0	0.7±0.6	2.8±1.4	3.0±1.9
	Rectal-CMP	4.3-7.7	NA	2.7±1.4	NA
Total duration of CMP per subject (min)	Low-frequency	15.3-39.3	7	70.3	26.7
	High-frequency	9.3-33.2	2.3	0.2	0
Total number of CMP pressure waves per subject	Low-frequency	45.6-92.5	38	207	58
	High-frequency	54.5-149.7	21	5	0

Table 4-3: Cyclic motor patterns in adult patients

The cyclic motor pattern data pertaining to 3 adult patients that demonstrated cyclic motor pattern occurrences. Normal range corresponds to the 95% confidence interval generated from the data presented in chapter 3 (Tables 2 and 3). Values below the control 95% confidence interval are blue; values above the control 95% confidence interval are red. Clear cells correspond to values within normal range, and NA marks instances where a particular parameter was absent

Discussion

The colon consists of haustral boundaries and intra-haustral activity may display rhythmicity, often at a low frequency between 2 and 6cpm, although high frequencies (7-15cpm) are also detected. Haustral boundaries may also be continuous pressure increases[6, 10, 12], displaying arrhythmic behavior. The intra-haustral activity can be propulsive with distinct propagation of pressure waves, or it generates a checkerboard pressure pattern characteristic of segmentation pattern. Haustral activity is present in both the proximal and distal colon, and it is present during all interventions. Both components of haustral activity are seen in proximity to cyclic motor patterns, high amplitude pressure waves and simultaneous pressure waves. Haustral activity was characterized in 21 healthy subjects and a set of control values were determined. In the patient sample, there was distinct heterogeneity in the presentations of haustral activity and cyclic motor patterns. Two contrasting groups of adult patients were noted amongst the 7 patients: high responders defined as those who elicited higher-than control haustral activity and presented evidence of cyclic motor patterns, and weak and non-responders who had a below-normal proportion of haustral activity and largely showed an absence of cyclic motor patterns.

Multimodal distribution of haustral activity amplitude

Haustral activity amplitudes reveal 2 distinct amplitude peaks between which the average amplitude of 8.70 ± 3.58 mmHg falls. The bimodal distribution in amplitude is apparent in both healthy subjects and adult patients; in 3 patients, haustral activity amplitudes are clustered at the high-amplitude peak of 11.2mmHg and 2 patients present close to the low-amplitude peak (5.3mmHg). A possibility that may explain the distinction between the 2 peaks is the difference in the diameter of the colon which would impact the amplitude[13] recorded by the catheter. Given that haustral activity is a low-amplitude motor pattern in comparison to high-amplitude pressure waves (75.3 ± 3.3 mmHg-109.3 ± 3.3 mmHg[14]), simultaneous pressure waves (12.0 ± 8.5 mmHg - 20.2 ± 7.2 mmHg[15]), cyclic motor pattern (16.5 ± 6.4 mmHg- 22.2± 8.7 mmHg[16]), colonic diameter

variations may have an inherent impact on haustral activity amplitudes captured while this impact may not be significant for the latter motor patterns.

Amplitude of haustral activity during baseline and following intraluminal bisacodyl

A study conducted in the same cohort of healthy subjects[14] concluded that bisacodyl administration was one of the most optimal stimuli to evoke HAPWs. HAPWs are a result of neural excitation, and by extension, haustral activity occurring in proximity to HAPW may produce higher amplitudes than those not associated. This phenomenon is evidenced in the overall increased haustral activity amplitudes of those near HAPWs versus those occurring in isolation (i.e. with distance greater than 2 min from the proximal-most appearance of HAPW). Bisacodyl is a stimulant laxative and used as a common means of pharmacologic provocation in colonic manometry inducing HAPWs[17], although its mechanism of action in the unprepared colon is still unclear[18]. The significantly higher amplitudes of HAPW during the bisacodyl period[14] may result in greater excitation influencing the amplitudes of haustral activity as evidenced in the overall higher amplitude of haustral activity during the HAPW-populated bisacodyl period. Similarly, during baseline the haustral activity in proximity to HAPW also showed an increase in amplitude when compared to those isolated from motor patterns in this period.

Impact of meal on haustral activity

When the durations of haustral activity were reported per 1hr period in healthy subjects, no significant differences in haustral activity overall or in the haustral boundary and intra-haustral activity were observed between baseline and other stimuli. Early reports on haustral activity using cinefluorography are suggestive of some increase in haustral activity following ingestion of meal compared to a period of rest [19, 20], however recent manometric findings did not find differences between baseline and following meal period[10], consistent with the findings reported in this study.

Functions of segmentation intra-haustral activity

The increased proportion of segmentation activity in healthy volunteers compared to the duration of propulsive activity in the haustra suggests that the haustral activity predominantly functions to promote water absorption, storage and stool formation and not transit. Circular muscle layer is primarily responsible for propulsive and segmenting activity[21]. Haustral activity is not peristaltic, defined as non-propulsive[22]; there are local movements of colonic content into neighboring haustra[23], however content is often 'returned' to the original haustra from which it was propagated, but also sometimes propagated further distally as noted in fluoroscopic studies[24]. In adult patients, segmentation-type intra-haustral activity is still more pronounced than propulsive intra-haustral activity. Segmentation is characterized by a checkerboard-type contraction pattern when confined to a haustrum. Barclay's[22] work using x-ray cinematography suggests the haustra work to turn and mix intestinal content; this was evidenced by the circular motion of the opaque, bismuth pills seen in the individual haustrum. While there is uncertainty around propagating haustral boundaries in humans[23], there is propagation of haustral boundaries in the anal direction observed in the rabbit[12, 25] and some in the guinea pig[24] which allow for the regular propagation of intraluminal content occurring simultaneously with the formation of stool into pellets and defecation. In the guinea pig, ultrasound imaging of the caecum suggested that the circular muscle contractions ultimately result in the mixing of colonic content[24, 26] separating the liquid from the solid components for absorption and uniform formation of stool.

Functions of propulsive intra-haustral activity

Distinctively propulsive-type intra-haustral activity was limited in controls with simultaneous propagation being the primary and significantly higher propagation type. Simultaneous pressure waves observed in manometry serve to propel content based on diameter maps in the rabbit colon[34]. Similarly, simultaneous pressure waves of sufficient amplitude likely facilitate emptying of the haustrum into the next haustrum; this may not lead to significant transit over many haustra[19] unless several haustra are synchronizing into CMPs. The synchronized activity within a haustrum (propulsive and simultaneous

pressure waves) likely also promotes absorption since there was never a consistent retrograde or antegrade period of propagation; it was always mixed with simultaneous pressure waves dominating. These mixing motor patterns create optimal exposure of the colonic content to the absorptive surfaces which in turn optimizes the digestion of content[33]. Adult patients showed no significant difference in the type of propagation for propulsive intra-haustral activity ($p=0.09$) when analyzed as a whole, however they demonstrated an overall increase in propulsive activity (when compared to segmentation) relative to controls. This increase in propulsive intra-haustral activity is driven by the strong responders presenting above average propulsive activity and conversely, a below average duration of segmentation activity. This feature holds promise in its contribution to the pathologies of the strong responders and is described later in the section.

Isolated haustral boundary activity

The function of haustral boundary contractions in the human colon is likely to retard content, possibly also to facilitate gas expulsion by simultaneous pressure waves while retaining solid content[15]. It would stand to reason that excessive haustral boundaries (number and/or duration) and/or excessive haustral boundary amplitudes could be a contributing factor in constipation. The amplitudes of haustral boundary may be more pronounced in a prepared colon, thus may have a clearer contribution to the underlying cause of constipation. We found in healthy subjects that total durations of isolated haustral boundary activity and intra-haustral activity did not differ. However, when the ratio of total durations of intra-haustral activity and isolated haustral boundary activity were determined, 62% of the subjects elicited greater proportions of intra-haustral activity. In contrast, in patients, the strong responder patient group (normal HAPW activity) showed a 45% and 90% increase in haustral boundary activity relative to the proportion of intra-haustral activity. In these strong responders who present HAPW with parameters comparable to controls, diminished colonic propulsive activity is not a cause of constipation, rather it may be excessive boundary activity. In these patients, it was also found that anal sphincter activity was not relaxing to the same extent as healthy subjects,

suggesting that constipation was due to retarding of content by haustral boundaries and poor relaxation of the anal sphincter (refer to **chapter 5**). The boundaries can be viewed as functional sphincters and they very likely have a different innervation from neighboring tissue; this would require the boundaries to be stationary and not propagating[23]. True sphincters have unique autonomic innervation, as seen in the anal sphincter[27, 28, 29], which may also apply to haustral boundaries, but this is a speculation that needs to be investigated.

ICC and haustral activity

In the human colon, ICC-SMP is proposed to be the omnipresent pacemaker with a frequency range of 2-6cpm, whereas the stimulus-dependent ICC-MP generates an electrical oscillation of ~20cpm[35, 36]. Based on this finding, the activity of the omnipresent ICC-SMP underlies the low frequency haustral activity, and ICC-MP may orchestrate the high-frequency haustral activity. Eighty percent of all rhythmic haustral activity presents low frequency, typically at 3cpm; majority of which occurs in isolation (not in the proximity of HAPW, SPW and cyclic motor patterns). The omnipresent nature of ICC-SMP likely results in the presence of haustral activity in the absence of other motor patterns that have a strong neurogenic dominance in contrast to ICC-SMP that is non-neurogenic[51]. Haustral activity is similar to the ripples observed in the rabbit colon; both have low-amplitude rhythmic activity orchestrated by the ICC-SMP[34]. Conversely, 30% of all rhythmic activity falls in the high-frequency range (>6cpm); elevated proportion of high-frequency rhythmic haustral activity occurs in close proximity to HAPWs. Neural stimulation is required for the generation of HAPW[39]; via the parasympathetic innervation into the myenteric plexus[37, 38], it is likely that extrinsic stimulation of the enteric nerves and ICC-MP during the generation of HAPW results in the high frequency haustral activity; this also explains the elevated haustral activity amplitudes when in proximity to HAPWs.

Elevated expression of low-frequency cyclic motor patterns in adult patients

The prominence of cyclic motor patterns and haustral activity in the low-frequency range in patients is attributed to the ICC in the submucosal layer. High-frequency cyclic motor patterns are largely absent in the two strong responders; its expression is well-below those observed in controls where 17 of 19 healthy controls presented high-frequency cyclic motor patterns. In contrast, subject C demonstrated evidence of both low- and high-frequency cyclic motor patterns and haustral activity, thereby suggestive of ICC expression from both the submucosal plexus and the myenteric plexus; this range of frequencies was otherwise absent in other adult patients. Subject C presented high-frequency cyclic motor patterns (9-10cpm) following an HAPW , illustrative of the rhythmic activity's myogenic origin (ICC-MP) but with neural regulation (for the generation of HAPW)[50].

Relationship between haustral activity and cyclic motor patterns in healthy subjects and adult patients

The presence of haustral activity near cyclic motor patterns that follow HAPWs has been noted. The cyclic motor pattern resolves into distinct and sequential haustra with lower amplitude. The transition of cyclic motor pattern to haustral activity is suggestive that the basic component of cyclic motor pattern is haustral activity. Sequential isolated haustral activity preceded and/or followed the cyclic motor pattern. The onset or an increase in amplitude of haustral activity in the proximity of HAPW signifies stimulation of the colonic region for the initiation of HAPW. When the HAPW terminates, residual stimulants (acetylcholine) results in the occurrence of cyclic motor patterns (reported in **chapter 3**) and/or haustral activity initiation/ increase in amplitude. In the intact human colon with choline ester injected, fluoroscopic observations revealed the disappearance of haustration in the left colon and increased propulsive activity in the proximal colon[32]. The disappearance of haustra and increased contractile activity can be inferred to be related to the occurrence of mass peristaltic events such as HAPW. Chapter 3[16] identifies the presence of 2 ICC networks with distinct frequency ranges (low-frequency (2-6cpm) and high frequency range (7-15cpm)); cyclic motor pattern following HAPWs appear to have

rhythmicity in the high frequency range. The elevated presence of high-frequency haustral activity in proximity of HAPW when compared to the proportion of high-frequency haustral activity occurring in isolation, is suggestive of the role of the ICC-MP. However, low frequency haustral activity is also evidenced following an HAPW-CMP. We hypothesize that while the contractile activity of the smooth muscle is under the influence of ICC-MP (i.e. the stimulus-dependent rhythmic depolarization of the ICC-MP sufficiently depolarizes the smooth muscle for the generation of action potentials), haustral activity will produce frequencies corresponding to the high-frequency pacemaker network. If the stimulation (e.g. from the acetylcholine release) is no longer sufficient for high-frequency contractile activity, ICC-SMP coordinates the low-frequency haustral activity. The frequency distribution of haustral activity occurring in close proximity (before or after) cyclic motor patterns needs to be further investigated to determine whether similar ICC networks are activated to produce both cyclic motor patterns and haustral activity.

In adult patients, each occurrence of cyclic motor patterns corresponds with the presence of intra-haustral activity and haustral boundary activity of at most 2 adjacent haustra in the same location preceding and/or following the cyclic motor patterns. This finding supports the hypothesis that intra-haustral activity in adjacent haustra synchronize to form cyclic motor patterns evidenced not only from the close temporal and spatial association of haustral activity and cyclic motor patterns, but through the similar frequency ranges observed.

Deficit of cyclic motor patterns

The deficit of cyclic motor patterns observed in the adult patients may prove to be a contributing factor to the pathology of chronic constipation. The function of cyclic motor patterns, a motor pattern commonly observed in healthy subjects, is for the mixing and absorption of colonic content; this motor pattern consists of non-propagating segmentation-type activity and propagation activity over short distances in the colon [16, 40,

[41]. Furthermore, the autonomic function assessed by the simultaneous HRV reveals an elevated sympathetic tone. The over activity of the sympathetic nervous system is hypothesized to cause a general lack of HAPW in response to physiological and stimulant conditions, and thus contribute to the overall reduction/lack of cyclic motor patterns, thereby contributing to these 4 patients' diagnosis of severe refractory constipation.

Elevated presence of cyclic motor pattern in the rectum

Hyperactivity in the distal colon is attributed to the pathophysiology of chronic constipation [41, 43]. The rectosigmoid brake hypothesis supported by studies from Lin et al [46] pointed to the prominence of retrograde propagation in the sigmoid and rectum. This motor pattern was very strong and persistent in the distal colon in patients after surgery involving right hemicolectomy [42] suggesting that it may serve to restore normal bowel function[46]. Subject F and G showed elevated low-frequency retrograde activity in the distal colon, which consistent with Lin's hypothesis, serves to prevent the untimely outflow of content; however the over expression of which serves to be a hindrance to transit of colonic content [47]. The rectosigmoid brake works to retard flow of content out of the rectum thereby keeping the rectum empty and preventing the onset of the defecation reflex initiated by rectal stimulation [48]. In an ambulatory coloinic manometry study, Rao et al [45] showed that patients with slow transit constipation showed a 50% increase in the cyclic motor pattern in the rectum, and the hypothesis was put forward that an increase in the rectal cyclic motor activity, as observed in subject F, may contribute to the pathogenesis of slow transit constipation.

Propagation distance of the cyclic motor pattern

The cyclic motor pattern propagated a shorter distance along the length of the colon than observed in healthy controls. The short propagation along the length of the colon may present a challenge in the local transit of intraluminal content as cyclic motor patterns play a role in local transit moving content from one region of the colon to the other in both directions [19,20,22]. Ritchie's cinefluorograms in 1968 [19], reveal the shortening of 3

adjacent haustra followed by the disappearance of their intra-haustral boundaries, and the subsequent serial transfer of barium content into the distally adjacent haustra. This serial transfer of barium into adjacent haustra almost synchronously appears to be similar to the synchronization of propulsive intra-haustral activity resulting in the generation of cyclic motor patterns. Consequently, the intra-luminal content moves in both the oral and aboral direction as observed in the mixed propagation of cyclic motor patterns. Despite the shorter propagation distance of cyclic motor patterns along the length of the colon, they may contribute to colonic transit as a secondary function.

Incongruences with findings from literature

Elevated distal colon activity is noted in patients undergoing hemicolectomies[42] both prior to and following surgery, but elevated distal colon activity was not seen in the majority of the adult patients that otherwise had 'quiet' distal colons. This finding may be the result of the heterogeneity in the underlying pathophysiology; whether the impairment is neurogenic and/or myogenic in origin. Despite all adult patients in this study being diagnosed with refractory constipation by their primary physician, those that showed evidence of cyclic motor patterns all presented HAPWs and autonomic functioning within normal range, while those with the absence of cyclic motor patterns displayed elevated sympathetic tone which were present during both the assessment during manometry and the pre-test. The presence of elevated sympathetic tone during the pre-test which does not pose significant stress on a subject signifies the inherent elevated sympathetic tone. As reported in chapter 3, a significant post-prandial increase in the low-frequency cyclic motor pattern became a benchmark for normal colonic response parameters[49, 44]; this feature was absent in 6 of 7 adult patients in this study. Furthermore, the adult patients in this study that did have cyclic motor patterns had variable responses to the meal period; this is consistent with the controls where an increased and decreased number of rhythmic pressure waves are observed following meal intake relative to the baseline period [16].

Implications of haustral activity in pathophysiology

Taken together, haustral activity is an integral component for the absorption of water evidenced from the pronounced segmentation activity. Haustral activity may participate in some propulsive activity that occurs as not all transit of colonic content is done by the infrequent mass peristaltic HAPWs. Much of the colonic activity consists of discrete movements of content in a slow and stepwise fashion over short distance[30], moving content in both antegrade and retrograde directions[31]. Ritchie[19] describes these discrete movements as the simplest haustral propulsion; these are systolic contractions of the haustrum resulting in the haustral contents being displaced into the next distal segment and continue distally. The mixed propagation of cyclic motor patterns may also contribute to the local propagation of colonic content. The lack of an ICC-orchestrated frequency gradient in the colon, and thus no distinct oral to aboral propagation, unlike the stomach and small intestine, is appropriate given that anally-directed propagation does not need to happen consistently in the colon. Absorption of water and nutrients, storage and formation of colonic content into stool occurs on an ongoing basis with local back and forth movement and mixing of content.

In patients, the elevated durations of haustral activity as isolated haustral boundaries or the mixed propagation of intra-haustral activity may contribute by being a physical hindrance and may retard flow of colonic content as demonstrated in the strong responders. Increased segmentation-type intra-haustral activity may result in increased colonic absorption of water resulting in hard stool. So far, we do not quite know the implications of below-average duration of haustral activity in the weak and non-responder patients, however we can hypothesize the lack of haustral activity results in the lack/impairment of critical functions performed by this motor pattern.

In this chapter, we provided the detailed characterization of haustral activity (haustral boundary activity and intra-haustral activity) on the parameters of frequency, amplitude, duration and propagation directions. This was the integral first step prior to any further comparisons to patients. However, there are still questions left to be answered. We hope

to analyze haustral activity and cyclic motor patters in pediatric patients in search for similarities and differences with adult patients. In doing, we hope to grasp a more definitive understanding of non-, weak vs strong responders when it comes to haustral activity and cyclic motor patterns.

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Chapter 5 : On the sphincter of O'Beirne and autonomous dyssynergia in chronic constipation

This study was submitted to Digestive Diseases and Sciences and is currently under review.

Introduction

Constipation is a common worldwide problem with a prevalence up to 35% in adults and up to 30% in children and contributes substantially to the financial burden of health care [1]. Constipation has a significant impact on quality of life, affecting both physical and emotional well-being [2], and should be considered a major public health issue both in the pediatric and adult population[1]. While in many cases, constipation can be treated successfully, symptoms can be chronic, difficult to treat, and debilitating [3]. Colonic motility testing is deemed important for identifying if constipation is caused by colonic motor dysfunction [4], yet it is rarely done in adults and hence treatment of severe constipation is often done empirically, and surgery, although rare, is still seen as an option. In fact, a study of 2377 colectomies for chronic constipation, obtained through the US Nationwide Inpatient Sample (1998–2011) showed that colectomy rates for constipation are rising, but are associated with significant morbidity and do not decrease resource utilization, leading the authors to raise questions about the true benefit of surgery for slow transit constipation [5]. This is consistent with the conclusion of Gladman and Knowles that in constipation, surgery should probably be avoided [6]. To make rational decisions about treatment options, better understanding of the pathophysiology of functional and organic constipation is needed [7]. In children, colonic manometry has been extremely valuable, but it is still uncommon in adults [7]. With the development of High-Resolution Colonic Manometry (HRCM), a new era of progress appears on the horizon with increased insight into the pathophysiology of constipation and substantially advanced options for diagnosis. In addition to high amplitude propagating pressure waves, other motor patterns are now recognized to play a role in colonic motor function such as simultaneous pressure waves [8–11] and cyclic motor patterns [12–14]. The aim of the present case study was to demonstrate how the use of HRCM identified motility

dysfunction in a patient who was considered to have refractory constipation due to an inert colon and who was slated for surgery. We show that HRCM identified targetable abnormalities; our results focus on the importance of the sphincter of O'Beirne and introduce the concept of autonomous dyssynergia as a potential contributing factor of chronic constipation, issues that would not have been corrected by colectomy.

CASE REPORT

A middle-age female patient with lifelong chronic constipation was referred for an assessment of pan-colonic motility using 84-channel water-perfused HRCM prior to a consideration of a colostomy or a colectomy due to significant progression in the last 5 years. The patient had 2-3 bowel movements (BMs) per month with passage of large and hard stool with excessive straining requiring high doses of laxatives, intermittent manual disimpactions and enemas and multiple emergency room visits due to large and hard stool impactions. Constipation worsened after 2 pregnancies with normal vaginal deliveries and a coccynx injury (all >15 years prior to our assessment) with ongoing coccygeal pain. Five years ago, she started to pass pencil-thin form stool or semi-liquid stool; no large caliber stool anymore. Physical examination revealed tenderness of the coccygeal region, otherwise unremarkable. Abdominal X ray showed dilated air-filled splenic flexure and moderate amount of stool in the cecum and ascending colon. Anorectal manometry revealed a borderline hypotensive anal sphincter with limited capacity to squeeze, and a failed balloon expulsion test; the recto-anal inhibitory reflex (RAIR) was present. A shapes study revealed shapes accumulating in the sigmoid colon. One colonoscopy and 2 flexible sigmoidoscopies were normal. Abdominal CT and spinal MRI were unremarkable. Prucalopride was discontinued due to palpitations. Linaclotide 145 mcg daily provided suboptimal effect and Linaclotide 290 mcg daily induced abdominal cramping. At the time of assessment, the patient did not have spontaneous bowel movements and laxative induced bowel movements lasted hours.

Methods

Please refer to chapter 3 methods section.

Nomenclature and abbreviations

The colonic motor patterns identified by High-Resolution Colonic Manometry (HRCM) were High-Amplitude Propagating Pressure waves (HAPWs), also called HAPS or HAPC [15–17], Simultaneous Pressure Waves [8,10,11], the cyclic motor pattern [13] and the sphincter of O'Beirne. The characteristics of the sphincter of O'Beirne in healthy subjects are shown in an accompanying paper [18].

Results

Baseline and the sphincter of O'Beirne

During the baseline period, the colon showed normal motor patterns but coordination between colonic motor patterns and the rectosigmoid sphincters appeared abnormal. The rectum was 8 cm long and at its proximal end, a high-pressure zone was present throughout the 6 hour recording at 15-30 mmHg which was at times increased by transient rhythmic contractions at 2-3 cpm (Figure 5-1). This was identified as the sphincter of O'Beirne [18]. The colonoscope passed this region/pressure band with significant resistance and the patient reported pain during its passage (Figure 5-1C). At baseline, the anal sphincter showed an average pressure at 60 mmHg; the sphincter of O'Beirne was prominent and rhythmically contracting at ~ 2.5 cpm generating an average pressure of 34 mmHg (Figure 5-1). The 3 cycles/min "cyclic motor pattern" was present with retrograde propagating short pressure waves proximal to the sphincter of O'Beirne. Two HAPWs started in the proximal colon and propagated to the splenic flexure and descending colon, switching into SPWs (Figure 5-2). The amplitudes of

SPWs reached the sphincter of O'Beirne there were contractions of the sphincter from 31.3 to 72.2 mmHg and from 32.4 to 77.7 mmHg. The SPW amplitudes were 35 and 37 mmHg. The SPWs did not penetrate into the rectum and following the SPWs, the anal sphincter contracted from 43.8 to 58.7 mmHg and from 44.5 to 57.8 mmHg (Figures 5-2,

3D), whereas the sphincters normally relax in healthy subjects [10].

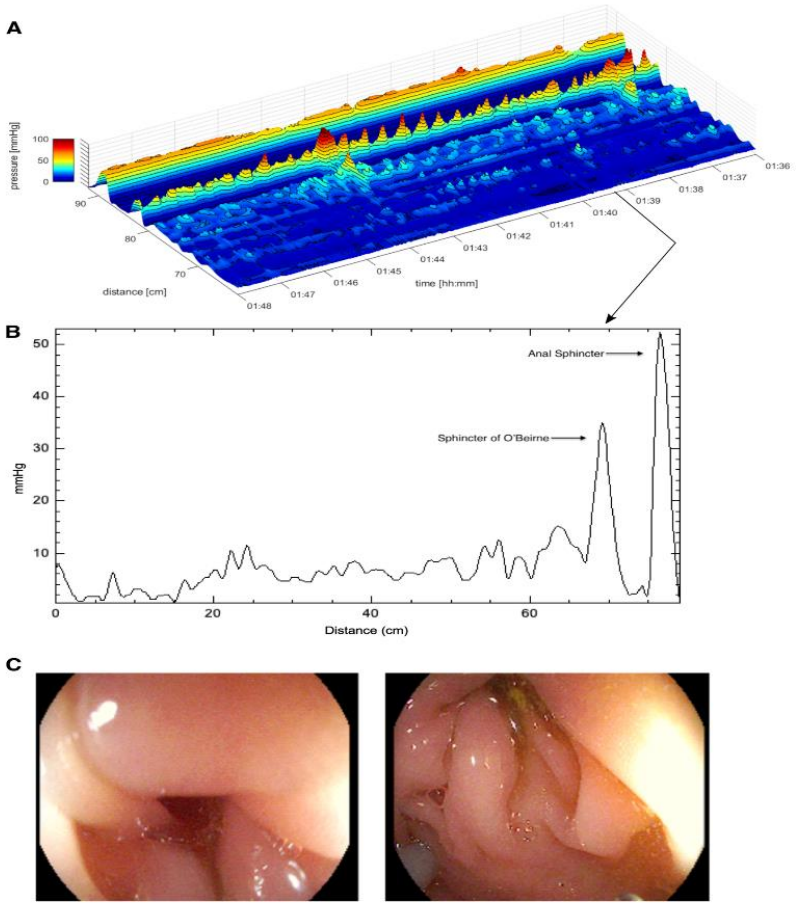

Figure 5-1 The prominence of the sphincter of O'Beirne

A. Baseline activity showing continuous anal sphincter pressure, and a continuous sphincter of O'Beirne at high pressure with rhythmic contractions at 3 cpm with an average amplitude of 34.2 mmHg. There was no activity in the rectum. A cyclic motor pattern was present proximal to the sphincter of O'Beirne at 3 cpm propagating orally.

B. The pressure profile from the proximal to distal colon at 1:39:30 during baseline. The first spike in pressure corresponds to the location of the sphincter of O'Beirne, which is located 10 cm from the anal verge, whereas the second spike in pressure corresponds to the anal sphincter. Smaller fluctuations in pressure closer to the distal colon correspond to the cyclic motor patterns.

C. Colonoscopy encountered a tight rectosigmoid junction that was about 10 cm above the anal verge. The patient experienced pain when the colonoscope tip passed through.

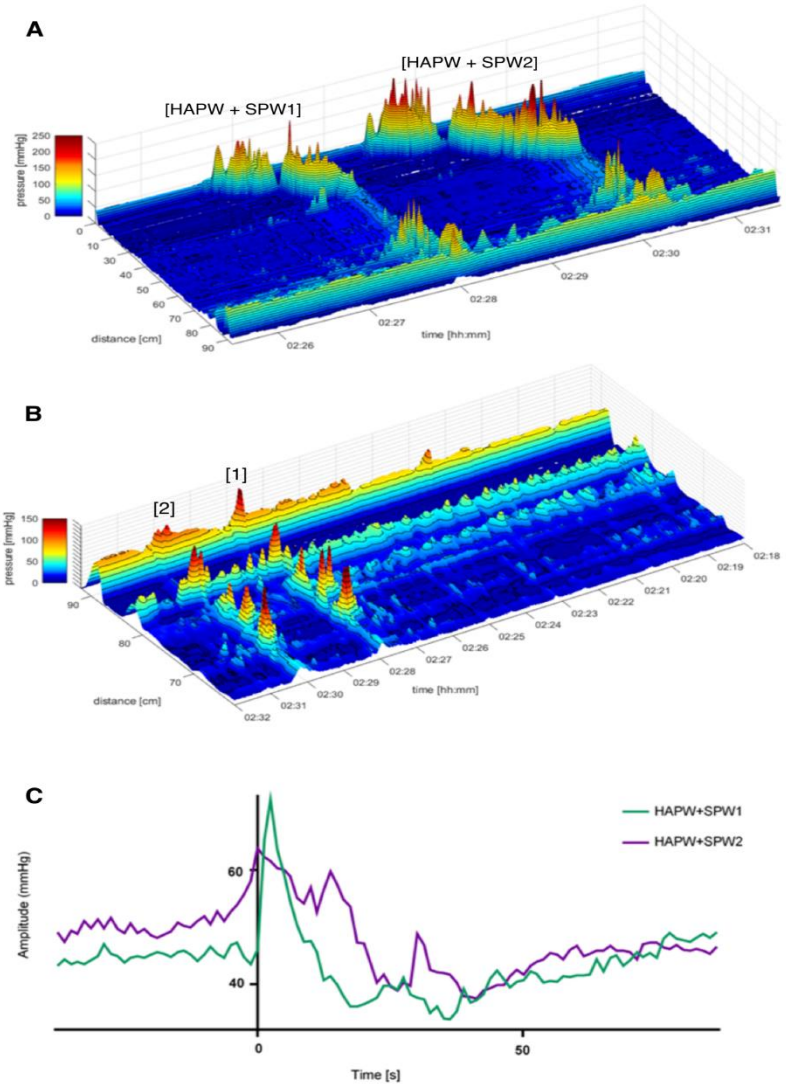

Figure 5-2: Motor patterns at baseline

A. Autonomous dyssynergia; Two HAPW-SPWs with paradoxical contractions of the anal sphincter and the sphincter of O'Beirne. The contraction of the sphincter of O'Beirne resulted in an increase of the average amplitude from 33.3 to 66.8 mmHg (200% increase with contraction).

B. This figure partially overlaps with Figure A and is turned 180 degrees, to show the cyclic motor pattern activity seen proximal to the sphincter of O'Beirne. It only shows the distal 25 cm. There is no activity in the rectum.

C. Anal sphincter pressure associated with the HAPW-SPWs, a ~ 25 mmHg contraction occurs instead of relaxation

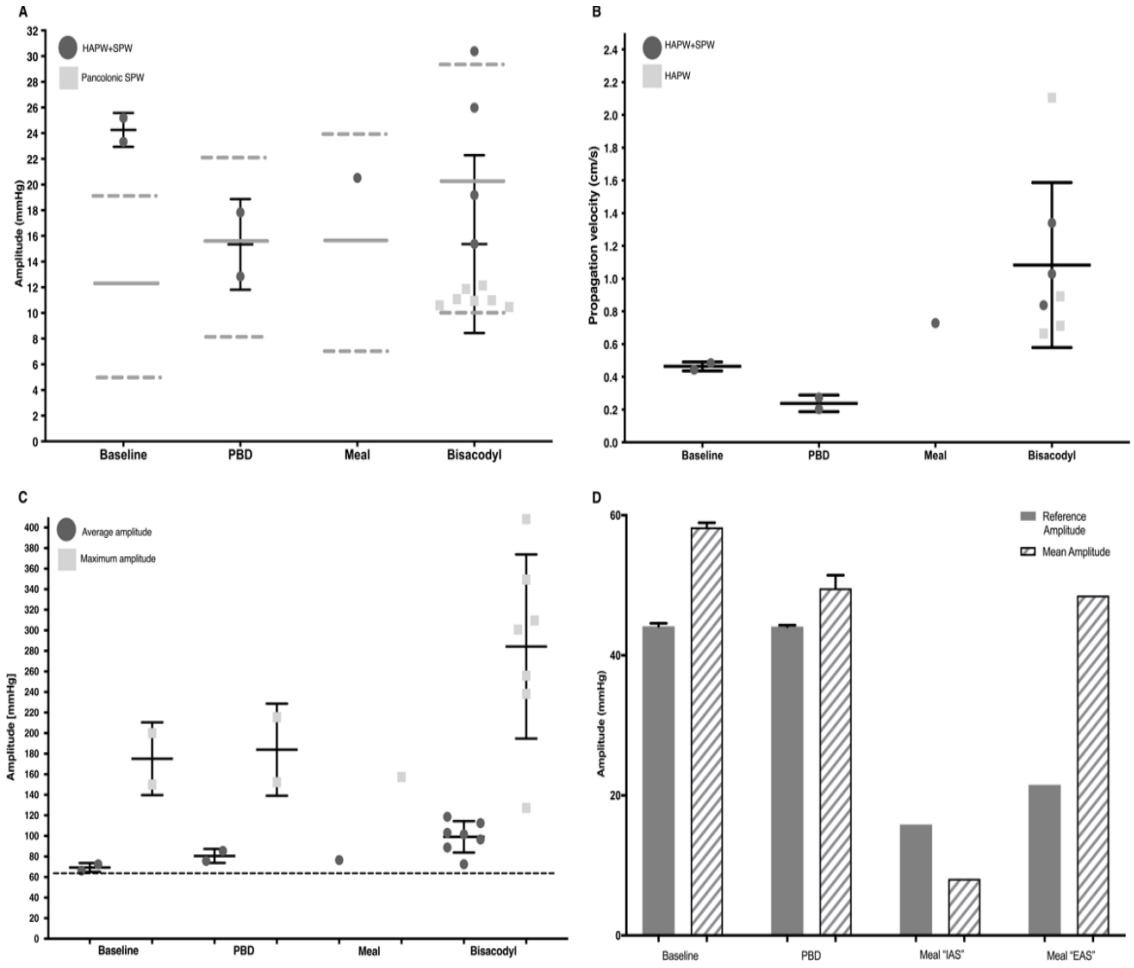

Figure 5-3: Motor pattern and anal sphincter quantification via intervention

A. Amplitude of HAPWs in response to the stimuli. Circles: Average amplitude of HAPWs, squares: Maximum amplitude of the HAPWs. Data show as mean ± SD. From the 15 HAPWs, 13 were followed by SPWs (HAPW-SPWs) whereas two were without SPWs.

B. Amplitude of pancolonic SPWs and the SPWs that are part of HAPW-SPWs. The solid gray lines are the average values obtained in healthy subjects, the dashed lines the SD values, as reported in Chen et al. [10]. The average duration of the SPWs was 21.5 s,

C. The propagation velocity of the HAPWs.

D. Quantification of anal sphincter responses to motor patterns by intervention. The reference amplitude is taken as the amplitude of the segment 3 minutes before the anal

sphincter response. At baseline and during proximal balloon distension (PBD) IAS and EAS were not distinguished. During a meal the upper, "IAS" and lower, "EAS" portion of the anal sphincter pressure were analyzed separately.

In this patient, a manometric pattern, consistent with the presence of the sphincter of O'Beirne, was evident and visible 96 % of the time during baseline and 63% of the time during the entire procedure contrasting with healthy controls where we see the sphincter of O'Beirne visible only 15.6 ± 12.2 % (n=116) of the time (Figure 5-4), based on Chen et al. 2019 [18].

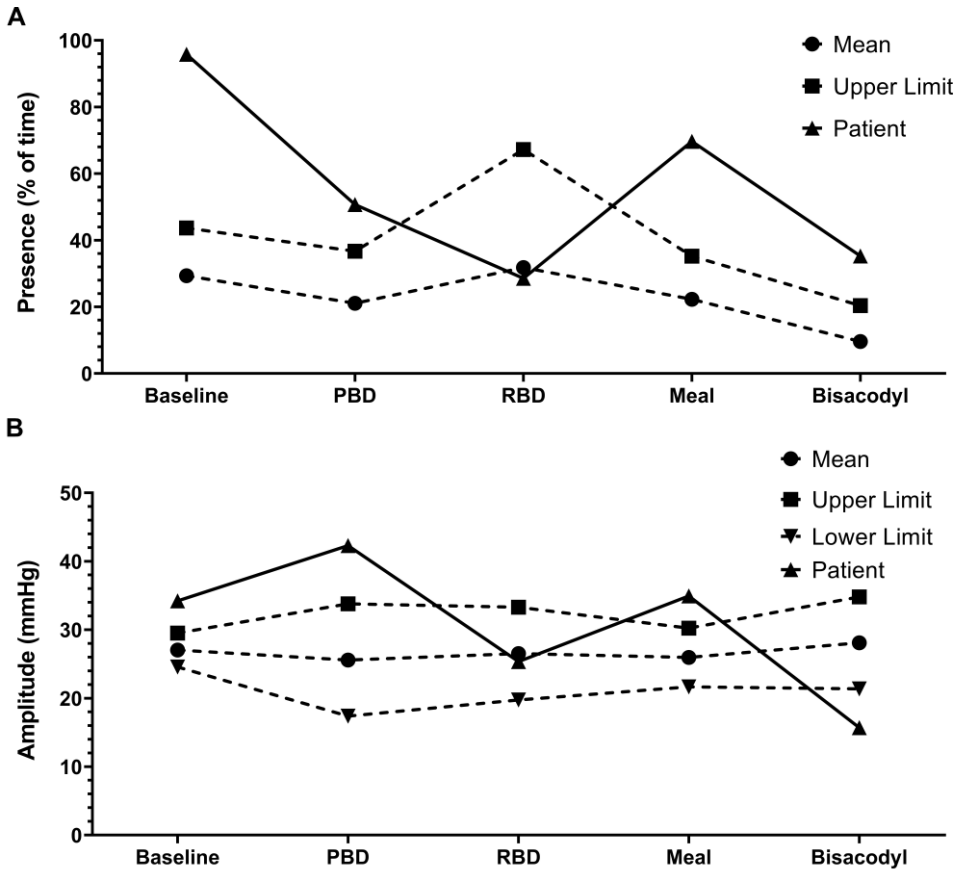

Figure 5-4: Comparison of the sphincter of O'Beirne's amplitude and % presence to healthy controls

A. % Presence; Comparison to control values [18]: The presence of the sphincter of O'Beirne was evaluated by intervention compared to healthy controls. The upper limit was calculated using a 95% confidence interval, the lower limit was taken to be 0, as the

sphincter is usually not present within the recordings of healthy controls. Overall, the sphincter of O'Beirne was present for 63.2% of the entire manometry recording contrasting with 15.6 ± 12.2% of the manometry reading in healthy controls. This patient had a higher presence recorded during baseline, meal and bisacodyl interventions. Proximal balloon distension was bordering the high side of the normal based on healthy controls, while the presence was closer to the low side of normal during rectal balloon distension.

B. Amplitude; comparison to control values: The amplitude of the sphincter of O'Beirne was evaluated by intervention compared to healthy controls. The upper and lower limits were calculated based on a 95% confidence interval. Overall the average amplitude across all interventions was 29.5 similar to an average amplitude of 27.6 mmHg found across interventions in healthy controls. The amplitude was higher than controls during proximal balloon distension, contrasting with an amplitude lower than controls during bisacodyl. All other interventions fell within the bounds of the upper and lower limit of heathy controls.

Response to stimuli

Proximal balloon distension induced a pair of HAPW-SPWs (Figure 5-5). The HAPWs started in the proximal colon and continued as SPWs at the splenic flexure. The patient reported abdominal pain (4/10) during the HAPW-SPWs. The HAPW average amplitudes were 75 and 85 mmHg, the SPW amplitudes were 17 and 23 mmHg (Figure 5-3). No relaxation of the sphincter of O'Beirne or the anal sphincters was observed in response to approaching SPWs (Figure 5-3D). The sphincter of O'Beirne showed rhythmic activity at ~ 2.5 cpm superimposed on a sustained pressure of 42 mmHg. The anal sphincter pressure was a sustained 50 mmHg.

Rectal balloon distension, up to 240 ml, did not induce anal sphincter relaxation. Instead, hypertensive sphincters were observed. The patient reported rectal pain and urge to defecate. The patient was not able to expel the balloon.

In response to the meal, a short HAPW at 84 mmHg occurred followed by a moderately strong SPW with amplitude of 25 mmHg. The sphincter of O'Beirne showed irregular contractile activity following the SPW from 30.2 to 59.9 mmHg (Figure 5-6). The external anal sphincter (the distal part of the anal canal) contracted from 20 to 50 mmHg

(Figure 5-6). The proximal part of the anal canal relaxed from 15 to 10 mmHg. Gas escaped with this motor pattern.

Bisacodyl in the rectum (a 10 mg suspension) induced a gradual decrease in the pressure of the anal sphincters and the sphincter of O'Beirne (Figure 5-7). The sphincter of O'Beirne started with an average amplitude of 34 mmHg during baseline but following the presence of rectal bisacodyl the amplitude reduced to an average of 15 mmHg. Seven pan-colonic simultaneous pressure waves (SPWs) occurred 5 minutes

later with an average amplitude of 14 mmHg and a frequency of 1/min. Initially, these SPWs were not associated with anal sphincter relaxation and there was no reported gas or liquid expulsion. In order to get definitive information, another 10 mg bisacodyl was given to the rectum. 3 HAPW-SPWs and then 4 strong HAPWs were observed with associated gas and liquid expulsion. The HAPWs were able to reach the distal sigmoid colon and anal sphincter relaxation was seen during the HAPWs. The average amplitude of HAPW-SPWs were 94 mmHg (HAPWs) and 29 mmHg (SPWs); the average amplitude of HAPWs without SPWs was 132 mmHg.

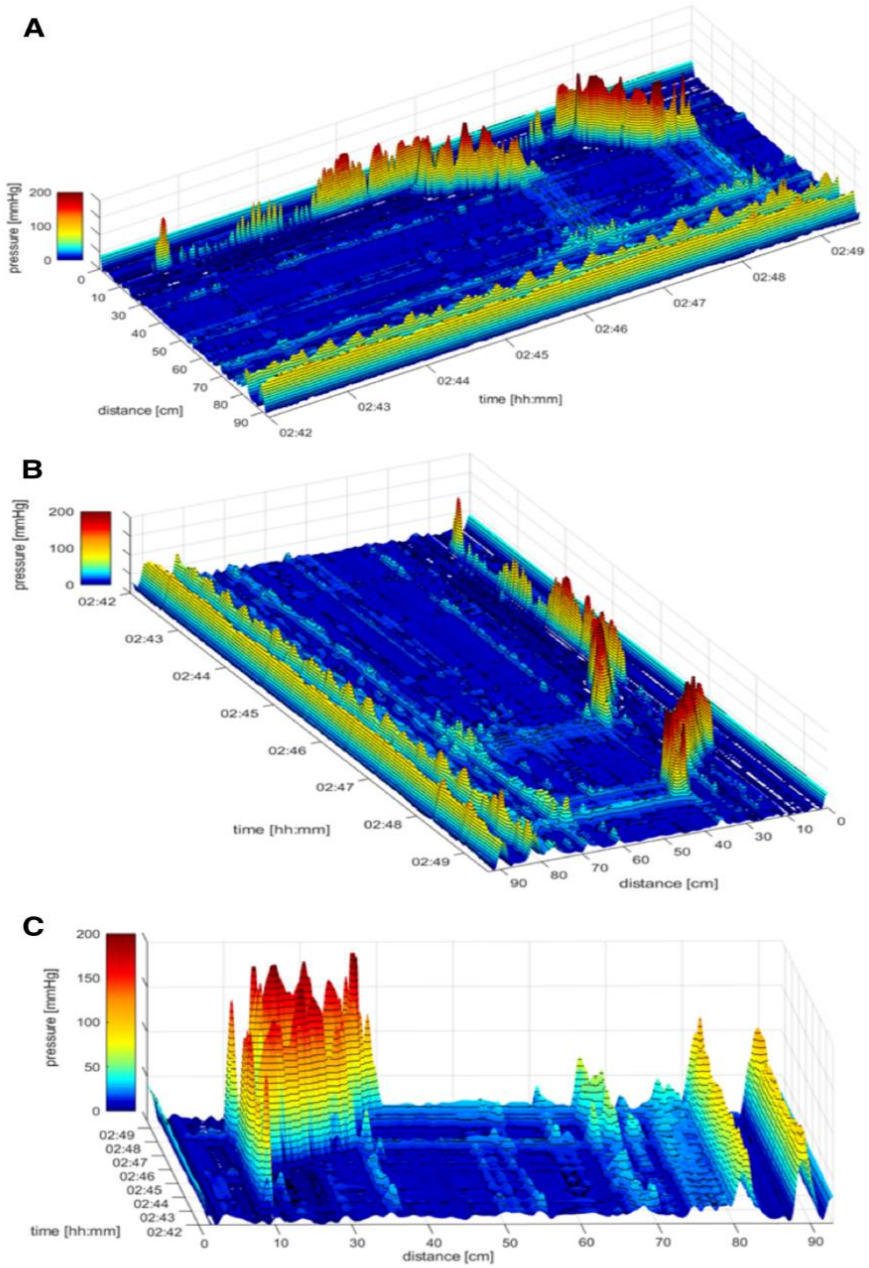

Figure 5-5: Motor patterns in response to proximal balloon distension

A. Two HAPW-SPWs developed without sphincter relaxations. The sphincter of O'Beirne is rhythmically contracting at 3 cpm superimposed on a high average tone of 31 mmHg.

B. Same as A but seen from a different angle.

C. Same as A but shown such that the pressure barriers are standing out.

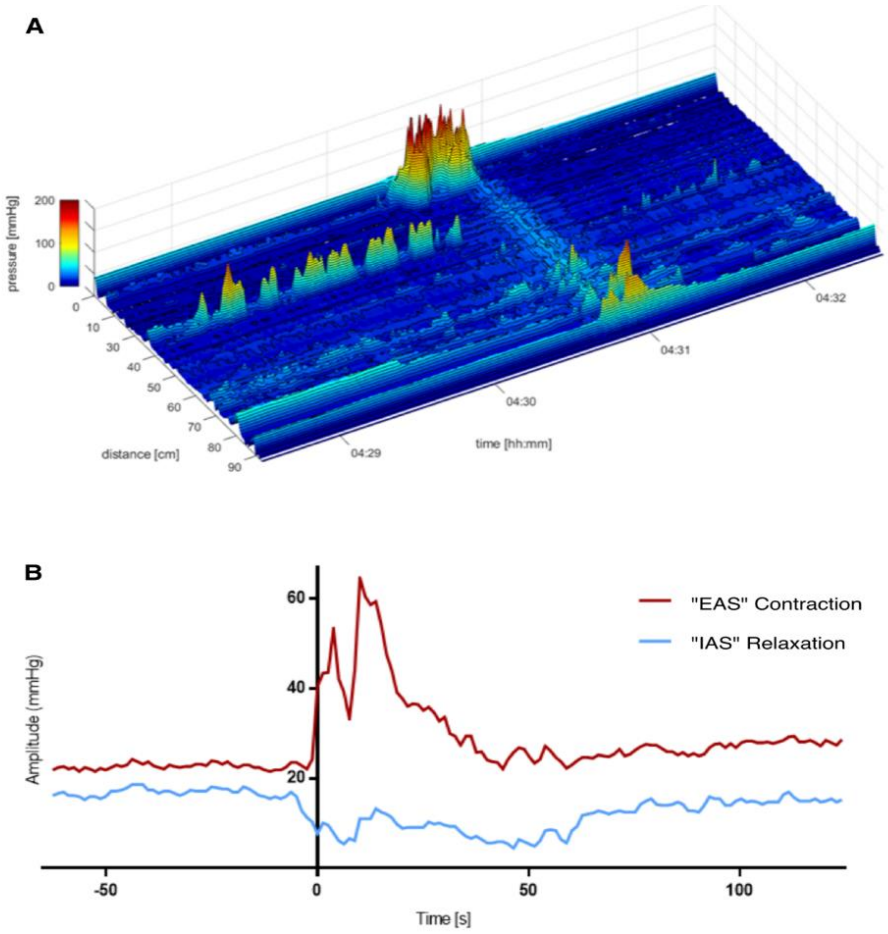

Figure 5-6: An HAPW-SPW in response to the meal

A. An HAPW-SPW progresses towards the rectum, is associated with transient but not full relaxation of the sphincter of O'Beirne prior to the arrival at the rectum. Upon arrival at the rectum, both the sphincter of O'Beirne and the external anal sphincter contract.

B. Plot profile of both the IAS (proximal part of the anal canal) and the EAS (distal part of the anal canal) of a single HAPW+SPW recorded during meal. The EAS shows a clear contraction during the SPW of the HAPW+SPW complex followed by a recovery in tone. The IAS relaxes during the SPW and recovers in tone demonstrating a dyssynergia within the SPW. Percent relaxation of the IAS was 72% (mean IAS relaxation amplitude: 8.1mmHg, min IAS relaxation amplitude: 4.4 mmHg, reference amplitude: 15.8 mmHg) whereas the EAS contraction demonstrated a 300% increase in amplitude compared to

before the HAPW+SPW complex (mean EAS contraction amplitude: 48.6 mmHg, maximum EAS contraction amplitude: 64.5 mmHg, reference amplitude: 21.5 mmHg).

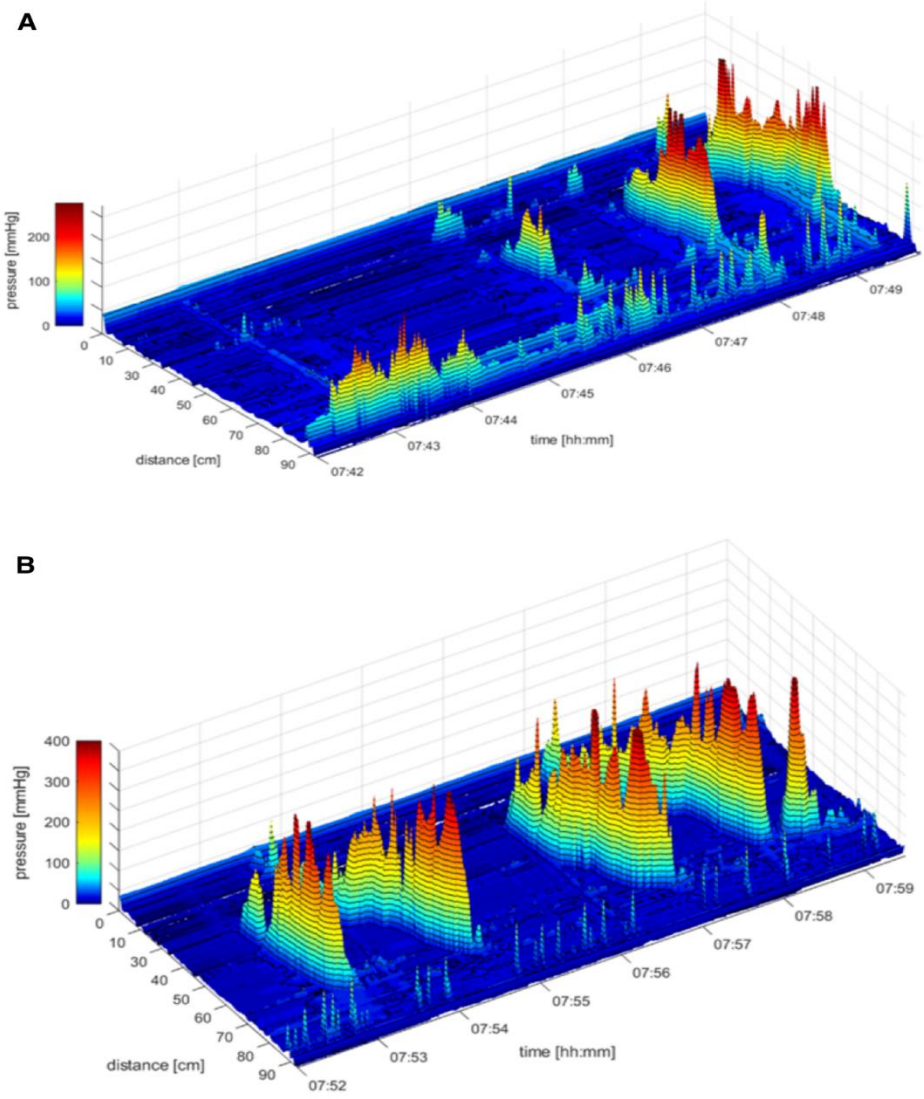

Figure 5-7: Motor patterns in response to rectal bisacodyl

A. 10 mg bisacodyl was administered to the rectum. Approximately 12 minutes following administration, HAPW-SPWs develop with increasing amplitude. The anal sphincter pressure was very high upon bisacodyl administration, but quickly diminishes and then was abolished except for transient contractions.

B. Subsequent to A, another 10 mg bisacodyl was given in the rectum and 4 more HAPWs emerged with complete relaxation of anal sphincters and the sphincter of O'Beirne. The HAPWs were not followed by SPWs. The HAPWs were associated with urge to defecate.

The cyclic motor pattern

The ~ 3 cycles/min cyclic motor pattern was prominently present 37% of the entire recording period; it was present during baseline, in response to proximal balloon distension, after the meal and in response to bisacodyl. It consisted of predominantly retrograde pressure waves at a frequency of 2.1 ± 0.2 cpm, at an amplitude of 16.8 ± 1.8 mmHg and a propagation velocity of 0.27 ± 0.03 cm/s, emerging proximal to the sphincter of O'Beirne that showed rhythmic contractions at the same frequency. The propagation length was between 4 and 6 cm at 4.5 ± 0.7 cm on average. The cyclic motor pattern occurred periodically with an average duration of 6.3 ± 2.6 min.

Discussion

This patient was given the diagnosis of "inert colon" based on the inability to generate spontaneous bowel movements, poor reaction to laxatives, together with the observation of slow transit shown by a Shapes study, prompting consideration of surgery. Although surgery for constipation is uncommon, it is usually performed without prior assessment of colonic motility. HRCM showed that the patient was able to generate normal motor patterns throughout all interventions as well as normal and complete sphincter relaxations in response to rectal bisacodyl. The reason for the inability to generate spontaneous bowel movements appears to be the absence of normal coordination between colonic motor patterns and anorectal function. When a HAPW-SPW propagated down the colon it encountered a spastic sphincter of O'Beirne, as well as a cyclic motor pattern proximal to this sphincter that, with its retrograde propagation, likely kept content away from the rectum [19]. There was almost no activity in the rectum, and the anal sphincters, just like the sphincter of O'Beirne, did not relax when a propulsive contraction came down towards the rectum, which contrasts sharply with observations in healthy volunteers [10,20]. Our interpretation is that this patient's severe constipation was due to the inability to relax the sphincter of O'Beirne and the anal sphincters in response to physiological propulsive colonic motility patterns. Instead, paradoxical contractions occurred. While the shapes

study identified slow transit, our results show that this was not due to the inability to generate propulsive contractions in the colon; the shapes study showed markers in the sigmoid colon after 6 days and none in the rectum consistent with our hypothesis that the sphincter of O'Beirne retarded content.

This patient had infrequent bowel movements since childhood with large stools requiring the occasional visit to the emergency department for disimpaction. Paradoxical contractions of the sphincter of O'Beirne and the anal sphincters may have existed since childhood and may have led to incomplete evacuation. Constipation was exacerbated by a coccyx injury which is not uncommon [21].

Dyssynergia is normally identified as the absence of relaxation of the internal anal sphincter upon rectal distension by a balloon or the act of bearing down, with or without "paradoxical" external anal sphincter contraction [2]. Hence, dyssynergia refers to an abnormal response to a conscious event. In this patient, autonomous relaxation of the sphincter of O'Beirne and the anal canal failed. Instead, involuntary contraction of the sphincter of O'Beirne and the anal sphincters occurred in response to colonic motor patterns coming down towards the rectum. This may be called: autonomous dyssynergia. Involuntary contraction of the external anal sphincter, mediated by the autonomic nervous system, is likely part of normal continence mechanisms for which Broens et al. [22] provided evidence. The normal response to rectal bisacodyl in this patient that, following rectal stimulation, evoked pancolonic motor activity that started in the proximal colon, indicates that the autonomic neural pathways are still intact. Bisacodyl affects rectal enterochromaffin cells, stimulating extrinsic autonomic sensory nerves that communicate with the sacral defecation center [23], to initiate a motor pattern- the HAPW, deemed essential for a normal defecation reflex. This same pathway is part of physiological activation of the defecation reflex as well since resection of the pelvic nerve in patients causes loss of rectal sensation and loss of the ability to defecate [24]. Under normal conditions, enteric nitrergic innervation is also involved in internal sphincter relaxation

and this might also be compromised although in this patient, the recto-anal inhibitory reflex (RAIR) was shown to be present at an earlier anorectal manometry test. Hence the inability to generate a normal defecation reflex in this patient appears to be due to a too weak activation of sensory and/or motor autonomic nerves. This is consistent with the fact that the patient has some difficulty with urination as well. It is likely that these reflexes involving the sacral nerves worsened after her coccyx injury.

This patient was able to generate a RAIR but failed the balloon expulsion test. This suggests that while nitrergic innervation of the internal anal sphincter was present, it was insufficient as part of the reflex to expel a balloon. Assuming that the physiology of innervation to the internal anal sphincter and the sphincter of O'Beirne is similar, the relaxation of both the internal anal sphincter and the sphincter of O'Beirne, in addition to intrinsic nitrergic nerves, is evoked by parasympathetic nerves from the inferior hypogastric plexus carrying acetylcholine acting on nitrergic and purinergic nerves, nerves releasing carbon monoxide, as well as sympathetic fibers releasing nor-adrenaline acting on beta receptors [25–27]. Although it is controversial whether or not pudendal nerves are stimulated by the autonomic nervous system, recent evidence suggests that this does occur and provides involuntary control of the external sphincter [22]. Roppolo showed that this is most likely done by parasympathetic nerves from the sacral defecation center which synapse in Onuf's nucleus [28] or via enkephalin positive interneurons in lamina X terminating on dendrites of pudendal motor neurons in Onuf's nucleus [29,30]. Hence weak parasympathetic innervation to the distal colon can be responsible for the absence of HAPWs in the descending colon as well as the lack of inhibition of the sphincters. Sacral nerve stimulation with implanted electrodes has shown promise in the treatment of severe refractory constipation [31]. The fact that rectal bisacodyl generates HAPWs in the distal colon as well as sphincter relaxation suggests that the sacral parasympathetic neural innervation is present but not sufficiently responding to colonic motor patterns evoked by physiological stimuli.

The predominantly retrograde propagating cyclic motor pattern, which was very prominently present in this patient in the sigmoid colon is similar in every respect to the cyclic motor pattern found in healthy volunteers [32] and deemed to function to keep the rectum empty [12,13,19]. The rectum appeared hyposensitive to distension but it had a normal sensitivity to bisacodyl. The rectum was exceptionally quiet throughout the entire procedure. An exceptionally quiet rectum in patients with constipation was also observed by Connell [33]. In most clinics that perform colonic manometry, the focus is on identifying pan-colonic HAPWs as a hallmark of normal colonic motility. We, and others [11], have recently demonstrated that another propagating motor pattern is more dominant in healthy controls, a HAPW that is followed by a simultaneous pressure wave, a HAPW-SPW. The HAPW-SPW is associated with gas expulsion, and liquid expulsion when a water-perfused catheter is used [10] but likely also contributes to defecation [34]. In the rabbit colon, we showed that SPWs are generated by fast propagating contractions which is likely the basis for the propulsive properties of SPWs [35]. In the present case study, the HAPW-SPW occurred frequently and under all conditions, identifying that the colonic musculature and the enteric nervous system are likely normal.

The sphincter of O'Beirne was described by James O'Beirne in 1834, and identified as a contributing factor in constipation [36,37]. O'Beirne identified the sphincter by digital examination. The existence of the sphincter in the present study on manometry found collaborating evidence in the fact that moving the colonoscope though the rectosigmoid junction was difficult and painful and the fact that the patient reported pencil thin stool. In the early 20th century, X-rays confirmed the sphincter of O'Beirne as "a hyper-pressure zone of 2-3 cm in length at the level of the rectosigmoid junction" where " a temporary stop of a barium column is generally observed on X-ray examination"; it was also called "sphincter of Moutier" [38]. They found that the sphincter could be rhythmic and retrograde peristaltic waves originated from it; it relaxed upon acetylcholine [39]. The latter would suggest similarity with the internal anal sphincter that also relaxes in response to acetylcholine since acetylcholine preferentially or dominantly acts on nitrergic nerves at

the sphincter [40,41]. The present study shows consistency with the findings in the early literature on the sphincter of O'Beirne; it shows prominent presence in a patient with constipation, it shows rhythmic contractile activity and from it, a cyclic motor pattern emerges that is predominantly retrograde that can act as a "braking mechanism" which can contribute to continence by moving content away from the rectum [42]. It may also contribute to constipation if it is too strong and fails to give way in response to a propulsive motor pattern. Low resolution colonic manometry did not advance our understanding since the sphincter will most often be missed when only 7-10 sensors are placed throughout the colon. Although the sphincter is often seen in cadavers [37] we believe that it is likely not a permanent physical barrier [18]. We sometimes do not see a pressure zone at the rectosigmoid junction in some volunteers during a 6-hour HRCM [18], and in the present case study, it is completely abolished by rectal bisacodyl. We hypothesize that the sphincter develops significant pressure in response to neural stimulation as part of continence reflexes that are probably more significant in an unprepared colon compared to an empty colon during colonic manometry.

O'Beirne focused on the rectosigmoid sphincter to treat chronic constipation. He wrote a treatise on the advantages of dilatation of the sphincter of O'Beirne if its excessive tone was the reason for constipation, and warned strenuously against making cuts in this sphincter which was commonly performed at the time [36,43]. These treatment suggestions make it clear that the sphincter was seen as a fixed stricture in patients with constipation. Here we show that bisacodyl readily relaxed the sphincter of O'Beirne hence dilatation or surgical intervention does not appear to be warranted. Because in this patient, rectal pharmacological stimulation can evoke a normal defecation reflex, that is: a strong propulsive motor pattern associated with rectosigmoid and anal sphincter relaxation, ways need to be discovered to restore neural reflexes to normal under such conditions.

It can be debated whether or not the sphincter of O'Beirne is a "real" sphincter. This is similar to a longstanding debate regarding the pyloric sphincter. Initially the junction

between the pylorus and duodenum failed to show a structural or functional sphincter based on manometric results, when looking for a closed high-pressure zone that would open or relax in response to a stimulus [39]. The pylorus gave insight into an open sphincter mechanism [39]. Similarly, extensive work done by Code et al [44] on the gastroesophageal junction led to the idea that a specific anatomical structure does not need to be present to deduce the presence of a functional sphincter. The open sphincter mechanism reveals that a sphincter can be open under many conditions but can shut tight to oppose the movement of solids and fluids.

Management of a patient as described here should include the prevention of hard stool, treatment of spinal nerve related pain and in addition this patient may benefit from non-invasive sacral nerve neuromodulation acting on the sacral extrinsic autonomic nervous system. This is consistent with some reports that implanting electrodes near the sacral defecation center can be effective [6,45] and reports of transcutaneous electrical neural stimulation that target the sacral defecation center [46]. After discussions with the patient, the option of surgery was not pursued. Instead the patient was offered non-invasive sacral neuromodulation, using transcutaneous electrical stimulation.

This study identifies autonomic dyssynergia, excessive contraction of the sphincter of O'Beirne and the external anal sphincter by the autonomic nervous system, as potentially important factors in the pathophysiology of severe and refractory constipation. We hypothesize that it results from impaired communication between the sacral parasympathetic nucleus and the distal colon and its sphincters. In this patient, in whom the colon appeared "inert" and who was destined for colectomy, HRCM identified this abnormality. We conclude that HRCM should be performed in all patients with severe constipation who are being considered for colectomy.

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Chapter 6 : General Discussion

The aim of this thesis project was to provide a detailed characterization of (1) cyclic motor patterns and (2) haustral activity in healthy subjects, and adult patients; and (3) a functional sphincter at the recto-sigmoid junction, the sphincter of O'Beirne, was also analyzed in a single patient and reported as a case report. Together with the patient's histories, the analysis of autonomic functioning (heart rate variability measures), and features of colonic motility (HAPWs, SPW and anal sphincter coordination, sphincter of O'Beirne), we gained insight into the contribution of cyclic motor patterns, haustral activity, and sphincter of O'Beirne on patient pathologies when assessed on an individualized basis. High-resolution colonic manometry (HRCM) is a valuable tool in providing clues about the mechanisms underlying colonic motility through the visualization of smooth muscle-generated pressure waves. Here, we studied the frequency distributions observed of both myogenic motor patterns to understand the workings of Interstitial cells of Cajal (ICC) in the generation of these motor patterns. Furthermore, the presence of distinct frequency ranges shared by both cyclic motor patterns and haustral activity allowed for the exploration of their relationship and their shared ICC-orchestrated origin.

Literature on a detailed description and quantification of haustral activity is largely absent in academic literature[1]. Most radiological and organ bath studies conducted on the haustra of colon in the late 19th century to late-20th century[2-8] provided valuable information regarding the formation haustra. Functions of taeniae, circular and longitudinal muscle contractions, and haustral folds were elucidated. Since no detailed analysis method was available, and since any published studies were insufficient to be used in HRCM analysis, I had to design analysis protocols before any interpretation was possible. The limited characterization performed on haustral activity using manometry[9] provided the basis for initial identification. Subsequent exposure to haustral activity using HRCM resulted in a thorough development of the criteria for haustral activity identification and categorization of features. With the details on colonic activity HRCM can provide, haustral activity

characterization described in chapter 4 is the first of its type. Based on our criteria of an active haustrum, we can indirectly quantify rhythmic circular muscular contractions through the visualization of rhythmic haustral boundary pressure transient. Furthermore, segmentation and propulsive intra-haustral activity are distinctly visible. Observations of haustral activity using HRCM allows us to integrate and evaluate the functions of haustral activity proposed by scientists whose discoveries we build on today.

Prior to drawing any conclusions about normality or abnormality, it was vital to comprehend various features and nuances of the motor patterns in healthy subjects, without which direct comparison to patients would not be possible. This step was often conjointly conducted with the analysis in patients to observe differences, which in turn highlighted specific features to analyze in depth. The distinct haustral activity observed in the manometry recording of strong responders laid the basis for the development of criteria for the identification of active haustra in healthy subjects. Once key features to analyze were determined, a structured approach in the identification, classification and analysis needed to be established. Chapter 2 provides a heavily tried-and-tested guide for future reference. This guide also ensures transparency in the criteria used to identify the motor patterns; the protocol for programs used for quantification and illustration of motor patterns was provided for future use. The detailed analysis of cyclic motor pattern and haustral activity provided control values enabling the comparison to patients. Adult patients were evaluated for the presence of cyclic motor patterns and haustral activity; each subject underwent the same protocol of parameters analyzed in controls. Patient values were analyzed individually given the heterogeneity in the pathophysiology; the average collective values were not reflective of the variations in the patient samples.

While contractile activity of the smooth muscle is a result of the coordination of multiple control systems, some mechanisms contribute more predominantly than others. Consequently, the rhythmicity present in cyclic motor patterns and haustral activity lends to those being categorized as myogenic motor patterns. The initial assessment of said

motor patterns revealed a prominent presence of low- and high- frequency activity which led to the categorization of the myogenic motor patterns largely based on the high and low frequencies they presented. The frequencies of the cyclic motor pattern largely were high-frequencies in the colon with a smaller proportion of slow wave frequency activity; whereas the majority of the haustral activity shared the same low-frequency range seen in the cyclic motor pattern. The low frequency rhythmic activity in the colon has been noted in the literature pertaining to both cyclic motor patterns and haustral activity as seen in manometry [9-12], however the high-frequency distribution is rarely reported on as this frequency range (10-15cpm) that is typically attributed to the respiratory artefact. Chapter 3 delves into providing sufficient evidence for the presence of the high frequency range associated with true colonic smooth muscle contractions rather than the breathing frequency. This was possible through the simultaneous heart rate variability and cardiac impedance measures conducted during the HRCM study. Based on electrophysiology studies on the gut smooth muscles in humans, assessing frequencies of electrical activity[23-25], two ICC networks with corresponding frequencies provided evidence for- an omnipresent ICC-SMP with a frequency range of 2-6cpm residing between the submucosa and inner circular muscle layer; and a stimulus-dependent ICC-MP with a high-frequency range (11-20cpm) found between the outer circular muscle and longitudinal muscle layers. These findings provide bases for ICC-SMP and ICC-MP as the underlying mechanism for both myogenic colonic motor patterns. The fluctuating nature of the frequency gradient in the colon speaks to its prominent non-propulsive function, carried out by haustral activity and cyclic motor patterns. Both patterns demonstrate predominant simultaneous and mixed propagation suggestive of mixing and some local transit. Segmentation primarily seen and exemplified in intra-haustral activity, serves to mix and breakdown content for separation of liquid from solid states; liquids are absorbed while solid content is formed into stool over its anally-directed slow propagation.

Standing on the shoulders of giants is a consistent trend in this thesis. James O'Beirne, in 1833, proposed a functional sphincter at the recto-sigmoid junctions which functioned to control the passage of stool into the rectum; the over-expression of which can contribute to chronic constipation[13]. Over time, clinical importance of spasmodic constrictions in the rectosigmoid junctions faded, re-emerging intermittently with those offering some support[14-16] and those in opposition[17]. The case report presented in chapter 5 on a patient with refractory chronic constipation shows the same characteristic pressure band at the recto-sigmoid junction accompanied with persistent retrograde propagation immediately proximal to the high-pressure zone as described in the review by Ballantyne in 1986[13]. This finding has led to the identification and thorough characterizations of the sphincter of O'Beirne in healthy subjects currently under review[18].

Sphincter of O'Beirne is hypothesized to be the last haustral boundary in the colon as it shares a great deal of characteristics with haustral boundary activity. Both boundaries function to retard the flow of fecal matter, demonstrate a pressure localized to 1-2cm, and operate primarily in the low-frequency range. Based on the data presented in the strong responders, the over-activation of both boundaries contributed to the pathophysiology of constipation. The case report presented in chapter 5 is for the same patient that demonstrated elevated haustral boundary activity and was reported on in chapter 4. This patient elicited several adjacent active haustra immediately proximal to the sphincter of O'Beirne. Collectively, the boundaries served as a hindrance to transit.

High-resolution colonic manometry with a 1cm distance between adjacent water-perfusion ports provides information that would be otherwise inaccurately described or be missed altogether in lower-resolution colonic manometry. However, haustral activity (activity confined to a haustrum) is a motor pattern that is relatively short in length (~4.5cm). When characterizing the type of intra-haustral activity, sometimes it would be difficult to distinguish between propagating pressure waves and those participating in segmentation. The proper characterization of intra-haustral activity would benefit from

higher resolution[19] to determine whether intra-haustral activity is presenting segmentation-type pattern or propulsive, and if it is propulsive, then having greater confidence in assigning the direction of the propagation (antegrade vs. retrograde propagation). The color map representation of colonic pressure proved easier to read, however it estimates the amplitude values between the sensors to generate a continuous representation of colonic activity. This leads to the possibility of some misclassification of intra-haustral activity in certain cases. In efforts to resolve this obstacle, haustral activity was also visualized as a line plot in instances where color maps made haustral activity difficult to analyze.

Water-perfusion is a cost-effective tool in assessing colonic pressure changes and produces comparable results when compared to solid-state catheters in the assessment of HAPWs and cyclic motor patterns[20, 21]. The use of the water-perfused catheter is criticized for the influence the introduction of water has on the amplitudes of colonic motor patterns [21]. The introduction of water into the colon has also been implicated in it generating colonic response, serving as a confounding variable when assessing the response of the colon to a stimuli. To mitigate the influence of the water introduced in the colon, a rectal drainage tube was placed in all subjects for the passive outflow of water. Furthermore, the background amplitudes of the manometry recordings were assessed for the change in amplitude across the recording period which overall revealed a change of 1-2 mmHg, an insignificant change in amplitude from the baseline period to just prior to intraluminal bisacodyl administration.

Extrinsic input regulates the expression of ICC-MP-mediated cyclic motor patterns. The residual acetylcholine from the generation of HAPW can serve as a stimulus for ICC-mediated rhythmic activity resulting in the cyclic motor patterns and/haustral activity in the vicinity of HAPW. Chapter 3 demonstrates the strong correlation of the amplitudes of the cyclic motor patterns (HAPW-CMP) with the amplitudes of the corresponding HAPW suggestive of extrinsic input. HAPWs result in the local transit of content and the

presence of cyclic motor patterns following such an event is integral for the restoration of the primary colonic functions: mixing, absorption and storage functions. In chapter 4, we note that active haustra often occur immediately following a CMP; this suggests that synchronization of haustral activity results in cyclic motor patterns. While haustral activity is responsible for non-propulsive functions, content has been observed in fluoroscopic studies to move distally[26]. The synchronization of adjacent active haustra into cyclic motor patterns may serve as the mechanism for local transit of colonic content. The colonic content moves in an oral or aboral direction allowing for the re-distribution of content in different haustra; this is evidenced by the re-establishment of individual active haustra following the termination of cyclic motor patterns. The presence of haustral activity following an HAPW or HAPW-CMP, works to reinforce the restoration of colonic function as well. This stepwise decline in motor patterns (HAPW to CMP to haustral activity) may occur on account of the declining concentration of a neural stimulus (for example a declining concentration of acetylcholine). Important questions remain such as: prior to the formation of a high frequency cyclic motor pattern, do adjacent haustral activities that typically present low frequency, operate at a high frequency prior to synchronization? To understand the possibility of this stepwise transition, correlation analysis should be conducted between the frequencies and amplitudes observed in cyclic motor patterns and haustral activity in the future. In doing so, we hope to assess whether neural excitation (e.g. in the proximity of HAPWs) can influence colonic motor patterns beyond the force of smooth muscle contraction and result in the differential activation of ICC networks.

Strong, weak, and non-responders are terms developed to classify patients based on HAPW and autonomic parameters[22]. This terminology was carried over and assessed for congruencies with respect to haustral activity and cyclic motor patterns. With the current patient sample size, we were able to confirm increased haustral activity relative to controls in 2 patients; these 2 patients were identified as being strong responders with respect to HAPW parameters. As of yet, weak and non-responders did not appear to

present any significant difference in haustral activity. This group's autonomic functioning revealed an increased sympathetic tone which correlated with the lack of cyclic motor patterns parameters, and collectively, they presented less haustral activity overall when compared to healthy controls. We hope to assess the robustness of this categorization (strong, weak and non-responders) more confidently with respect to haustral activity and cyclic motor patterns. If this type of categorizations shows promise, this will allow for the clinical classification of an otherwise heterogeneous patient population. Instead of using average values in a very heterogeneous sample size, the grouping of strong, weak and non-responders will group patients based on various features of colonic motility (HAPW, anal sphincter activity, haustral activity, cyclic motor patterns), and will serve to separate patients with different etiology to provide evidence based treatment

In conclusion, this thesis demonstrates the value of HRCM in assessing myogenic motor patterns in both healthy subjects and patients. Development of identification and analysis techniques suited to the cyclic motor pattern and haustral activity was documented; the execution of these techniques allowed for the detailed characterization of cyclic motor patterns and haustral activity. Without the analysis in healthy controls, the comparison to patients, as presented in chapter 4, would not have been possible. The analysis on the relationship between haustral activity and the cyclic motor pattern reveals that synchronization of haustral activity results in the formation of cyclic motor patterns; both activities function to mix colonic content and are implicated in some propulsive activity. Furthermore, the similarities between haustral activity and a distinct sphincter at the recto-sigmoid junction is suggestive of it being the last haustral boundary in the colon. The work summarized in this thesis, has made it possible to give detailed information about important motor patterns identified and quantified in healthy subjects for the subsequent analysis in patients with colonic motility disorders.

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Appendix

1A: Calibration and prep for HRCM checklist

1. Biopsy Prep

*********This is done any number of days in advance.*

- □ Ensure the following are present in the lab for the biopsy prep:

- ○ 5 x Eppendorf Tubes- Safe-Lock Tubes 2.0mL
- ○ 5 x 1.0mL Micro-Centrifuge Tubes
- ○ 4% PFA in 0.1M PB (refrigerator)

- □ Label each set of 5 tubes with the date of the HRCM procedure, initials of volunteer/patient and with the letter A, T, D, S, R representing the samples obtained from the ascending colon, transverse colon, descending colon, sigmoid and rectum, respectively, on the top and side of the vials.
- □ Weigh and record the Eppendorf Tubes- Safe-Lock Tubes on excel file labelled "biopsies".
- □ Using a pipette (P1000), add in each 1.0mL micro-centrifuge tube 1mL 4% PFA in 0.1M PB.
- □ Place these tubes in the test tube rack labelled "Biopsy Prep" alongside the empty Eppendorf Tubes in the refrigerator until required on day of HRCM procedure.
- □ Ensure liquid nitrogen is available for the day of HRCM procedure (Verdu Lab- 3N49)

2. HRCM-MMS Prep

*********This is done 1-2 days in advance. Preparations are conducted in the Dictation Room in the Endoscopy Unit. The sterilization procedure is conducted before and after the procedure if the procedure is spaced over more than a week.*

- □ Ensure the following components are present for the HRCM prep:

- 2-3 Bottles of Pure H_2O
- H_2O_2
- 20mL syringe
- Gauze
- Tape
- Scissors
- Water filter (syringe filter)
- Catheter attached to manifold
- Flowrestrictor (purple tube)
- DTX plus transducer (blue tube connector)
- Pliers
- Electrode Sensor
- Blue pad
- Clean sheets to cover the system
- Containers (clear rectangular one to contain the water under the water perfusion system and a circular one containing the catheter)
  - □ Plug the MMS machine.
  - □ Fill the 2 pure water containers pure water (1L of pure H_2O in each container).
  - □ Add 10mL of H_2O_2 in each container (1% H_2O_2) using a syringe. Ensure the containers are properly closed to allow for adequate pressure build-up.
  - □ Ensure back-up battery is being charged.
  - □ Detach the catheter from the manifold and then attach the catheter with the corresponding 84 channels on the HRCM machine.
  - □ Ensure the balloon connector is kept dried (use gauze and tape to cover until later use) and away from the water perfusion channels (red attachment). Attach the gauze-covered balloon channel on the left side of the electrode sensor.
  - □ Place the end of the catheter in a large clear, circular container with a blue pad underneath for the water to drain into.
  - □ Place a clear container under the water perfusion system to contain any water leaks.

- □ Open the MMS (Medical Measurement System) Investigation and Diagnostic Software. Find a 'Test' file. Click on 'New Investigation', then click on 'HRCM 84 CH 1 Balloon'.
- □ Unclip the clips at the bottom of the HRCM that drain the pure water, to ensure the pure water flows out of the channels into the clear rectangle container first.
- □ Upon secure attachment of all components, click 'Start Investigation' and run the system for 10 minutes. Make sure that the pressure is 1000mBar for the system to have sufficient pressure. If this is not the case, then check the water containers are sealed tightly shut (undo the connectors on the container and reconnect attachment points as well as retightening the cap to the container).
- □ Once the flow of pure water (with H_2O_2) into the clear rectangle container has become laminar, close the clip.
- □ Ensure all the holes on the catheter corresponding to the 84 channels have consistent flow of water. This should not be an area of much concern as this is assessed when the catheter is being sterilized.
- □ Once the system has been running for sufficient time, click 'zero', raise the circular container containing the catheter high for a few seconds, and then place back on the floor.
- □ On the MMS recording, ensure that all 84 channels are responding and displaying pressure waves. If a channel or few channels appear to display no/insufficient signal, follow the prompt outlined in the file "HRCM Checklist Appendix".
- □ Check periodically to see if the containers contain adequate pure water. If water is running low, then click on button on top left of screen that prompts water refill. This will ensure that the pressure in the system is zero prior to opening the container and the recording will be paused. Quickly fill the container with water and then click 'continue' on screen to re-establish the pressure and to resume MMS recording.
- □ Continue running the system until the pure water + H_2O_2 has completed running through the HRCM system.
- □ Attach 2 water filters (syringe filters) to the left most channels in the 1st and 3rd row (Channel 1 and 49). Then check if all the channels are still working with the filter on, in particular channel 1 and 49.
- □ Refill the containers with pure H_2O only (follow the prompts to change the water) and re-run the system only filling 500mL in each container.
- □ Once system has finished running, the investigation is ended and the program is closed.

- Cover the 84-channel sensor with clean sheets, and wrap and secure with tape.

1B: HRCM protocol checklist

1. HRCM Procedure Day- Prior to Procedure Commencement

****The HRCM equipment is brought to the endoscopy procedure room.*

- □ Ensure the following components are present for the HRCM on the procedure day:

- Flowrestrictor (purple tube)
- DTX plus transducer (blue tube connector)
- H_2O_2
- 75% alcohol
- Catheter
- Electrode Sensor
- Drainage Tube
- Pure H_2O (at minimum 6 bottles)
- Syringe (60mL, 20mL, 10mL)
- 3-way stopcock with Swivel Male Luer Lock
- Bisacodyl solution
- Prucalopride
- Tape
- Clip
- Remote controller for patient
- Fish wire
- Scissors
- Gauze
- Gloves
- Timer
- Blue pad
- Spoon for meal
- Zip ties
- Diaper
- Blankets
- Sheets
- Containers (clear rectangular one to contain the water under the water perfusion system and the circular tray for the catheter)

- □ Ensure that the HRCM machine is plugged in to an electrical outlet.
- □ Ensure backup battery is charged.
- □ Make sure the clip connected to the tubing is closed.

- □ Keep the HRCM covered with the sheets for the entire duration of the endoscopic procedure to avoid contamination.
- □ Ensure the balloon connector/channel is kept covered to avoid any liquids from penetrating as this is exclusively an air channel.
- □ Check to see if the balloon on the catheter is working properly (inflating and deflating) prior to procedure.
- □ Add 2 bottles of pure H_2O in the water containers (1L per container); record amount and time of water added in notes.
- □ Wrap the length of the catheter (minus the extreme distal end) in the blue pad secured with tape.
- □ Place the catheter on a blue pad in the circular tray.
- □ Turn on the MMS software and create new patient profile with the name, D.O.B, and patient number.
- □ Click "New Investigation" under this volunteer/ patient profile and then click on 'HRCM 84 CH 1 Balloon'.
- □ Change perfusion pressure to 1000mmHg for the calibration phase.
- □ Have the bed lowered.
- □ Run the system for 10 minutes at least and check if all channels are working as outlined in '**HRCM-MMS Prep**' section.
- □ Once all channels are well-calibrated, reduce the perfusion pressure to 300mmHg until the colonoscopy procedure finishes.
- □ Click "Start Investigation" closer to commencement of colonoscopy.
- □ Add comment in the MMS recording the colonoscopy "start time" and time-match (record in the MMS comment).

2. HRCM Procedure Day- Vial Acquisition

****Approximately once the scope is in the descending colon, go to retrieve supplies for biopsy acquisition.*

- □ Ensure the following components are present for the HRCM on the procedure day:

- 5 x Eppendorf Tubes- Safe-Lock Tubes 2.0mL
- 5 x 1.0mL Micro-Centrifuge Tubes containing the 1mL 4% PFA in 0.1M PB
- Forceps
- Thermos
- Liquid Nitrogen
- Gloves

- □ Wear gloves to pour the liquid nitrogen in the thermos (Verdu Lab- 3N49). Pour halfway and do not tighten the lid on the thermos to prevent pressure build-up.
- □ Bring the aforementioned materials to the endoscopy procedure room.

---

3. HRCM Procedure Day- Biopsy Acquisition and Storage

****This occurs during the colonoscopy, upon attachment of catheter in the colon (using clip).*

- □ Obtain a red pin to transfer the biopsy samples
- □ Use the red pin with gloves on to place each of the samples into the corresponding Eppendorf Tubes- Safe-Lock Tubes 2.0mL and 1.0mL Micro-Centrifuge Tubes (2 samples from each region obtained).
- □ Place the samples in the PFA solution in the test tube rack.
- □ Hold the Safe-Lock Tubes containing the sample using forceps and first suspended in the liquid nitrogen until the sample turns white in appearance and then the entire vial is dropped into the liquid nitrogen thermos.
- □ Discard the red pins into the biohazard container after use.
- □ Go to the Immunity Lab (3N 11) to store the 5 frozen biopsy samples in the Ratcliff freezer in the box labeled "Colonoscopy Biopsies Huizinga lab". The key to the lab in found in the 1st drawer in the Huizinga lab.
- □ Go to Huizinga lab to discard the liquid nitrogen by placing it in a beaker to evaporate.
- □ Place the TFA tubes containing the 5 biopsy samples in the refrigerator in the test tube rack containing other biopsy samples.
- □ Remind lab technician to change the PFA within 1 week of biopsy acquisition.

---

4. HRCM Procedure Day- Bisacodyl Prep

****This is conducted in the Huizinga Lab.*

- □ Ensure the following components are present for Bisacodyl prep conducted on the procedure day:

- ○ 2 x 5mg tablets Apo-Bisacodyl
- ○ Mortar and pestle
- ○ Distilled water
- ○ Small beaker
- ○ Plastic pipette
- ○ Funnel
- ○ 15mL centrifuge tube

- Electronic timer clock

  - Take 2 tablets of 5mg APO-BISACODYL and grind using a mortal and pestle for 5 minutes using an electronic timer clock.
  - Obtain some distilled H_2O in a beaker, and using a plastic disposable pipette, add some distilled H_2O to the grinded bisacodyl in the mortar.
  - Wash the remaining residues of bisacodyl from the pestle.
  - Use a funnel to transfer the solution into a 15mL centrifuge tube.
  - Add additional distilled H_2O until the volume is 15mL.

5. HRCM Procedure Day- During the HRCM Recording

****The HRCM equipment is brought to the endoscopy procedure room.*

- Immediately following the end of the colonoscopy, increase the perfusion pressure back to 1000mmHg.
- Lower the bed.
- Add comment on MMS recording to mark the end of the colonoscopy and time match.
- Following the end of the colonoscopy and prior to baseline period commencement, add comments in the MMS recording for the following information:
  - Location of catheter with respect to the location of the clip.
  - Location of catheter with respect to the anorectal position.
  - Location of the balloon.
  - Medication and quantity used during the colonoscopy (e.g. type and quantity of sedation).
  - Vitals (blood pressure and heart rate).
  - Patient condition (awake or sleeping).
- Immediately prior to baseline period starting (after the drainage tube is placed), add comment in MMS recording pertaining to catheter location with respect to the anorectal position
- Provide the volunteer/patient with the remote control providing instructions on using it (e.g. pressing button to signify pain, discharge, urge to defecate, etc)
- Where appropriate (i.e. volunteer/patient breathing in steady, relaxed fashion), ask volunteer to hold their breath. Add comment on MMS recording when this was done.

- □ Where appropriate (i.e. volunteer/patient breathing in steady, relaxed fashion or volunteer/patient asleep), use electronic timer to measure their breathing over the course of one minute. Add comment on MMS recording when this was done.
- □ Throughout the experiment, ensure to keep the containers filled with an approximate refill time of 1 hr. To fill containers, click on button on top left of screen that prompts water refill. Resume the recording as soon as possible.
- □ Record amount of pure H_2O used as well the time.
- □ Time match approximately every 30 minutes, **and** before and after water containers refilled.

Insert comments on the MMS recording and pay attention to note the following:

- Gas
- Body movement
- Bowel sound
- Talking
- Meal begins
- Meal ends
- Medication (specify type)
- Drinking
- Balloon distention period as well as the volume of air injected
- Deflation of balloons
- Urination
- Pain and/or cramping
  - Pain level out of 10
- Discomfort
- Urge to defecate
- High Amplitude Pressure Wave (HAPW)
- Liquid out

- □ Following bisacodyl administration, ask volunteer/patient to squeeze anus once and then twice.
- □ Following bisacodyl period, Valsalva maneuver is conducted 5 times.
- □ Before the X-Ray is taken, fill some air in the balloon.
- □ Add comment on when X-ray was taken
- □ Mark end time of the procedure upon catheter extubation.
- □ Click on "Stop Investigation" twice to properly save the recording.

1C: Post-HRCM protocol checklist

1. HRCM Procedure Day- Clean-up Post HRCM Recording

****The cleaning facility part of the endoscopy procedure room.*

HRCM Equipment and Room Clean-up:

Ensure the following components are present for bisacodyl prep conducted on the procedure day:

- H_2O_2
- Syringe (10mL)
- Pure H_2O
- Catheter manifold
- Clean green and waterproof blue sheets
- Clear rectangular container for MDRD department

- □ On MMS, open a "Test" file, click "New Investigation".
- □ Follow appropriate prompts to release the perfusion pressure in the water containers.
- □ Add H_2O_2 into the container (10% concentration relative to remaining pure H_2O levels).
- □ Start the investigation and run the system with the pure H_2O with H_2O_2 for at least 10 minutes.
- □ Run the system with pure H_2O (~400mL in each container) and open the clips at the bottom at the end.
- □ Remove the blue filters and dispose.
- □ Run the system with air for at least 20 minutes.
- □ Remove bedding and pillow case. Place these in the laundry bins.
- □ Wipe all surfaces (HRCM system, bed, pillow, counters) in the endoscopy procedure room with disinfectant wipes.
- □ Dry the electrode sensor region.
- □ Once the HRCM system has completed running, close the investigation. Shut down the HRCM system.
- □ Retrieve the catheter manifold and detach catheter from the electrode sensor.
- □ Re-attach manifold to each of the 84 channels on the catheter.
- □ Place catheter end into a pure H_2O bottle (half filled)
- □ Remove the gauze and tape from the balloon channel.

- □ Remove the T-clip on balloon channel. Place the T-clip in the HRCM machine drawer.
- □ Return HRCM system to Endo-Unit Dictation Room covered and secured with clean sheets.

2. Catheter Cleaning Procedure

- □ Once the catheter extubation has occurred, follow the cleaning instructions as outlined by **Silicone Colonic HRM Water Perfused Catheter Cleaning Instructions Prior to Autoclave Sterilization** (1^{st} wash enzyme, 2^{nd} wash pure H_2O, 3^{rd} wash pure H_2O, 4^{th} air).
- □ Dry the catheter with towel.
- □ Place the sterilized catheter in the clear container and wrap with green sheet which is placed in a waterproof blue sheet (as supplied by the MDRD).
- □ Fill and attach the appropriate form into the plastic bag attached to the container.
- □ Go to the MDRD department (yellow elevators for equipment and patients, 1^{st} floor) to drop off catheter while informing the attendant that it has been cleaned.
- □ After 1-3 days, pick up catheter from MDRD department and leave it in the Dictation Room in the Endoscopy Unit.

2: Haustral activity data collection guide

Use the excel file HA_Events which contain the pertinent data collection template.

2A: Tab 1 - Study_Session_Information

1. Acquire the comments files from the MMS machine for subjects and note down the times of the intervention periods for baseline, PBD, DBD/RBD, Meal and bisacodyl.

2. All units for time will **always** be in mins. There are quick excel ways to convert time in hh:mm:ss to just mins.

3. Ensure you add info about any missing interventions under **notes_intervention** row.

4. Often PBD and DBD/RBD are repeated thus I have added **PBD (1)** and **PBD (2)**, likewise for **DBD/RBD**.

5. For bisacodyl, I understand that the dosages and routes of administration vary, so state **yes** or **no** (all in lowercase) for the **rectal administration** and **proximal administration**; input the final dosage in the **total dosage**.

6. Provide the total duration of the study by summing the durations of the stated interventions.

7. The last few rows try to get an idea of the catheter location; add any available information obtained from the comments file and the HRCM folder for the subject.

8. The last row, **catheter movement**, is to record any drastic shifts in catheter and if so, specify approximately the time and number of sensors. This can be filled out at the end of the **HA_Selections**.

9. **Catheter type**: A has 1 cm inter-sensor distance and 2 balloons; B has 1cm inter-sensor distance and 1 balloon; C is the 41 sensor catheter with 1cm inter-sensor distance and no balloons; and D is the 1 (starting at sensor 48) to 1.5cm (sensor 1-48) with 1 balloon. Catheter A, B, and D contain water-perfusion ports corresponding to 84 sensors.

2B: Tab 2 – Image_Pixel_Size

1. Specify the final Overlay pixel size. Keep the width pixel size the same as the number of seconds contained in the image file (it specifies a size in seconds). When

you adjust the size, un-tick the maintain aspect ratio, and for the length, use 400 pixels.

2. When you save the image with your ROIs in a .tiff format and measurement output from ImageJ is recorded, be sure to have a consistent naming format and record that in the same tab.

2C: Tab 3 – HA_Selection

1. When all the ROIs for a subject are selected, select and paste the ImageJ measurement output into a spare tab in the excel file, and then sort the BX values in an ascending order ensuring the ROIs are all in chronological order. Copy the entire re-organized output and then paste it into the HA_Selection tab.

2. Time Start is the BX value converted into hh:mm:ss format.

3. Duration (mins) is the difference between Time Start and Time End converted into min (2 decimal places) format.

4. State the intervention next. They will always be all Caps for BASELINE, PBD, DBD/RBD, MEAL, and BISACODYL. Make sure to always use this notation (e.g. even if it is only RBD, still use DBD/RBD (no spaces)).

5. For any intervention repeated, place a consistent symbol of your choice in the Repeated intervention column so we know to treat them separately.

6. Sensor Start is BY, Sensor End is BY + Length, and Sensor length is the difference of Sensor Start and Sensor End; these values are rounded to whole numbers.

7. Length is calculated based on catheter specifications and utilize the sensor information.

8. Category: (1) corresponds to isolated haustral boundary activity, and (2) is intra-haustral activity with or without haustral boundary activity.

9. Rhythmic will always be answered with YES or NO; blank values are only reserved for ROIs later omitted and for the spaces between successive patients.

10. For Frequency try to provide specific frequency values, but if there are multiple frequencies (e.g. in a range of 4-6cpm), then be sure to keep them in the same frequency range (e.g. 4-6cpm is fine because it is in the low-frequency range, but something like 5-8cpm is 2 frequency ranges (note: Low-frequency range is 2-6cpm and high-frequency range is 7cpm and higher. If there is a scenario where there are truly both low- and high- frequencies, color that ROI a specific color, copy the ROI and paste below so that the frequencies can be separated.

11. Frequency HiLo is where you would categorize the frequency as HIGH or LOW (use this notation).

12. Erratic/Tonic will be always answered with YES or NO.

13. Propulsive and Segmentation can be YES or NO only exclusively (i.e. if propulsive is YES, then segmentation is NO; unless it a haustral boundary activity thus both fields would be NO). Propulsive+Segmentation is for instances where both are present in a single instance of haustral activity.

14. For Behavior use the GT, BA, TM and Comb (the last one for any combination of the standard 3).

15. Check your data at the end for any categories that have unnecessary blanks for where you could have YES or NO.

16. Color any interesting/ exemplary ROIs a specific color for later viewing.

3: Data collection for cyclic motor pattern (sample excel file format)

A

A	B	C	D	E	F	G	H	I	J	K	L
	Data from MMS										
Volunteer / Patient	Time start	Time end	Duration (min)	Intervention	Sensor Start	Sensor End	Location (Proximal)	Location (Distal)	Motor Pattern Association	Frequency estimate (manual)	

B

N	O	P	Q	R	S	T	U	V	W	X	Y	Z
ImageJ Output (Macros)				Calculations per Event from ImageJ Macro Output								
Event (ROI)	Interval	Length	Mean Amplitude	Maximum Amplitude	Average Amplitude	±	Standard Deviation (Amplitude)	Average Inverse Interval	±	Standard Deviation (Interval)	Average Length of Pressure Wave (PW)	Min Length of PW	Max Length of PW

C

AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ	AK	AL	AM	AN	AO	AP	AQ	AR
Propagation Velocity ImageJ Output & Calculations											MatLab Line plot & Calculation						
Antegrade	Retrograde	ROI	BX	BY	Width (s)	Height (cm)	Angle	Length (cm)	Balloon?	Velocity (cm/s)	# of Simultaneous (S) PW	# of Antegrade (A) PW	# of Retrograde (R) PW	Total # of PW	% S PW	% A PW	% R PW

Data collection table for cyclic motor patterns. Screenshot of the data collection table. A-C are sequential in that they appear in a single row. The details of these features are found in chapter 3 results.

4: Data collection for haustral activity (sample excel file format)

A

Image_J_Output											
Subject	ROI#	Area	Mean (mmHg)	Min (mmHg)	Max (mmHg)	BX	BY	Width (s)	Height (cm)	IntDen	RawIntDen

B

Time					Location						
Time Start	Time End	Duration (Mins)	Intervention	Repeated intervention	Sensor Start	Sensor End	Sensor distance	Length (cm)	General Location	Specific Location	Proximal vs Distal haustral #

C

	Rhythmic behavior		
Category #	Rhythmic	Frequency	Frequency HiLo

D

Haustral activity						
Erratic/ Tonic	Propulsive	Majority Antegrade	Majority Retrograde	Mixed/S	Segmentation	Propulsive+ Segmentation

E

Haustral activity and Other Motor Patterns				
Isolated (>2min from MP)	HAPW-Associated	HAPW-SPW Associated	SPW Associated	Behavior

Data collection table for haustral activity. Screenshot of the data collection table. A-E are sequential in that they appear in a single row. The details of these features are found in chapter 4 results.