ASSESSMENT OF HEALTHY COLONIC MOTILITY PATTERNS, COLONIC DYSMOTILITY, AND ITS ASSOCIATION WITH AUTONOMIC NERVOUS SYSTEM DYSFUNCTION

By

Natalija Milkova, BSc

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TITLE: **ASSESSMENT OF HEALTHY COLONIC MOTILITY PATTERNS, COLONIC DYSMOTILITY, AND ITS ASSOCIATION WITH AUTONOMIC NERVOUS SYSTEM DYSFUNCTION**

SUBMITTED BY: Natalija Milkova

STUDENT NUMBER: 001420094

SUPERVISOR: Dr. Jan D. Huizinga

SUPERVISORY COMMITTEE: Dr. Ji-Hong Chen

Dr. Elyanne Ratcliffe

EXTERNAL EXAMINER: Dr. Mehran Anvari

Abbreviations

HRCM: high-resolution colonic manometry

HAPW: high-amplitude propagating pressure wave

SPW: simultaneous pressure wave

HAPW-SPW: a proximal HAPW followed by SPW

RAIR: recto-anal inhibitory reflex

ANS: autonomic nervous system

HRV: heart rate variability

RSA: respiratory sinus arrhythmia

SI: Baevsky's stress index

ENS: enteric nervous system

CNS: central nervous system

NTS: nucleus tractus solitarius (spinosolitary tract)

ICC: interstitial cells of Cajal

LLLT: low-level laser therapy

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Chapter 1: General Introduction

The colorectal area has the complex task of mixing content within the colon, facilitating absorption, and propagating the waste material in an overall anal direction for waste expulsion. Successful completion of these tasks results in defecation, and this process requires complex coordination between multiple systems which influence colonic motility. With the complexity of this process, it is not surprising that colonic motility and the mechanism of defecation remain incompletely understood, although an increasing number of studies continue to give clues with regards to the control of defecation. Colonic motility control can generally be divided into 3 different systems: the muscular apparatus featuring the pacemaker cells- Interstitial Cells of Cajal (ICC), the enteric nervous system also known as intrinsic innervation, and the autonomic nervous system which is the extrinsic innervation [1].

While the ICC cells are important for slow-wave activity in the colon, they are not responsible for high-amplitude propagating activity due to the absence of a frequency gradient in this location of the gastrointestinal tract [2]. This is why propulsive activity within the colon is deemed neurogenic and largely relies on the enteric nervous system (ENS) [1,3]. In fact, absence of innervation by the ENS leads to absolute loss of propulsive activity, and it is something that is observed in patients with Hirschsprung's disease [3]. However, although the ENS can largely function independently and is often referred to as the 'second brain', it also receives essential influence from the autonomic nervous system (ANS) branch of the central nervous system (CNS) which modulates the ENS through sympathetic and parasympathetic inputs [1,3,4].

Sympathetic innervation of the colon comes from the thoraco-lumbar area of the spinal cord, ending at L2 level [4]. The sympathetic nervous system works to inhibit colonic activity and contract the anal sphincter, and likely contributes to the resting anal sphincter tone [1,4].

Parasympathetic innervation of the colon comes from two different areas: cranial which is associated with the vagus nerve, and sacral which comes from the sacral defecation center at S2-S4 levels of the spinal cord [1,4]. The cranial portion of parasympathetic

innervation only reaches the more proximal parts of the colon up to the splenic flexure, while the sacral defecation center is responsible for the distal colon and anorectal region [1,4]. The parasympathetic branch of the ANS works to stimulate activity in the colon, and relax the anal sphincter such that waste expulsion can occur [1,4]. Mechanical distention of the colon can stimulate extrinsic sensory neurons which activate parasympathetic motor neurons that stimulate contractile activity in the colon [1].

The most prominent characterized contractile event in the colon is the High-Amplitude Propagating Pressure Wave (HAPW) [1,5–7]. HAPWs can propel colonic content over long distances in the aboral direction and can lead to rectal filling. Filling and distention of the rectum stimulates activity of the parasympathetic sensory innervation which sends feedback to the sacral defecation center, stimulating HAPWs in the distal parts of the colon leading to more rectal filling and distention [1]. At the same time, this information is also relayed up the spinal cord, projecting to areas such as Barrington's nucleus [1,7,8]. Barrington's nucleus then sends information through the vagus nerve to more proximal areas of the colon as well, triggering HAPWs in the ascending and transverse colon as well, thus pushing more waste in the anal direction [8].

The importance of HAPWs in defecation has been implicated in many studies, which show an increase in their amplitude and frequency in the pre-defecatory and defecatory phase [5,6,9–11]. Their importance in the process of defecation, and the ease with which they are distinguished have made HAPWs the main motor patterns of interest in measurements of colonic motility both with low and high-resolution colonic manometry for the purpose of diagnosing constipation [12–14]. However, although its importance is highly recognized, this motor pattern is not well characterized in the literature, and there is no consensus on the HAPW parameters which indicate normal motility. Some classify HAPWs to be any propagating motor pattern with an amplitude greater than 75 mmHg, which propagates in the anal direction for more than 15 cm [11,15,16]. Other studies consider HAPWs to be motor patterns with an amplitude of more than 100 mmHg at two and more than 80 mmHg at a third sensor [17]. Furthermore, even studies which have similar cut off values for what defines an HAPW have different methods of measurement of these values. Some studies take the average pressure of the entire

motor pattern as its amplitude [11], others take the maximum amplitude [15], while some studies divide the colon into segments and define the pressure of the HAPW within each segment of the colon [17]. Consensus is also lacking in definition of what an HAPW is in health. Pediatric consensus reports state that a normal HAPW should begin in the proximal colon, propagate all the way to the rectum, and if it is terminated earlier, that is considered an indication of abnormal motility [18]. In contrast, adult literature suggests that HAPWs originating in the proximal areas of the colon rarely propagate to the rectum [5] and instead in the pre-defecatory phase they start around the transverse or descending colon, with a proximally shifting origin and an increase in amplitude and frequency as defecation approaches [1,11]. The hypothesis behind this spatiotemporal organization of the HAPWs is that smaller motor patterns act in coordination with one another to move content from the proximal colon to the rectum as opposed to one large HAPW performing this task [1,11]. In addition to the lack of consensus about the motor pattern itself, little to no emphasis has been placed in the assessment of the extrinsic control of colonic motility by the ANS, when assessing constipation pathophysiology in patients.

Constipation presents itself through infrequent bowel movements, unsatisfactory and incomplete evacuation of stool, straining, and need for manual disimpaction among other symptoms, which are present for more than 3 months [19]. Between 3% and 27% of the population are diagnosed with constipation, however, with lack of consensus on normal motility and assessment methods its pathophysiology is largely unknown [20]. Patients with constipation are often treated only based on clinical history, and when physiologic testing is performed, tests have significant limitations such as focusing on the anorectal region of the colon only [19]. This leads to unsatisfactory treatment of constipation which can also lead to unnecessary invasive interventions such as surgery.

Therefore, the overall objective of my work is to improve understanding of High-Resolution Colonic Manometry (HRCM) features in health and constipation, understand how autonomic dysfunction is related to HRCM observations in patients, and evaluate the effect of sacral neuromodulation on colonic function, with the final goal of improving understanding of constipation pathophysiology and assessing non-invasive methods of

treatment as well as indications for their use as an alternative to invasive surgeries. Each study, shown in the following chapters, starts with an introduction specific for that study.

My studies had 4 overall aims:

1. (Chapter 3) Characterize High-Amplitude Propagating Pressure Waves (HAPWs) which can be used to identify normal motility, optimize their method of initiation during HRCM, and develop a quantifiable assessment method

HAPWs which are the strongest contractions in the colon are used to propagate content in the anal direction, and they precede defecation. As such, their presence has been used as the gold standard by pediatric gastroenterologists to diagnose motility-related causes of constipation in children using HRCM. However, they are rare motor patterns that occur rarely without stimulation in a 4-6-hour timespan which would be used for HRCM assessment. Additionally, despite its importance, this motor pattern has not been analysed in such depth to create a consensus or guidelines which can be used to diagnose issues with motility both in adult and pediatric patients with constipation. My specific objective was to categorize all HAPWs with regards to their location in the colon, devise a qualitative assessment method for the motor pattern, and optimize a stimulation method which would give the highest likelihood observing the rare motor pattern using healthy volunteers. Three categories of HAPWs were identified:

1. Proximally originating
2. Proximal continuing
3. Transverse/descending colon originating

50 mmHg was found the be the cut-off amplitude for separating HAPWs from low-amplitude propagating contractions. Additionally, the HAPW index was developed to provide a method of quantitative analysis and symbol maps were developed to show the variability in response to each intervention between individuals. Distention of a balloon in the proximal colon, and instillation of bisacodyl in the rectum were found to be most reliable in inducing HAPWs in subjects.

2. (Chapter 4) Characterize motility in patients undergoing HRCM, and use knowledge obtained from healthy volunteers as well as correlation with autonomic nervous system assessment to elucidate possible constipation pathophysiologies

The specific objective of this study was to apply the newly acquired knowledge of normal HAPWs, to HRCM analysis of patients with constipation and identify HAPW features which could serve as biomarkers for diagnosis of abnormal motility leading to constipation. From the symbol maps in the previous study of healthy volunteers, we observed variability in responses between individuals. Additionally, unlike healthy volunteers, patients undergoing HRCM study have a wide range of relevant medical histories. To take this into account, I have been analyzing patient motility data on a case-by-case basis. I have also compiled the data of all the patients to identify any features present in all patients which stand out from healthy controls.

Heart rate variability (HRV) data has also been obtained for both patients and healthy volunteers undergoing HRCM to assess ANS function. In this study, HRCM and HRV data will be compiled together to compare constipation patients to healthy volunteers. The purpose of this study is to evaluate whether there are any features in patient motility which give indication to constipation pathophysiology, and whether there are features in ANS function which relate to potential impairments in motility observed in the patients.

3. (Chapter 5) Demonstrate the value of comprehensive assessment of patients by integrating motility as assessed by HRCM, patient history, and the autonomic nervous system as assessed by HRV

The objective of this study was to show the utility of HRCM in diagnosis of motility dysfunction in patients. Patients such as the one in the study are often diagnosed with inert colon and are slated for surgery, however, in this case report we show that through the use of HRCM we can identify specific targets for treatment and therefore avoid unnecessary surgery. The study also identifies the sphincter of O'Beirne as well as distal colon dyssynergia as potential contributors to constipation pathophysiology, and factors which would not be corrected through surgery.

4. (Chapter 6) Assess the effect of one-time Low-Level Light Therapy (LLLT) on colonic motility and autonomic nervous system

My primary purpose of this study was to assess whether stimulation of the defecation center in the sacral area of the spine with low level red and infrared lights leads to any increase in colonic motility. Effects of LLLT on colonic motility are evaluated by performing LLLT during sessions of HRCM

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Chapter 2: Materials and Methods

Note: most methodologies were not established prior to the studies conducted. I contributed to optimization of the calibration and maintenance methods for the HRCM machine, to prolong the lifetime of machine parts and avoid malfunction of sensors with progression of an individual study. I also contributed to optimization of the HRCM protocol through modification of the interventions administered as well as their order and timing, to make the process more efficient and fit the study within a given timeline. In collaboration with other members of the lab, I helped to optimize a more standardized way of measuring HAPW amplitude using a plug-in in ImageJ software, and devised a way to calculate an HAPW Index. I was also involved in the development of the Low-Level Laser Therapy (LLLT) protocol with regards to finding the most optimal placements of the device and finding the most effective timing and protocol.

2.1 Calibration of the High-resolution colonic manometry (HRCM) machine

Calibration should begin 1 to 2 days before the scheduled manometry. Calibration begins with attachment of the catheter to the transducers. If calibration is started earlier than the day before manometry (recommended), then one of the spare catheters should be used each time until the day before manometry. The spare catheter can stay attached at all times until the day before manometry, which is when the catheter being used can be attached. When attaching the catheter being used, a separate disposable medication delivery catheter should be threaded through, and if the loops are far apart catheter should also be secured with parafilm. Once the catheter is attached water tanks should be filled with sterilized water and 10% hydrogen peroxide. For this step only 500 ml of water and 5 ml hydrogen peroxide are needed. Containers should be closed tightly and seals should be wetted with sterilized water prior to closing to prevent air leakage and pressure drop. A test investigation can be started, and it should run for 15 minutes. After 15 minutes, the two valves at the bottom of the transducers should be opened to let air bubbles out until laminar flow is achieved, and water tanks are almost empty. Tanks should then be refilled with sterilized water only. Catheter should be ‘zeroed’ then lifted to check for non-functional sensors. When fixing sensors, cap from catheter should be taken off and the blue transducer should be tapped gently to ensure

that an air bubble is not causing the issue. If this does not work, the purple transducer can be taken off and checked to see if water comes out of it. If there is no water from purple transducer it should be replaced. If purple transducer is functional, this means problem is in the blue transducer and it should be replaced. Once all sensors are functioning, if it is the day before manometry air filters should be placed on channels 1 and 49 and some time should be given for water to start running through filters before channels start working again. If calibration is done multiple days before HRCM, 3% hydrogen peroxide should be added to water, such that the hydrogen peroxide makes up 10% of the solution. If it is the day before, regular sterilized water can be kept in the system.

2.2 High-resolution colonic manometry (HRCM)

HRCM is performed on a custom-made platform (Medical Measurement Systems (MMS); Laborie, Toronto, Canada). In the past, one of two 84-sensor water-perfused catheters were used (diameter: 8.0 mm; Mui Scientific, Mississauga, Canada) that included a balloon either between sensors 10 and 11, or both sensors 10 and 11 as well as sensors 40 and 41. Both balloons were 10 cm long and no sensors were placed within the 10 cm sections, hence all recordings have a gap of 10 cm that is indicated in the figures by a white line. Currently, a catheter which has a balloon between sensors 7 and 8 is being used. A separate rectal balloon is also inserted following colonoscopy, and it is removed after rectal balloon distention.

The catheter is inserted with minimal sedation (Fentanyl IV 50-100 mcg and midazolam IV 2-5 mg) with the assistance of a colonoscope after a bowel cleaning procedure using an inert osmotic laxative (PEG-Lyte, Pendopharm, Quebec, Canada) but no stimulant laxatives such as bisacodyl. 3 L of PEG (70 g/L) are taken between 4 and 6 p.m. the day before the procedure, with more water consumed if needed to have all solids removed. The next morning, 1 L is taken at 4 a.m. The tip of the catheter is clipped to the mucosa via a fish line tied to the tip of the catheter, a few centimeters distal to the caecum. The anal sphincter is recorded across 2-4 sensors because the sensors are 1 cm apart from each other and the sphincter is about 2.5-4 cm in length. Although catheter displacement is rare, movement of the catheter could be detected since even if

one sensor moved away from the high-pressure zone the other sensor(s) would remain and visibly move up or down allowing us to detect shifting of the catheter as opposed to a false anal sphincter pressure change. The catheter is made of 100% silicone; after use, a hospital approved cleaning procedure is executed, including sterilization with an autoclave. A disposable dual lumen stomach tube (3.3 mm x 91 cm; Salem Sump^{TM}, Covidien Ilc, USA) is placed in the rectum for passive liquid drainage for the duration of the study. All subjects are in the supine position during the entire recording with the exception of the intake of meal when they were seated up at a 45-degree angle. The subjects are instructed to report all events such as gas or liquid expulsion, cramping, and nausea. The subjects are asked to refrain from preventing or promoting gas or liquid expulsion, by increasing abdominal pressure or contracting the external anal sphincter should an urge arise, and to instead let their colonic motor activity proceed uninterruptedly and spontaneously. All body movements such as changing body position, talking, coughing, laughing, and urination should be noted immediately into the data acquisition files in order to remove pressure artifacts.

2.3 Protocol

A 90-minute recording of baseline activity is started 30 minutes after the colonoscope is withdrawn. The response to a 5 min balloon distension at the proximal colon and/or the rectum is then investigated. The balloon is initially inflated until first sensation is reported. This is followed by incremental increases in balloon volume by 60 mL until the maximum tolerated volume is achieved which is usually between 250-400 ml air. In each of these periods, the volume is sustained for a short period (between 2-3 min). The extent of the balloon inflation is determined by the subject's level of discomfort in response to the distension. Inflation is stopped when the discomfort reaches 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 is deflated. Analysis of the response to balloon distention is performed on the 5 min period of sustained distention as well as a 15 min period after deflation. Next, a meal is given to induce the gastrocolic reflex. Meal can be anything which reaches 1000 kcal. Its effect is observed for 90 minutes. Next, 4 mg of prucalopride is administered through medication catheter for proximal or drainage tube

for rectal instillation in 0.5 mg doses and its effect are observed for 30 minutes. Following prucalopride, a 30 mg bisacodyl suspension in 5 mg doses (Dulcolax; Boehringer Ingelheim, Sanofi Canada, Quebec) is injected in the rectum via a syringe or in the proximal colon via medication catheter and its effect studied for 30 min. The bisacodyl suspension is made in saline by crushing 6 tablets, 5 mg each, with a pestle and mortar for 5 minutes. At the end of the study, an X-ray is taken using a portable X-ray machine. The current catheter has radiopaque markers which helps to visualize its placement using X-ray.

2.4 Analysis

The manometric recording is first inspected visually on the measurement system itself, to identify all motor patterns and artefacts. Artefacts due to cough, position change or straining should be removed from analysis. An HAPW is identified as a motor pattern that propagates slower than 2.5 cm/s, has an average pressure of more than 50 mmHg and is not part of a cyclic motor pattern. An SPW was identified as a pressure transient which occurs simultaneously at all sensors [1]. The scan is then transferred to ImageJ for quantitative analysis. To analyze all the motor patterns, an Event Series plug-in is used in ImageJ, which converts the data from the manometry scan into a spatiotemporal plot and allows us to use the tools provided by ImageJ to measure various parameters. When first opening up the scan in the Even Series plug-in it is important to use that Save As feature from the plug-in and not ImageJ, so that the saved image can later on be manipulated.

<u>Amplitude</u>

To measure HAPW amplitude, the freehand tool is used to outline the general area around the pressure wave. A 20-mmHg isobaric contour line can then be set using a Contourer plug-in, which automatically selects the area of the HAPW and calculates the mean amplitude of all the points within the outline, as well as the maximum value. To do this, once the area around the HAPW is selected, click bind on the Contourer plug-in, then back on the image, and set the minimum pressure to 20 mmHg.

Velocity

To measure the velocity, the line tool is used to draw a line from the beginning of the pressure wave to the most distal end. From the line tool we obtain the length and width of the contraction, then calculate the velocity using length/width. Pressure waves are categorized according to points of origin and cessation in the colon, as well as the intervention during which they occurred. The exact positioning of the catheter within the colon is determined based on an X-ray taken at the end of the study to identify the ascending, transverse, descending, and rectum. If the HAPWs begin proximally to the balloon, which with the current catheter would be up to 10.5 cm from the top of the recording, 10 should be added to length when calculating the velocity to account for the gap from the balloon.

Anal sphincter relaxation

HAPWs are paired with their associated percentage of anal sphincter relaxation which is measured using ImageJ. Its rectangular selection tool is used to obtain the mean amplitude of the relaxation as well as the anal sphincter amplitude 3 minutes before the relaxation occurred (reference amplitude). To measure the mean amplitude of the relaxation pressure, the relaxation area is selected and then the plot profile option in ImageJ is used to narrow the selection to only encompass the lowest area of pressure associated with the HAPW. This area is taken as the area of relaxation. The box profile is used to obtain pressure values for each second of the selected area. If the mean amplitude of the 'relaxation' is less than the mean amplitude of the area 3 minutes before then it is considered a true relaxation, and the minimum value from the box profile macro is taken as the relaxation pressure. If the mean pressure of the selected area is higher than the 3 min. before area, then there is a contraction and the maximum value is taken as the relaxation pressure. To measure the reference pressure, the area 3 minutes before the HAPW is selected and it's mean value is used as the reference pressure. If HAPWs occur at a higher frequency, or if there is another motor pattern occurring close before the HAPW, the resting pressure that is available between the 2 consecutive relaxations is taken as the reference. The % relaxation was calculated using the formula $100 - [\left(\frac{Relaxation\ amplitude}{Reference\ amplitude}\right) * 100]$.

2.5 Generation of symbol maps

To generate symbol maps, one first needs to know the range of time which encompasses a single intervention. The time of each motor pattern can then be labelled as minutes from the beginning of the intervention (ex: if bisacodyl was administered at 4:20:00 and an HAPW is observed at 4:25:00 then the time for that HAPW is 5 minutes). To create the actual maps an empty scatter plot should be created with a visible grid, where the x-axis represents time and the y-axis represents one subject. Shapes representing each motor pattern can then be individually placed on the symbol map, according to the previously determined time, as well as the subject. The size of the HAPW shapes should also be adjusted to account for the duration of the HAPW. When finished, include a legend indicating what each symbol represents.

2.6 HAPW index calculation

In esophageal high-resolution manometry, topographical maps are used to calculate an index of motility called the distal contractile integral (DCI) [2–4]. The DCI assesses the vigor with which a contraction occurs, and it is measured by multiplication of the amplitude, length and duration of the pressure wave (mmHg.cm.s) [5–7]. It is used in combination with other factors to ascertain whether a patient is suffering from a certain deglutitive disorder [5]. The study in chapter 1 set out to determine a similar index to be used for the assessment of the HAPWs-the HAPW Index. It was found that HAPWs of highest likelihood to be propulsive are those of high amplitude and a longer duration [8]. The HAPW index is the product of the amplitude, length, and duration of the HAPW. To calculate it, parameters which have already been obtained from previous measurements can be used. Amplitude can be taken from the above-explained measurement method. Length and duration can be taken from the measurements of velocity using the line tool. The duration in the line tool is represented by width. It is important to remember to add 10 cm to the length when necessary to account for the balloon. After multiplying the amplitude, length and duration, a value with the units mmHg.cm.s is obtained. Divide that value by 100 to obtain a value with the units mmHg.m.s.

2.7 Heart Rate Variability (HRV) electrode placement and assessment protocol

Placement of the electrodes for heart rate variability should be performed based on diagram in figure 1, which demonstrates placement of electrodes for ECG. The protocol for heart rate variability assessment is as follows:

Supine: 6 minutes

Sitting: 6 minutes

Standing: 6 minutes

Walking at their own regular pace: 6 minutes

Sitting: 6 minutes

Recovery in supine position: 6 minutes

IMPORTANT: when placing the electrodes it is important that the wire is looped at the point of attachment to the body and the loop is secured with 3M Tegaderm™ film to reduce artifacts. It is also important that the electrodes are immobilized while the subject is walking, as movement of the wires can cause artifacts. This can be done by looping the wires and asking the subject to hold then up in their hands such that they are not being moved with movement of the legs.

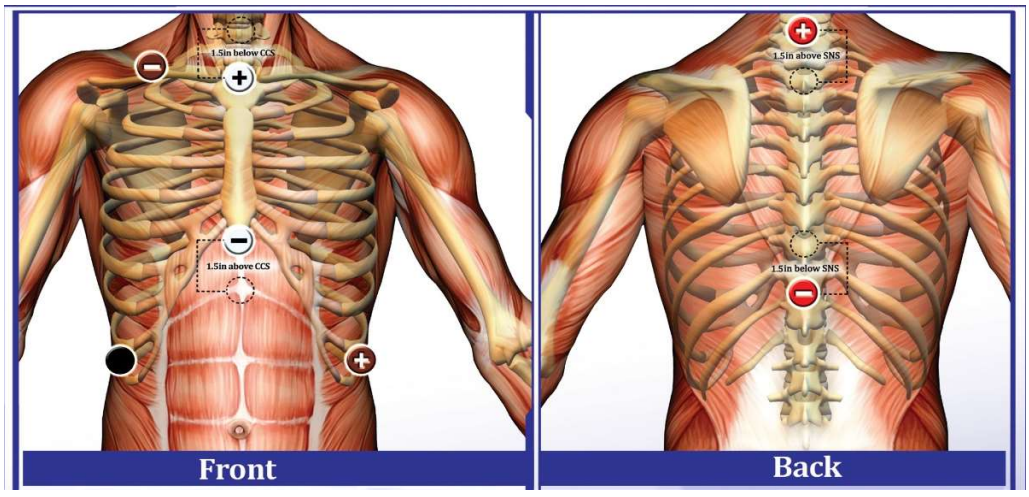

Figure 1. Placement of electrodes for Heart Rate Variability. For placement of the white positive electrodes it is important to note that to avoid artifacts from speech the electrode could be slightly offset to the left or right. Image was adapted from MindWare Technologies LTD.[9]

Two different values are calculated from heart rate variability and used to assess the activity of the sympathetic and parasympathetic branches of the autonomic nervous system: RSA and SI

Respiratory sinus arrhythmia (RSA): representation of the parasympathetic regulatory activity of the autonomic nervous system [10]

Baevsky's Stress index (SI): representation of the sympathetic regulatory activity of the autonomic nervous system [10]

2.8 LLLT placement

Placement of the LED arrays should occur in the following order: A & C, B & D (Figure 2). The timing protocol for each placement is as follows:

A & C red: continuous for 5 minutes

A & C infrared: 20 Hz, 50% duty cycle for 5 minutes

B & D red: continuous for 5 minutes

B & D infrared: 20 Hz, 50% duty cycle for 5 minutes

IR probe: continuous for 10 minutes

The IR probe should not be kept at one position for longer than 10 seconds. It can be applied starting at level L2 of the spine and moving down in a 'Z pattern' all the way to the tailbone. To find the L2 level, follow the last rib all the way to its origin I the vertebrae. This will take you the T12. Two vertebrae down from that is L2.

IMPORTANT: when using the probe, it is essential that all present in the room wear safety glasses before the probe is plugged into the unit, and the signs for laser use are placed outside the door of the room.

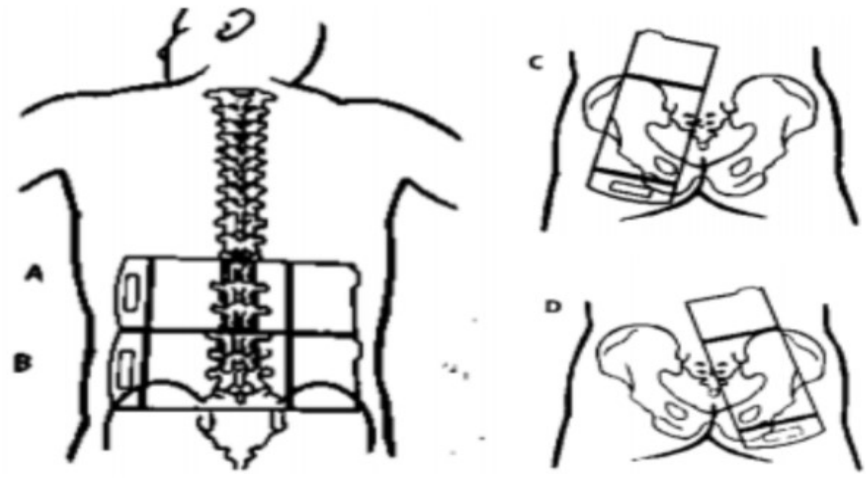

Figure 2. Placements of LLLT LED arrays. Placement of the arrays begins at L2 level of the spine. For placements C and D, it is important that the array covers both the sciatic nerve and the respective side of the sacral spinal cord [11]

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Chapter 3: On the Nature of High-Amplitude Propagating Pressure Waves in the Human Colon

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ABSTRACT

Characterization of High-Amplitude Propagating Pressure Waves (HAPWs or HAPCs) plays a key role in diagnosis of colon dysmotility using any type of colonic manometry. With the introduction of high-resolution manometry, more insight is gained into this most prominent propulsive motor pattern. Here we employ a water-perfused catheter with 84 sensors with intervals between measuring points of 1 cm throughout the colon, for 6-8 hours, in 19 healthy subjects. The catheter contained a balloon to evoke distention. We explored as stimuli a meal, balloon distention, oral prucalopride, and bisacodyl, with a goal to optimally evoke HAPWs. We developed a quantitative measure of HAPW activity, the "HAPW Index". Our protocol elicited 290 HAPWs. 21% of HAPWs were confined to the proximal colon with an average amplitude of 75.3 ± 3.3 mmHg and an average HAPW Index of 440 ± 58 mmHg.m.s. 29% of HAPWs started in the proximal colon and ended in the transverse or descending colon with an average amplitude of 87.9 ± 3.1 mmHg and an average HAPW Index of 3344 ± 356 mmHg.m.s. 49% of HAPWs started and ended in the transverse or descending colon with an average amplitude of 109.3 ± 3.3 mmHg and an average HAPW Index of 2071 ± 195. HAPWs with and without Simultaneous Pressure Waves (SPWs) initiated the colo-anal reflex, often abolishing 100% of anal sphincter pressure. Rectal bisacodyl and proximal balloon distention were the most optimal stimuli to evoke HAPWs. These measures now allow for a confident diagnosis of abnormal motility in patients with colonic motor dysfunction.

INTRODUCTION

Chronic colonic motility disorders are treated or undergo surgical intervention, most often without proper diagnosis of motor dysfunction, yet, all consensus reports indicate that colonic manometry is essential for diagnosis of colon motor dysfunction [1–4]. Colonic manometry is considered of uncertain usefulness because of our limited knowledge of

normal colon motor patterns and normal reaction to stimuli. Diagnosis of esophageal dysfunction has changed due to high-resolution manometry, from measurements of isolated points along the esophagus due to a low number of sensors, to a detailed characterization of esophageal motility. This, amongst other improvements, led to increased sensitivity to detect achalasia and it allowed for subclassification of achalasia leading to improved guidelines for treatment [5,6]. The equivalent of the esophageal peristalsis propagating contraction in the colon, is the high-amplitude propagating pressure wave (HAPW) [7,8], also known as high- amplitude propagating contraction (HAPC) [9,10], or high-amplitude propagating sequence (HAPS) [2,11]. Guidelines for colonic manometry indicate that the most important feature that should be achieved is the ability to conclude that a patient's motor function is normal [1]. However, we do not yet have criteria to confidently identify normal HAPWs and no consensus exists as to which protocol to utilize to elicit HAPWs for diagnostic purposes. Also, an adequate healthy control data set is essential for interpreting an abnormal test [1] and such a data set is not yet available. HRCM may achieve this and the present study provides an important advance towards this goal. Previous studies have demonstrated the relevance of appreciating the regional distribution of propagating waves in the colon. It was found that in the early pre-defecatory phase, the origin of HAPWs shifts distally [9]. This coordinated spatiotemporal pattern has been suggested to play an important role in the shifting of colonic content in the rectal direction to prepare for defecation, as most individual HAPWs do not span the entire colon [12]. The innervation of the colon also shows regional differences [13], and functional differences related to transit and storage are well documented [14–16]. There have also been indications in the pediatric literature that HAPWs are not normal unless they span the entire colon [4], which makes it important to study regional HAPWs. Therefore, the first objective of this study was to characterize HAPWs in healthy subjects using 84 sensors throughout the colon based on the site of origin and site of termination and quantify their features, so as to assess in future studies potential regional dysfunction in patients. HAPWs generally occur between 4 and 10 times per 24 hours in the unprepared colon [11,17,18]. In a short manometric study, they may not happen without a stimulus, and are usually evoked by various stimuli including a meal and proximal bisacodyl. However, in healthy subjects, a

meal may not evoke HAPWs, and rarely bisacodyl may not either [10]. Hence the second objective was to identify optimal stimuli that will reliably evoke HAPWs in healthy subjects. A third objective was to develop a quantitative assessment of normal HAPW activity.

METHODS

Please refer to chapter 2.1 to 2.6

RESULTS

A total of 19 healthy subjects underwent HRCM which generated 290 HAPWs (Figures 1-3). HAPWs, independent of location or type, had an average amplitude higher than 50 mmHg, and a velocity between 0.2 cm/s and 2.2 cm/s. HAPWs were associated with an average anal sphincter relaxation of 66% (range 61 – 100%), from an average resting anal sphincter pressure of 52.8 ± 2.0 mmHg (range: 48.9 - 56.8 mmHg) measured in the 3 min period prior to the HAPW. All HAPWs propagated in antegrade direction.

HAPW Categories

The HAPWs were classified in 3 different categories based on their origin and termination in the colon, starting with activities that were initiated in the proximal colon.

Category 1. Proximal HAPWs: HAPWs originating in the ascending colon which did not propagate beyond it (21%; N=12, n=62)

Examples are shown in Figure 1. The average amplitude of the HAPWs in this category was 75.3 ± 3.3 mmHg. The normal range based on the 95th percentile was 46.5-145.2 mmHg. The mean velocity was 0.88 ± 0.11 cm/s with a range of 0.32-2.2 cm/s. The mean HAPW Index was 440 ± 58 mmHg.m.s. and its range was 87-1540 mmHg.m.s. The average anal sphincter relaxation for this group was 47.5 ± 3.1%.

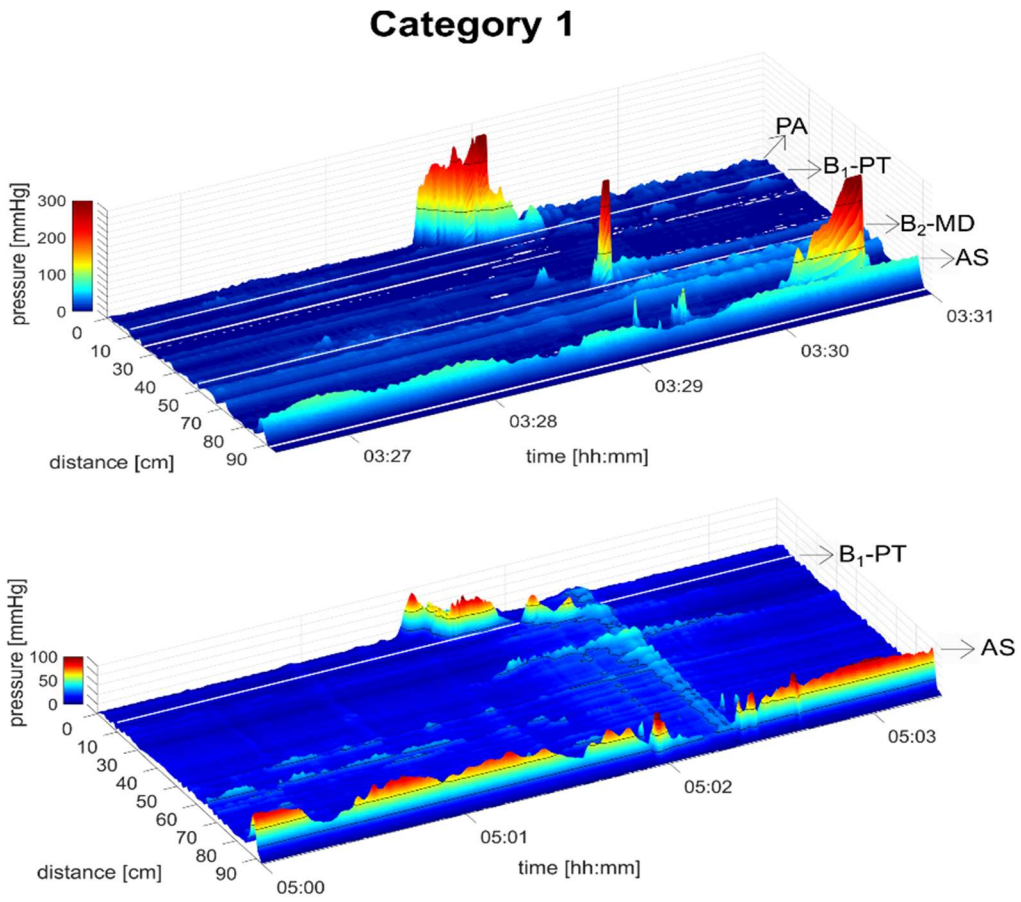


Figure 1. Category 1: proximal HAPWs. HAPWs originate in the ascending colon and also terminate within it. They may A) terminate fully, or B) transform into an SPW. A) was observed during meal and B) was observed during rectal bisacodyl. White line represents 10-cm balloon. PA=proximal ascending; PT=proximal transverse; MD=mid-descending; AS=anal sphincter

89% of the HAPWs in this category were associated with relaxation of the anal sphincter of > 20%. 64% of the HAPWs in this category transformed into SPWs; 88% of these were associated with anal sphincter relaxation. In this category, there were no significant differences in amplitude, velocity, or index between HAPWs with or without SPWs.

Category 2. Proximal continuing HAPWs: HAPWs originating in the ascending colon and terminating in the transverse, descending, or sigmoid (29%; N=13, n=85)

HAPWs originating in the proximal colon and terminating beyond it were the second most prominent category (Figure 2). Their mean amplitude was 87.9 ± 3.1 mmHg with a

normal range between 52.5-141.9 mmHg. The mean velocity of this category was 0.79 ± 0.05 cm/s, range: 0.29-1.50 cm/s. Their mean index was 3344 ± 356 mmHg.m.s., range: 368-12189 mmHg.m.s. 92% of the HAPWs in this category were associated with significant anal sphincter relaxation of more than 20% from resting pressure.

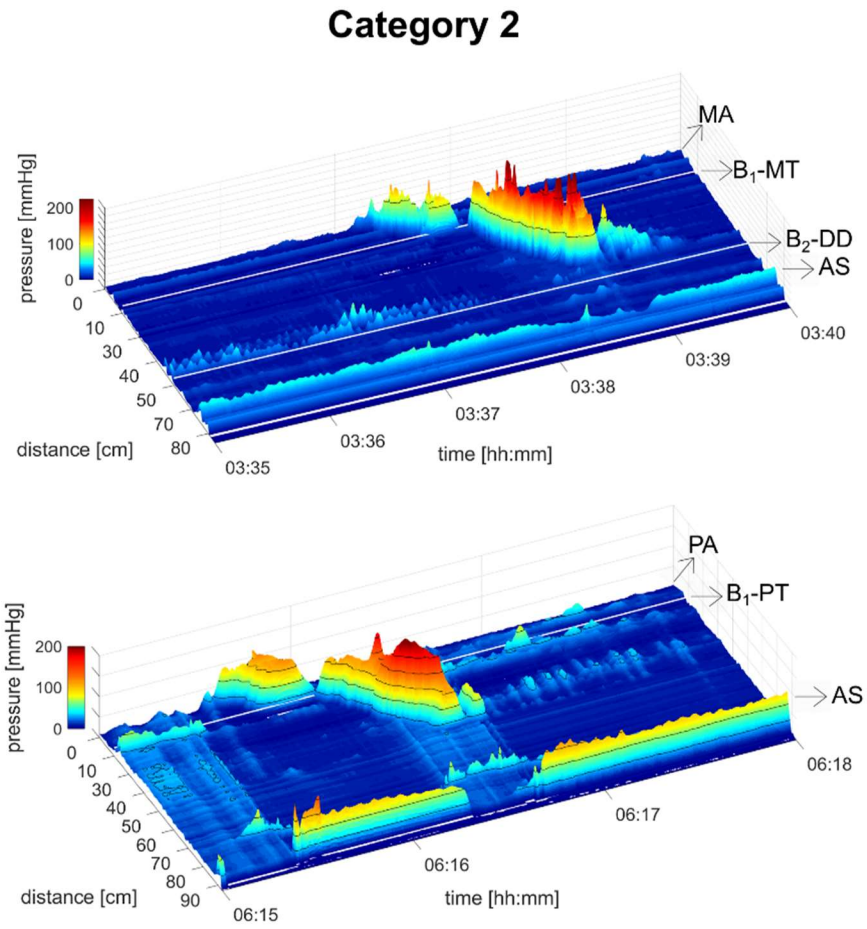


Figure 2. Category 2: proximal continuing HAPWs. HAPWs originated in the ascending colon, and may terminate in the transverse, descending, sigmoid colon, or rectum either A) fully, or B) into an SPW. A) was observed during meal and B) was observed during rectal bisacodyl. White line represents 10-cm balloon. PA=proximal ascending; MA=mid-ascending; PT=proximal transverse; MT=mid-transverse; DD=distal descending; AS=anal sphincter

68% of the HAPWs in this category terminated in the transverse or descending colon by transforming into SPWs. 97% of the HAPWs without SPWs were associated with anal sphincter relaxation. In this category, HAPWs with SPWs had significantly higher amplitude ($p<0.0001$) and index ($p<0.0001$). HAPWs with SPWs had a significantly higher velocity ($p=0.0062$).

Category 3. Transverse / descending HAPWs: HAPWs originating in the transverse or descending colon (49%; N=18, n=143)

This category of HAPWs was the most prominent (Figure 3). Their mean amplitude was 109.3 ± 3.3 mmHg, range: 48.0-183.5 mmHg. The mean velocity of this category was 0.60 ± 0.03 cm/s, range: range: 0.22-1.15 cm/s. The average HAPW Index was 2071 ± 195 mmHg.m.s, range: 155-7492 mmHg.m.s.

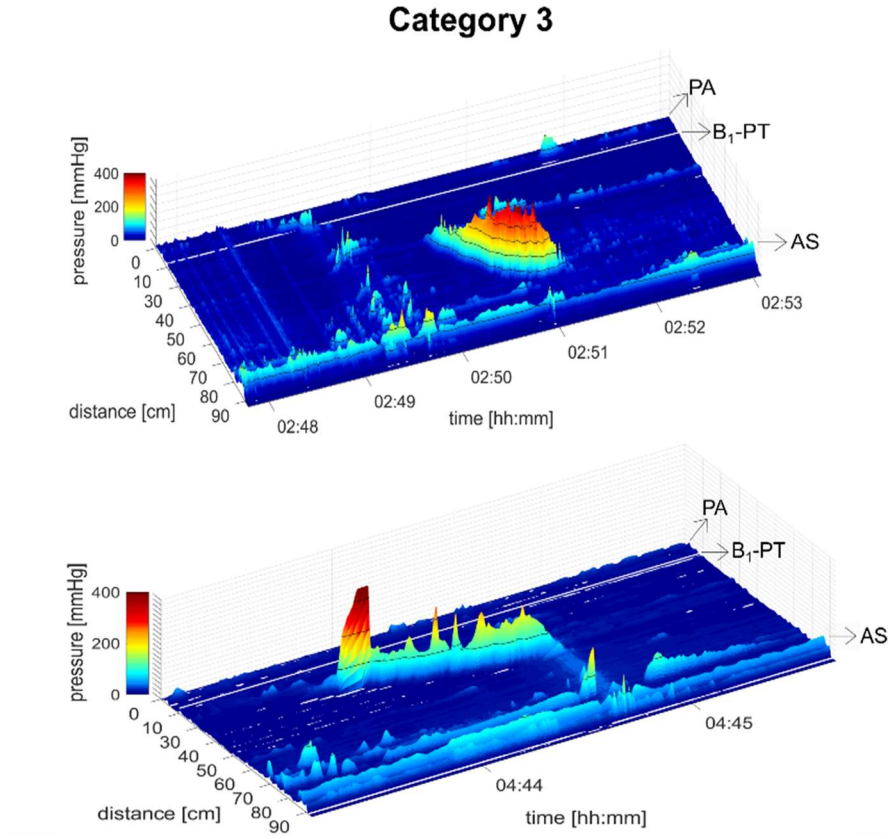


Figure 3. Category 3: transverse/descending HAPWs. HAPWs originate in the transverse or descending colon and may terminate A) fully or B) into an SPW. A) was observed during meal and B) was observed during oral prucalopride. White line represents 10-cm balloon. PA=proximal ascending; PT=proximal transverse; AS=anal sphincter

95% of HAPWs in this category were associated with relaxation of the anal sphincter of more than 20% from resting pressure. 39% of the HAPWs in this category transformed into SPWs in the descending colon. 96% of HAPWs with SPWs in this category were associated with anal sphincter relaxation or with a contraction. In this category, HAPWs

without SPWs had significantly higher amplitude ($p<0.0001$) and index ($p<0.0001$), however, velocity was not significantly different.

Spontaneous relaxations of the anal sphincter

The anal sphincter was seen to relax spontaneously, that is without association of a motor pattern in 24 instances observed across 10 of the 19 subjects. Hence 9 of the subjects had no independent anal sphincter relaxations. The anal sphincter was occasionally seen to relax rhythmically at 1 cpm as reported previously [19]. The average percent anal sphincter relaxation during the independent relaxations (50.0%) was significantly lower compared to that of relaxations associated with motor patterns ($p<0.0001$). Additionally, none of the independent relaxations reached 100%, while complete relaxation of the anal sphincter was observed in association with 12% of the HAPWs.

Comparison between HAPW subgroups

Amplitude

Transverse / descending HAPWs (category 3) had the highest average amplitude (109.3 mmHg), which was significantly higher than both categories 1 ($p<0.0001$) and 2 ($p<0.0001$). Categories 1 and 2 were also significantly different from one another ($p=0.0179$), with proximal HAPWs having the lowest amplitude of all three.

Velocity

The category with the highest amplitude HAPWs had the lowest mean velocity. Category 3 was significantly lower than both categories 1 ($p=0.0332$) and 2 ($p=0.0076$). Categories 1 and 2 were not significantly different from each other.

HAPW Index

Proximal continuing HAPWs (Category 2) has the highest HAPW Index. It was significantly higher than both categories 1 ($p<0.0001$) and 3 ($p=0.0059$). Categories 1 and 3 were also significantly different from each other ($p<0.0001$), with category 1 having the lowest index.

Site of origin and termination

The majority of HAPWs terminated at the descending colon (66%), with another 6% terminating at the splenic flexure. 21% of HAPWs propagated to the transverse colon, with an additional 2% terminating at the hepatic flexure. 5% of the HAPWs entered the rectum, 1% of which were proximally originating, and the rest originated in the transverse or distal colon. There was no significant difference between the number of HAPWs which originated in the proximal colon, compared to those which originated in the transverse/descending (51% and 49% respectively). The proximally originating HAPWs did propagate a longer distance than the transverse-descending originating ones (27.0 cm and 23.8 cm respectively; $p<0.05$). No significant difference in average anal sphincter relaxation was observed between any of the 3 categories.

Response to Interventions.

Baseline (90 min), N=19

33 HAPWs were observed during baseline, in 8 individuals (Table 1) dominated by proximal HAPW-SPWs and transverse - descending HAPWs. The symbol maps show that isolated SPWs are the dominant motor pattern as reported on previously [19,20] (Figure 4A). Only 16% of the subjects did not have any HAPW or SPW at baseline. The HAPW Index for baseline was 1432 ± 215 mmHg.m.s, and its range was 175 to 4549 mmHg.m.s (Figure 6). The amplitude during baseline was 89.1 ± 4.9 mmHg, with a range of 79.1 to 99.1 mmHg. The velocity ranged between 0.61 cm/s and 0.80 cm/s with an average of 0.71 ± 0.05 cm/s.

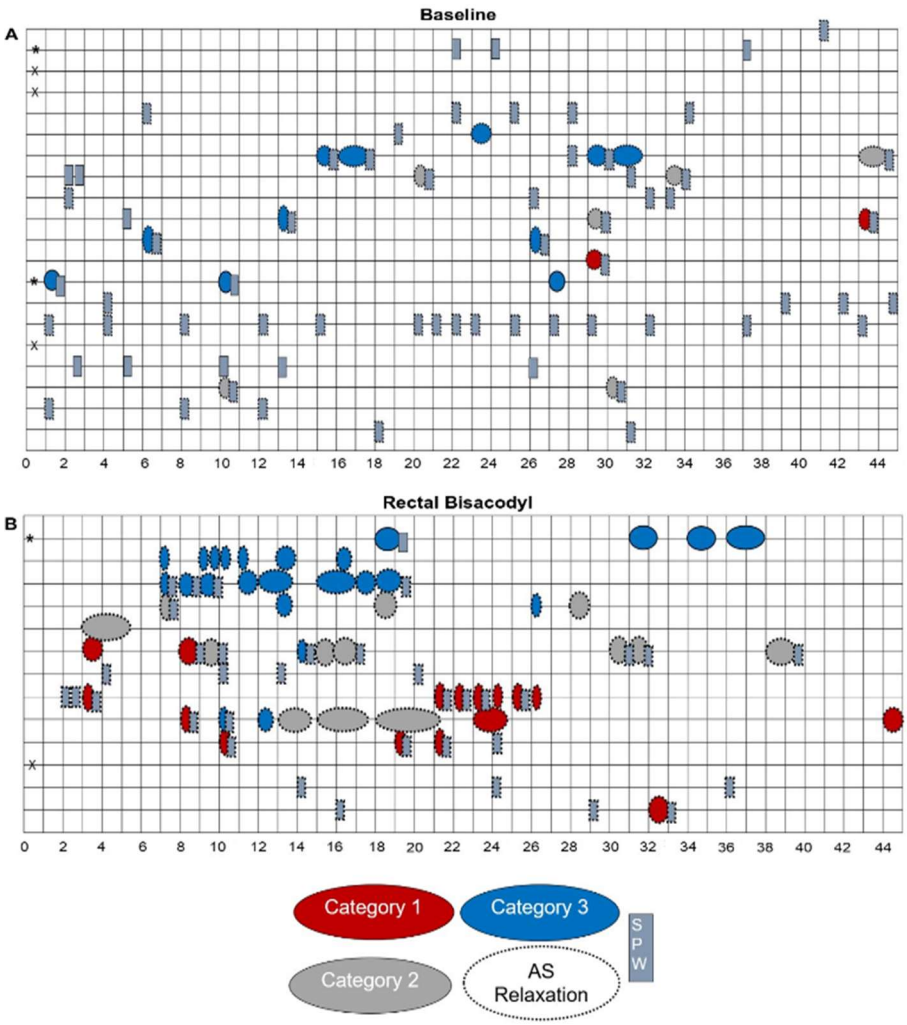


Figure 4. Symbol Maps of A) baseline, and B) rectal bisacodyl. Symbol map of baseline includes the second half of the 90-min baseline recording, 45 minutes before the subsequent intervention. Each row represents a single volunteer. X represents a lack of response, * represents no visualization of the anal sphincter as a consequence of all sensors being oral to the sphincter. A dotted outline around a symbol indicates that the motor pattern is associated with anal sphincter relaxation. There was a relatively uniform response across the subjects, dominated by SPWs during baseline. Rectal bisacodyl induced an overall strong response dominated by HAPWs, with great variability in the type of HAPWs that were observed.

Proximal balloon distention (20 min), N=19

44 HAPWs were observed during proximal balloon distention, in all individuals (Table 1) (Figure 5), dominated by HAPWs from categories 2 and 3. The average HAPW Index from proximal balloon distention was 2973 ± 445 mmHg.m.s, and its range was 128 to

11156 mmHg.m.s (Figure 6). The mean HAPW amplitude during the intervention was 105.2 ± 5.1 mmHg with a range of 94.8 to 115.5 mmHg. The velocity ranged between 0.40 and 0.55 cm/s with a mean of 0.47 ± 0.04 cm/s.

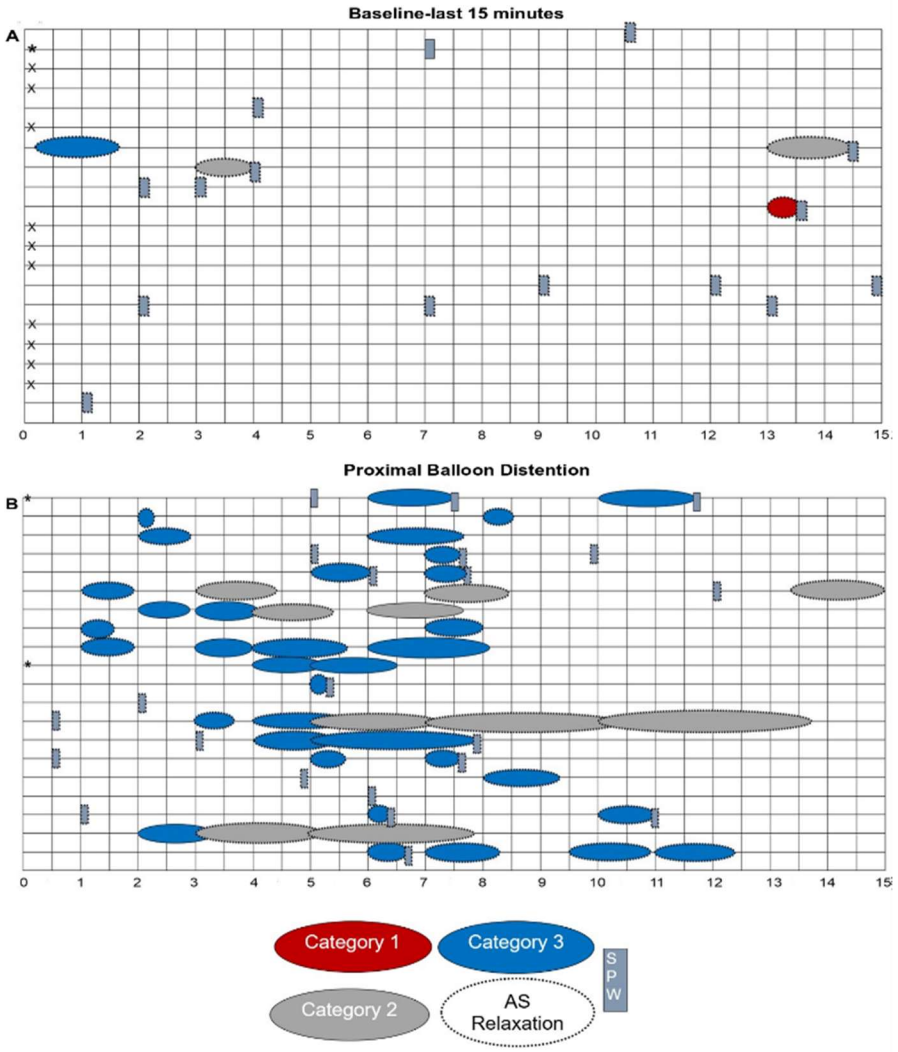


Figure 5. Symbol Maps of A) last 15 minutes of baseline and B) proximal balloon distention. Each row represents a single volunteer. X indicates a lack of response, while * represents no visualization of the anal sphincter during HRCM. A dotted outline around a symbol indicates association of a motor pattern with anal sphincter relaxation. During proximal balloon distention, all subjects showed a response, and it was dominated by HAPWs, dominated by those originating in the transverse/descending colon.

Meal response (90 min), N=19

49 HAPWs were observed after meal intake (Table 1) in 13 subjects, showing a large intersubject variability in the generation of the HAPWs and also in the time they

appeared after intake of the meal (Supplementary Figure 1). Although the exact timing of the gastrocolonic reflex is difficult to determine because it cannot be excluded that some HAPWs would have appeared even without meal, the start of the gastrocolonic reflex took an average of 24.1 ± 4.6 min, range 7 to 62 min. Hence, to make sure the reflex has materialized, an observation time of at least 60 min since beginning of meal intake is essential. 32% of healthy subjects did not generate HAPWs but did respond to the meal with SPWs [19,20] (Supplementary Figure 1). The mean amplitude of HAPWs during this intervention was 77.2 ± 3.3 mmHg, and the mean velocity was 0.77 ± 0.05 cm/s. The average HAPW Index was 950 ± 130 mmHg.m.s and the normal range was 91 to 2768 mmHg.m.s (Figure 6).

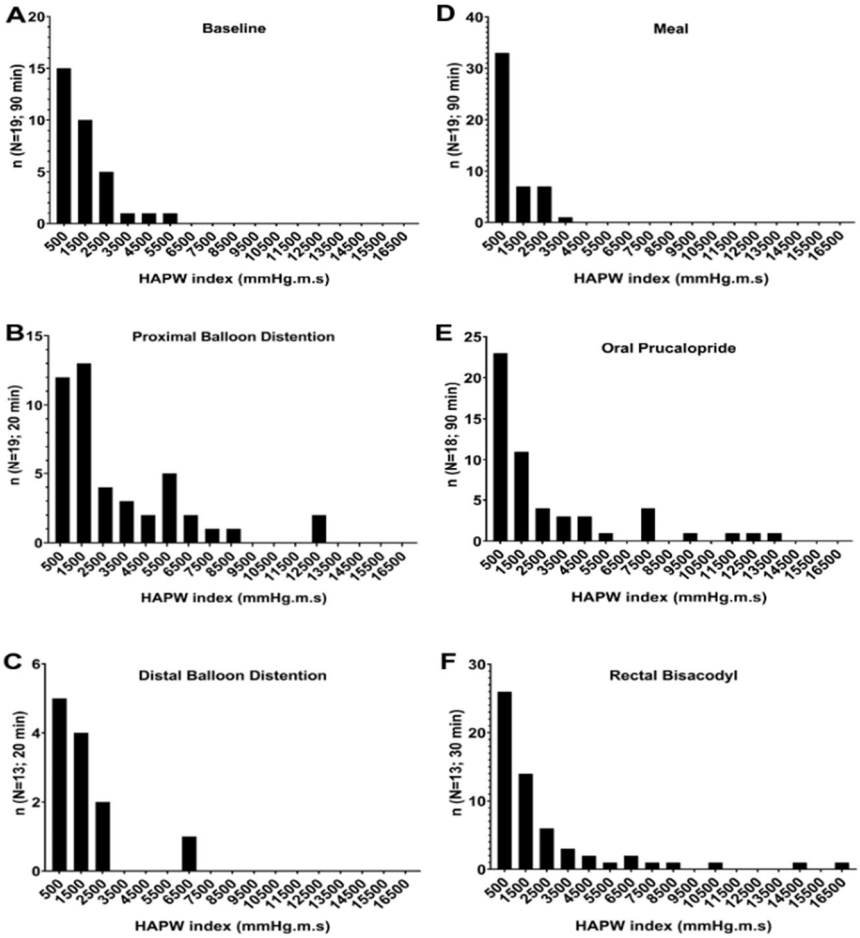


Figure 6. HAPW Indexes separated based on interventions. Shown are baseline, proximal balloon distention, distal balloon distention, meal, oral prucalopride, and rectal bisacodyl (N=19, n=290). Center of bin is shown on X-axis.

Oral prucalopride (90 min), N=18

52 HAPWs were observed in 10 individuals after oral prucalopride intake (Table 1) (Supplementary Figure 2). The response to prucalopride (4 mg) was variable both in onset time, as well as type of response. The average HAPW Index for this intervention was 2624± 460 mmHg.m.s, and the normal range was 130 to 11969 mmHg.m.s (Figure 6). The amplitude of HAPWs during this intervention was 94.7 ± 6.5 mmHg, ranging between 81.7 and 107.7 mmHg. The average propagating velocity was 0.69 ± 0.04 cm/s and it ranged between 0.61 to 0.76 cm/s.

Rectal bisacodyl (20 min), N=13

58 HAPWs were observed in 12 individuals in response to rectal bisacodyl (Table 1), Rectally administered bisacodyl induced an early response and the greatest number of HAPWs, belonging to each of the three categories (Table 1). This was the only intervention where pancolonic HAPWs were observed, entering the rectum. The symbol map for this intervention shows a large variability with regards to the type of HAPWs that can be observed (Figure 4). Only a single subject had no response to this intervention, and only 2 responded with SPWs alone. Most subjects responded within the first 10 min of administration (Figure 4); only 2 subjects exceeded that time by a few minutes. HAPWs during this intervention had an amplitude of 104.1 ± 5.2 mmHg, ranging between 93.8 and 114.5 mmHg. The propagating velocity ranged between 0.64 and 0.82 cm/s, with an average of 0.73 ± 0.05 cm/s. Rectal bisacodyl HAPWs had an index of 2337 ± 436 mmHg.m.s, with a range of 86 to 10470 mmHg.m.s (Figure 6). Figure 7 shows a response to bisacodyl (10 mg) illustrating the gradual increase in excitation of the musculature represented by a gradual increase in the HAPW Index.

Comparison between interventions (Table 1).

Proximal balloon distention was the intervention during which the highest average HAPW amplitude was observed. The second-highest amplitude was observed in response to rectal bisacodyl. The meal was the intervention which induced the lowest mean amplitude HAPWs. The opposite was true with regards to velocity, where the meal was observed to have induced HAPWs at the highest velocity on average, and

proximal balloon distention induced the lowest velocity HAPWs. In addition to amplitude, proximal balloon distention induced HAPWs with the highest HAPW Index, and similarly, the meal showed the lowest HAPW Index.

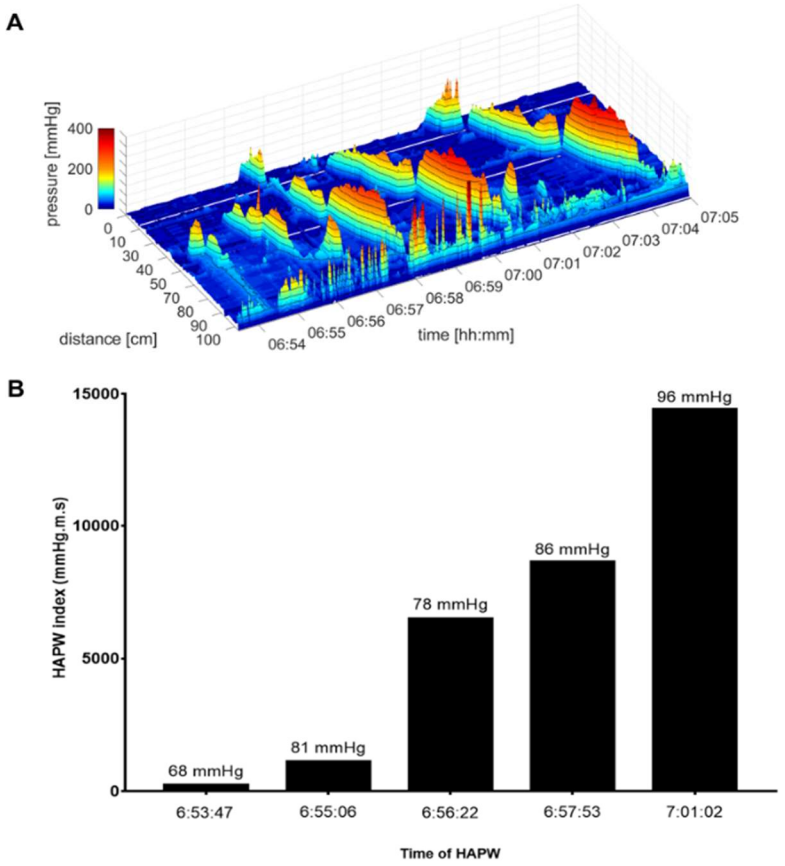


Figure 7. HAPW Indexes of individual HAPWs in response to rectal bisacodyl. A gradual increase in the HAPW Index can be observed that corresponds with the increase in excitation caused by the intervention, and the size and propagation length of the HAPWs

Assessing normal HAPW activity (Table 2).

Protocols with only baseline and a meal, or baseline and rectal bisacodyl have a high probability (6 out of 19) of exhibiting non-responding subjects. A meal, baseline and rectal bisacodyl revealed 5 out of 19 non-responders. Proximal balloon distention was observed to be a superior intervention with a low probability of non-responders even when used alone. When proximal balloon distention was used with meal or rectal bisacodyl, each of these combinations of interventions only had 1 volunteer who did not respond with HAPWs, although the occurrence of HAPWs oral to the most proximal

Table 1. HAPW categories and interventions

	Total HAPWs $N = 19$; $n = 290$	Proximal *Category 1*	Proximal Continuing *Category 2*	Transverse/Descending *Category 3*
		Baseline		
Occurrence	$N = 8$; $n = 33$; $n/N = 4.1$; n/total $N = 1.7$	$N = 2$; $n = 2$	$N = 5$; $n = 9$	$N = 6$; $n = 22$
Amplitude, mmHg	89.1 ± 4.9 46.1–132.8	64.5 ± 15.4 49.1–79.9	68.9 ± 5.9 44.8–100.0	99.7 ± 5.7 47.9–136.4
Velocity, cm/s	0.71 ± 0.04† 0.32–1.1	0.64 ± 0.03 0.62–0.67	0.82 ± 0.10 0.34–1.2	0.66 ± 0.05 0.28–1.1
Index, mmHg·m·s	1,432 ± 215†† 175–4549	247 ± 83 164–330	1351 ± 430 286–4198	1573 ± 264 211–5063
		Proximal balloon distention (PBD)		
Occurrence	$N = 16$; $n = 45$; $n/N = 2.8$; n/total $N = 2.4$	$N = 0$; $n = 0$	$N = 4$; $n = 10$	$N = 16$; $n = 16$
Amplitude, mmHg	104.0 ± 5.2* 41.4–166.8	N/A	123.9 ± 7.3 93.4–154.4	98.3 ± 6.0 38.0–174.8
Velocity, cm/s	0.49 ± 0.04††‡**## 0.32–1.1	N/A	0.38 ± 0.04 0.24–0.61	0.52 ± 0.05 0.15–1.2
Index, mmHg·m·s	2,973 ± 445†††‡‡ 128–11,156	N/A	7,012 ± 988 3,984–12,670	1,819 ± 283 111–5,781
		Distal balloon distention (DBD)		
Occurrence	$N = 6$; $n = 12$; $n/N = 2$; n/total $N = 0.6$	$N = 1$; $n = 2$	$N = 5$; $n = 6$	$N = 2$; $n = 4$
Amplitude, mmHg	90.9 ± 9.0 45.2–156.3	51.7 ± 6.3 45.4–58.0	86.4 ± 9.3 53.4–122.8	117.2 ± 13.5 05.7–156.3
Velocity, cm/s	1.4 ± 0.38 0.17–3.9	1.7 ± 0.52 1.2–2.2	1.4 ± 0.53 0.34–3.5	1.2 ± 0.92 0.17–3.9
Index, mmHg·m·s	1,667 ± 478 89–6269	93 ± 4 89–96	2,218 ± 839 875–6,269	1,626 ± 435 625–2,746
		Meal		
Occurrence	$N = 13$; $n = 48$; $n/N = 3.7$; n/total $N = 2.5$	$N = 3$; $n = 10$	$N = 6$; $n = 19$	$N = 8$; $n = 19$
Amplitude, mmHg	77.2 ± 3.3*# 47.6–122.7	91.3 ± 8.7 51.2–125.7	65.3 ± 2.45 51.2–87.11	81.7 ± 5.5 43.2–125.9
Velocity, cm/s	0.77 ± 0.05‡ 0.44–1.6	0.87 ± 0.15 0.43–2.0	0.83 ± 0.09 0.41–1.7	0.65 ± 0.03 0.48–0.96
Index, mmHg·m·s	950 ± 130‡‡***### 91–2,768	329 ± 74 61–708	1375 ± 232 253–3,761	851 ± 183 71–2,660
		Oral prucalopride		
Occurrence	$N = 11$; $n = 53$; $n/N = 4.8$; n/total $N = 2.8$	$N = 8$; $n = 21$	$N = 4$; $n = 15$	$N = 4$; $n = 17$
Amplitude, mmHg	94.7 ± 6.5 49.5–195.1	66.1 ± 5.5 40.9–155.8	77.8 ± 4.5 55.6–107.9	144.9 ± 11.4 61.3–200.7
Velocity, cm/s	0.69 ± 0.04** 0.21–1.3	0.67 ± 0.05 0.30–1.3	0.77 ± 0.09 0.29–1.5	0.63 ± 0.06 0.29–1.2
Index, mmHg·m·s	2,624 ± 460*** 130–11,969	479 ± 122 125–2,295	3,502 ± 831 480–11,952	4,500 ± 985 135–13,044
		Rectal bisacodyl		
Occurrence	$N = 12$; $n = 59$; $n/N = 4.9$; n/total $N = 3.1$	$N = 5$; $n = 16$	$N = 5$; $n = 16$	$N = 8$; $n = 27$
Amplitude, mmHg	103.2 ± 5.2# 50.9–189.7	80.5 ± 6.0 50.7–148.7	97.6 ± 7.8 71.0–200.7	120.0 ± 8.5 49.3–196.2
Velocity, cm/s	0.78 ± 0.09## 0.29–1.7	1.2 ± 0.28 0.23–5.0	0.73 ± 0.06 0.29–1.2	0.63 ± 0.05 0.27–1.4
Index, mmHg·m·s	2,337 ± 436### 86–10,470	325 ± 74 58–1,300	5,232 ± 1,101 1,210–16,416	1,813 ± 467 97–9,282

Values are expressed as averages ± SE values and 95th percentile normal ranges. N refers to number of subjects, while n refers to the number of HAPWs. *$P = 0.0005$; #$P = 0.0007$; †$P = 0.0069$; ‡$P = 0.0005$; **$P = 0.0036$; ##$P = 0.0304$; ††$P = 0.0317$; ‡‡$P = 0.0008$; ***$P = 0.0108$; ###$P = 0.0371$.

sensor cannot be excluded. A combination of baseline, proximal balloon distention and meal was able to induce HAPWs with the highest amplitude and index. Combining baseline, proximal balloon distention, meal, and rectal bisacodyl gives a high likelihood of observing HAPWs, with only 1 non-responder.

Table 2. Response to intervention combinations

Intervention	Baseline + PBD	Baseline + Meal	Baseline + RB	Baseline + PBD+ Meal	Baseline + Meal + RB	Baseline + PBD + RB	Baseline + PBD + Meal + RB
HAPWs/ subject	4.2 ± 0.73*	4.5 ± 1.1	5.1 ± 1.2	6.9 ± 1.1	7.7 ± 1.7	7.4 ± 1.1	9.6 ± 1.3*
Average amplitude, mmHg	97.7 ± 3.7†	82.1 ± 2.8†#‡**	98.2 ± 3.8#	89.9 ± 2.8	91.0 ± 2.9	100.1 ± 3.1‡	94.2 ± 2.5**
	45.3–151.3	46.8–125.9	49.0–181.0	45.8–147.8	49.2–175.8	48.6–180.2	48.9–171.8
Average Index, mmHg·m·s	2,321 ± 285††	1,146 ± 119††‡‡***	2,012 ± 293	1,799 ± 192	1,648 ± 202	2,328 ± 247‡‡	1,970 ± 191***
	153–7,328	152–3,289	153–7,894	151–5,855	132–6,315	149–8,521	137–7,029
Number of HAPW nonresponders	2	6	6	1	5	1	1

Average amplitude and index are presented as means ± SE. Symbols are noted at the two values that are compared: *$P = 0.0185$; †$P = 0.0174$; #$P = 0.0146$; ‡$P = 0.0005$; **$P = 0.0280$; ††$P = 0.0043$; ‡‡$P = 0.0005$; ***$P = 0.0055$.

Symptoms and other events

HAPW in all three categories were most commonly not associated with symptoms or gas or liquid expulsion (Figure 8A). HAPW-associated liquid expulsion was seen most commonly in transverse/descending HAPWs. Gas expulsion was most commonly reported with HAPWs from category 1 (Figure 8B). Urge to defecate was the most common symptom, it was most often reported with transverse/distal HAPWs. Nausea was only reported with 2% of HAPWs and was never associated with those from category 2. In 3 of the 19 subjects, vomiting occurred with category 1 and category 2 HAPWs, but not with transverse/descending HAPWs; 56% of vomiting episodes occurred in the 90-min period after oral prucalopride.

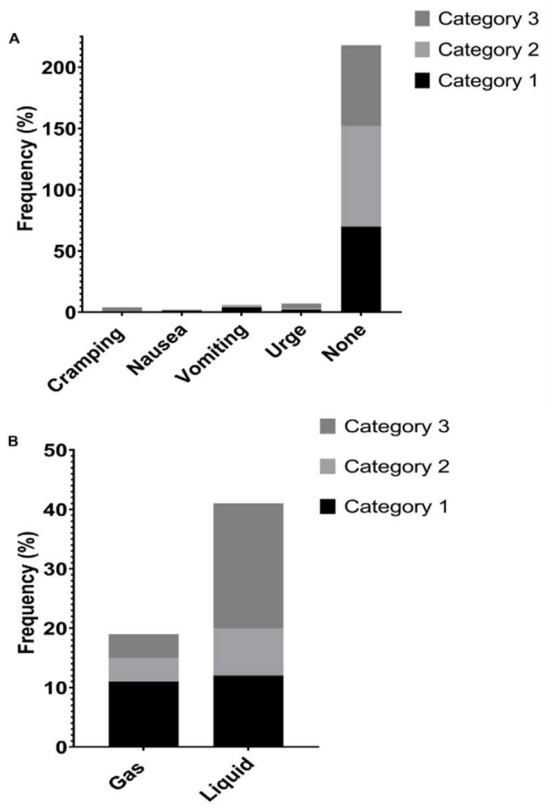


Figure 8. Symptoms and transit events associated with HAPWs. A) most HAPWs were not associated with any reported symptoms. B) Frequency of HAPW-associated liquid expulsion was similar across all 4 categories of HAPWs. Gas expulsion was most commonly reported with HAPWs starting in the proximal and terminating in the descending colon. (N=19, n=290)

DISCUSSION

Overall features of HAPWs

Here we present a comprehensive assessment of the HAPW using 84 sensors, 1 cm apart, throughout the colon. We show that HAPWs in the healthy adult (18 years and older) can be restricted to the proximal colon, can start and terminate in the transverse and descending colon, and are rarely pan-colonic. Previous manometric data in adults already indicated that HAPWs do not necessarily progress as HAPWs towards the rectum [10] but this contrasts with studies in the pediatric population where only pancolonic HAPWs are considered normal, with the understanding that no data are available for healthy children [4]. In the USA, pediatric patients include ages 18-20 years. In the present study, 3 subjects were 20 years of age and they showed all categories of

HAPWs. Here we show that 52% of HAPWs transform into a simultaneous pressure wave and in this way, they reach the rectum. We therefore recommend that the assessment of HAPWs should include the recognition of HAPW-SPWs.

The colo-anal reflex

HAPWs are associated with anal sphincter relaxation, defined as the colo-anal reflex [2,21–24]. Here we show that this relaxation occurred with all categories of HAPW and was on average 66% of its baseline anal pressure. We also show that the relaxation often amounts to 100% indicating that the relaxation involves the external anal sphincter. Hence, the relaxation of the external anal sphincter involves spinal autonomic nerves likely acting on the efferent nerves in Onuf's nucleus [25,26]. The colo-anal reflex is probably an essential component of defecation, involving autonomic sacral neural pathways [27]. It is not assessed by anorectal manometry which tests the recto-anal inhibitory reflex (RAIR) in response to rectal balloon distention. The RAIR only involves enteric nitrergic relaxation of the IAS [22,28] and usually 24 mmHg of resting pressure remains during balloon distention [4]. Consistently, in children with disrupted continuity of the colon which abolishes RAIR, the colo-anal reflex was preserved indicating that it is mediated by a different pathway from the RAIR, likely an extrinsic neural pathway [23]. In dogs, anal relaxation upon proximal colon distention was mediated by sympathetic nerves [29]. Anal sphincter relaxation also occurs in response to the Simultaneous Pressure Wave [19,20,30] (see the symbol maps in Figures 4-5, Supplementary Figures 1-2) hence the term colo-anal reflex should be defined as the autonomous relaxation of the anal sphincters in response to propulsive colonic motor patterns.

When should HAPWs be described as "Low Amplitude Propagating Pressure Waves" (LAPWs)?

Here we show that the amplitude of HAPWs in healthy subjects had an average value > 50 mmHg and the lowest maximal pressure was 88 mmHg; hence we suggest that in patients with HAPWs < 50 mmHg, the HAPWs may be of insufficient force and the motor pattern should be referred to as LAPWs. This was also proposed by Bassotti et al. although a consensus report suggested a cut-off of 75 mmHg based on low-resolution manometry [1,31,32] and based on studies that used only the maximum

amplitude of a single measuring point of the HAPWs. We agree with Bampton et al. [9] and Bassotti et al. [33] that the activities are on a continuum and that HAPWs and LAPWs should not really be seen as different motor patterns. LAPWs should be seen as possibly inefficient HAPWs. With regards to velocity of the HAPWs we observed them to range between 0.2 cm/s and 2.2 cm/s, consistent with values obtained from previous studies [11,21,34]. Motor patterns of low amplitude have been associated with myogenic dysfunction [35], but it is also possible that insufficient neural excitation is the underlying dysfunction.

The creation of symbol maps.

When assessing normal occurrence of HAPWs under baseline conditions or in response to a meal, it is custom to present the average value of the number of HAPWs as well as a normal range. However, decisions about abnormality should not be made solely by comparing features of a patient's HAPWs with average values from healthy persons. Here we introduce the symbol map to give an overview of baseline activity and responses to interventions of all subjects, with details about the HAPW category, their length of propagation, their association with SPWs and anal sphincter relaxation, and the time they occur relative to the start of the intervention. The dramatic development of HAPWs in response to balloon distention (Figure 5B) and bisacodyl becomes immediately obvious (Figure 4B) but the variability in response to stimuli in healthy subjects is also clear. This large variability is what makes diagnosing colon dysmotility more difficult compared to esophageal dysmotility.

A comprehensive quantitative assessment, the HAPW Index

The present study introduces the HAPW Index as a quantitative measure of the strength of the HAPW. In clinical assessments of colonic motility thus far, only the HAPW amplitude and velocity are quantified. Figure 7 shows clearly that the HAPW Index better represents the strength of the HAPW compared to the amplitude alone. We show that the average HAPW Index centers around 1400 mmHg.m.s at baseline, ~ 950 mmHg.m.s after a meal, ~ 2600 mmHg.m.s after oral prucalopride, ~ 3000 mmHg.m.s during proximal balloon distention, and ~ 2300 mmHg.m.s in response to rectal

bisacodyl. It is clear from these data that a single index value does not appear to be useful, the index should be linked to baseline or a specific intervention.

Development of optimal stimulus parameters

We show here that rectal bisacodyl and proximal balloon distention are stimuli that have a high chance of evoking all types of HAPWs. These stimuli are rarely performed but are highly effective. Rectal bisacodyl evoked HAPWs that started in the proximal colon after about 10 min. Hence bisacodyl will activate extrinsic sensory nerves that communicate with the spinal cord neurons that ultimately evoke vagal responses to initiate proximal HAPWs [36,37]. A positive response to rectal bisacodyl confirms intactness of critical neural reflexes. When a HAPW develops it also shows normal colonic musculature and enteric neural circuits. In patients who do not have spontaneous bowel movements, rectal bisacodyl may evoke HAPWs; hence although bisacodyl activates physiological reflexes, it is a powerful pharmacological substance that does not necessarily mimic a physiological rectal stimulation; nevertheless a positive response shows that the spinal and vagal innervation, as well as the communication between autonomic nerves and the colon are present and intact [38].
Rectal stimulation will become more important in the future since solid state catheters and fibre optic catheters do not have the ability to deliver a stimulus to the proximal colon, a stimulus that was routinely given using water perfused catheters. Although both proximal and rectal bisacodyl can induce HAPWs that start in the proximal colon, a different mechanism of action may underly it, as the proximal and distal colon are predominantly innervated by the vagus and sacral nerves respectively [19,36,37,39].
Although proximal bisacodyl can evoke HAPWs, rectal bisacodyl may be more relevant for the testing of the rectal reflex to initiate HAPWs. Our data are consistent with early studies from Preston and Lennard-Jones who looked at bisacodyl instilled within the recto-sigmoid area, and found that in healthy controls there was a marked increase in anally-progressing propagating waves [40].
We show here that proximal balloon distention is a very good stimulus to evaluate if the colon is capable of generating propulsive motor patterns. The stimulus evoked all 3 categories of strong HAPWs that were of a high amplitude, but a slower velocity

compared to baseline (Table 1), which, based on other studies, are likely able to propel content [34]. There were only 2 subjects who did not respond to proximal balloon distention with HAPWs, but they did respond with SPWs (Table 2, Figure 5B). Proximal balloon distention activates sensory receptors in the proximal colon that can initiate motor patterns starting proximal to the stimulus, likely mediated by both extrinsic vagal pathways as well as the enteric nervous system [41]. It likely imitates food entering the colon, and in our study it evoked mostly HAPWs with origin in the transverse or descending colon. Similarly, Kamm et al. [42] observed HAPWs in response to proximal balloon distention that expelled isotope, although these HAPWs entered the rectum which never happened in our studies.

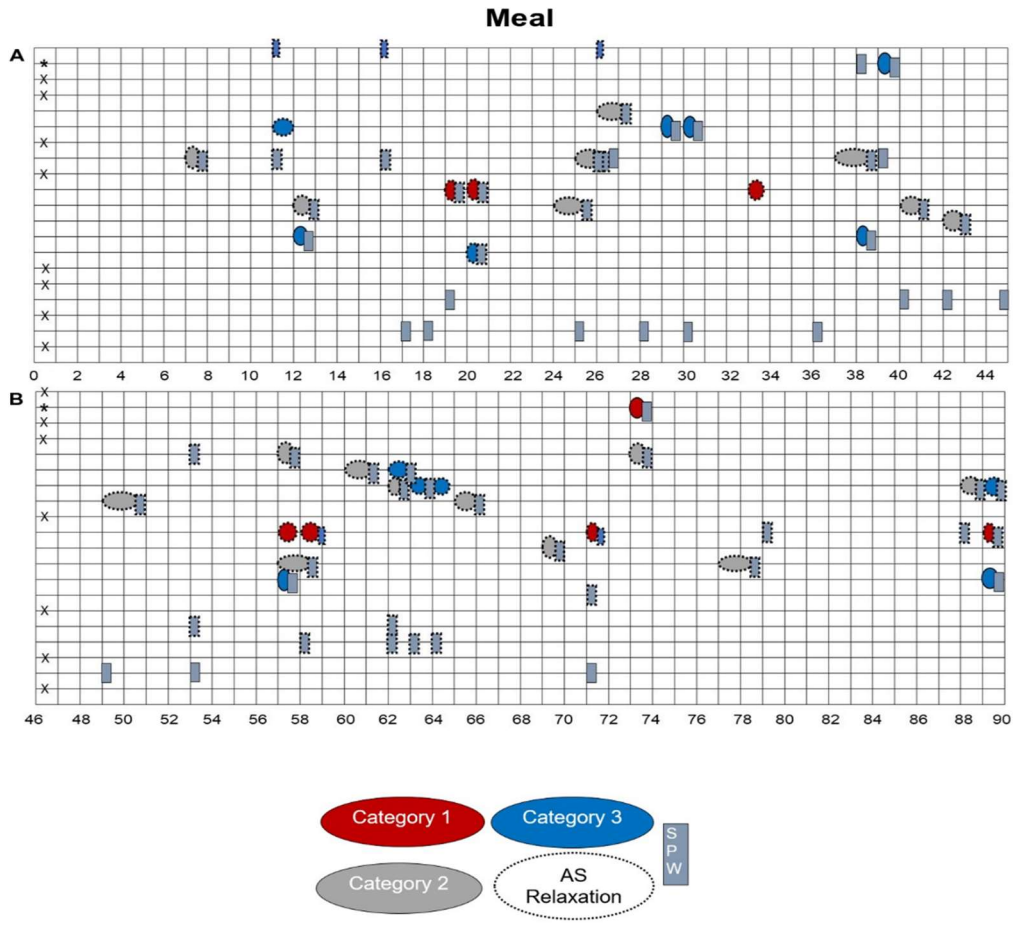
It may be useful to test the response to a meal, although it has a lower chance of evoking HAPWs compared to the above-mentioned stimuli. A colonic response to a meal signifies the gastro-colonic reflex, a vagally mediated “awakening” of the colon [9,43,44]. The present study shows that a meal can evoke HAPWs which have amplitudes on the lower end of the spectrum but with a higher velocity. It is also evident that the response is highly variable with many healthy subjects showing no or a late response to the intervention and others showing an increase in SPWs but no HAPWs, as shown in the symbol maps. We defined the presence of the gastrocolonic reflex as an increase in propulsive motor patterns compared to baseline following a meal [45]. In Considering our observation that healthy subjects may not exhibit a response to meal, in patients, a positive response, whether it is HAPWs or SPWs, suggests intactness of vagal innervation. However, no response, by itself, does not necessarily identify pathophysiology.

Based on a previous study [46] we hypothesized that oral prucalopride, once entered the stomach, would activate the numerous 5-HT_4 receptors on the luminal surface of epithelial cells [47], releasing 5HT from enterochromaffin cells to activate vagal sensory nerves that might lead to a gastro-colic reflex. In the present study, 5 out of 17 subjects who took oral prucalopride showed a HAPW response within 15 minutes, possibly due to this gastro-colic reflex. Since the response was not consistently observed, the clinical value of giving prucalopride as a diagnostic tool during HRCM is questionable. HAPWs

generated after prucalopride intake did show a significantly higher amplitude compared to the meal response; in addition, they were most commonly associated with vomiting compared to other interventions. This may be related to prucalopride's stimulating effects of 5-HT_4 vagal afferents which send signals to stimulate the vomiting center in the brain [48].

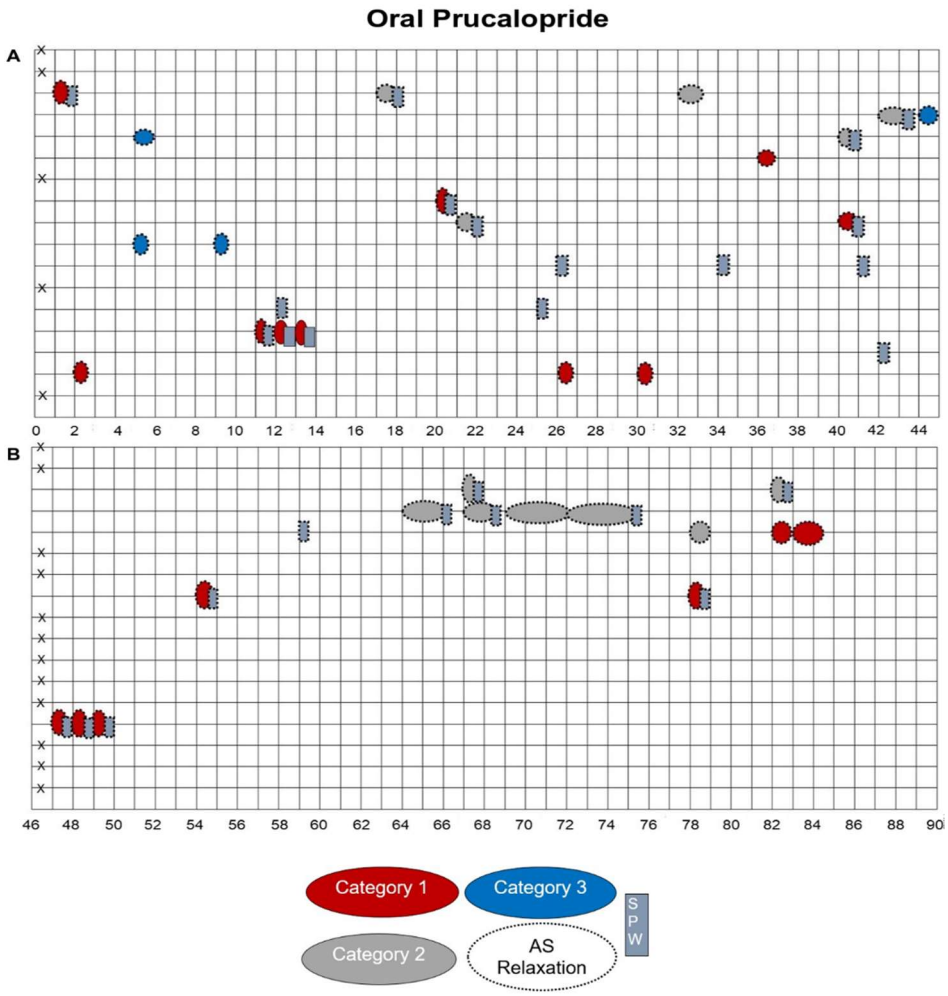
Based on our experience, an optimal protocol to assess colon function, including the gastrocolonic reflex is baseline period, a meal, proximal balloon distention and rectal bisacodyl. If the only objective is to observe HAPWs and there are time constraints, then proximal balloon distention and/or rectal bisacodyl may be sufficient.

SUPPLEMENTARY FIGURES



Supplementary Figure 1. Symbol maps of response to meal. Symbol map shows A) first 45 minutes and B) last 45 minutes of meal. Each row represents a single volunteer. X represents las of response, *

represents no visualization of the anal sphincter. Meal exhibited a variable response regarding presence/absence, time of response, and types of motor patterns generated.



Supplementary Figure 2. Symbol maps of response to oral prucalopride. Symbol map shows A) first 45 minutes after taking oral prucalopride and B) last 45 minutes of the intervention. X represents lack of response, * represents no visualization of the anal sphincter. Great variability was observed following administration of oral prucalopride, with many non- or late responding subjects. Two of the subjects showed a response to oral prucalopride after the 90-minute mark for the intervention (not shown)

ACKNOWLEDGEMENTS

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study. Data for HAPWs and anal sphincter relaxation was analyzed by me, using ImageJ plug-ins designed by Sean P. Parsons. I also interpreted the results of each experiment and devised HAPW categories and HAPW index in collaboration with Dr. Huizinga and Dr. Chen. 3D images were prepared by me using MatLab code created by Sean P. Parsons. I created a first draft of the manuscript which was then edited and revised to reach a final version by myself and all other authors.

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Chapter 4: HAPW activity and autonomic dysfunction in patients with chronic constipation

INTRODUCTION

Although studies in healthy volunteers have provided a vast amount of important information regarding colonic motility, there is still no consensus as to what constitutes normal motility. This has made diagnosis of patients with constipation difficult. Many patients are often diagnosed with constipation, but do not respond to treatment and are referred for surgery although only 3% are truly good candidates and would benefit from it [1]. Currently, the main focus is placed on the HAPW as a marker for a healthy colon, due to its distinct shape and high amplitude which makes it easy to identify [2]. Emphasis is also placed on this motor pattern because of its importance in colonic transit. Due to its high amplitude and propagating nature, the HAPW is responsible for movement of content in the anal direction and rectal filling, and it precedes defecation [2]. However, there are some issues with using the HAPW as a marker for healthy motility. Although it is a very distinct motor pattern, the HAPW is very rare. HAPWs are often observed upon waking or following meal, though it is not uncommon even in healthy volunteers that they are not observed after eating. They occur between 4-10 times on average in a 24-hour period in an unprepared colon [3], however, during shorter diagnostic recordings of 4-6 hours, it is not uncommon that they are not observed at all unless strong stimulation is provided. Attempts have been made to create consensus statements on HRCM assessment and what constitutes a normal HAPW during this assessment such as the consensus statement created by Camilleri et al [2]. They used 75 mmHg as a cut-off amplitude for HAPWs [2]. However, they have not been able to provide any definitive features which should be observed in normal HAPWs, as different studies have often arbitrarily used different amplitudes obtained from different ways of measurement to define HAPWs. Additionally, most studies thus far have only considered HAPWs in isolation from the remaining activity in the colon, thus ignoring any other possible causes such as outlet dysfunction based on HRCM or impairment of the colo-anal reflex which presents as lack of coordination between motor patterns in the colon and the

response of the anorectal region which consists of contraction of the rectum and relaxation of the anal sphincter.

Furthermore, little emphasis has been placed on the control mechanisms of colonic motor activity. Colonic motility is generated as a response to stimuli which are mediated by intrinsic and extrinsic innervation of the colonic musculature [4–7]. The extrinsic innervation is responsible for communication between the brain and the colon and it plays an important role in colonic motility by mediating aspects of it such as the defecation reflex [8,9]. The defecation reflex is initiated through activation of the sacral sensory nerves in the sacral defecation center which is in response to rectal stimulation. These nerves are part of the sensory portion of the autonomic nervous system (ANS) [10,11]. From here signals are sent to the brain which activate motor neurons of the ANS that act to orchestrate colonic motor activity [10,11]. The importance of the role of the ANS has been demonstrated on multiple occasions through studies of spinal injury patients who demonstrated loss of the defecation reflex due to damage of sacral parasympathetic innervation [12]. Activity of the sympathetic and parasympathetic branches of the ANS can be measured through heart rate variability (HRV), and changes in their activity in association with motor patterns observed during HRCM have already been observed in healthy volunteers [13].

In the previous chapter, we were able to establish that HAPWs fall into one of 3 general categories based on their points of origin and termination: proximally contained, proximally originating and terminating in the transverse or descending colon or rectum with or without SPW, and transverse/descending originating. We also found based on the amplitude frequency distribution of HAPWs in healthy volunteers, that the minimum cut-off to differentiate between them and low-amplitude propagating pressure waves (LAPWs) which can still propel content but may not be as effective as HAPWs is 50 mmHg. Coordination between activity in the proximal colon and the anorectal region was observed even when the activity was contained within the ascending colon, such that even proximal only HAPWs were associated with more than 20% of anal sphincter relaxation. Therefore, the objective of this study is to combine motor pattern characteristics from HRCM in healthy volunteers as well as what is known about

changes in ANS associated with colonic motility, and apply this to constipation patients to search for the pathophysiology underlying the constipation as an essential part of its management.

METHODS

Please refer to chapter 2.1 to 2.7

RESULTS

HRCM results

A total of 15 patients underwent diagnostic HRCM. Of the 15 patients, 9 were pediatric ranging from 6 to 17 years of age. The adult patients ranged between the ages of 23 to 61. A total of 140 propagating motor patterns were identified across 13 patients. From the 140 propagating motor patterns 25 were LAPWs and the remainder (115) were HAPWs. Five patients showed no propagating activity, and in one patient there was propagating activity but only in the form of LAPWs. Four patients showed normal HAPW activity, two of which were adults and the other two children.

The general distribution between the 3 spatiotemporal categories was similar between patients and volunteers. Of the 115 HAPWs, the most prevalent were those originating in the transverse or descending colon, making up 58% of the HAPWs compared to 49% in healthy volunteers. These were followed by HAPWs which originate in the proximal colon and terminate in the transverse or descending colon with or without SPWs. These comprise 26% of the HAPWs observed in patients while in volunteers they made up 29% of the HAPWs. Finally, the least prevalent category were HAPWs which were contained within the proximal colon either with or without SPWs, and they made up 16% of the HAPWs in patients compared to 21% in volunteers.

In comparison to healthy volunteers, patients showed a significantly lower HAPW amplitude during proximal balloon distention ($p<0.0001$), meal ($p=0.01$), and rectal bisacodyl ($p<0.0001$) (Figure 1).

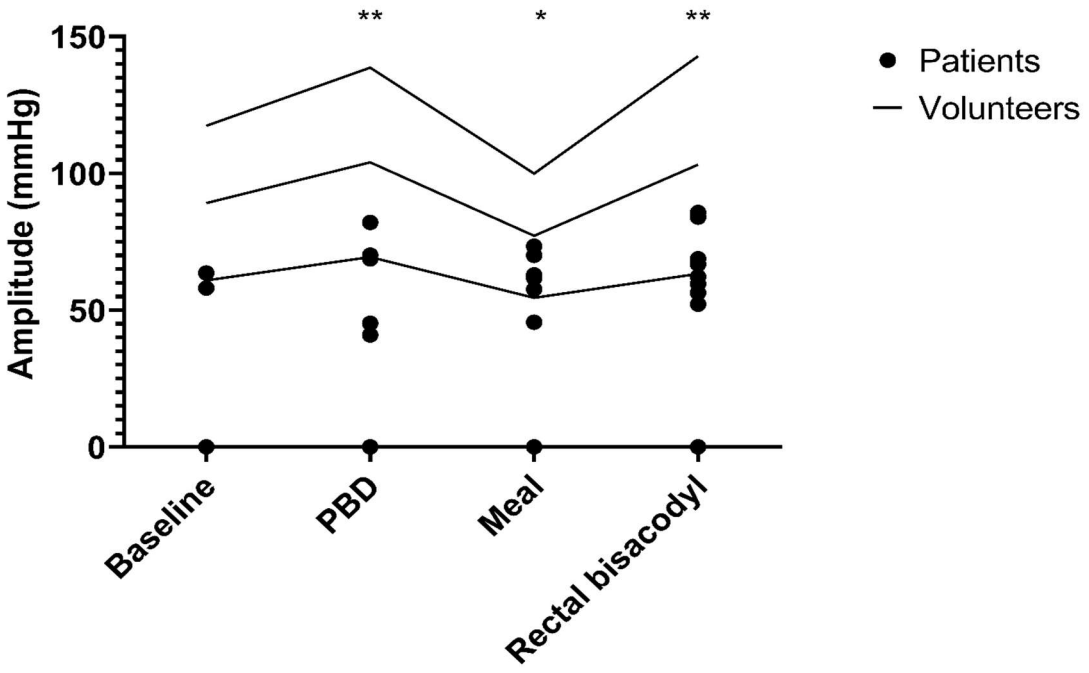

Figure 1. Average ± 1SD amplitude of healthy volunteers, and amplitudes of individual patients. All patients show amplitudes lower than the average amplitude of healthy volunteers for all interventions. PBD, meal, and rectal bisacodyl average patient amplitudes are significantly different than those of healthy volunteers. *p=0.01, **p<0.0001

Five patients showed some response, but it was classified as weak compared to healthy volunteers. A response was classified as weak if it mainly consisted of LAPWs but still showed some HAPWs, it occurred during only one or two sessions of HRCM, only included one type of HAPW, or the amplitude of the HAPWs was more than 1 SD below the mean.

Overall, patients could be classified into strong responders, weak responders and non-responders.

Strong responders

Four patients belonged to this category (P1, P2, P7, and P8) (Figure 2). This group of patients was characterized by response to all of the interventions with one or more categories of HAPWs, with or without response during baseline (Table 1) (Box 1).

Box 1. Summary of case report related to P1

P1 had history of extensive sacral spinal cord injury. Assessment of the motility of this patient showed a colonic response corresponding to that of healthy volunteers, but with absence of coordination between proximal areas of the colon, and the sigmoid and anorectal regions. The absence of coordination is also supported by the supine and orthostatic HRV (Table 1) which showed high sympathetic and low parasympathetic tone, as well as HRV assessment during manometry where occasional high sympathetic and to a higher extent low parasympathetic activity was observed. Observations from this patient point to the importance of the parasympathetic innervation of the sacral defecation to the colon, which when impaired leads to constipation even in the presence of normal motility as it is seen in P1.

Table 1. Patient response to interventions during HRCM and supine and orthostatic HRV and comparison to healthy controls. Normal healthy control values are ± 1SD from the mean. Green columns indicate strong responders, yellow are weak responders and orange are non-responders. Blue text indicates lower and red indicates higher than normal range

	Healthy Controls	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8	P 9	P 10	P 11	P 12	P 13	P 14	P 15
HAPW amplitude* (mmHg); Type of HAPW in brackets; HAPWs with improper relaxation or contraction/all HAPWs																
Baseline	60.9-117.4 (1, 2, 3) 0/33	63.6 (2) 0/2	-	-	-	-	-	58.1 (3)	-	-	-	-	-	-	-	-
Meal	54.5-99.9 (1, 2, 3) 0/48	61.7 (1) 1/1	70.0 (1,2,3) 0/5	57.6 (2) 0/2	-	-	-	62.8 (3) 1/4	62.9 (2,3)	-	-	45.6 (1) 0/1	-	73.4 (3) 0/3	-	-
PBD	69.4-138.6 (2, 3) 8/45	70.1 (3) 1/2	82.1 (3) 0/2	-	-	-	-	45.2 (3) 0/3	68.7 (1,2,3) 4/7	-	-	-	40.9 (2) 0/1	-	-	-
Bisacodyl	63.3-142.8 (1, 2, 3) 5/59	84.0 (2,3) 1/7	85.7 (3) 0/7	-	-	62.2 (3)	-	52.2 (3) 1/6	66.8 (2) 1/4	56.4 (2) 0/6	-	68.8 (1) 0/4	-	59.6 (3) 1/2	-	-
Supine and Orthostatic HRV																
Supine	RSA 5.4-7.8	6.8	6.9	7.5	6.9	5.7	5.8	7.0	7.0	8.0	6.7	6.0	6.2	6.1	6.0	7.3
	SI 7.9-49.8	23.9	48.7	14.8	46.2	71	50.3	19.4	85.2	21.7	31.5	61.5	25.3	79.9	73.1	23.4

	HR 55-76	60.6	64.9	52.3	69.8	75	73	73	101	67.6	70	73	62	80	87	62.9
Supine to standing	RSA 4.2-6.6	3.2	5.9	5.9	5.2	3.3	3.9	5.3	5.9	7.4	5.3	4.2	4.6	5.0	5.4	5.3
	SI 27.5-84.9	96.1	70.1	37.3	192.2	125	166.5	47.0	146.8	42.1	56.4	219.5	42.9	89.1	97.3	36.7
	HR 70-93	73.2	79.9	66.7	88.3	87	88	80	107.3	82.1	84	105	79	93	105	86.5

Although patients in this category responded to all interventions, their mean HAPW amplitudes were on the lower of the normal range when compared to healthy volunteers. With regard to amplitude, P1 and P2 showed completely normal motility with average amplitudes within normal ranges during all interventions. P7 and P8 had mean amplitudes which were still considered within the HAPW as opposed to LAPW range, however, during some interventions the amplitudes were outside of normal range when compared to healthy volunteers.

Only P2 from the strong responders showed entirely normal motility including normal coordination of colonic motor patterns with relaxation of the anal sphincter. In this patient there were no HAPWs during which there was a contraction or lack of relaxation of the anal sphincter. In P1 and P8, a lack of coordination was observed between occurrence of the HAPWs and relaxation of the anal sphincter. This lack of coordination was observed in association with at least one HAPW per intervention, and during meal and PBD half or more of the HAPWs showed this pattern.

With their normal motility and coordination, P2 also showed normal supine and orthostatic HRV indicating normal autonomic function. Both P1 and P8 who had lack of coordination between their HAPWs and anal sphincter relaxation also showed significantly increased Si during their supine and orthostatic HRV (Table 1), indicating higher than normal sympathetic nervous system tone and reactivity.

Taking into account the HRCM and HRV data, the diagnosis of these patients is normal motility, with impaired anorectal coordination to which impairment in ANS regulation is a contributor.

Weak responders

Five of the patients were categorized as weak responders (P3, P5, P9, P11, and P13) (Figure 3) (Box 2). Characteristic response of patients in this category included response to only 1 or 2 of the interventions administered, and response with only one type of HAPW with a consistent point of termination in each individual patient (Table 1). Three of the five patients only responded to one intervention during their individual assessment. In two of the instances the response was to rectal bisacodyl and in one instance it was to meal. In each of the three patients who only responded to one intervention the average

Box 2. Summary of case report related to P5

The history of P5 included significant pain in the lumbar area of the back, as well as lifelong constipation and abdominal pain. HRCM assessment of the motility of this patient revealed weak motility with the only HAPW response observed during rectal instillation of the potent stimulator bisacodyl. The patient's weak motility is supported by their significantly high SI during autonomic pre-assessment (Table 1), as well as the constantly high SI during all interventions of HRCM. High SI is an indication of increased sympathetic nervous system regulatory activity, which is known to inhibit colonic motility. Observations from this patient show the extent of motility impairment which can occur in the presence of dysautonomia and emphasize the importance of ANS assessment in the diagnosis of constipation pathophysiology.

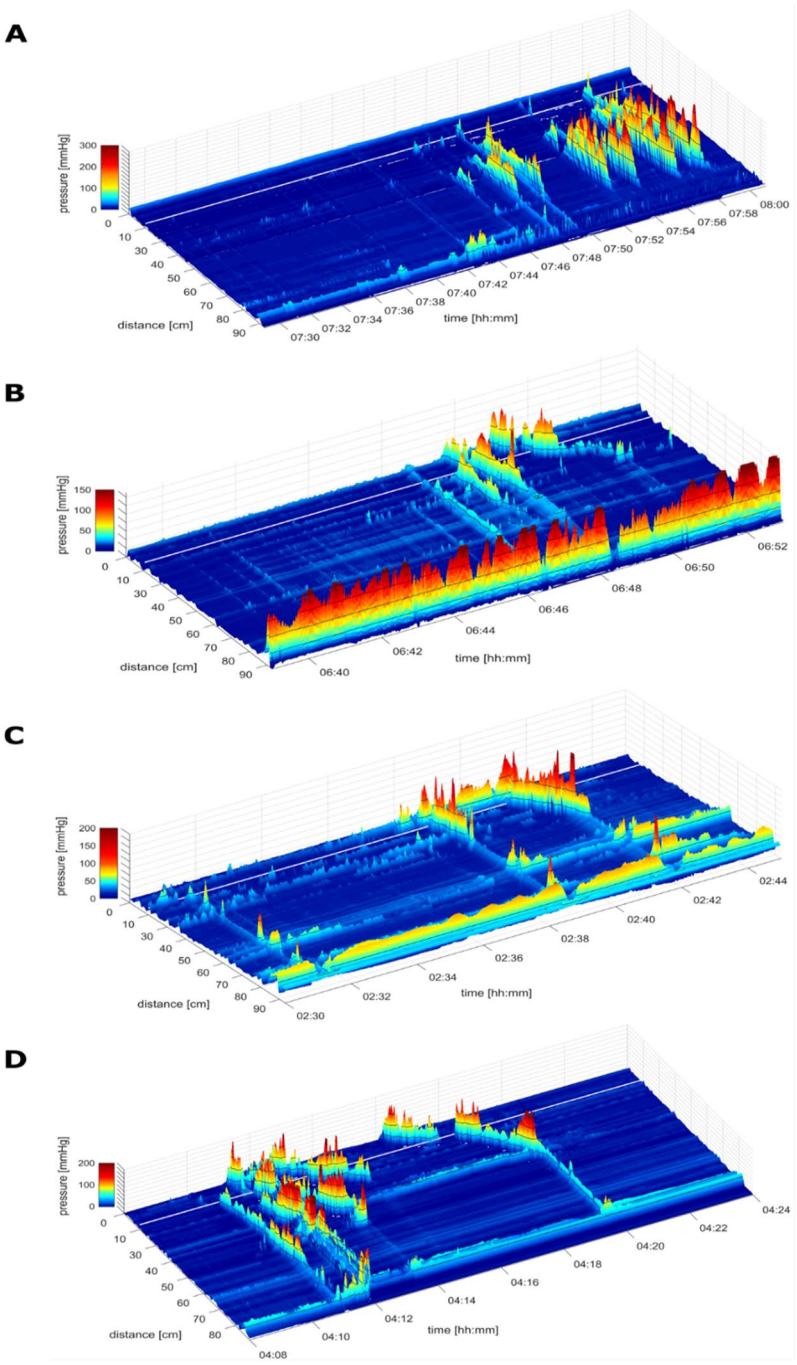

Figure 2. Responses of the strong responder group. Four of the patients assessed showed normal motility with regards to HAPW amplitude and propagation. A and B are adult patients, while C and D are pediatric. A and C show the response of the patients during an entire session of rectal bisacodyl, while B and D show the response during an entire session of PBD. White line represents a 10-cm balloon

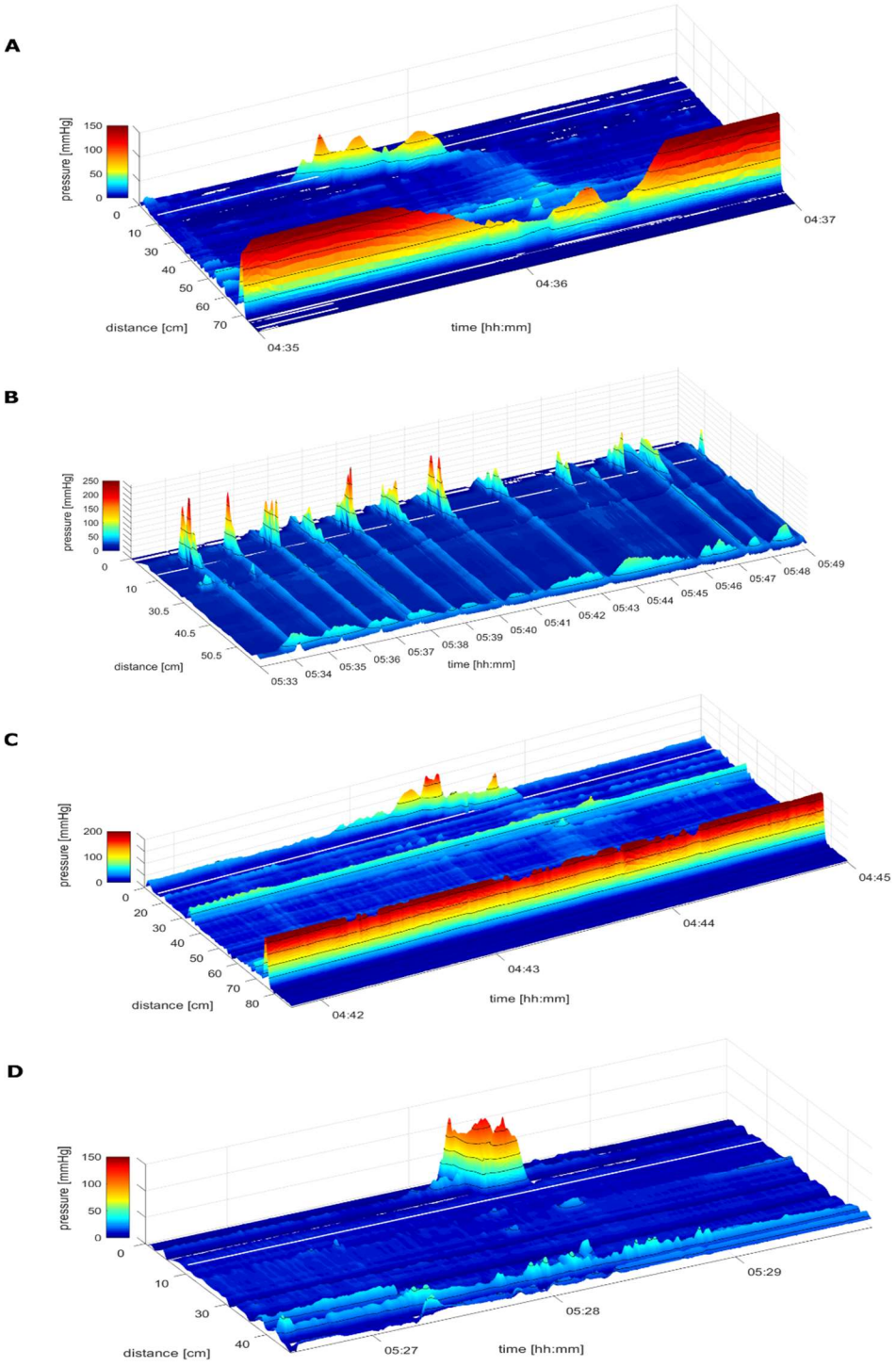

Figure 3. Responses of patients in the weak responder group. A and B show responses in adults, while C and D show responses in pediatric patients. A and C show response to meal, and B and D show response to rectal bisacodyl. In all patients HAPWs are of low amplitude and short propagation length. White line represents a 10-cm balloon

amplitude was lower than the normal range determined by healthy volunteers. Two of the patients responded to two interventions, and in both instances those interventions were meal and rectal bisacodyl. In both patients one intervention was outside the normal and the other intervention was within the normal range. In one of the two patients the average amplitude was lower than 50 mmHg, and this occurred during meal.

Despite the lower HAPW amplitude, all patients had normal coordination between the HAPWs and anal sphincter relaxation.

60% of the patients in this category showed a highly elevated stress index both during supine and orthostatic HRV, indicating a high level of sympathetic inhibition.

Considering the HRCM and HRV assessment of the patients in this group, the overall diagnosis is presence of some residual motility albeit weak, in association with high sympathetic nervous system activity as shown by elevated SI.

Non-responders

Five patients belong to this category (Table 1) (Box 3). One of the five patients responded to proximal balloon distention, however, it was only with a single LAPW. The other 4 patients had no HAPWs during any of the interventions.

Three of the patients showed high stress index levels during their supine and orthostatic HRV assessment.

Box 3. Summary of case report related to P4

This patient had a tendency of constipation since childhood, which worsened after injury to the sacral spinal cord with inability to achieve bowel movement for up to 28 days. Assessment of the motility and autonomic nervous system activity in this patient revealed high sympathetic nervous system activity which is known to inhibit motility, along with decreased propagating motility in the colon consistent with the slow transit shown by the shapes study. This may be secondary to the injury and pain in the tailbone region which could have led to damage of nerves in the sacral defecation center, which innervate the distal areas of the colon where there is diminished activity in this patient.

DISCUSSION

In the present study we observed that 60% of the chronic constipation patients who underwent HRCM were able to generate HAPWs in response to at least one of the interventions administered to them. This observation is only slightly lower compared to previous literature on patients which has found that only about 71% of patients are able to generate HAPWs, whereas in healthy volunteers at least one HAPW can be observed in every individual [14]. From the 40% of patients who did not respond with HAPWs, two were adults and four were children one of which did have propagating motor patterns in the form of LAPWs. The patients who did respond could be classified into 2 different categories: strong responders with ability to generate normal HAPWs, and weak responders.

In the strong responder category of patients were 2 adults, and 2 pediatric patients. The strong responders could be categorized by HAPW response to all interventions administered to them. Two of the strong responders were even able to generate spontaneous HAPWs without any stimulation during baseline. Based on parameters determined in the study of healthy volunteers in chapter 1 such as the 50 mmHg cut-off amplitude for normal HAPWs, these patients have normal colonic motility despite experiencing constipation. Studies of patients in literature thus far, have come to a general consensus that constipation is marked by an absence of HAPWs over a 24-hour recording, weakened response to pharmacological stimuli, and decease in antegrade propagating activity [2,14]. Additionally, some studies have found there is a significant increase of retrograde propagating activity, and the antegrade propagating waves are of low amplitude and a very short propagating distance [14]. However, in the present study of chronic constipation patients we show that those are not consistent markers of constipation which can be generalize to all patients. Therefore, it is necessary that more factors such as coordination of the colo-ano-rectal region are taken into consideration in addition to the nature of a patient's propagating motor patterns.

Another commonality in these patients is that the coordination between their motor patterns and their anorectal region is poor which indicates an impairment in the colo-anorectal reflex. In these patients HAPWs are not always associated with relaxation of

the anal sphincter, and in fact, contractions of it to up to 250% from the resting pressure were observed in association with HAPWs.

Although the gut has been considered to be largely controlled by the enteric nervous system (ENS) which is thought to be independent of the central nervous system, the ENS does not act autonomously and it works in concert with the sympathetic and parasympathetic branches of the ANS [10,15]. The sympathetic branch of the ANS innervates the colon from the sympathetic chain located between L2 to L5 of the spinal cord, and it provides inhibitory signals [16,17]. Parasympathetic innervation of the colon which promotes motility is dual, arising both from the vagus nerve which innervates the proximal and transverse colon, as well as the sacral defecation center located between S2 to S4 of the spinal cord which innervates the descending colon and rectum [8,9,15–17]. The recto-spinal pathway contains a neuronal loop which sends sensory information to the sacral spinal cord and provides immediate motor feedback to the distal colon and rectum thus initiating the defecation reflex [8,9,15]. While this reflex is short and fast, there is also parasympathetic sensory information sent from this area to a region in the brainstem called the spinosolitary tract (NTS) [16]. Here, sympathetic and parasympathetic information is integrated from both the spinal and vagal pathway, and motor information is sent back to more proximal areas of the colon [16]. This integration of vagal and spinal parasympathetic, and sympathetic input facilitates the second, long defecation reflex which allows for generation of more proximally originating motor patterns with stimulation of more distal areas of colon to push more content in the anal direction.

The strong responders in the present study have normal sensory function in both the short and long defecation reflexes, as they are able to generate normal HAPWs throughout the entire colon after stimulation. However, despite their normal motor patterns they show a lack of coordination between the HAPWs they are generating and the anal sphincter, a poor colo-anal reflex, and paradoxical contractions of the sphincter reaching double the resting pressure. Localized dysfunction of the anorectal region in these patients may point to impairment in the parasympathetic motor input from the

spinal defecation center. The high sympathetic index in these patients may also point to localized sympathetic inhibition of the recto-anal area only.

Patients in the weak responder category had responses which were more consistent with what has been described in the literature thus far [14, 17]. Their motility was categorized by response to only one or two stimuli, with only one of the three categories of HAPWs [18], and a consistent point of HAPW termination. Integrating their HRCM and HRV results, and the neuroanatomy of extrinsic innervation of the colon, it appears that this group of patients may have impairments in the longer defecation reflex along with sympathetic inhibition. In studies of healthy humans where proper coordination of all sources of innervation occurs, it has been shown that there is a spatio-temporal pattern which exists between HAPWs preceding defecation [19]. In this spatio-temporal pattern, there is a constant shift in the origin of HAPWs such that none of them individually span the entire colon, however, placed together in a sequence they cover the entire length to move content aborally in a step-wise manner, as it is observed in the healthy volunteer of figure 7 in chapter 3 [15]. However, in studies in patients, an adynamic zone was described in the middle of the colon due to short HAPW propagation length [14]. This is consistent with the observations in patients of the weak responder category, where HAPWs consistently originated and terminated at the same points in the colon, creating an adynamic zone following the termination point where there is no propagating activity that can move content in the anal direction. This may point to a disruption in the coordination between the vagal and sacral sources of innervation in the colon, as the shift between the two occurs around the adynamic region described. A disruption between the two sources could lead to initiation of the sequence of HAPWs in the proximal region, and a disruption when the sequence reaches a shift in innervation. As it has been previously proposed, this would allow for preservation of proximal propulsive motility in the colon [20], although it would be insufficient for normal defecation. Despite this, preservation of proximal motility in the colon could point to the ability of a patient's colon to still generate motor activity. This would steer more patients away from invasive unnecessary surgical methods as only 3% of them are good candidates and would benefit from it [1] and more towards non-invasive methods which make use of the knowledge of extrinsic colonic innervation such as neuromodulation.

In conclusion, integration of motility data along with data on autonomic nervous system activity and extrinsic innervation of the colon has allowed for the beginning of more detailed constipation pathophysiology. Previous studies of constipation patients have largely focused on the motility of the colon, including length and amplitude of HAPWs, but not much focus has been placed on the role of the extrinsic innervation of the colon. Focusing on this aspect of control of motility will not only shed light on previously unidentified constipation pathophysiologies, but it will also allow for more focused treatments such as modulation of the extrinsic innervation as opposed to symptom-based treatments such as osmotic laxatives and prokinetics.

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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.

HRCM procedure and protocol

Please refer to chapter 2.1 to 2.4

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].

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 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 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 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 2). The amplitudes of

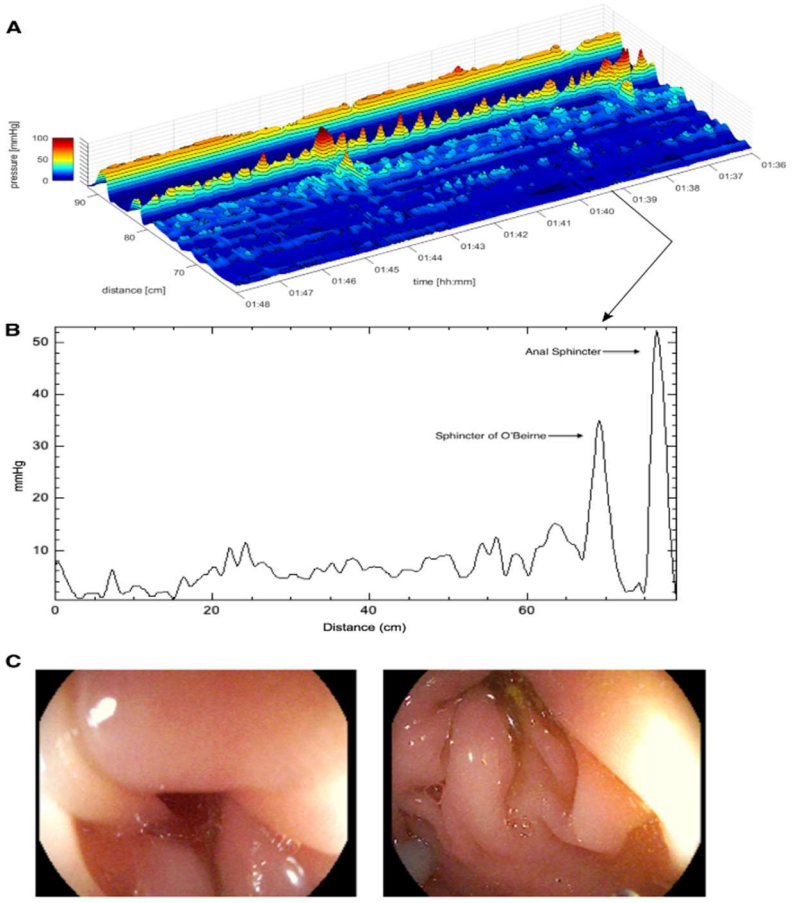

Figure 1. The prominence of the sphincter of O'Beirne

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.

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 2, 3D), whereas the sphincters normally relax in healthy subjects [10].

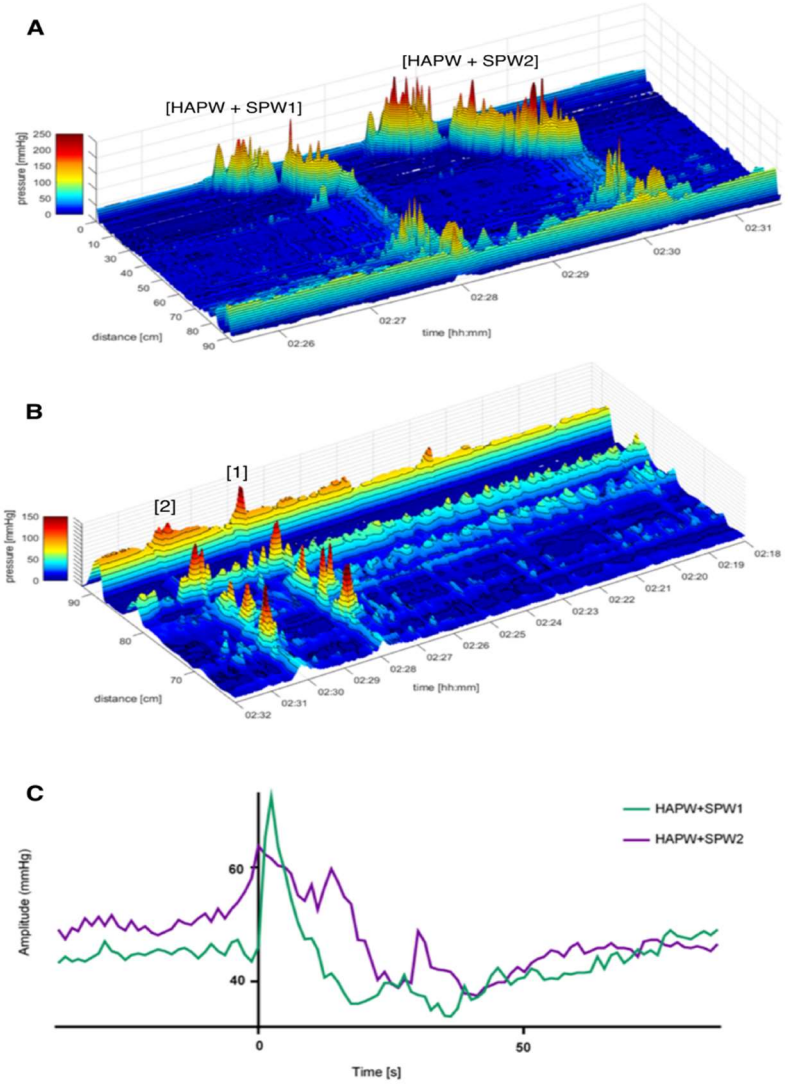

Figure 2. Motor patterns at baseline

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

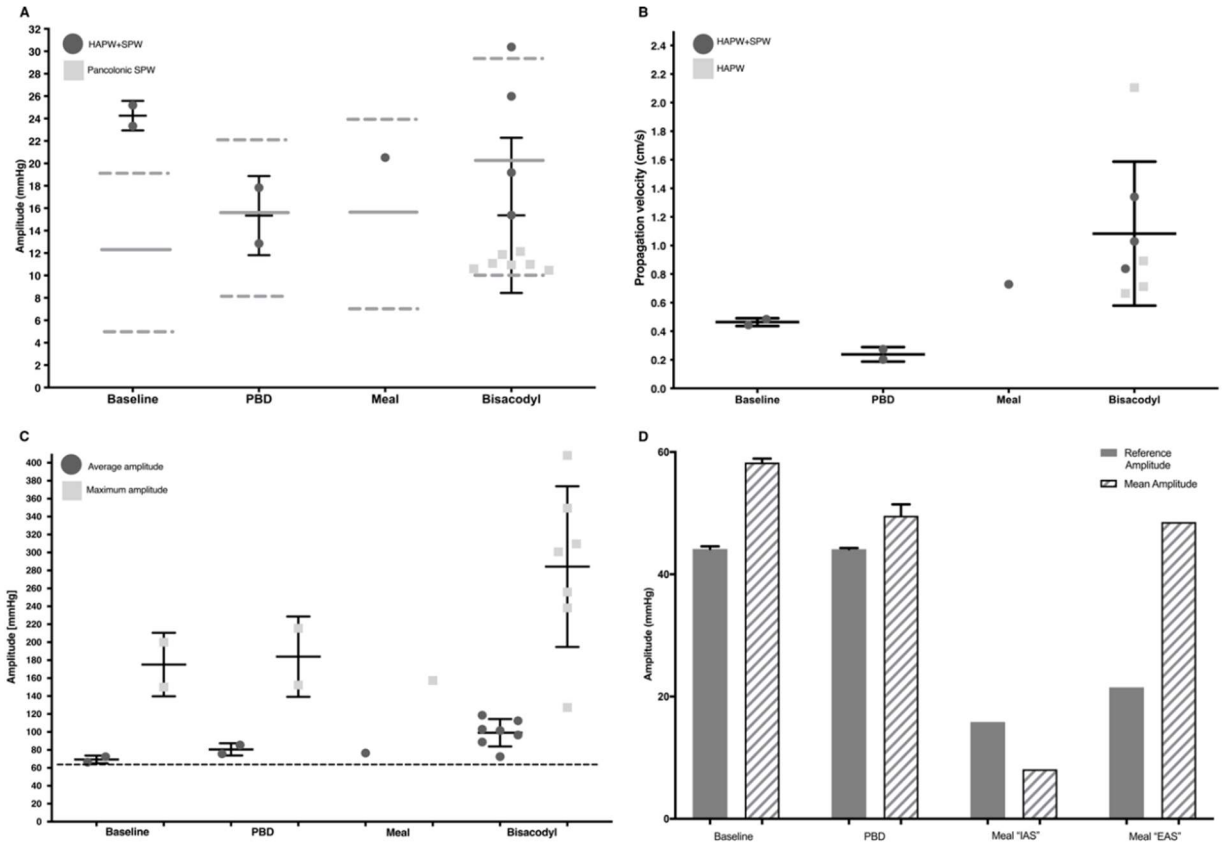

Figure 3. Motor pattern and anal sphincter quantification via intervention

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 4), based on Chen et al. 2019 [18].

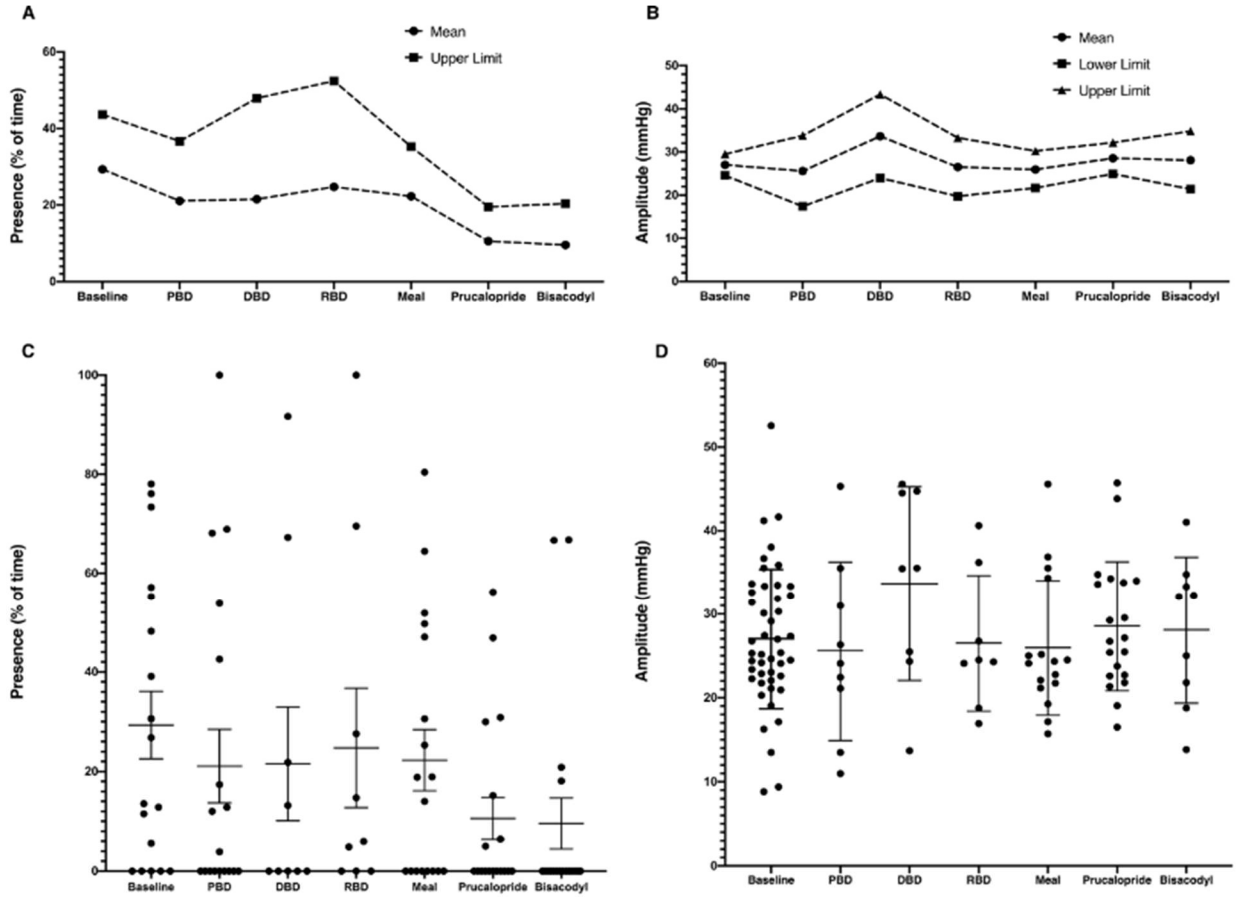

Figure 4: Comparison of the sphincter of O'Beirne's amplitude and % presence to healthy controls

A. Percentage of time the sphincter of O'Beirne is present by intervention. The average value was calculated based on 18 healthy volunteers. The upper limit is based on a 95% confidence interval. Distal balloon distension: n=9; rectal balloon n=7.

B. Average amplitude of the sphincter of O'Beirne via intervention. The mean was taken for each intervention. The upper and lower limits were determined based on a 95% confidence interval. The average value was calculated based on 18 healthy volunteers. Baseline n= 46 (90 mins), Proximal Balloon distension n=9 (15 mins), Distal Balloon Distension n=8 (15 mins), rectal balloon distension n=8 (15 mins), meal n=16 (90 mins), Prucalopride n=20 (90 mins), Bisacodyl n=9 (30 mins).

C. Percentage of time the sphincter of O'Beirne was present by intervention with standard deviation error bars from the mean.

D. Amplitudes of the sphincter of O'Beirne via intervention with standard deviation error bars from the mean

Response to stimuli

Proximal balloon distension induced a pair of HAPW-SPWs (Figure 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 3). No relaxation of the sphincter of O'Beirne or the anal sphincters was observed in response to approaching SPWs (Figure 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 6). The external anal sphincter (the distal part of the anal canal) contracted from 20 to 50 mmHg (Figure 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 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

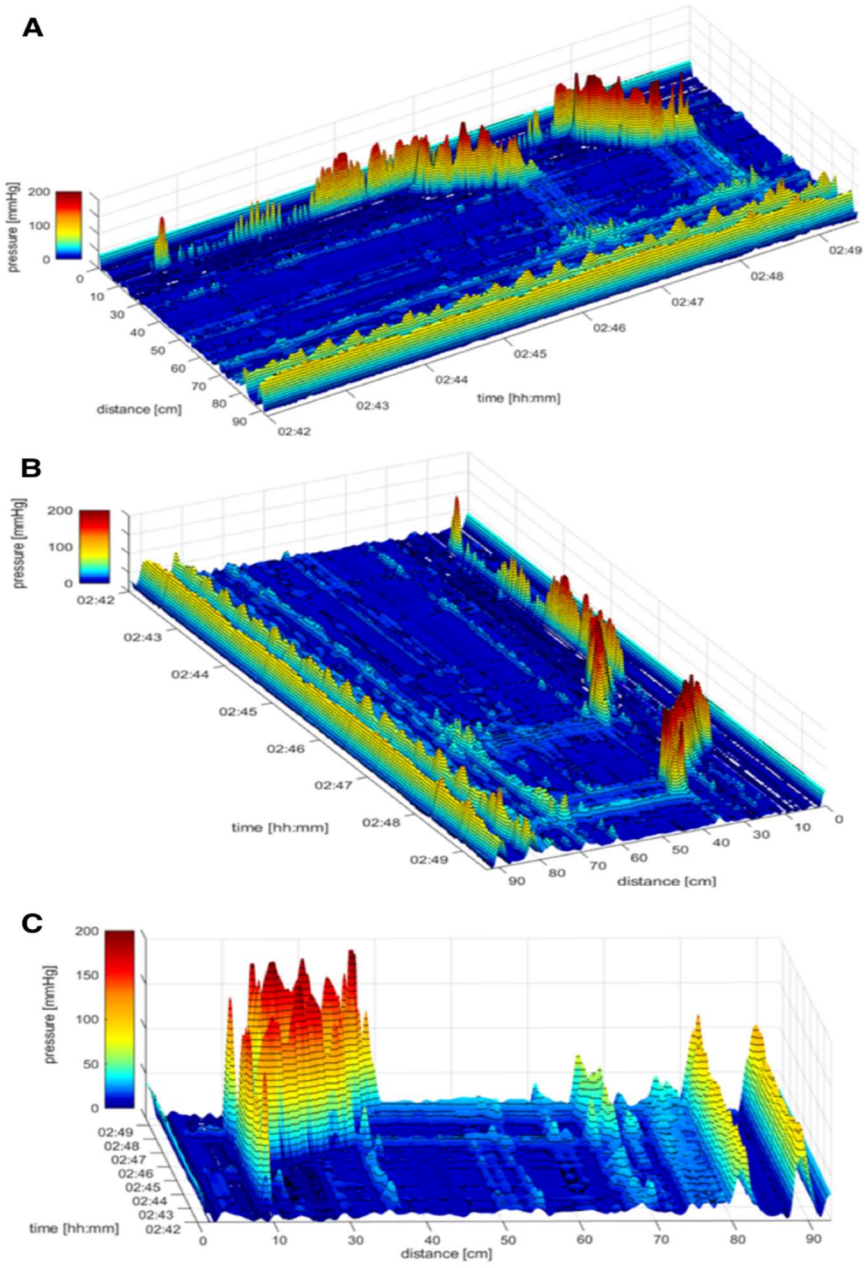

Figure 5. Motor patterns in response to proximal balloon distension

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.

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.

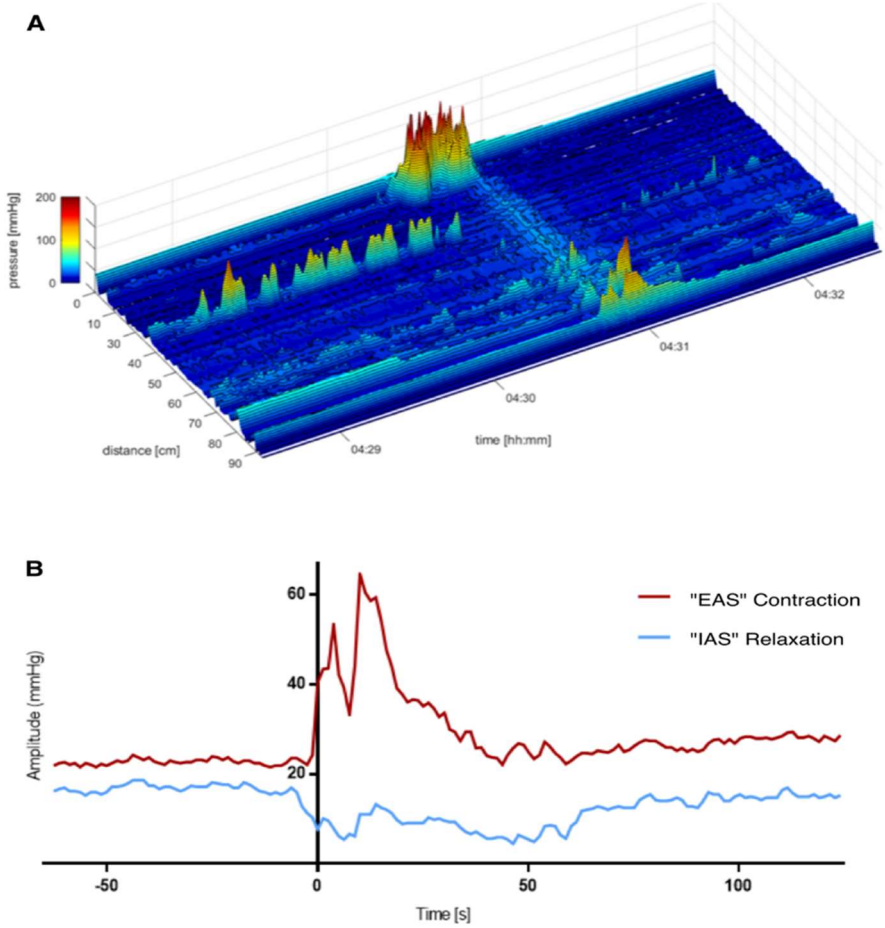

Figure 6. An HAPW-SPW in response to the meal

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).

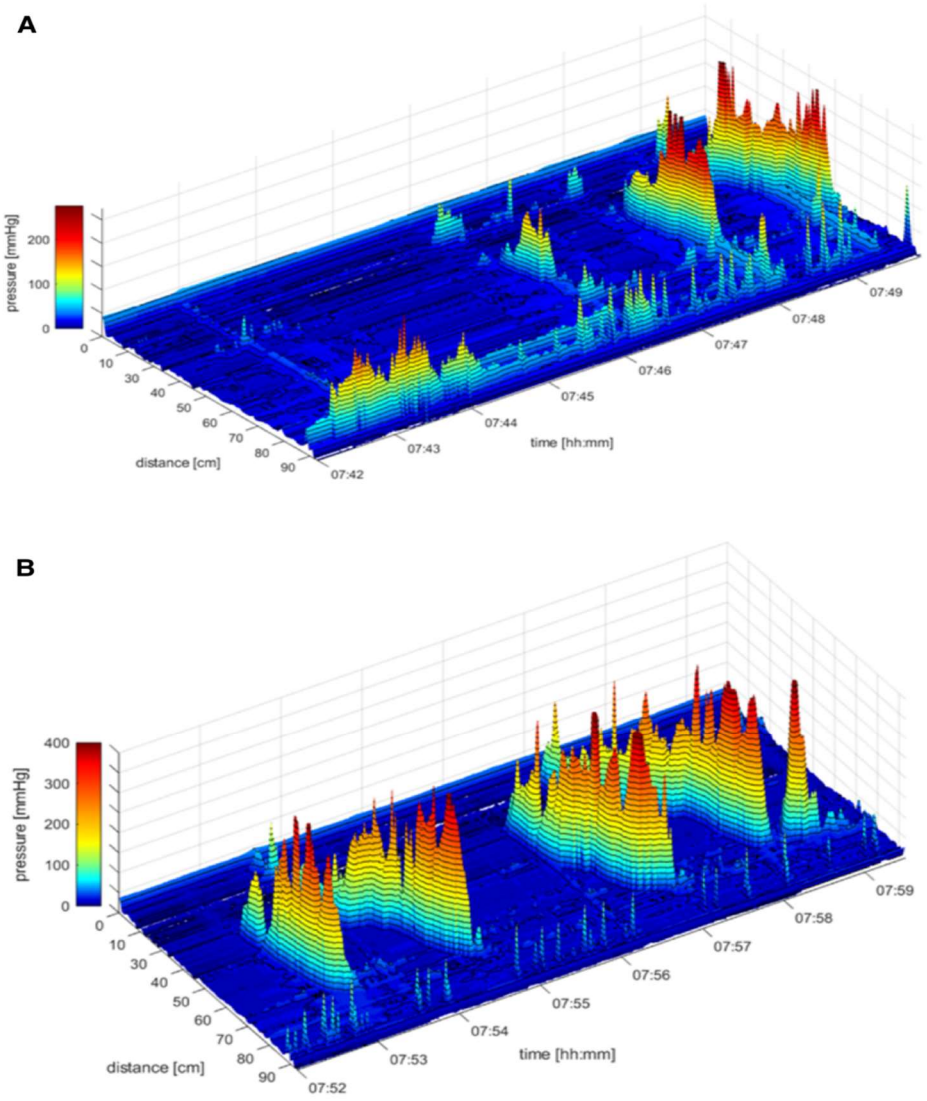

Figure 7. Motor patterns in response to rectal bisacodyl.

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 $\pm$ 0.2 cpm, at an amplitude of 16.8 $\pm$ 1.8 mmHg and a propagation velocity of 0.27 $\pm$ 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 $\pm$ 0.7 cm on average. The cyclic motor pattern occurred periodically with an average duration of 6.3 $\pm$ 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: Effects of an acute, one-time, Low-Level Laser Therapy session as Assessed by High-Resolution Colonic Manometry

INTRODUCTION

The study of the autonomic nervous system's (ANS) role in healthy colonic motility [1], as well as the patient case reports from the previous chapter outline the importance of autonomic control on colonic motility. Hence, when autonomic dysfunction occurs due to physical stress or injury of the lumbosacral defecation center, steps could be taken to neuromodulate the ANS and restore its normal function. Through studies of the rectal mucosal blood flux which is heavily affected by the extrinsic autonomic innervation, it has already been proven that electrical stimulation of the sacral region of the spinal cord can lead to improvements of spinal innervation to the colon; through symptom reporting of patients it has also been shown that it can lead to improvement of defecatory issues and quality of life [2,3]. One current method includes surgical implantation of electrodes in the sacral spinal cord which is highly invasive and can lead to side effects such as pain, inflammation, battery failure etc. Other methods of non-invasive nerve stimulation include various forms of transcutaneous electrical nerve stimulation (TENS) [4–6]. We are the first to explore the application of low-level laser therapy (LLLT) to this particular condition although it has been used successfully in the treatment of painful diabetic neuropathy as well as other forms of neurorehabilitation [7–9]. LLLT which is also known as photobiomodulation, involves application of specific frequencies of light to tissues to promote their regeneration and healing. LLLT has a photochemical effect, meaning that the application of light and its absorption cause a chemical change in the tissue [10,11]. There is ongoing research about the cellular and molecular mechanisms through which LLLT promotes healing. However, the current understanding is that the wavelength of light which is used during LLLT interacts with chromophores-specifically cytochrome C oxidase (Cox) [9–11]. This increases ATP production in the cell giving it more energy, while also increasing production of reactive oxygen species (ROS) [9]. Although ROS are often seen as damaging, they are a normal product of cellular respiration, and in lower

concentrations can be beneficial to cells [12]. In the case of treatment with LLLT, ROS activate redox-sensitive transcription factors such as NF-κB, which lead to upregulation of stimulatory and protective genes [9–11]. Increased ATP production from LLLT also upregulates production of nitric oxide (NO) which is a potent vasodilator and allows for increased blood flow and therefore nutrient delivery to the areas being stimulated [9–11]. The neuro-reparative effect through photobiomodulation of LLLT based on the previously described mechanism has thus far been proven in painful diabetic neuropathy, and various other neurological conditions [7–9]. The purpose of this study is to examine whether one-time stimulation with LLLT leads to acute changes in colonic motor activity which would provide evidence for communication between stimulated nerves and colonic motor activity. However, the parameters used are the same as those used for longer term treatment protocols.

METHODS

Please refer to chapter 2.4 and 2.8

RESULTS

9 patients received LLLT during their HRCM. In 6 of these patients, motor patterns were observed during this intervention. There were 21 SPWs associated with stimulation by array, as well at one LAPW-SPW and these were distributed across 5 patients. Stimulation with the probe, showed 5 SPWs and one LAPW in 4 patients. When compared to the 30 minutes of baseline, 5 showed no changes in their motility, while in the other 4, increase in motility from baseline was observed. In the 3 patients that did not show any activity during LLLT stimulation, this was also true during baseline. The increase was in the form of an increased number of SPWs, or a shift from no or simultaneous motor patterns to propagating ones. Although HAPWs were not observed in any of the patients, there were 2 LAPWs in 2 different patients (Figure 1, Figure 2). One of the LAPWs had an amplitude of 35 mmHg and was followed by a SPW (Figure 1). The LAPW in the second patient had an amplitude of 36 mmHg and it was contained within the ascending colon (Figure 2).

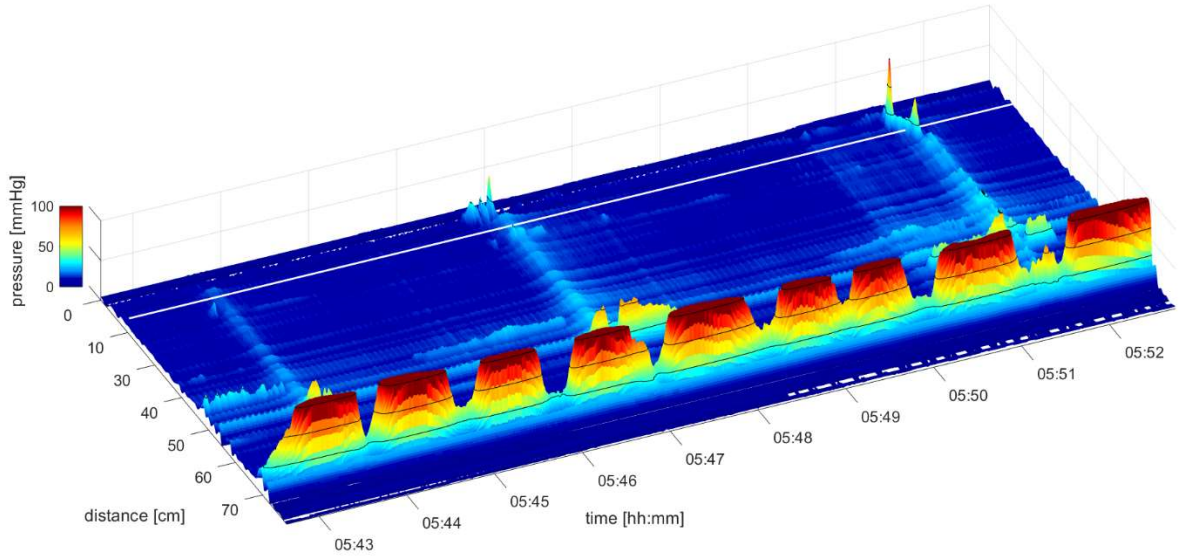

Figure 1. Low-amplitude pressure wave in response to stimulation by LLLT array. Patient responded with 2 SPWs followed by a low-amplitude propagating pressure wave which transformed into SPW. Anal sphincter relaxation with a longer duration than that of the rhythmicity was observed in association with the propagating pressure wave.

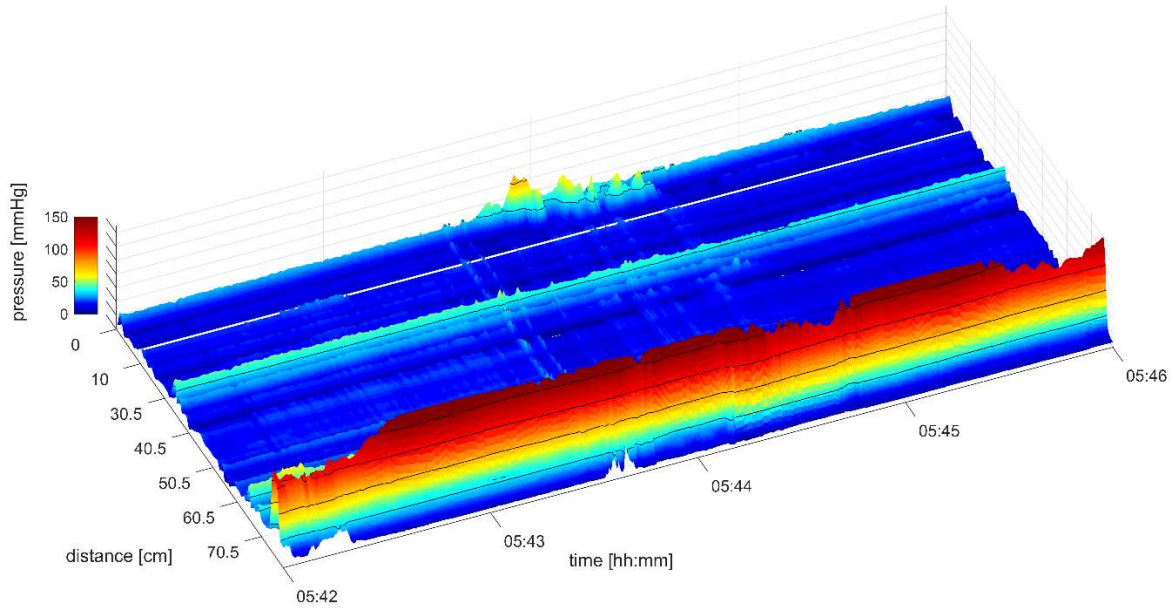

Figure 2. Low-amplitude pressure wave in response to LLLT laser probe. Pressure wave starts in the ascending colon and terminates within it as well. There is no anal sphincter relaxation associated with the pressure wave

The response to LLLT was dominated by SPWs (Figure 3). In the 4 patients who had an increase in motility compared to baseline there were a total of 16 SPWs in periods of 30 min. compared to 4 SPWs during an equal amount of time at baseline. The mean amplitude of the SPWs was 20.9 ± 1.5 mmHg compared to 22.3 ± 0.7 at baseline. From the 16 SPWs, only 2 were associated with relaxation of the anal sphincter of more than 20%, in comparison to baseline where 3 out of the 4 SPWs were associated with significant relaxation. During 3 of the SPWs the anal sphincter was not visualized, and in the rest there was either no change in anal sphincter rhythmicity or a contraction of it. However, even in patients who did not have an increase in motor activity there was still SPW-dominant activity. Considering all 6 patients who had some motor activity during stimulation, there was a total of 27 SPWs observed. The mean amplitude of the SPWs was 20.6 ± 0.9 mmHg compared to 19.9 ± 1.0 mmHg during all of the baseline sessions. From the 27 SPWs, 8 of them were associated with anal sphincter relaxation, 17 were not associated with any changes or with a contraction of the sphincter, and in 3 SPWs the anal sphincter was not visualized. The average SPW-associated relaxation of the anal sphincter (taking only those data into account where > 20% relaxation was observed) was 37.2 ± 5.0%.

When separating array stimulation from probe stimulation it was found that from the 27 total SPWs observed, 5 were in response to probe and 22 were in response to array. The 2 LAPWs which were observed were distributed evenly between array and probe, with the LAPW-SPW being observed during array and the solitary LAPW observed during probe. The average amplitude of array-associated SPWs was 21.8 ± 0.9 mmHg, and the average amplitude of probe-associated SPWs was 15.6 ± 2.2 mmHg.

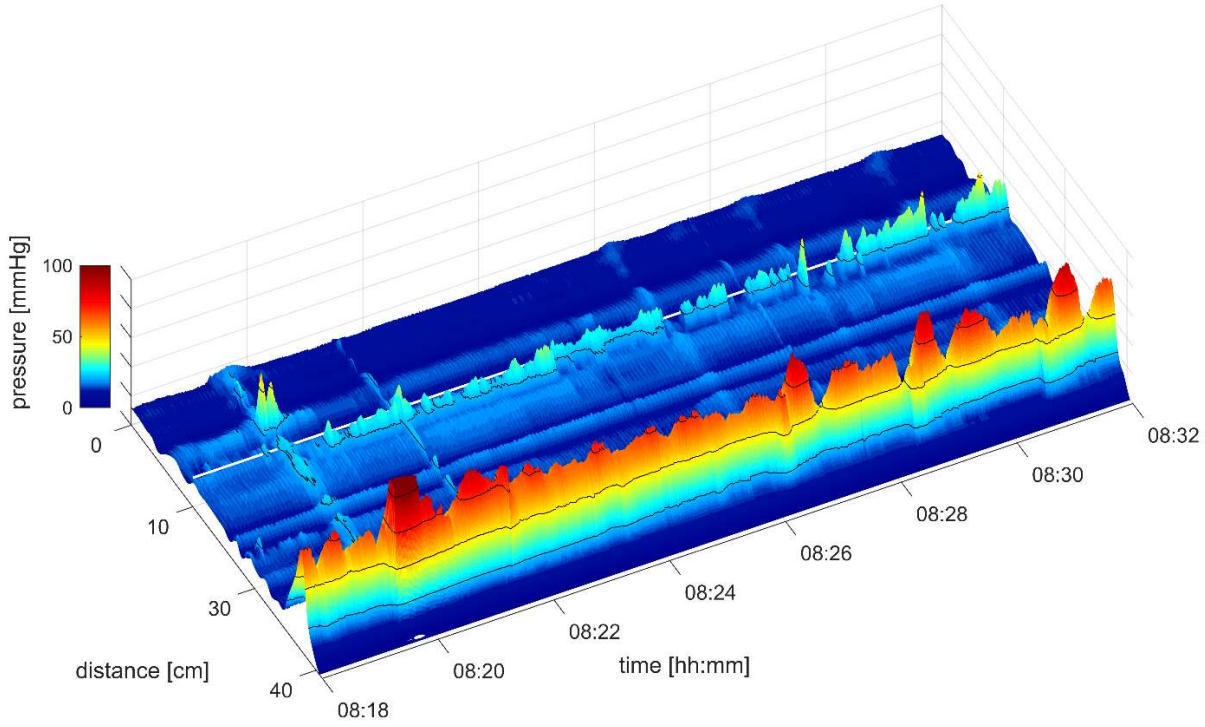

Figure 3. SPWs in response to treatment with LLLT array. SPW amplitudes range between 20-26 mmHg. All SPWs are associated with transient relaxations of the anal sphincter with the exception of the first which is associated with a contraction of the sphincter to 190% from rest.

DISCUSSION

Based on the data from 9 patients who received one-time stimulation with LLLT, this method shows promise in stimulation of colonic activity through photobiomodulation of the nerves in the lumbo-sacral spinal cord in some patients.

Distal parts of the colon receive parasympathetic innervation through afferents from the lumbo-sacral area of the spinal cord which contains the sacral defecation center [13].The cell bodies of these afferent neurons lie within the dorsal root ganglia (DRG) of the lumbar and sacral portions of the spinal cord [13]. When the sacral area receives stimulation, these neurons can be activated and can generate feedback. This feedback returns to the distal portions of the colon through the defecation reflex, activating motor patterns in the descending colon as well as stimulating the rectum and relaxing the internal anal sphincter in preparation for defecation [13]. At the same time, this information also may project to Barrington's nucleus through spinal pathways [13,14]. Barrington's

nucleus can then project the information to the vagus nerve through the dorsal motor nucleus of the vagus [14]. The vagus nerve innervates more proximal areas of the colon, thus information from Barrington's nucleus would stimulate it to invoke motor patterns in the ascending and transverse parts of the colon, thus transporting more colonic content in the anal direction [13,14]. Hence, stimulation of defecation center nerves with cell bodies in the DRG of the sacral spinal cord can trigger a full and a sacral defecation reflex and invoke motor patterns in proximal and distal areas of the colon.

A study of hippocampal neurons of rats, showed that irradiation with low-power infrared light has the potential to activate neurons and trigger action potentials, through changes in local temperature of the neuron's cell membrane [15]. This local change in temperature was seen to increase the activity of voltage-dependent Na and K channels, which would sometimes lead to depolarization of the hippocampal neurons [15]. This study also proposed that in addition to hippocampal neurons, the DRG which expresses temperature-sensitive transient receptor potential (TRP) channels should be sensitive to stimulation by infrared light [15]. A later study confirmed this, showing that even stimulation with an infrared pulse of 20 milliseconds emitting 1875 nm light is able to trigger depolarization, or action potentials in sensory neurons of the DRG through transient changes in the local temperature of the membrane, though they did not find that depolarization is due to presence of TRP channels [16]. In this study action potentials were achieved in about 20% of the neurons stimulated [16]. Both studies show that the temperature changes in the cell membranes are reversible and non-damaging, unlike what is observed with heat-emitting devices and high-power lasers [15,16]. Both of these studies show that there is promise in stimulating neurons using low-level laser as a treatment method for neurological issues.

We used a single session of a typical LLLT protocol to target the DRG of the sacral area of the spinal cord which innervates the distal colon and is an integral part of the defecation reflex. Based on the response in our patients, we can see that we were likely able to trigger nerve action potentials in this area, which resulted in stimulation of the colonic musculature and/or the enteric nervous system to generate SPWs and LAPWs observed during the intervention. However, no HAPWs were triggered in these patients

which would give us an indication of strong stimulation of the defecation center as well as a strong response in the colon. This may be due to the fact that the intervention was performed in patients, who already have impaired colonic motility and may have a dampened response to all interventions not just LLLT. The observed response may also be due to the intensity of the light used, as both previous studies used higher light intensity with shorter stimulus time [15,16]. The study on the hippocampal neurons used a light intensity of 970 nm, while the DRG study used a light intensity of 1875 nm with which they still only observed action potentials in only about 20% of neurons [15,16]. In our study, we used lights with intensity of 660 nm and 840 nm, for 5 minutes at each placement. This lower intensity may have resulted in a lower level of stimulation of the DRG at any one time, ultimately leading to absence of strong contractions such as HAPWs. It should be noted that the goal of LLLT is not necessarily the acute generation of strong contractions but rather to neuromodulate the circuitry of the autonomic nervous system so that normal reflexes are restored, and this study using acute stimulation shows at the very least, indirectly, that neural activity is generated.

With the target of this treatment being the sacral parasympathetic innervation of the colon, it would be worth exploring the heart-rate variability (HRV) of the 9 patients who received the intervention during HRCM which would give an indication of the sympathetic and parasympathetic activation. This may give an idea of the level of stimulation provided by LLLT to each patient, which may also correlate to the response observed. Additionally, there is the potential of elucidating certain autonomic nervous system profiles of each patient which may relate to the level of response observed in them. Something which should be considered during this analysis are the possible additive effects of all other interventions administered during HRCM before LLLT and their effect on the autonomic nervous system as well as LLLT response.

Another avenue which is worth exploring is the possible presence of additive effects of multiple treatments with LLLT. As we are currently using a lower level of stimulation, the thermal effect and low-intensity neuronal stimulation of LLLT may have a cumulative effect with its photochemical effect. Hence, stimulation with LLLT may stimulate neurons to fire more action potentials strengthening their synapses, while also stimulating

increased energy production and nutrient delivery through vasodilation; this would allow for recovery of the functionality of the neurons in the sacral defecation center and ultimately help in the restoration of the defecation reflex.

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Dr. Huizinga and Dr. Chen designed this study and contributed to data collection, analysis and interpretation as well as writing of the manuscript

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Chapter 7: General Discussion

The aim of this thesis project was to improve the understanding of HRCM as a tool for diagnosis of motility disorders leading to constipation using healthy controls and patients, while also exploring the relationship between autonomic dysfunction and constipation pathophysiology, as well as the possibility of its treatment using sacral neuromodulation.

Constipation is an issue which affects up to 27% of North Americans, and while many respond to pharmacological interventions, there are those who do not or eventually become unresponsive [1]. The most common option for patients with chronic idiopathic constipation has been surgical intervention, for which there is a variable success rate due to the fact that only about 3% of patients electing to undergo surgery are truly good candidates for it [1,2]. A large contributor to this issue is the lack of standardization of assessments in the area of colonic motility, as well as lack of consensus on what constitutes normal motility. Hence, the first aim of this thesis was to characterize normal motility in healthy volunteers using 84-sensor HRCM, as well as establish the best assessment tools to use with HRCM and find a way to quantify motility. This was the first study to use such high resolution within the colon with 84 sensors and only 1-cm side-hole spacing to attempt characterization of normal motility. Most studies of colonic motility use a spacing between 1 and 15-cm apart, and even with 1-cm spacing, the number of recording side-holes ranges between only 1 to 16 recording sites [3–5]. This spacing was previously known to create issues in identification of colonic motor activity, as many studies identify HAPWs to be those which propagate across 3 or more sensors, and with a spacing of 10-cm, the minimum length of a detectable motor pattern would be 20-cm [6]. Hence, any shorter motor events such as HAPWs belonging to category 1 from chapter 1 would be missed. Additionally, in studies comparing sensor spacing it was found that increase in the distance between recording channels not only led to increased chances of missing a motor pattern, but also mislabelling it. These studies showed that an increase of the spacing even from 1 to 2-cm halved the number of propagating pressure waves which were detected, and an increase to 3-cm led to a 30% chance of incorrectly labelling a propagating pressure wave [6,7]. Thus, the data

obtained from the 19 healthy volunteers in chapter 1 is of high value with regards to its contribution towards characterization of normal motility and standardization of HRCM.

In addition to healthy volunteers, this thesis project also looked at the motility of chronic constipation patients, to both apply the knowledge obtained from chapter 1 and also obtain more data that would contribute to elucidating the pathophysiology of chronic constipation. Chapters 2 and 3 looked to combine multiple aspects of colonic motility as well its extrinsic control, to characterize different constipation pathophysiologies, but also highlight the variability present between patients and the importance of individualized analysis. Variability in colonic motility even in healthy volunteers already became evident through the symbol maps constructed in chapter 1, therefore it was essential that there is a study which looks at patients on a more individual basis and considers the presence of different pathophysiologies. This study is not the first to compare healthy volunteers to patients. Many previous studies have done this and drawn the general conclusions that in comparison to healthy volunteers, patients tend to have more quiescent colons with decreased or lack of presence of HAPWs which in general are also of lower amplitude [8–10]. Even in the clinical setting, diagnosis of constipation is done in a very general manner based on symptoms classification using the Rome IV criteria. Patients are most often classified into either the slow-transit or functional outlet obstruction category [11], and with the lack of personalization in diagnosis, treatment of their constipation is not based on their personal pathophysiology either. However, recently there has been a shift in this general view of constipation away from just symptoms and HAPWs and more towards the integration of colonic motility with all of its other controlling factors. Specifically, studies have started to focus more on the extrinsic control of colonic motility and its role in constipation [12–14], as well as the personalized assessment of patients [11]. The studies in chapters 2 and 3 provide valuable information for this new integrative view on colonic motility and constipation. The strength of these studies comes from the consideration of multiple components of motility within the colon itself as well as the integration of one of its major sources of control-the sympathetic and parasympathetic extrinsic innervation. These are some of the first studies which provide a detailed look at the changes in autonomic activity during the occurrence of colonic motor patterns, as well as the correlation between

autonomic dysregulation and dysmotility. Another strength of these studies is the case-by-case analysis of each patient, which has been essential in elucidating the variety of pathophysiologies which can contribute to constipation. The ability to identify these individual pathophysiologies will lead to great advances in more personalized treatments for constipation patients which will focus on addressing their specific issues. Another major finding of the studies was the re-introduction of the sphincter of O'Beirne as a possible contributor to constipation. The sphincter had previously been identified by James O'Beirne as well as described by a few others [15,16]. However, due to its functional nature and absence in some individuals, many have since rejected its presence [17,18] and it has therefore been largely ignored when looking at constipation pathophysiology. Owing to the individualized assessment of patients in this thesis project, in chapter 3 the sphincter of O'Beirne was once again described and was identified as a possible contributor to constipation in some patients.

Throughout all the studies in this thesis, in addition to motility of the colon great emphasis was placed on its ANS control and how impairments in it can lead to patients' symptoms. Specifically, the sympathetic and parasympathetic inputs were the main focus, as they can have significant impact on motility but can also be more easily affected both positively and negatively compared to other control mechanisms such as the enteric nervous system. In the study of patients and their motility, it was evident that impairment of ANS activity in the form of sympathetic inhibition played a role in the pathophysiology. Study of patient motility and ANS also drew attention to the defecation reflexes mediated through the sacral defecation center which not only controls the distal colon and anorectal region, but also sends sensory information to the proximal areas of the colon through the brainstem and vagus nerve to regulate proximal colon motor activity [19]. Due to its role in defecation control this sacral area has gained attention when it comes to non-pharmacological treatment of constipation. Many studies have now explored electrical stimulation of this area with implanted electrodes to improve constipation, and this has been done with some success [20,21]. However, due to the invasive nature of this treatment and the associated side effects, patients have displayed limited compliance and satisfaction with this treatment [22,23]. Hence, the results from one-time LLLT stimulation in chapter 4 are valuable in not only providing evidence

for sacral control of colonic motility and the induction of motor patterns with its stimulation, but also a step towards a novel non-invasive treatment for intractable constipation. One limitation of this study is the fact that the treatment is only applied one time, and while its acute effect can be observed during manometry, it is difficult to predict what the long-term implications of this intervention would be. This warrants a more long-term study of sacral neuromodulation with LLLT, to assess its effects on patient symptoms, as well as autonomic nervous system activity and anorectal function. Additionally, the study on one-time laser stimulation was not accompanied by a placebo treatment, hence the conclusions from the study should be considered preliminary.

The assessment of long-term LLLT in patients would also address a second limitation which was present in all studies within this thesis project, and that is the additive effect of all interventions. In the study of one-time LLLT, the intervention was applied following proximal balloon distention, and meal administration. Therefore, it is difficult to eliminate the possibility of these two interventions having an influence of the response to LLLT. The same is true for all of the other interventions which were assessed both in patients and healthy volunteers. To address this concern, future HRCM studies in patients and volunteers could be performed in randomized order with regards to administration of interventions such that the effect of interventions on one another could later be elucidated.

Another possible criticism is the use of water-perfused catheter. Water-perfused manometry has been the method of choice in most pediatric and adult colonic manometry studies [24–27]. However, with the advent of solid-state high-resolution manometry, water-perfusion has received much criticism due to the introduction of fluid inside the colon. There have been conflicting reports in the comparison between solid-state and water-perfused manometry. Some studies show that there are advantages to using solid state, as it was observed in some studies to be more sensitive compared to water-perfused; though the HAPWs observed with solid-state were also observed with water-perfused [28]. However, other studies show that there is no significant difference between these two types of catheters [25,27]. In all, it is still unclear whether or not this method of measurement affects physiological motility of the patient or measurement of

the pressure waves. Currently methods are chosen based on cost, equipment available, and study design [24], therefore depending on differing views this may be viewed as a limitation or it may not.

The final limitation of this study is the use of a prepared colon. Retrograde placement of the manometry catheter in the prepared colon using colonoscopy has been the most widely-used method in both pediatric and adult studies [10,24,29–31]. The advantage of using this procedure is that it provides the easiest access to the colon, the catheter can usually be clipped as far as the caecum, and it is well tolerated by participants [24]. Additionally, when the bowel preparation is performed in the same manner the starting point for all participants is standardized [24]. However, a criticism has been the use of colon preparation for colonoscopy, as this renders the colon empty, which is not its physiological state, and additionally, the preparation even without the use of stimulant laxatives may alter the manometry response. Some studies have found that the use of a prepared colon leads to an increased number of HAPWs, as well as a disruption in their spatio-temporal organization [32,33]. As the importance of spatio-temporal organization of HAPWs in the colon was highly emphasized in the study of chapter 1, it would be worthwhile to perform the same study in the unprepared colon of healthy volunteers and compare the data between the two. Performing a study in the unprepared colon would also give the advantage of the ability to study the correlation between individual HAPWs, their features and colonic transit. This can be achieved by performing HRCM on the day after the insertion of the probe, but this requires the ability to keep patients overnight.

In conclusion, this thesis provides valuable information regarding HRCM assessment of colonic motility in both healthy volunteers and patients. It also provides the basis for a more integrative and individualized assessment of constipation pathophysiology in patients by linking together motility with its autonomic control. This data served as a step closer towards the discovery of different pathophysiologies linked to constipation as well as novel methods of non-invasive treatment of it such as LLLT.

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Conferences

November, 2018 – McMaster Innovation Showcase (poster)

February, 2019 – Medical Sciences Research Day (poster)

May, 2019 – Health Sciences Graduate Research Plenary (poster)

May, 2019 – Farncombe Family Digestive Health Research Institute: Research-in-Progress (oral)

March, 2020 – Farncombe Family Digestive Health Research Institute: Research-in-Progress (oral)