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DIFFERENTIAL EFFECTS OF SHORT-CHAIN FATTY ACIDS ON MOTILITY OF
GUINEA PIG PROXIMAL AND DISTAL COLON

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of
Science at Virginia Commonwealth University.

by

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Virginia Commonwealth University
Richmond, Virginia
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Abstract

DIFFERENTIAL EFFECTS OF SHORT-CHAIN FATTY ACIDS ON MOTILITY OF GUINEA PIG PROXIMAL AND DISTAL COLON

By Norman Reynolds Hurst

A thesis submitted in partial fulfillment of the requirements for the degree of Master of
Science at Virginia Commonwealth University.

Virginia Commonwealth University, 2013.

Major Director: John R Grider, PhD
Professor, Department of Physiology

INTRODUCTION: Colonic bacteria produce short-chain fatty acids (SCFAs) by fermentation of dietary carbohydrates and fiber. The production of SCFAs is greatest in proximal colon where propulsion is likely to be highly dependent on chemical/nutrient stimuli. Unabsorbed SCFAs entering the distal colon are likely to modify peristalsis initiated by fecal pellet-induced distension. **AIM:** To determine the effect of individual SCFAs on propulsive contractions in guinea pig proximal colon and on pellet propulsion in distal colon. **METHODS:** Proximal colon was excised, cannulated and placed in Krebs buffer in an organ bath. After equilibration, the colon was distended with 1ml of Krebs

buffer alone or containing sodium salts of acetate, butyrate, or propionate at 10-100mM. Motility was video recorded, spatiotemporal maps generated, and the number of full-length propulsive contractions during a 5 min period was determined. The distal colon was removed from guinea pig and placed in an organ bath containing Krebs buffer. Following equilibration, video-tracking software was used to measure the velocity of propulsion of a clay pellet placed in the oral end of a segment. Krebs buffer alone or containing individual SCFAs at 30mM was perfused caudad to the pellet at 0.1 ml/min. **RESULTS:** The basal rate of propulsive contractions in Krebs buffer was 2.9 ± 0.7 per 5 min. Butyrate and propionate had concentration-dependent and opposing effects on propulsive contractions; acetate had no effect. Butyrate significantly increased propulsive contractions (maximal increase of 207% ($p \leq 0.05$) at 30 mM) whereas propionate abolished propulsive contractions (91-100% inhibition at 10-30mM). Control Krebs buffer containing 10-100mM NaCl had no effect. The mean rate of pellet propulsion during perfusion of Krebs buffer alone was 1.5 ± 0.2 mm/sec. Addition of sodium butyrate (30 mM) to the perfusate increased the velocity of pellet propulsion by $40 \pm 4\%$ ($p < 0.05$) whereas addition of 30mM sodium propionate decreased velocity of pellet propulsion by $75 \pm 8\%$. Acetate had no significant effect in either proximal or distal colon. Mixtures of SCFAs showed a general decrease in overall motility. **CONCLUSION:** SCFAs have differential effects on propulsive contractions in the proximal and distal colon of the guinea pig, with butyrate being excitatory and propionate being inhibitory. These studies suggest that the movement of feces in the colon depends not only on distension but the chemical (i.e. dietary) composition of the feces.

1. Introduction

1. A) The Anatomy of the Gastrointestinal Tract

The gastrointestinal (GI) tract is a tubular organ that traverses the thoracic and abdominal cavity, beginning at the mouth, continuing through the esophagus, stomach, small (duodenum, jejunum, and ileum) and large (cecum and colon) intestine, into the rectum and finally ending at the anus. It serves two primary functions, to absorb nutrients from ingested food and to serve as a barrier between ingested material from the outside environment and the interior systems of the body. The anatomy can be divided into two general structures: the muscular layers and the mucosal layer.

A1) Characteristics of the Muscular Layers

The GI tract consists of an outer longitudinal muscle layer, which is closely associated with the more interior circular muscle layer. Each of these muscle layers serves a role in mixing and propelling luminal contents in an anal (caudad) direction. The longitudinal layer contracts in the orad and caudad direction along the tract, while the

circular layer contracts and results in occlusion of the luminal space. These layers contract in a coordinated fashion in order to propel contents. This coordination is due to the neural networks that are collected in the myenteric plexus, a layer of neurons that are situated between the longitudinal and circular muscle. The neurons of the myenteric plexus extend projections into both muscle layers and release a multitude of neuropeptides and neurotransmitters. This complex patterning of neural release regulates the various motility patterns observed in the GI tract.

There are two primary motility patterns that are observed in the gut. The first is a reflex known as peristalsis, which results in the propulsion of luminal contents in a caudad direction by coordinated contraction of the circular muscle orad and relaxation caudad of a bolus in the lumen of the GI tract. (45) The second pattern of motility is known as segmentation, and is considered to be a non-propulsive contraction of the circular muscle layer in order to mix luminal contents to facilitate more effective digestion and absorption of luminal contents. (47) **(Figure 1A)**

A2) Characteristics of the Mucosal Layers

Closer to the lumen of the GI tract lies the submucosa, a layer of connective tissue that contains a large vascular and lymphatic network, which serves to transport nutrients and hormones to and from the mucosa. The mucosa is a layer of epithelial cells that are in direct contact with the lumen of the GI tract, and are responsible for absorption of nutrients. Many cells of the submucosa and mucosa are closely associated with axons of

neurons that lie in the submucosal plexus, which lies between the submucosa and circular muscle layer. This neural network receives signals from epithelial cells in the mucosal layer via afferent neurons that control and influence the motility patterns of the muscle layers by synapsing with both the muscle layers as well as the neurons of the myenteric plexus. **(Figure 1A)**

A3) The neural reflex of motility

Despite the level of diversity regarding all the neurons of the enteric nervous system, much work has been done to piece together the basic reflex related to peristalsis. (1, 43, 44, 46) **(Figure 1B)**. Beginning on the luminal surface of the epithelium, chemical or physical stimulation from chyme facilitates 5-HT (serotonin) release from enteroendocrine cells. 5-HT activates calcitonin-gene regulated peptide (CGRP) containing afferent neurons in the submucosa, which then signal into the myenteric plexus. At this point, a series of interneurons are activated and ultimately stimulate the release of substance P (SP) and acetylcholine (ACh) oral and vasoactive intestinal peptide (VIP) and nitric oxide (NO) caudad. These molecules cause oral contraction and caudad relaxation of the circular muscle, respectively. (1, 43, 46) Simultaneously, the opposite muscular effect is observed in the longitudinal muscle layer. Together, this results in the coordination propulsion of luminal contents caudally through the GI tract. Distension of the intestinal wall is also capable of initiating peristalsis via an extrinsic afferent neuron that relays through the dorsal root ganglia. (46)

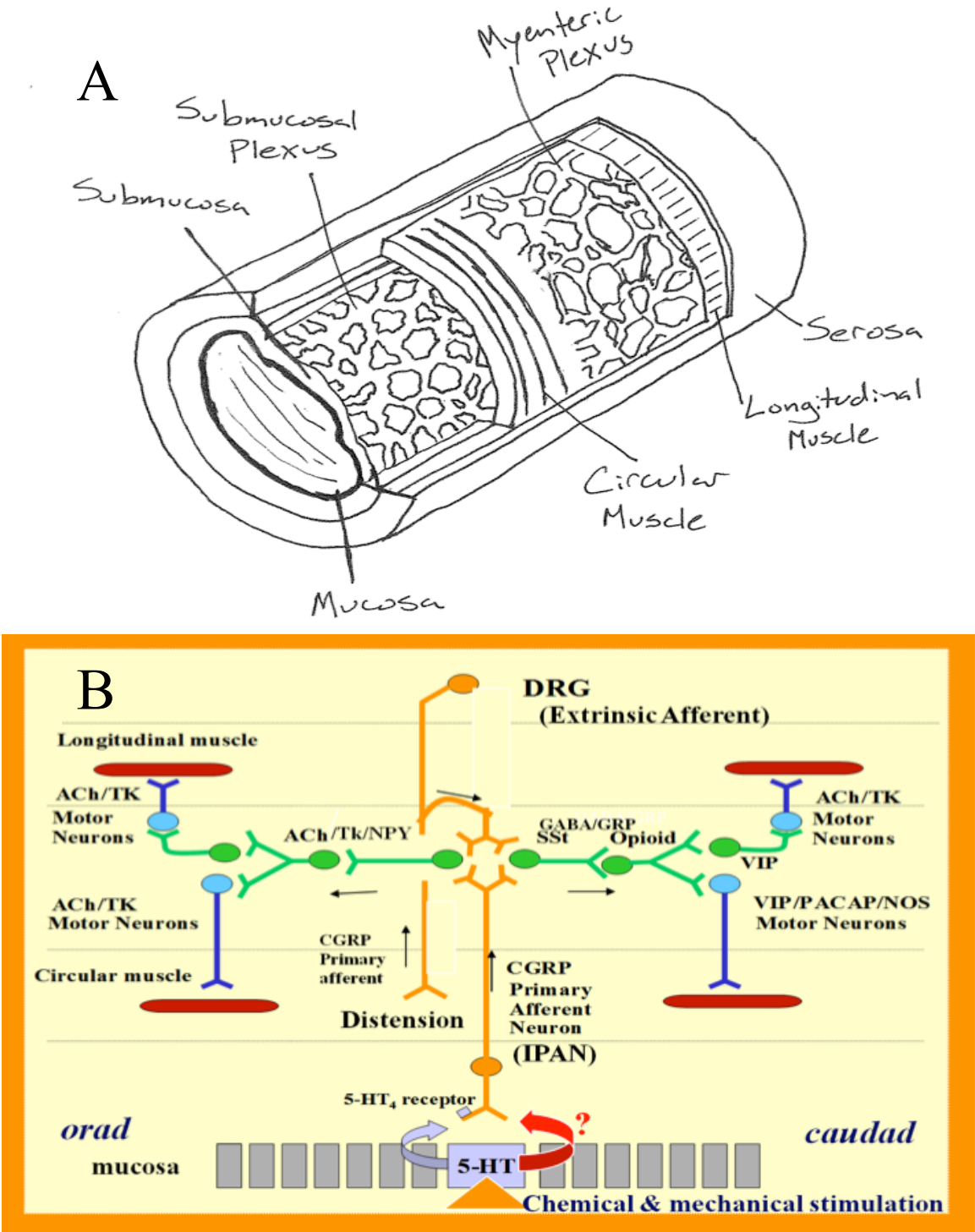
1. B) Function of the Gastrointestinal Tract

Once ingested contents exit the stomach, they are sufficiently broken down for further digestion and absorption in the small intestine. Proteins are efficiently broken down by peptidases and absorbed, and fats are emulsified with bile, digested, and absorbed into the lymphatic system. Simple carbohydrates are digested by amylases and absorbed through transporters on the surface of the epithelium. Certain compounds, such as cellulose and other carbohydrate chains (termed as fiber), are not digestible by the enzymes secreted into the lumen of the GI tract. These compounds reach the ileum and are propelled into the cecum through the ileocecal valve. At this point, luminal contents are condensed into feces and are fermented by bacteria that reside in the large intestine. These resident bacteria have been referred to as the microbiome.

Figure 1. Diagram of the GI Tract and the Canonical Pathway of the Peristaltic Reflex

A) The layers of the GI tract move out from the lumen to include the mucosa, submucosa, submucosal plexus, circular muscle layer, myenteric plexus, longitudinal muscle layer, and serosa. **B)** Either mechanical or chemical stimulation of the intestinal mucosa causes release of 5-HT from enteroendocrine cells. 5-HT in turn is released on the basolateral side and activates CGRP-containing neurons which then activate a series of interneurons in the myenteric plexus. These interneurons activate motor neurons, which release acetylcholine (Ach), tachykinin (TK), and substance P (SP, not pictured) orad to the luminal stimulus and cause contraction of the circular muscle and relaxation of the longitudinal muscle. Vasoactive intestinal peptide (VIP) and nitric oxide (NO) are released caudad to the luminal stimulus and stimulate circular relaxation and longitudinal contraction. This coordinated activity results in the caudal propulsion of luminal contents via peristalsis. (Yellow = Afferent Neurons; Green = Interneurons; Blue = Motor Neurons)

Figure 1. Diagram of GI Tract and the Canonical Pathway of the Peristaltic Reflex



1. C) Characteristics of the Gut Microbiome

The microbiome is a massive, diverse population of commensal bacteria that reside on all the external body surfaces, including the vagina and colon. In the colon the microbiome consists of ~1000 species. (50) They are first introduced to newborns through the secretion of the birth canal as well as through mother's milk (2). This initial population develops in the colon of the growing organism and serves to ferment any undigested material, as well as provide an immunomodulatory effect in the gut and prevent pathogenic bacteria from growing in the anaerobic environment of the colon. (3, 48)

The microbiome is also modified by various dietary inputs. (51, 52) The composition of carbohydrates and fibers within different foods has been shown to change the population of bacteria that make up the microbiome. (4, 51, 52, 53) Use of antibiotics has also been shown to severely limit the growth and diversity of bacteria in the colon. (5) Reductions of diversity in the microbial population have been correlated to various GI pathologies, such as inflammatory bowel diseases (IBDs) and irritable bowel syndrome (IBS). (6-9) While no specific organism has been attributed to all onsets of IBDs, however, *Mycobacterium paratuberculosis*, *M paramyxovirus*, *Listeria monocytogenes*, and *Helicobacter hepaticus* have been implicated. (6) In IBS, the ratio of *Firmicutes* to *Bacteroidetes* has been shown to be altered in some cases (49) Recently, changes in the microbiome have even been associated with mood disorders and given rise to the idea of the microbiome influencing the gut-brain axis. (10)

Commensal bacteria play a role in fermenting luminal contents that reach the colon. Some modify the bile salts that reach the colon (11). Others generate hydrogen, hydrogen sulfide, and methane. (12, 13) Many that digest the fiber and carbohydrate in the colon excrete short-chain fatty acids as a byproduct of their metabolism.

1. D) Short-Chain Fatty Acids

Short-chain fatty acids (SCFAs) are fatty acids that consist of 2 to 6 carbons (acetate, propionate, butyrate, valerate, and caproate, respectively). In the colon they are the metabolic byproducts of anaerobic bacterial fermentation of carbohydrates and fiber that are not digested in the preceding regions of GI tract. The three most abundant in the colon are acetate, propionate, and butyrate. (14) Total concentrations of SCFAs vary depending on species, diet, as well as population and diversity of the microbiome, but their concentration is general thought to range between 70-140mM. Acetate is the most abundant, followed by butyrate and propionate, though the precise ratio is dependent on the same variables mentioned above. (15)

The concentrations of SCFAs are highest in the cecum and proximal colon, where most of the bacteria reside and where most undigested material is held in the colon. As material moves caudally, the SCFA concentration decreases due to their uptake and utilization as a nutrient source for colonocytes. Butyrate is considered to be the primary nutritive SCFAs for colonocytes. (16) It has been known for decades that SCFAs also play a role in the contractility of gastrointestinal smooth muscle, and several groups have previously studied the effect of SCFAs on motility of the distal ileum and colon. (17-22)

These studies have focused on such measurements as myoelectrical activity and transit time, as well as peptide release. These findings will be discussed in detail in the discussion section.

1. E) Gastrointestinal Motility Monitor (GIMM)

Measuring more detailed aspects of motility i.e. peristalsis and segmentation, has been relatively qualitative and difficult to assay in a precise manner. For instance, measuring luminal pressure and volume changes indicates nothing regarding different motility patterns. To attempt to solve this dilemma, video technology began to be utilized to record isolated tissue preparations for more detailed analysis. These analyses generate spatio-temporal maps of the video, which measure the distance from one lateral edge of the tissue to the other, and thus indicate either distension or occlusion of the intestinal lumen. (23, 54) In the decade since the inception of this method, it has been developed commercially and sold as a Gastrointestinal Motility Monitor (GIMM). (24)

The primary use of GIMM by several groups has been to monitor propulsion of artificial fecal pellets in distal colon. Mawe has utilized this technique for studying the dysmotility of pellet propulsion in several animal models of inflammatory bowel diseases, particularly ulcerative colitis. (55,56) Others have used the technique to analyze peristalsis and segmentation by perfusing fluid intraluminally in the small and large intestine. (25-27) More recently, investigators working in Bornstein's lab have tested the influence of nutrients on motility in the small intestine, and have attempted to show the role of the enteric nervous system in its coordination. (28,29,31) They have found that nutrient

induced motility is neurally mediated and can be blocked by tetrodotoxin, a sodium channel blocker; hyoscine/scopolamine, a muscarinic receptor antagonist; and hexamethonium, a nicotinic receptor antagonist. (28) Various methods of quantification have been employed, however, no consistent method has been agreed upon to define each motility pattern observed. The use of GIMM analysis has not yet been employed to investigate the role of SCFAs in motility.

1. F) Short-chain fatty acid receptors and potential downstream signals.

There are two receptors known to detect SCFAs. Both are G-protein-coupled receptors (GPCRs) with seven-transmembrane domains. G-protein-coupled receptor 43 (GPR43) and 41 (GPR41) have been shown as part of a family of GPCRs that detect free fatty acids. (35) The receptors have been renamed from GPR43 and GPR41 to free fatty acid receptor 2 (FFA2) and 3 (FFA3), respectively. Both of these receptors are expressed together in various cell types, such as adipose tissue, inflammatory cells, enteroendocrine L-cells in the GI tract, as well as pancreatic β cells. For this reason, investigation of the receptor effects via knockout animals has been difficult. (35) Recent pharmacological studies have shown that both of these receptors bind all SCFAs with varied potency. Propionate has the highest potency to FFA2, followed by acetate and butyrate, which are equipotent. Propionate and butyrate are equipotent at FFA3, whereas acetate is less potent at FFA3 compared to propionate and butyrate (33, 35) Both receptors are also able to couple with $G\alpha_{i/o}$ and thus inhibit adenylate cyclase, but only FFA2 is able to couple to $G\alpha_q$ and activate phospholipase C (PLC) and increase intracellular calcium levels. Based

on these known pathways, it is possible that SCFAs can affect several pathways in GI epithelial cells such as PYY release from L-cells as well as smooth muscle cells contraction and thus FFA2/3 are now under investigation as therapeutic targets. (32, 35, 36, 37)

1. G) SCFAs and the microbiome in health and disease

Because the microbiome is sensitive to changes in diet and antibiotics, the potential exists for changes that can result in dysbiosis and facilitate the development of pathologies in the colon. Indeed, the role of the microbiome has come under the attention of clinical gastroenterology in recent years. (5,6,9, 38, 39) Studies have looked for correlations between changes in the microbiome, SCFA levels, and onset of certain diseases. Particularly, IBDs such as ulcerative colitis (UC) have been investigated clinically. (38, 39) In UC patients, there was a significantly reduced microbial population compared to non-UC individuals. In regards to active versus inactive mucosal inflammation in UC patients, a shift in the microflora was also observed. Part of this rationale is focused on the bacterial production of SCFAs being diminished in UC or other IBDs. Because SCFAs are nutritive for colonocytes, a lack of SCFA production could result in a cellular stress response and cause the development of pathology. Even with adequate SCFA production, products from sulfur reducing bacteria (which are overgrown in UC) can block butyrate utilization by colonocytes. (38)

The microbiome has also been implicated in development of obesity. (38, 39) Again, many environmental factors play a role in the diversity of the microflora. Because

of this, it is more useful to assay the relative concentration of SCFA production as an indicator of certain bacterial populations. In one such study, two bacterial groups—*Firmicutes* and *Bacteroidetes*—were assayed in lean and overweight and obese patients. (40) Additionally, relative SCFA levels were measured in the feces of patients. Compared to lean patients, overweight and obese patients showed a relative increase in *Bacteroidetes* and showed a relative increase in total SCFAs, with propionate being the most increased. (40) More recent studies have indicated that a normal level of SCFAs may be preventative toward obesity. (39) Through the activation of FFA2 in particular, the release of PYY or GLP-1 may be increased, which regulate digestive enzyme secretion and satiety among other factors. (41, 42) Activation of FFA2 on adipose tissue also inhibits lipolysis and increases adipogenesis while stimulating leptin secretion. (39) Due to these findings, as well as the potential role in IBDs, pharmacological studies have been designed to attempt to synthesize agonists for FFA2/3 as potential therapeutic targets. (35)

Rationale:

Given that dysbiosis contributes to several GI pathologies, including IBDs, IBS, and even diseases such as obesity, the effect of SCFAs is of clinical importance. However, the majority of current studies are focused on the cellular mediators and downstream effects that result from SCFA action on FFA2/3. Previous studies focusing on SCFAs in the colon have focused on smooth muscle characteristics and related this to motility, but lacked the technology to study motility in a whole tissue preparation in detail. With the development of the GIMM, this becomes possible. Owing to this experimental advancement, and the fact that dysmotility is also present in the above pathologies (38, 39), studying the connection between SCFAs and specific motility of the colon can help further characterize the role of SCFAs in gut function, and suggest implications for gut health and disease.

Hypothesis:

SCFA effects differ depending on location in the colon (proximal or distal), and also on chain length.

2. Materials and Methods

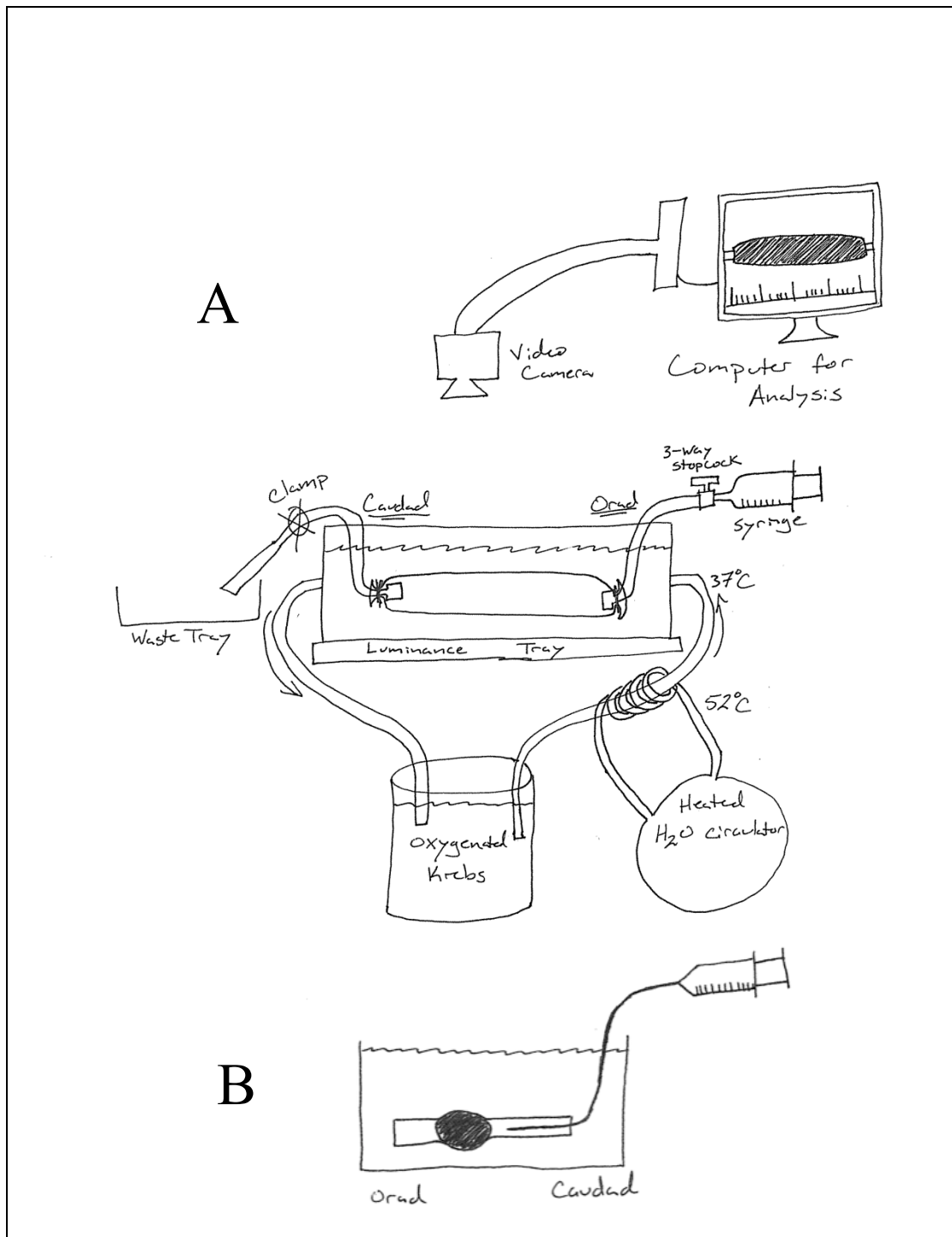
2A. Reagents and Equipment

Acetate ($\text{C}_2\text{H}_3\text{O}_2\text{Na}$, 82.03 g/mol, $\text{pK}_a = 4.76$), Propionate ($\text{C}_3\text{H}_5\text{O}_2\text{Na}$, 96.06 g/mol, $\text{pK}_a = 4.87$) and Butyrate ($\text{C}_4\text{H}_7\text{O}_2\text{Na}$, 110.1 g/mol, $\text{pK}_a = 4.82$), Sodium Chloride (NaCl , 58.44 g/mol), Potassium Chloride (KCl , 74.55 g/mol) and D-glucose ($\text{C}_6\text{H}_{12}\text{O}_6$, 180.2 g/mol) were purchased from Sigma-Aldrich, St. Louis, MO. Potassium Phosphate (KH_2PO_4 , 136.09 g/mol) and Calcium Chloride (CaCl_2 , 147 g/mol) were purchased from Fischer Scientific, Pittsburgh, PA. Magnesium Sulfate (MgSO_4 , 246.48 g/mol) was purchased from JT Baker, Phillipsburg, NJ. Sodium Bicarbonate was purchased from Midwest Scientific, St. Louis, MO. All were kept at room temperature and were no less than 99% pure. The Gastrointestinal Motility Monitor (GIMM) was purchased from Catamount Research and Development, St. Albans, VT. The GIMM consists of several organ baths, which are placed on top of luminance plates to silhouette an intestinal segment, which is then recorded from above using a video camera connected to a computer running GIMM software. This allows for computer analysis of a modified Trendelenburg preparation as well as tracking of artificial fecal pellet velocity. (**Figure 2**)

Figure 2. Diagram Indicating Setup of the Gastrointestinal Motility Monitor.

A) The segment of intestine is excised and placed in an organ bath with circulating Krebs buffer (pH 7.4, 37°C). In the proximal colon the oral and caudal ends are cannulated with glass tubes, tied with suture, and attached to the walls of the organ bath by molding clay. The organ bath sits on a tray that emits light from below and causes the tissue to be silhouetted in view of the video camera situated above the preparation. This camera is attached to a computer that records and analyzes the motility patterns of the tissue over a specified timeframe. In order to distend the proximal colon a caudal clamp is closed and fluid is added via an oral syringe. These together are used to control the volume of fluid in the lumen. **B)** In the distal colon the setup is identical, but the cannulas are removed and a fine catheter (PE 10) is inserted into the anal end and advanced about half way into the colon towards the pellet. Experimental fluids are infused through this catheter at a rate of 0.1 ml/min via a syringe and syringe pump.

Figure 2. Diagram Indicating Setup of the Gastrointestinal Motility Monitor.



2B. Animals

Hartley guinea pigs of either sex and 150-200g weight were purchased from Charles River Laboratories International and euthanized by asphyxiation with carbon dioxide as approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University. The animals were housed in the animal facility administered by the Division of Animal Resources, Virginia Commonwealth University. All procedures were conducted in accordance with the Institutional Animal Care and Use Committee of the Virginia Commonwealth University.

2C. Preparation of Tissues and Equipment

Following euthanasia, the abdominal cavity was dissected and the proximal and distal colon were removed and placed in warmed Krebs buffer (pH 7.4) which contained (in mM): 118 NaCl, 4.75 KCl, 1.19 KH_2PO_4 , 1.2 MgSO_4 , 2.54 CaCl_2 , 25 NaHCO_3 , 11 Glucose. Krebs buffer was bubbled with 95% oxygen and 5% carbon dioxide.

Proximal colon was cut to approximately 8 cm, flushed of feces and cannulated using custom-built glass tubes on the orad and caudad ends. Following this, the proximal colon and cannulas were placed in one of the GIMM organ baths. The baths contained Krebs buffer and were continuously perfused with fresh oxygenated Krebs buffer held at 37°C. To achieve 37°C in the organ bath, the perfused Krebs buffer was sent through a series of heating coils circulating water from a perfusion pump held at 52°C. Krebs buffer of the same oxygenation and temperature was also injected into the lumen of the proximal colon through the orad cannula and left to equilibrate for approximately 15

minutes. During this period the caudad cannula was left open to allow the tissue to clear itself of contents as a result of native propulsive motility.

Distal colon was cut into 3-4 segments of approximately 5 cm, placed into the GIMM organ baths, pinned at the orad and caudad ends at their *in situ* length, and allowed to expel natural pellets for approximately 10 minutes, after which point any remaining pellets were flushed out with Krebs buffer. Following expulsion of pellets, distal colon was left to equilibrate for 5-10 minutes.

The GIMM cameras were then calibrated in the dark to record the colon segments. Calibration involved adjustments of distance (mm) as well as camera focus and aperture to properly visualize the segments against the background luminance plates. Proper adjustment yielded a silhouette of the proximal colon such that it was the only dark area on the computer screen, absent of any marks or bubbles that were present in the organ bath. Proper adjustment for recording of distal colon yielded a “washed out” visual display such that the tissue was hardly visible. This allowed for proper tracking of the dark artificial pellet that would later be used in the experiment.

2D. Proximal Colon Response to Intraluminal Distension

Following equilibration, the caudad cannula was clamped shut to prevent fluid from being expelled and the segment was injected with Krebs buffer via the orad cannula. Because the orad syringe and caudad clamp resulted in a closed system, all excess injected Krebs buffer volume was displaced into the lumen of the proximal colon, resulting in distension of the tissue. The volume was increased in 0.5ml increments from 0.0-2.0ml every 5 minutes and recorded continuously throughout the 25-minute

experiment. Video was later analyzed using GIMM software to determine the minimum-effective distension to visualize motility of the proximal colon. Throughout following experiments with treatment of SCFAs, the initial control was used to determine the minimum effective distension for each segment used. It was found that in all segments of approximately 8cm, distension of 0.5-1.0ml was sufficient to visualize motility. (**Figure 3**)

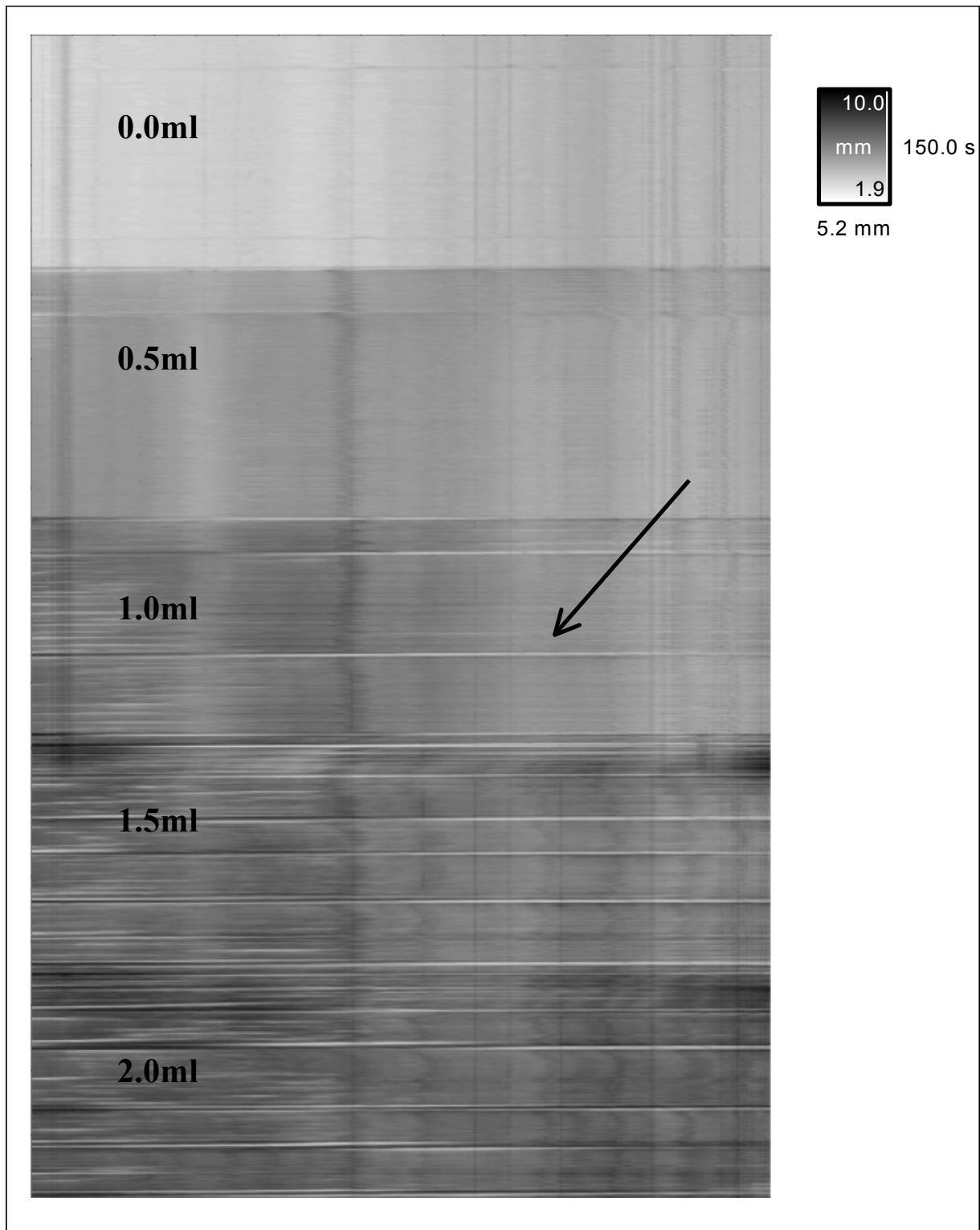
2E. Proximal Colon Response to Experimental Treatments

In all treatment experiment trials, the segments were distended and recorded for 10-minute periods. During analysis, the last 5 minutes of each video were used. Each 10-minute trial was followed by removing the clamp, allowing the proximal colon to empty and then pushing Krebs buffer through the orad cannula and tissue to clear the system of the treatment solution. The segment was then left to rest for 10-15 minutes until the next trial. Each experimental treatment was preceded by a control distension of Krebs buffer in order to establish a baseline of motility patterns for the proximal segment. Following this, several experimental conditions were tested:

Figure 3. Distension-Response Map of the Guinea Pig Proximal Colon

The proximal colon was recorded continuously for 1500 seconds (25 minutes) during which time distension volumes are increased from 0.0 – 2.0 ml in 0.5 ml increments every 300 seconds (5 minutes). The incremental darkening of the map indicates the increase in overall diameter of the segment as distension occurs. Each white band (arrow) that slopes from the right (oral) side of the map to the left (caudad) side of the map indicates a decrease in diameter (contraction) of the segment which propagates in the caudad direction. These were termed “full propagations” This is most clear at 1.0 ml (middle section) and is thus considered the minimum effective distension volume of the segment. The small key next to the spatiotemporal map indicates the diameter of the tissue based on shading. The key is also representative of $1/10^{\text{th}}$ of the Y-axis (time) and the X-axis (length of segment within view of the camera).

Figure 3. Distension-Response Map of the Guinea Pig Proximal Colon



2E.1) Treatment with single SCFAs (dose-response)

Acetate, propionate, or butyrate were added to Krebs buffer to make 10, 30, and 100mM solutions which were then used to distend the proximal colon for 10 minutes of video. A Krebs washout and rest period followed each dose before adding the next dose. Because of the time used for the experiments (1.5-2 hours), doses were shuffled at random in order to determine motility changes were dose dependent and not time dependent. Time controls (i.e. repetitive trials with Krebs buffer only) were also performed for this experimental scheme to verify that there was no time dependence on motility.

2E.2) Perfusion with 100mM NaCl

Because Na^+ is included with all of the SCFAs, the possibility exists that any observed change could be the result of an equimolar amount of sodium being added to the Krebs buffer (10, 30, 100mM respectively). For this reason, several control experiments were performed using a 10-minute distension using Krebs buffer with 100mM NaCl added following a baseline trial of standard Krebs buffer. These control experiments showed no difference for an additional 100mM NaCl versus standard Krebs buffer.

2E.3) pH variations due to SCFAs and buffer shift

Given that the pKa values of the SCFAs occur between 4.7 and 4.9, it is not expected that these would have any effect on the pH of the buffer in the concentrations used. However, the pH values of SCFA solutions as well as perfused Krebs buffer

without SCFAs were tested at random. No difference was observed between the pH of Krebs buffer alone or with added SCFAs at any concentration. However, throughout the experiment the pH of the Krebs buffer would shift slightly from 7.4 to 7.6

2E.4) Mixtures of SCFAs

Two mixtures of all three SCFAs were used following a 10-minute control recording with Krebs buffer alone. Both mixtures were used in a single experiment, but their order was randomized in order to discern if there was a marked difference from each other. The two mixtures consisted of an equivalent dosage ratio (10mM Acetate:10mM Propionate:10mM Butyrate) and physiological ratio which mimicked that reported to often occur in colonic samples (15mM Acetate:10mM Butyrate:5mM Propionate). These dosages were chosen based on the initial dose-response experiments with each individual SCFA, and were formulated to carry equivalent total SCFAs between the two mixtures (30mM vs 30mM).

2F) Distal Colon Propulsion of Fecal Pellets

For these experiments, the chamber, transillumination, videorecording technique, Krebs' buffer solutions, and temperature/oxygenation/pH monitoring were identical to those described for fluid-induced motility experiments described above. Following equilibration, an artificial clay pellet designed to mimic native pellets in size (on average 12 x 5 mm) and shape was inserted into the oral end of the distal colon and its movement throughout was recorded using the GIMM video camera. This was repeated at least 3 times in order to establish a consistent baseline velocity. Trials were recorded for as long

as it took to propel the pellet, but would be ended after 5 minutes if propulsion had not completely expelled the pellet. At this point the pellet would be forced out using injection of a small amount of Krebs buffer to flush the lumen.

Upon completion of the control pellet propulsion videorecording, a 10ml syringe containing a Krebs buffer solution with an individual SCFA at 30mM would be placed in a syringe pump and set to push 0.1ml/min through a flexible plastic tube (PE 10) attached to the syringe and inserted in the caudad end roughly to the midpoint of the distal colon segment. This allowed for solution to be perfused into the lumen ahead (i.e. just caudad) of the artificial fecal pellet which was inserted into the orad end. The same period of time for videorecording of pellet movement was used as in the control trials. In control studies, pellet movement was videorecorded in the same segment without perfusion of Krebs buffer and during perfusion of Krebs buffer alone in the same system (PE 10 tubing; perfusion at a rate of 0.1ml/min). There was no difference in pellet propulsion in the absence and presence of Krebs buffer perfusion indicating that this rate did not affect motility via a mechanical effect. The velocities were 1.35 ± 0.1 mm/s and 1.36 ± 0.2 mm/s respectively.

2G) Video Recording and Quantification of the Proximal Colon

Using the 10-minute (600 second) videos, the GIMM software was used to analyze the motility of the proximal colon in response to treatment at the minimum effective distension during the last 5 minutes (300 – 600 seconds). The software does this by detecting the lateral edges of the tissue segment and measuring the distance between them. Increased distance between edges (i.e. relaxation) is represented as darkening and

decreased distance between edges (i.e. contraction) as lightening on a 2-dimensional spatiotemporal map in which the X-axis represents distance from orad to caudad and the Y-axis represents time from approximately 300 – 600 seconds of the video recording.

(Figure 4)

Two main motility patterns were differentiated using the spatiotemporal maps. The first is a propulsive, propagating wave of contraction that moved caudad throughout the segment. In quantifying this, propulsive waves were separated into short-propagations and full-propagations. This was determined based on the propagation length in the segment: if greater than half the distance, it was called a full-propagation; if shorter than half the distance, it was called a short-propagation. Full-propagations therefore began in the orad half of the segment. Typically, short propagations would begin in the caudad half of a segment. However, any propagation that began in the orad half and propagated less than half the distance of the segment would be quantified as a short-propagation. These values were also combined to determine the total propagations within a 300 second period. **(Figure 5)**

Figure 4. Example of Spatiotemporal Map of Proximal Colon

A typical example of a spatiotemporal map showing approximately 300 seconds (5 minutes) of motility in the proximal colon. Propagating contractions are seen as light bands which angle from the right (orad) side to the left (caudad) side. The dark black bulb at the end of the propagation is an artifact distension that results from luminal fluid being pushed against the resistance of the clamp on the caudad cannula. Non-propagating contractions of various lengths are observed as short white bands interspersed between the propagating contractions.

Figure 4. Example of Spatiotemporal Map (Caudad – Orad)

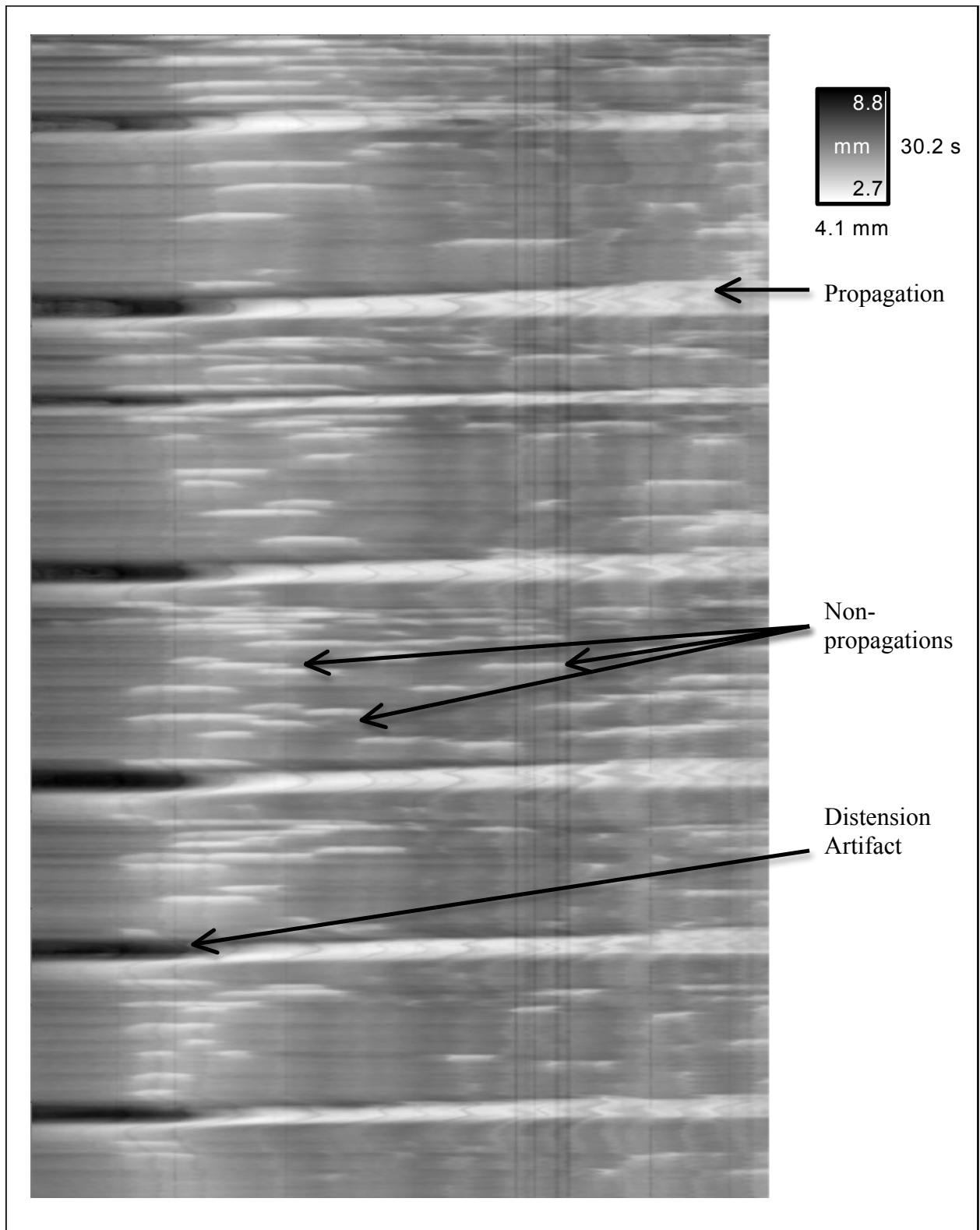
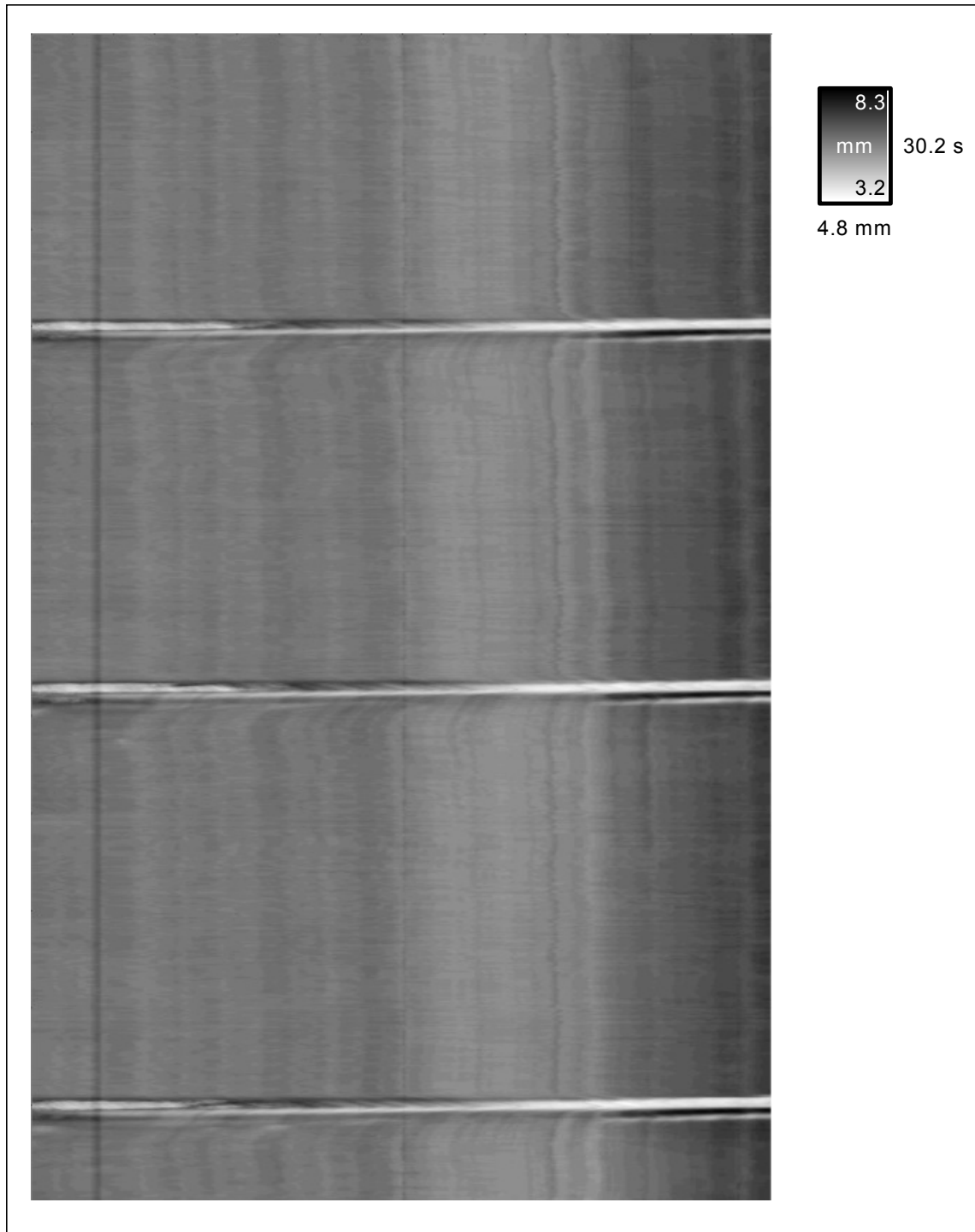


Figure 5. Example of Solitary Propagating Contractions in Proximal Colon

Pictured are three propagating contractions seen against an otherwise quiescent section of proximal colon. The various horizontal dark bands within each propagation are indications of artifact that is the result of the clamp on the caudad cannula, which inhibits the expulsion of luminal fluid. This results in an apparent fractionated propagation on the spatiotemporal map, but does not introduce a confounding distension effect to the motility of the segment. This is indicated by the quiescence present following the propagating contraction.

Figure 5. Example Spatiotemporal Map of Propagating Contraction
(Caudad – Orad)



The second main motility pattern observed was a non-propagating, mixing contraction. This contraction pattern rarely completely occluded the lumen and would often be seen on the mesenteric side of the proximal colon only. Typically this contraction was 1-3mm in length but could be as large as 8mm. However, given that it is difficult to be this precise using the spatiotemporal maps, a maximum cutoff of approximately 10mm (or 1/5th of the segment length) was used. In some cases, small ripple-like patterns would be observed and move between 5 and 15mm and appear as a small propagation on the spatiotemporal map. However, because these ripple-like patterns did not result in occlusion of the lumen, were observed infrequently, and only occurred on the mesenteric side of the proximal colon, they were counted as a single contraction in the total pool of non-propagating contractions. (**Figure 6**)

Despite the above methods for quantification, some aspects of motility in the proximal colon were not able to be numerically counted due to the level of variance observed from one segment to the next. The primary example of this was seen during equilibration and throughout most of the control trials using Krebs buffer at the beginning of every experiment. This motility pattern is best described as a “twitch” that involved contraction of nearly the entire segment in unison. This is considered qualitatively in addition to the propagating and non-propagating contractions to define “overall motility” of a segment. (**Figure 7**)

2H) Velocity Analysis of Artificial Fecal Pellets in the Distal Colon

Velocity analysis was performed using the pellet tracking method in the GIMM software, in which the pellet is darkened compared to the rest of the video and tracked

from the oral to caudal end. Velocity is calculated and displayed in mm/sec. In using velocity values, and minimum travel distance of 20mm was determined, as it was not common to see a pellet propel continuously farther than this distance. The velocities for each condition were averaged each day, yielding two mean values from each experiment indicating a control velocity and a treatment velocity. **(Figure 8)**

B8) Statistical Analysis

Prism GraphPad statistical software was used to analyze the data. Two-tailed t-tests and One-Way ANOVA were the two tests used depending on the number of conditions per experiment, with a Dunnett's multiple comparisons post-hoc test being performed on significant ANOVAs. Graphical representations of the results represent the Mean \pm SEM with significance denoted with * and representing $p \leq 0.05$

Figure 6. Example of Non-Propagating Contractions in Proximal Colon

Non-propagating contractions are pictured as isolated white bands between 1 - 10mm. These contractions can be singular or occur simultaneously with other contractions along the length of colon. In the figure, both are seen. The majority of the non-propagations are coordinated with others, which results in the checkerboard like pattern displayed in the map

Figure 6. Example Spatiotemporal Map of Non-Propagating Contraction

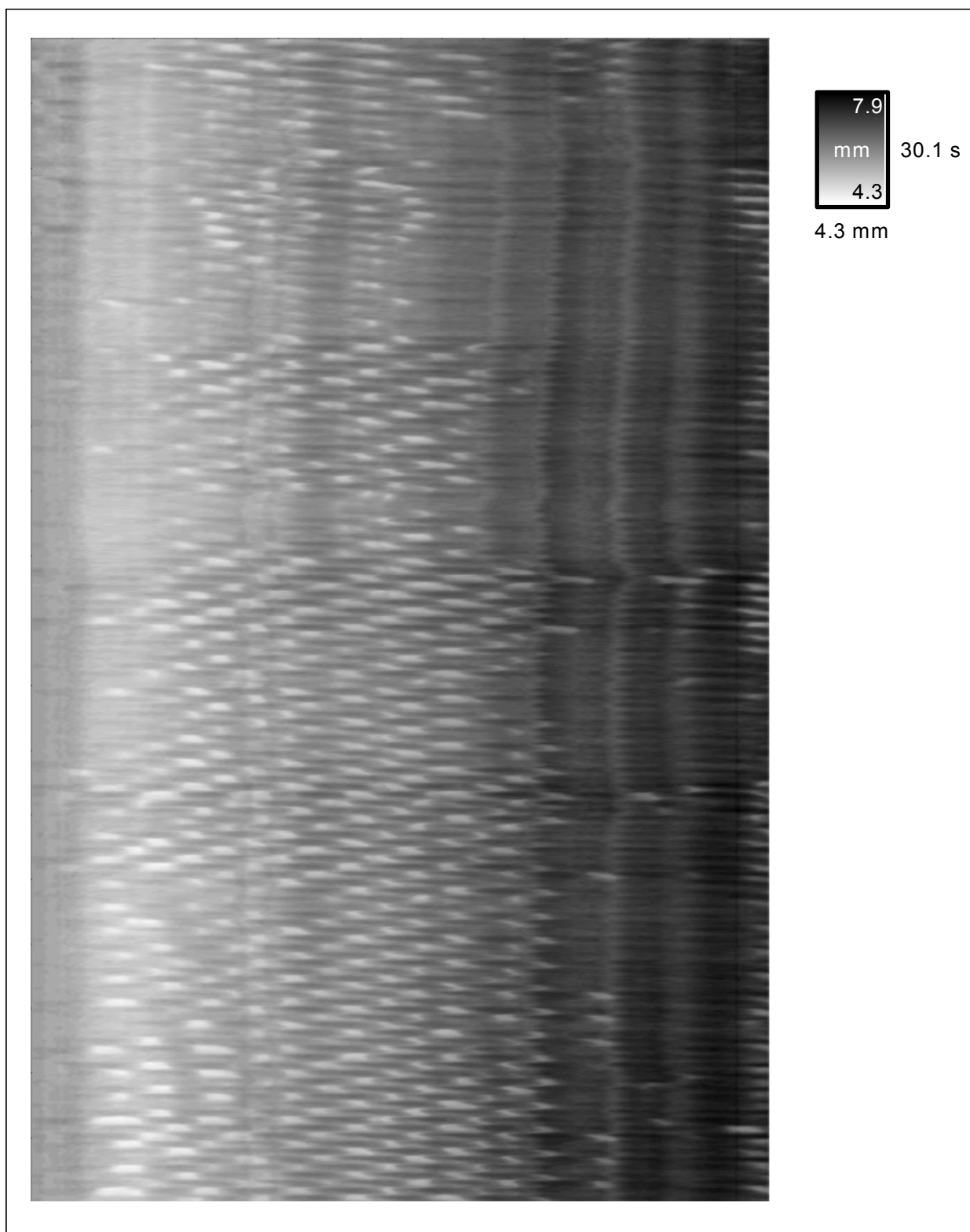


Figure 7. Example Spatiotemporal Map Displaying “Twitch” Motility

Many control trials displayed a spatiotemporal map that was similar to this figure. This is a profound example of the propagating and non-propagating contractions, which are buried in rapidly occurring bands that resemble a twitch of the entire segment. In most cases, propagations were still clearly distinguishable; however, non-propagations were more difficult to accurately quantify. For this reason, an indication of changes in “overall motility” from control versus any experimental solution was included in the results.

Figure 7. Example Spatiotemporal Map Showing “Twitch” Motility

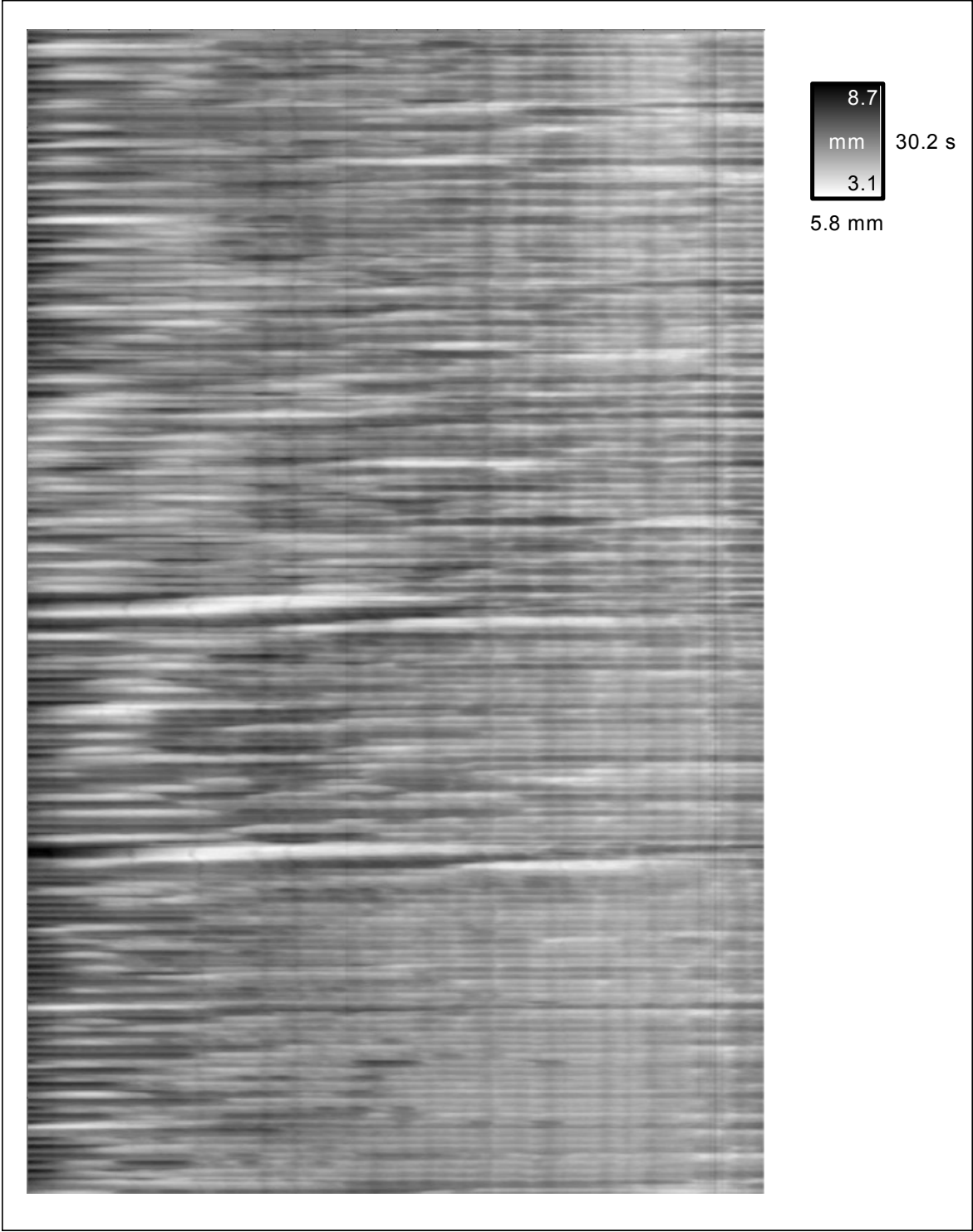
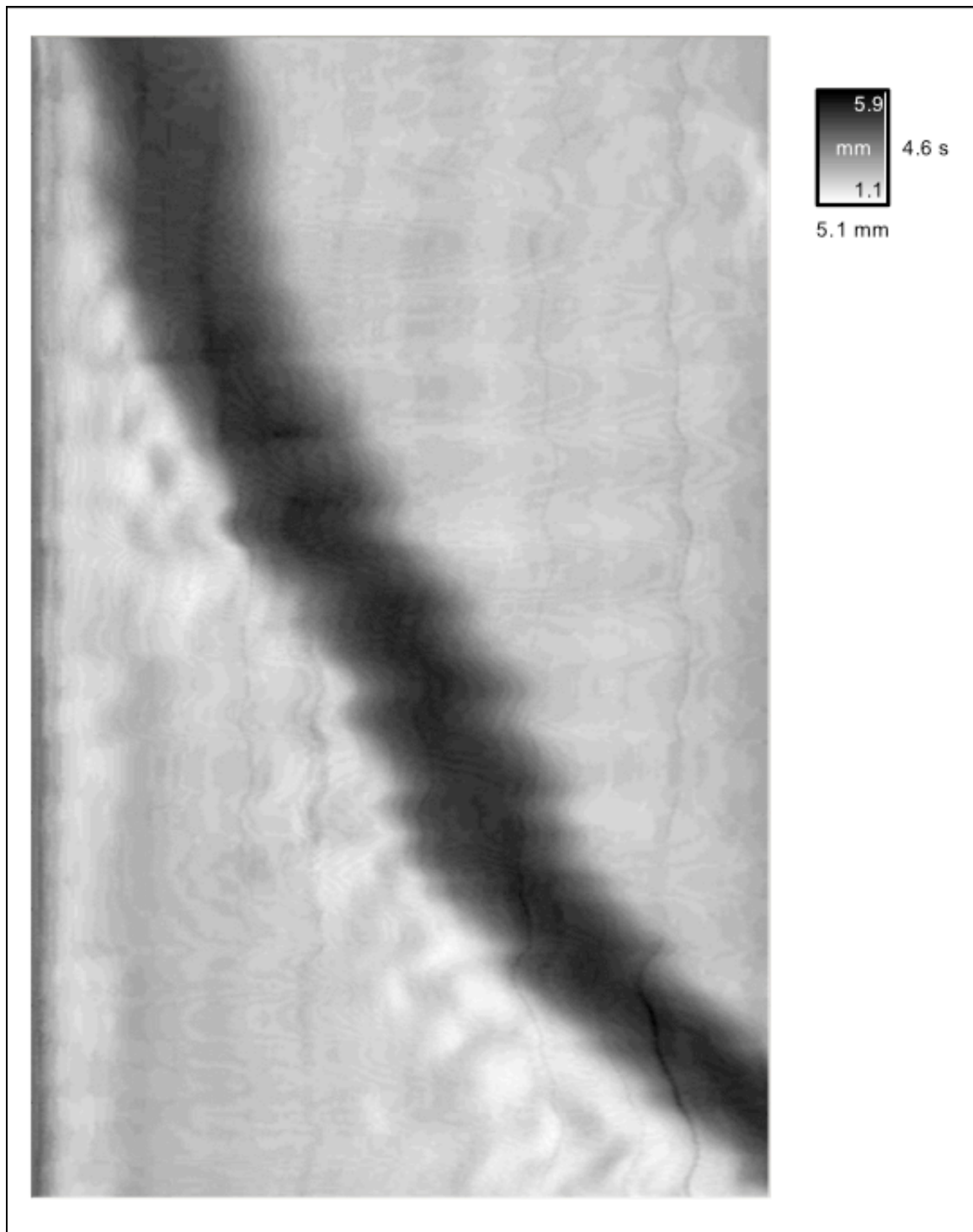


Figure 8. Example Spatiotemporal Map Showing Pellet Movement in Distal Colon

Shown is a spatiotemporal map illustrating the distension of the distal colon induced by a fecal pellet as it is propelled from the oral end more caudally. Using the Y-axis of time (seconds) and X-axis of distance (millimeters), it is possible to calculate the velocity of pellet movement (mm/s). The GIMM software contains pellet-tracking software that allows for velocity calculations without the generation of a spatiotemporal map. This figure is included only to demonstrate the differences between methods in the proximal versus distal colon, and how this is recorded by the computer.

**Figure 8. Example Spatiotemporal Map Showing Pellet Movement in Distal Colon
(Orad – Caudad)**



3. Results

3A) Response of Proximal and Distal Colon to Butyrate

In the proximal colon, four series of quantification were taken for each dose of butyrate that was perfused into the lumen. Full propagations and half propagations were measured separately and then combined for determine total propagations. Non-propagating contractions were counted and recorded.

Full propagations were the clearest to distinguish on the spatiotemporal map. The control baseline frequency of full propagations in response to distension with Krebs buffer in seven proximal colons from seven guinea pigs was 2.0 ± 0.6 per 5-minute period. 10mM butyrate solution increased the frequency of full propagations by 178% of control to a value of 5.6 ± 0.8 per 5-minute period. 30mM butyrate solution also increased the frequency of full propagations by 207% of control to 6.1 ± 0.5 per 5-minute period. 100mM butyrate solution increased the frequency of full propagations by 121% of control to a value of 4.4 ± 0.6 per 5-minute period. (**Figure 9A**)

Short propagations had a control baseline frequency of 3.3 ± 1.2 per 5-minute period. 10mM butyrate increased the frequency of short propagations by 13% of control to 3.7 ± 1.7 per 5-minute period, but 30mM decreased the frequency of short propagations by 65% from control to 1.1 ± 0.5 per 5-minute period. 100mM butyrate further decreased the frequency of short propagations by 87% from control to 0.4 ± 0.4 per 5-minute period. (**Figure 9B**)

Total propagations were summations of both the full and short propagations, which showed a baseline frequency of 5.3 ± 1.4 per 5-minute period. 10mM butyrate increased the frequency of total propagations by 75% of control to 9.3 ± 2 per 5-minute

period, while 30mM butyrate increased the frequency of total propagations by 35% of control to 7.1 ± 0.7 per 5-minute period. 100mM butyrate decreased the frequency of total propagations by 8% from control to 4.9 ± 0.8 per 5-minute period. **(Figure 9C)**

Non-propagating contractions had a control baseline frequency of 68.6 ± 8.9 per 5-minute period. 10mM butyrate increased the frequency of non-propagations by 46% of control to 100.6 ± 26.0 per 5-minute period. 30mM butyrate increased the frequency of non-propagations by 26% of control to 86.7 ± 11.0 per 5-minute period. 100mM butyrate decreased the frequency of non-propagations by 58% to 29.0 ± 9.0 per 5-minute period. **(Figure 10)**

Overall motility of all seven proximal colons increased following perfusion of butyrate at 10mM and remained increased at increasing concentrations.

Propagating motility was more prominent than non-propagations following perfusion of butyrate at 100mM. Propagating contractions also increased in duration of contraction (occlusion) compared to control, as indicated by the thickness of the bands on the spatiotemporal maps. **(Figure 11)**

In the distal colon, artificial fecal pellets were inserted into the orad end and the velocity was tracked using GIMM software. The baseline velocity of three segments from three guinea pigs was 1.22 ± 0.1 mm/s. Following treatment of 30mM butyrate via intraluminal perfusion at 0.1ml/min caudad to the pellet, the velocity increased 30% to 1.6 ± 0.1 mm/s. **(figure 12)**

Figure 9. Effect of Butyrate on Propagating Contractions in Proximal Colon

Butyrate in solution in Krebs buffer (0.5 to 1.0 ml) was perfused into the lumen of proximal colon closed on the caudad end and was used to cause minimal distension. (A) At all doses, butyrate increased full-length propagations with the greatest increase at 30mM. (B) With increasing concentration, short propagations were diminished in favor of full-length propagations. (C) The opposing effects of butyrate on full-length vs. short propagations resulted in a non-significant change in total contractions. Data represents number of propagated contractions per 5 min period (n=7, Mean \pm SEM, $p < 0.05$)

Figure 9. Effect of Butyrate on Propagating Contractions in Proximal Colon

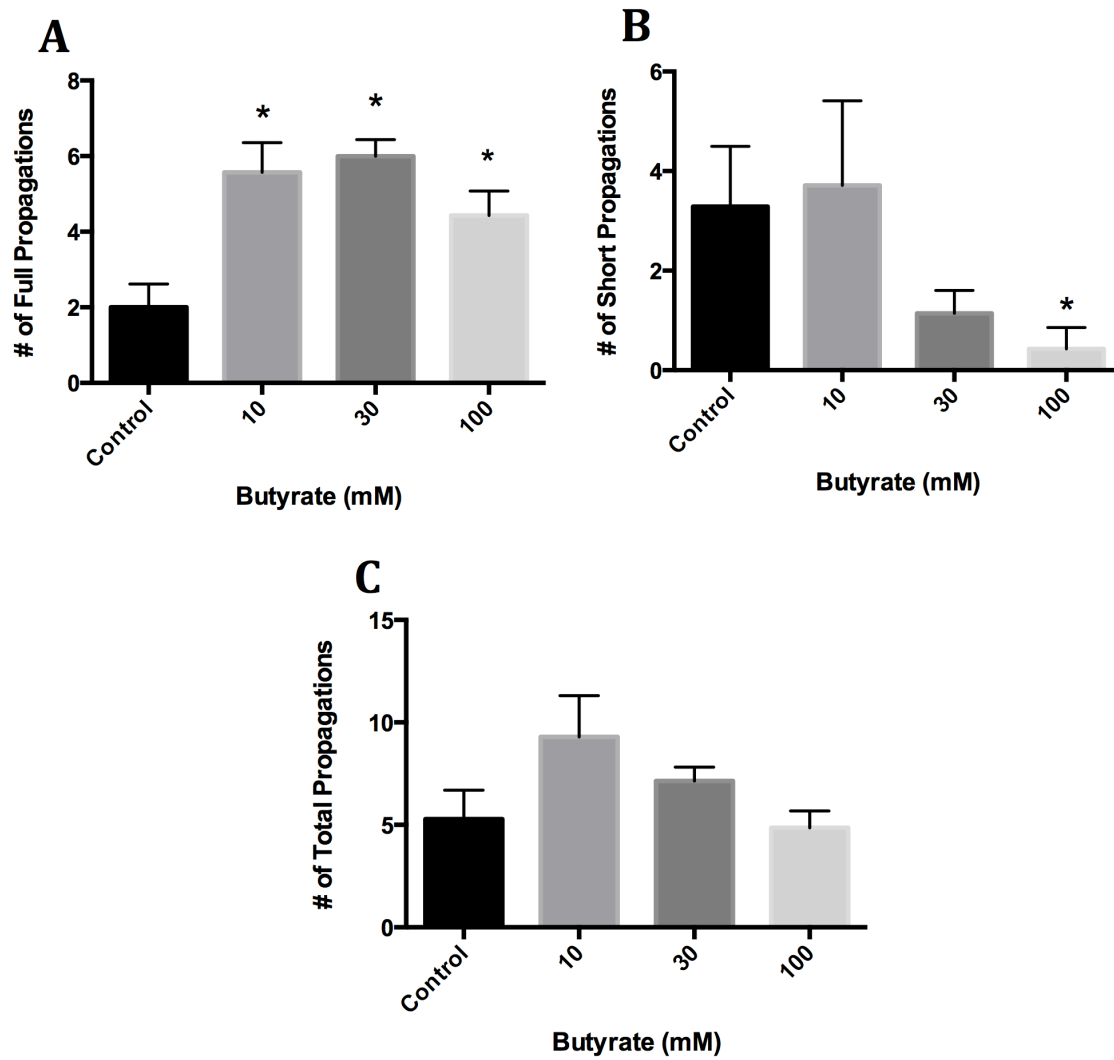


Figure 10. Effect of Butyrate on Non-Propagating Contractions in Proximal Colon

Following perfusion and distension using a solution of butyrate in Krebs buffer, non-propagations were quantified in the proximal colon. At 10mM, butyrate increased non-propagating contractions. As the concentration increased, non-propagations decreased. At 100mM, these non-propagations were significantly decreased. Data represents number of non-propagated contractions per 5 min period (n=7, Mean \pm SEM, $p < 0.05$)

Figure 10. Effect of Butyrate on Non-Propagating Contractions in Proximal Colon

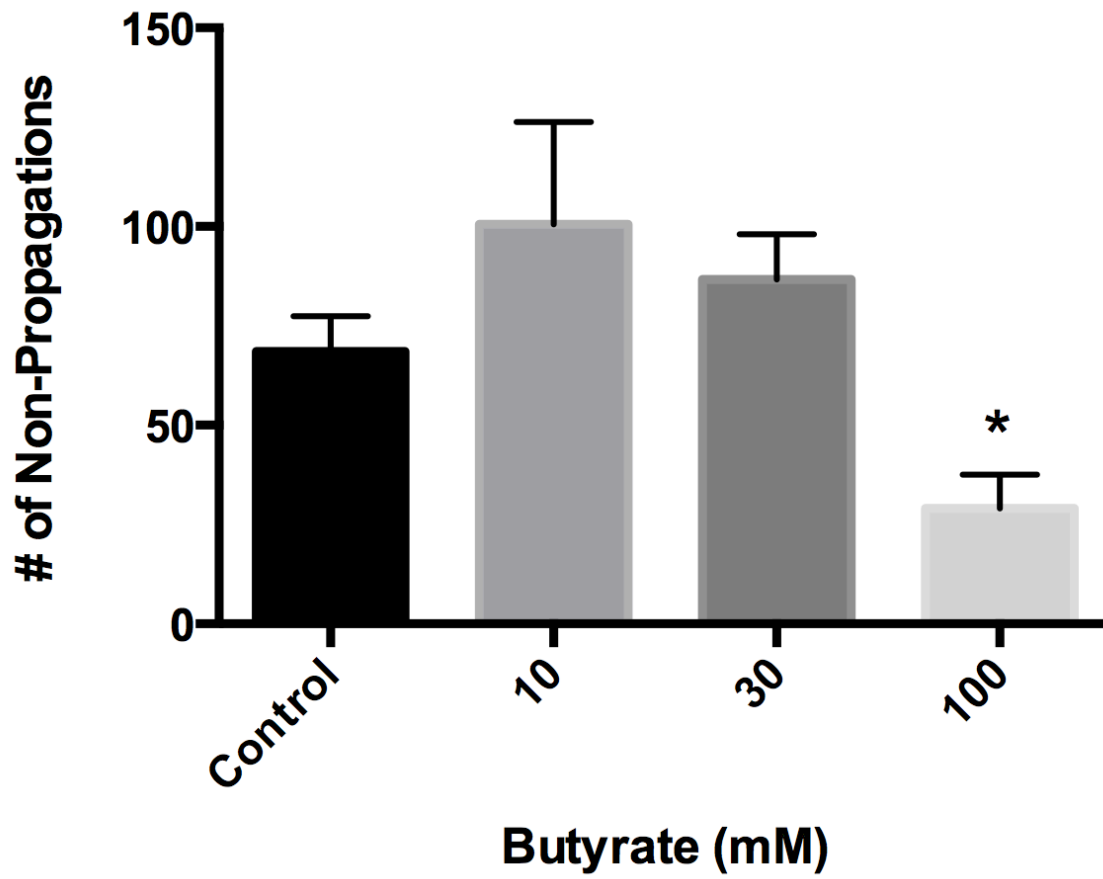


Figure 11. Overall Motility of Proximal Colon Following Butyrate Treatment

Compared to control, an overall increase in motility in the proximal colon was observed following luminal perfusion of butyrate solution. As the concentration of butyrate increased, non-propagations were decreased in favor of propagating contractions. The propagated contractions were of increased duration compared to control, as indicated by band thickness.

Figure 11. Overall Motility of Proximal Colon Following Butyrate Treatment

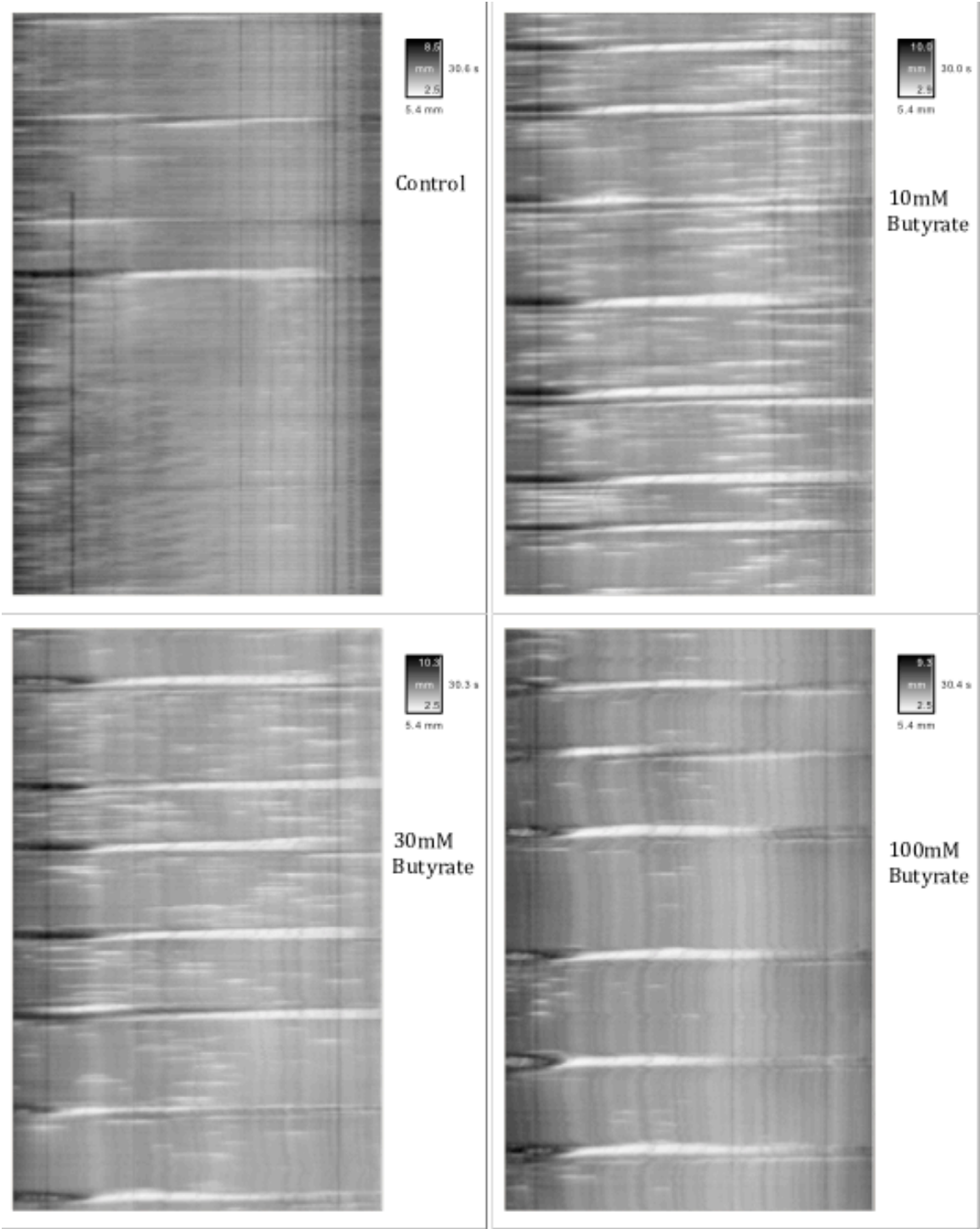
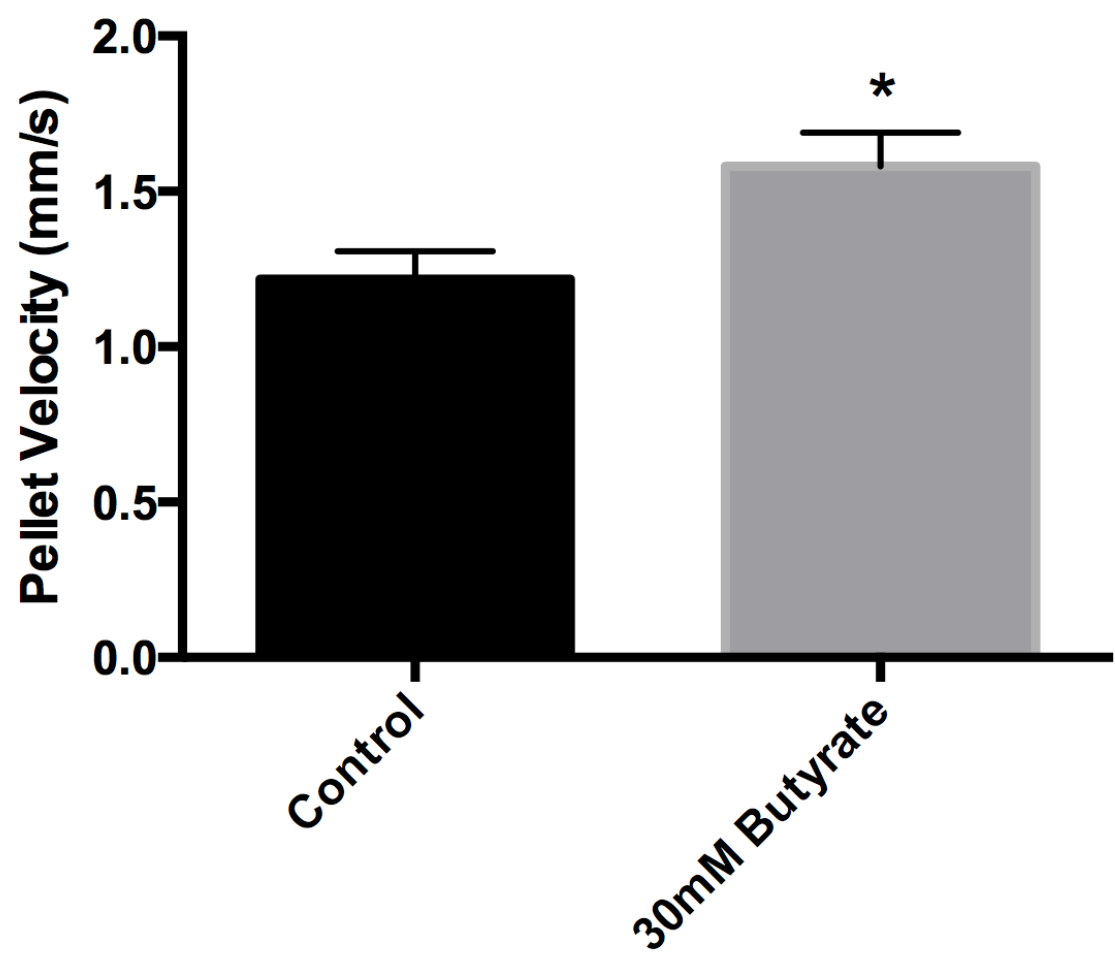


Figure 12. Mean Pellet Velocity Following Caudal Perfusion of 30mM Butyrate

Following perfusion of 30mM butyrate via a flexible catheter (PE 10 tubing) inserted in the caudal end of distal colon, pellet velocity was increased compared to control velocity. Control velocities were 1.2 ± 0.1 mm/s and increased to 1.6 ± 0.1 mm/s following 30mM butyrate intraluminal perfusion. (n=3, Mean \pm SEM, $p < 0.05$)

Figure 12. Mean Pellet Velocity of Artificial Fecal Pellet Following 30mM Caudal Perfusion in Distal Colon



3B) Response of Proximal and Distal Colon to Propionate

Full propagations in the proximal colons of nine guinea pigs had a baseline frequency of full propagations of 3.0 ± 1.0 per 5-minute period. These propagations were completely abolished at 10mM propionate. At 30mM propionate, the frequency of full propagations were decreased by 96% from control to a value of 0.2 ± 0.2 per 5-minute period. 100mM propionate decreased the frequency of full propagations by 54% from control to a value of 1.4 ± 0.4 per 5-minute period.

(Figure 13A)

Short propagations were similarly decreased from a baseline frequency of short propagations of 2.0 ± 0.4 per 5-minute period. At 10mM concentration, short propagations were completely abolished. 30mM propionate decreased the frequency of short propagations by 89% from control to a value of 0.2 ± 0.2 per 5-minute period. At 100mM, propionate decreased the frequency of short propagations by 33% from control to a value of 1.3 ± 1.0 per 5-minute period.

(Figure 13B)

Total propagations were summations of both the full and short propagations, which showed a baseline control frequency of 4.8 ± 1.2 per 5-minute period. 10mM propionate completely abolished propagations, while 30mM decreased the frequency of total propagations by 90% from control to a 0.4 ± 0.3 per 5-minute period. 100mM propionate decreased the frequency of total propagations by 42% from control to 2.8 ± 1.3 per 5-minute period. **(Figure 13C)**

Non-propagating contractions had a baseline frequency of 76 ± 24 per 5-minute period. At 10mM, propionate decreased the frequency of non-propagations

by 68% from control to 24 ± 12 . per 5-minute period. 30mM propionate increased the frequency of non-propagations by 52% of control to 115 ± 40 per 5-minute period. 100mM propionate further increased the frequency of non-propagations by 64% of control to 124 ± 45 per 5-minute period. **(Figure 14)**

Overall motility in all nine proximal colons was decreased following perfusion of propionate at 10mM, with a return of non-propagating motility at 30mM and 100mM. Propagating motility increased with propionate concentration, but remained slightly diminished from control. **(Figure 15)**

The propulsion of artificial pellets through the distal colon was recorded from four segments from four guinea pigs and the velocity was calculated. The control baseline velocity was 1.7 ± 0.2 mm/s. Following treatment with 30mM propionate via intraluminal perfusion at 0.1 ml/min caudad to the pellet, the velocity decreased by 75% to 0.4 ± 0.2 mm/s. **(Figure 16)**

Figure 13. Effect of Propionate on Propagating Contractions in Proximal Colon

Propionate in solution in Krebs buffer (0.5 to 1.0 ml) was perfused into the lumen of proximal colon closed on the caudad end and was used to cause minimal distension. (A) At all doses, propionate decreased full-length propagations versus control, with the greatest decrease at 10mM. (B) Short propagations were diminished at lower concentrations, and abolished at 10mM. (C) The inhibitory effect of propionate on propagations showed an overall decrease in total propagations. Data represents number of propagations per 5 min period. (n=9, Mean \pm SEM, $p < 0.05$)

Figure 13. Effect of Propionate on Propagating Contractions in Proximal Colon

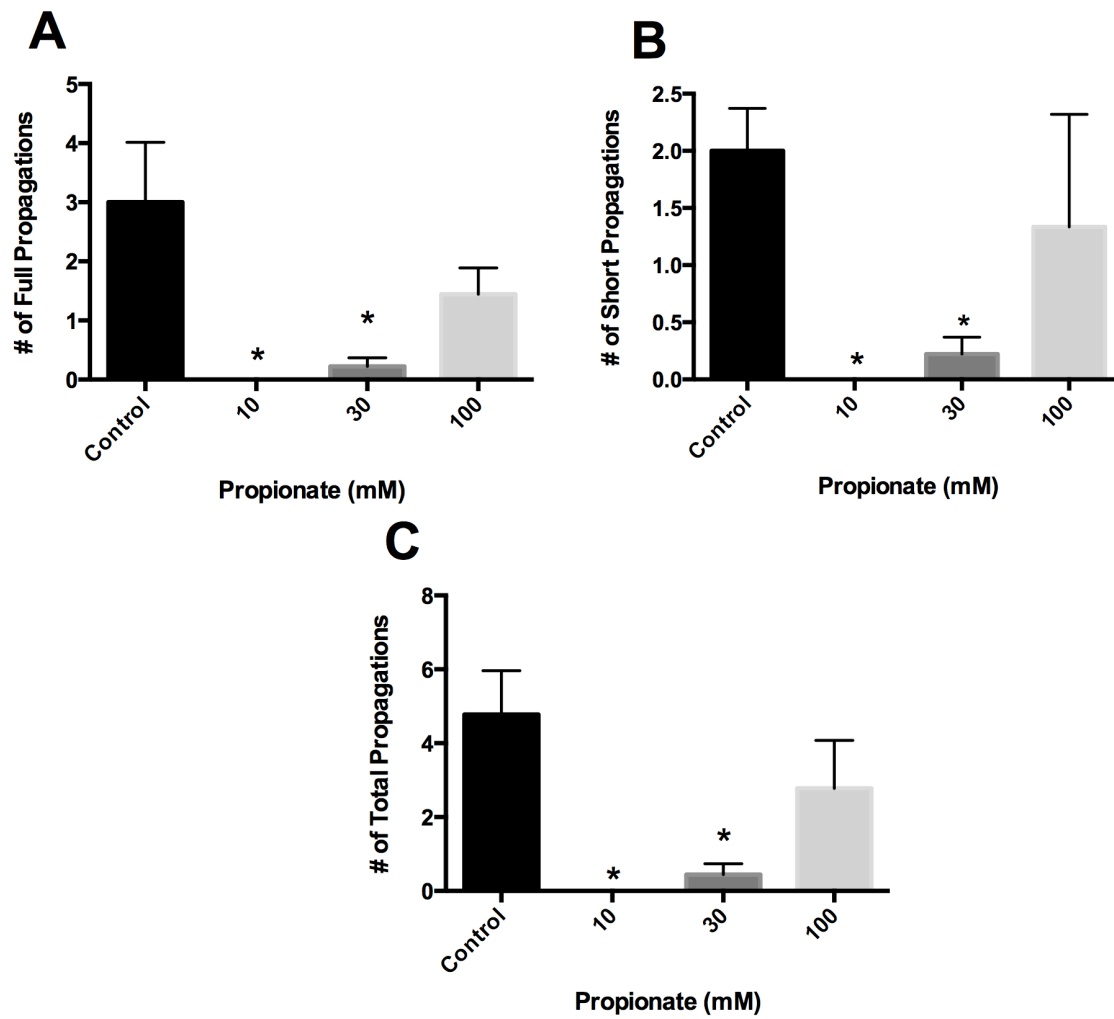


Figure 14. Effect of Propionate on Non-Propagating Contractions in Proximal Colon

Following perfusion and distension using a solution of propionate in Krebs buffer, non-propagations were quantified in the proximal colon. At 10mM, propionate decreased non-propagating contractions. As the concentration increased, non-propagations increased. This increase in non-propagations was generally opposed to the effect on propagations above 30mM. Data represents number of non-propagated contractions per 5 min period. (n=9, Mean \pm SEM)

Figure 14. Effect of Propionate on Non-Propagating Contractions in Proximal Colon

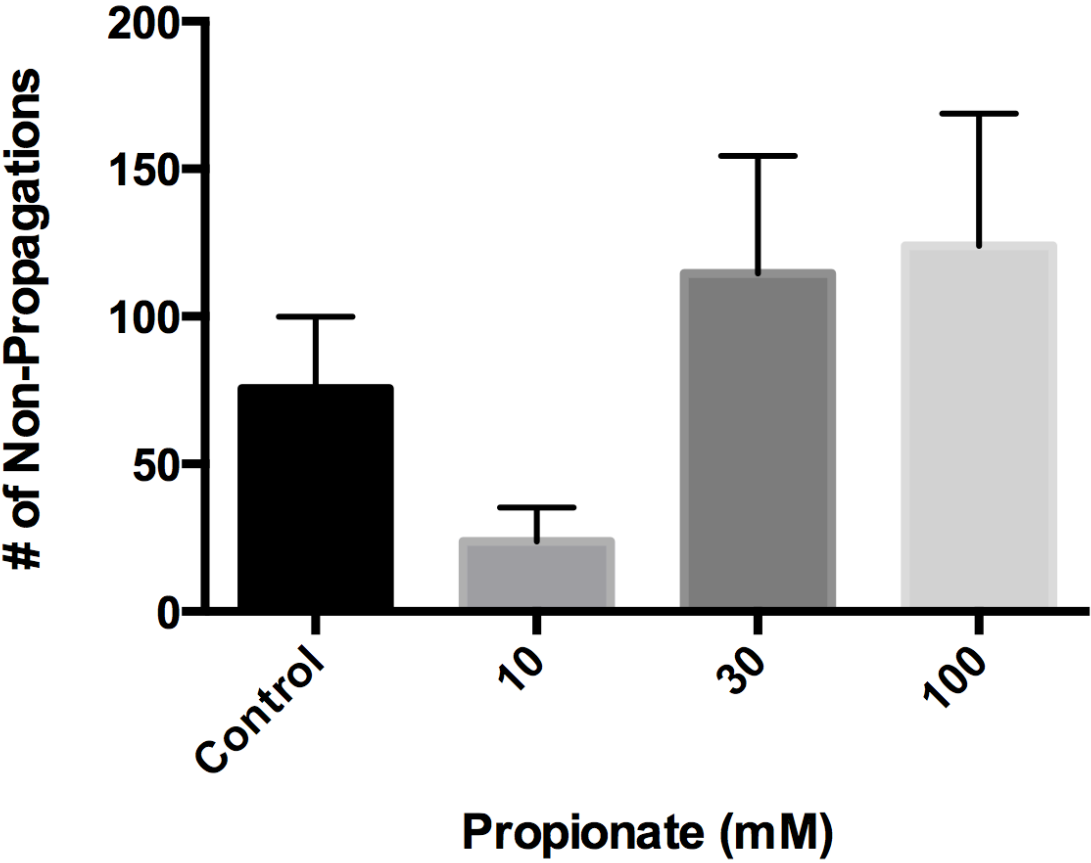


Figure 15. Overall Motility of Proximal Colon Following Propionate Treatment

Compared to control, an overall decrease in motility in the proximal colon was observed following luminal perfusion of propionate solution. As the concentration of propionate increased, motility increased but remained low compared to control. Non-propagations were more frequent at higher concentrations of propionate compared to propagating contractions.

Figure 15. Overall Motility of Proximal Colon Following Propionate Treatment

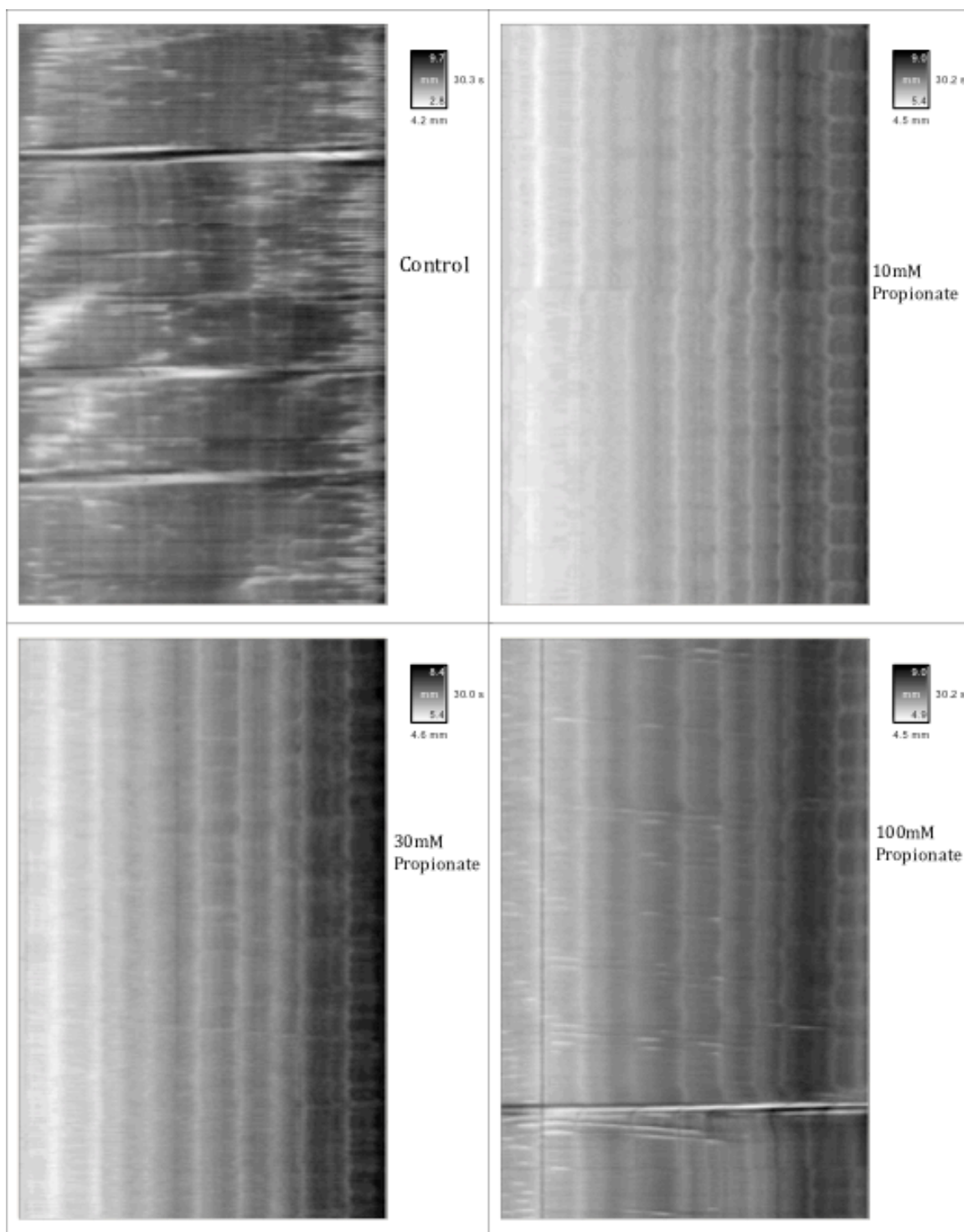
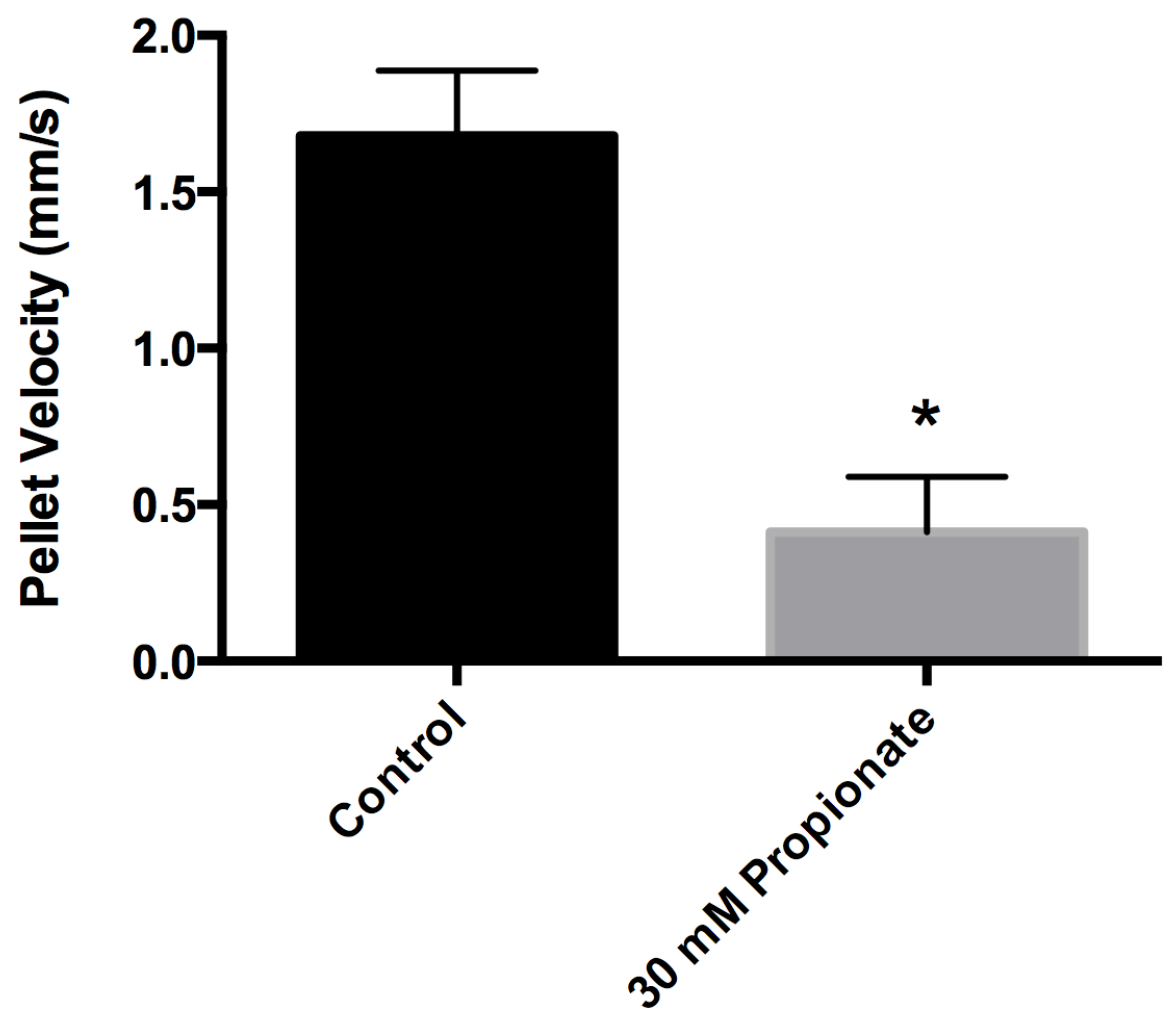


Figure 16. Mean Pellet Velocity Following Caudal Perfusion of 30mM Propionate

Following perfusion of 30mM propionate via a flexible catheter (PE 10 tubing) inserted in the caudal end of distal colon, pellet velocity was decreased compared to control velocity. Control velocities were 1.7 ± 0.2 mm/s and decreased to 0.4 ± 0.2 mm/s following 30mM butyrate intraluminal perfusion. (n=4, Mean \pm SEM, $p < 0.05$)

Figure 16. Mean Pellet Velocity Following Caudal Perfusion of 30mM Propionate



C3) Response of Proximal and Distal Colon to Acetate

Full propagations had a baseline frequency of 3.3 ± 0.7 per 5-minute period. At 10mM, acetate decreased the frequency of full propagations 40% from control to 2.0 ± 0.58 per 5-minute period. 30mM acetate decreased the frequency of full propagations 30% from control to 2.3 ± 0.3 per 5-minute period, while 100mM acetate increased the frequency of full propagations 10% of control to 3.7 ± 0.9 per 5-minute period. **(Figure 17A)**

Short propagations showed an overall decrease from a baseline frequency of 7.0 ± 2.1 per 5-minute period. At 10mM, acetate decreased the frequency of short propagations 24% from control to 5.3 ± 1.5 per 5-minute period. 30mM acetate further decreased the frequency of short propagations 57% from control to 3.0 ± 1.2 per 5-minute period, and 100mM acetate decreased the frequency of short propagations 81% from control to 1.3 ± 0.9 per 5-minute period. **(Figure 17B)**

Total propagations were summations for full and short propagations with a baseline frequency of 10.3 ± 1.4 per 5-minute period. 10mM acetate decreased the frequency of total propagations 29% from control to 7.3 ± 1.3 per 5-minute period. 30mM acetate further decreased the frequency of total propagations 45% from control to 5.7 ± 1.4 per 5-minute period, and 100mM decreased the frequency of total propagations 51% from control to 5.0 ± 1.7 per 5-minute period. **(Figure 17C)**

Non-propagations had a baseline frequency of 99.7 ± 23 per 5-minute period. 10mM decreased the frequency of non-propagations 49% from control to 50.7 ± 15 per 5-minute period. 30mM acetate decreased the frequency of non-propagations

40% from control to 59.3 ± 12 per 5-minute period. 100mM acetate decreased the frequency of non-propagations 56% from control to 43.7 ± 6.4 per 5-minute period. **(Figure 18)**

Overall motility was decreased in all three proximal colons following perfusion of acetate and continued to decrease as concentration was increased. Both propagations and non-propagations showed an overall decrease compared to control. **(Figure 19)**

The propulsion of artificial pellets through the distal colon was recorded from two segments from two guinea pigs and the velocity was calculated. The control baseline velocity was 1.8 ± 0.1 mm/s. Following treatment of 30mM acetate via intraluminal perfusion at 0.1ml/min caudad to the pellet, the velocity decreased 47% to 0.95 ± 0.3 mm/s. **(Figure 20)**

Figure 17. Effect of Acetate on Propagating Contractions in Proximal Colon

Acetate in solution in Krebs buffer (0.5 to 1.0 ml) was perfused into the lumen of proximal colon closed on the caudad end and was used to cause minimal distension. (A) At all doses, acetate had no significant effect on full-length propagations versus control. As dose increased, full propagations tended to increase back to control values. (B) Short propagations were diminished at higher concentrations. (C) The overall inhibitory trend of acetate on propagations showed a decrease in total propagations. Data represent number of propagations per 5 min period. (n=3, Mean \pm SEM)

Figure 17. Effect of Acetate on Propagating Contractions in Proximal Colon

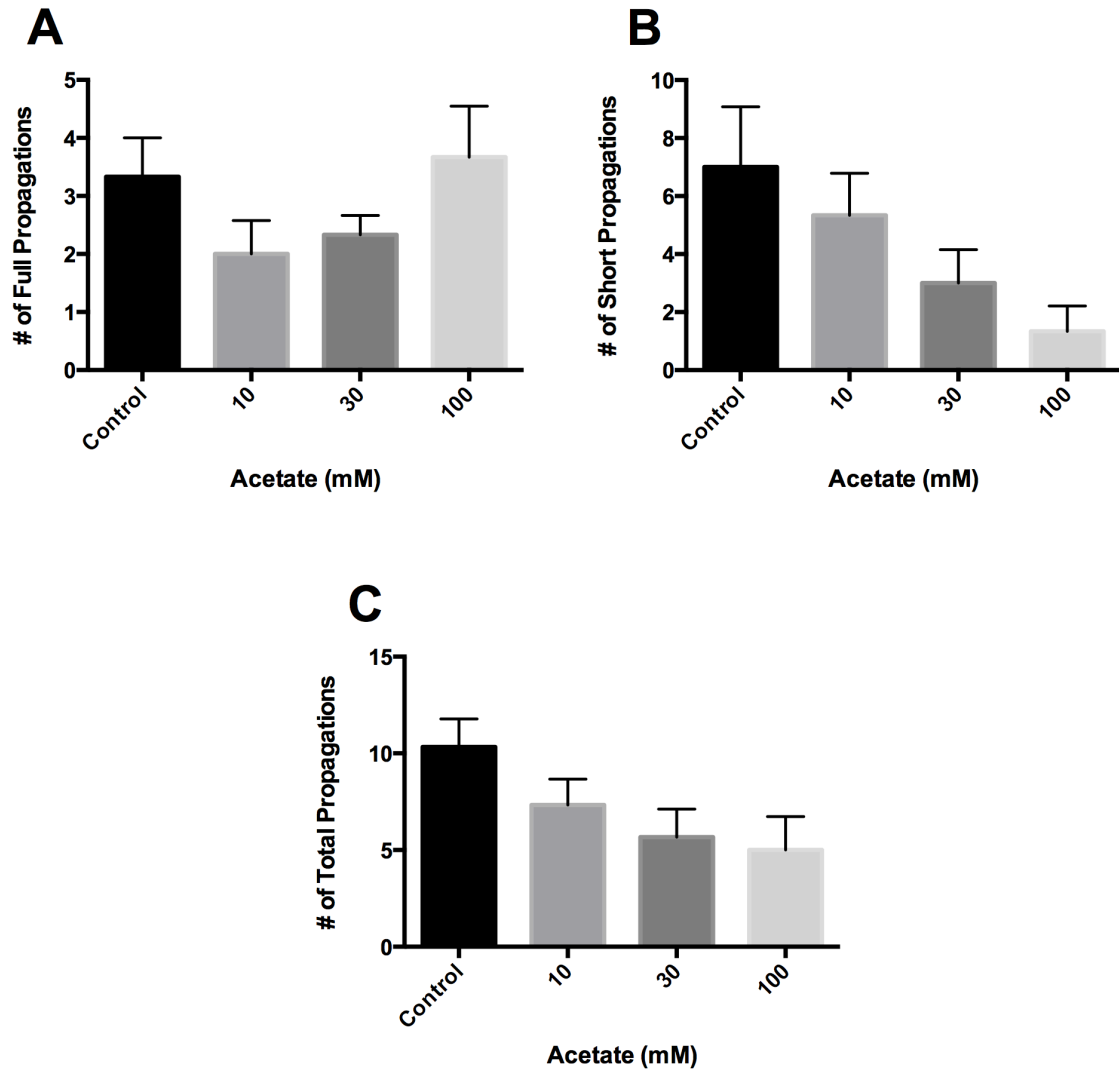


Figure 18. Effect of Acetate on Non-Propagating Contractions in Proximal Colon

Following perfusion and distension using a solution of acetate in Krebs buffer, non-propagations were quantified in the proximal colon. At all doses, acetate decreased non-propagating contractions versus control. Data represent number of non-propagating contractions per 5 min period. (n=3, Mean \pm SEM)

Figure 18. Effect of Acetate on Non-Propagating Contractions in Proximal Colon

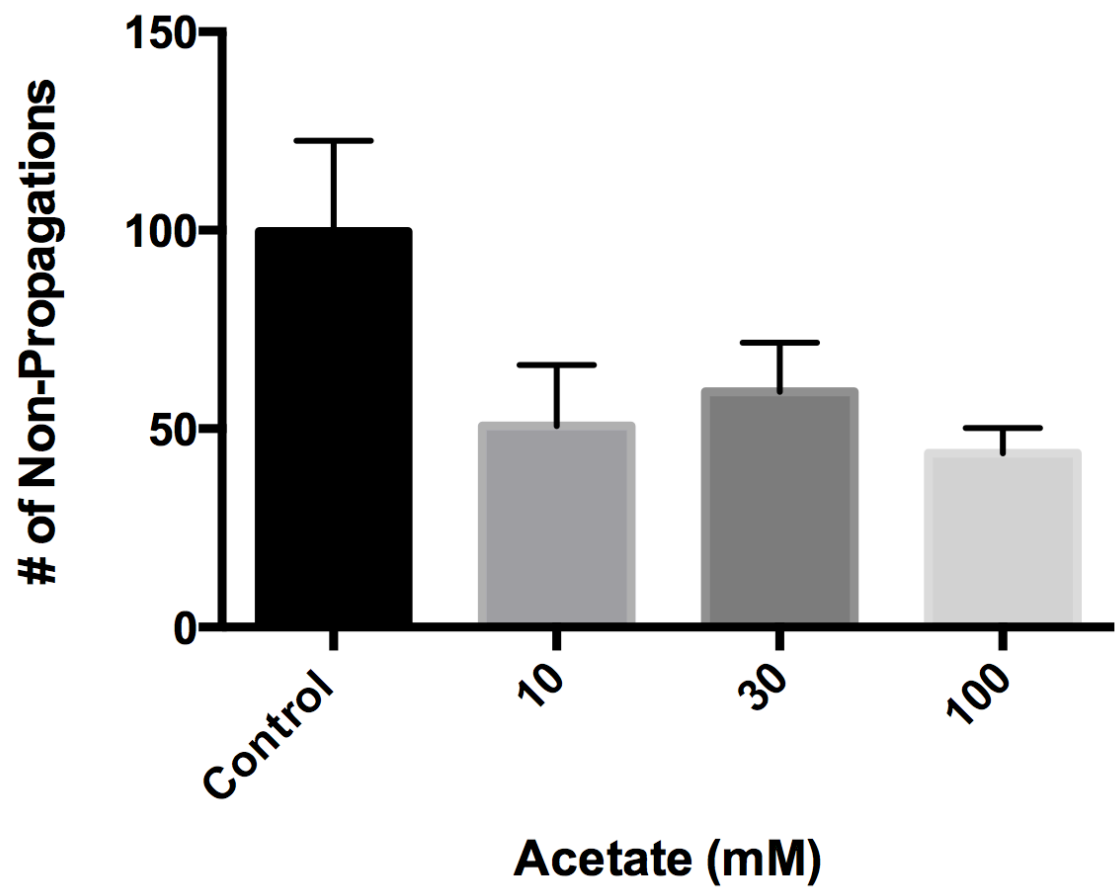


Figure 19. Overall Motility of Proximal Colon Following Acetate Treatment

Compared to control, an overall decrease in motility in the proximal colon was observed following luminal perfusion of acetate solution. Both propagations and non-propagations decreased slightly with increasing concentration of acetate.

Figure 19. Overall Motility of Proximal Colon Following Acetate Treatment

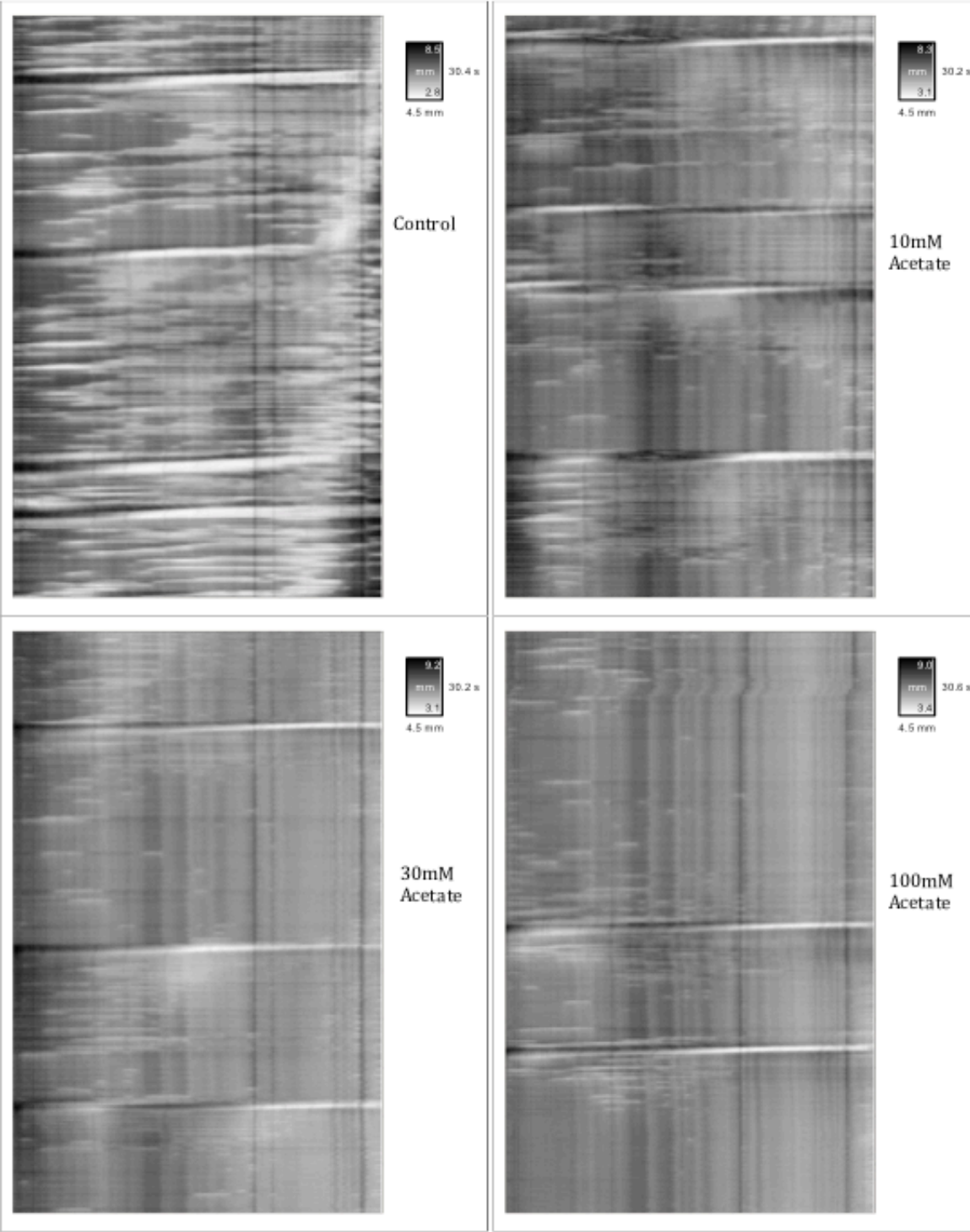
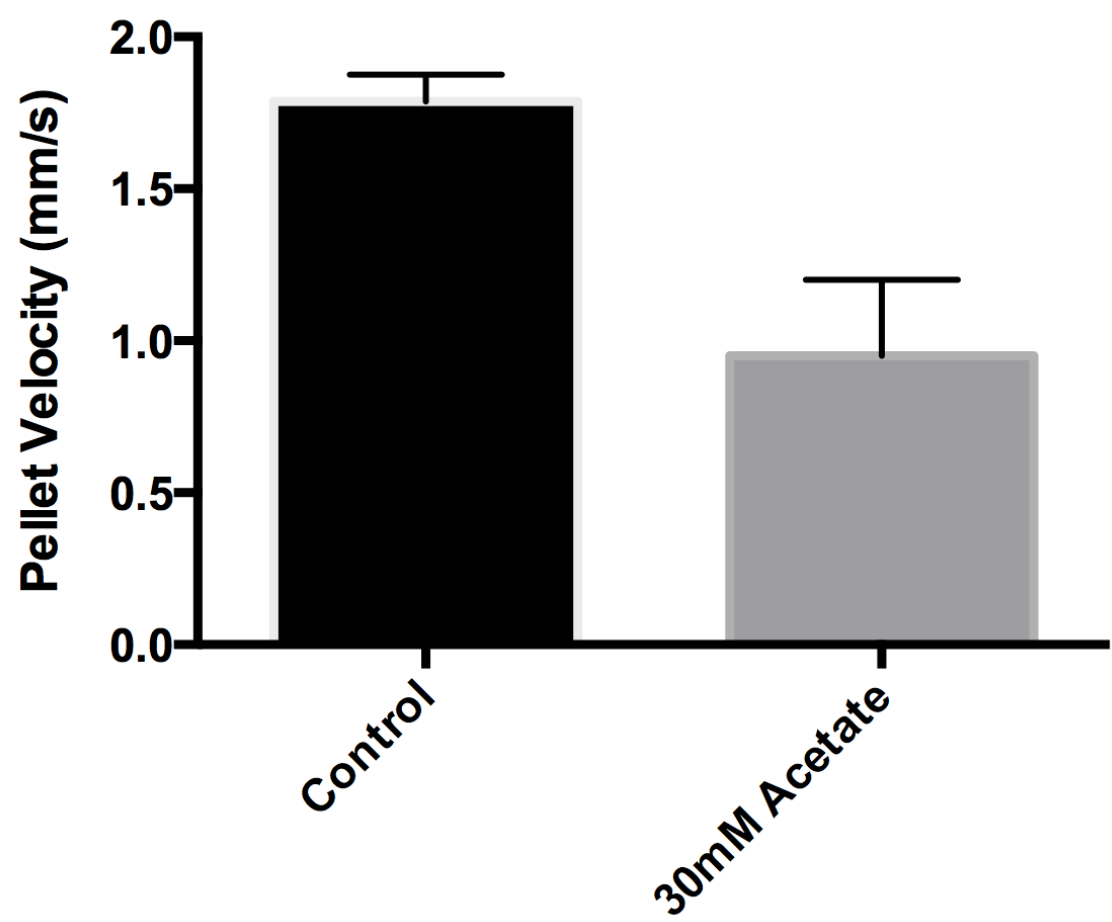


Figure 20. Mean Pellet Velocity Following Caudal Perfusion of 30mM Acetate

Following perfusion of 30mM acetate via a flexible catheter (PE 10 tubing) inserted in the caudal end of distal colon, pellet velocity was decreased compared to control velocity. Control velocities were 1.8 ± 0.1 mm/s and increased to 0.95 ± 0.3 mm/s following 30mM acetate intraluminal perfusion. (n=2, Mean \pm SEM)

Figure 20. Mean Pellet Velocity Following Caudal Perfusion of 30mM Aceate



C4) Response of Proximal Colon to Equimolar and Physiological Ratios of SCFAs

Five proximal colons from five guinea pigs were used to determine the effect of mixtures of SCFAs on colonic motility. In two cases, an equimolar ratio (10mM acetate, 10mM butyrate, and 10mM propionate) was perfused into the colon first. Following a 10-minute wash with Krebs buffer, a second perfusion was done using an equivalent total of SCFAs at 30mM but in a ratio more resembling physiological concentrations (15mM acetate, 10mM butyrate, 5mM propionate). In the remaining three trials, the order of mixture perfusion was reversed.

Neither full propagations nor short propagations behaved differently depending on the order of SCFA ratios used, and for this reason were counted together. Full propagations were no different from control to either mixture, with a baseline frequency of full propagations of 2.0 ± 0.8 per 5-minute period. **(Figure 21A)** In all cases, short propagations were completely abolished from control, which had a baseline frequency of short propagations of 2.2 ± 0.7 per 5-minute period. **(Figure 21B)**

In the two trials that perfused equimolar amounts of SCFAs first, non-propagations were abolished from a baseline frequency non-propagations of 37.5 ± 8.5 per 5-minute period and did not return after a luminal washout with Krebs buffer followed by a physiological ratio of SCFAs. However, in the three trials that started with a physiological ratio of SCFAs and were followed by an equimolar ratio, there was no change in the frequency of non-propagations from a baseline frequency of non-propagations of 70.7 ± 24 per 5-minute period. **(Figure 22)**

Four of the five segments showed an overall decrease in motility from control, which persisted throughout the remainder of the experiment. **(Figure 23)**

Figure 21. Effects of Combinations of SCFAs on Propagating Contractions in Proximal Colon

Whether SCFAs were combined in 30mM total concentration in equal or physiological ratio (10mM acetate, propionate, and butyrate; or 15mM acetate, 10mM butyrate, and 5mM propionate) there was no difference in their effect. (A) Full-length propagations were not modified with either combination. (B) Short propagations were completely abolished with either combination. (C) Overall, a slight decrease in total propagations were observed versus control in both mixtures. (n=5, Mean \pm SEM, $p < 0.05$)

Figure 21. Effects of Combinations of SCFAs on Propagating Contractions in Proximal Colon

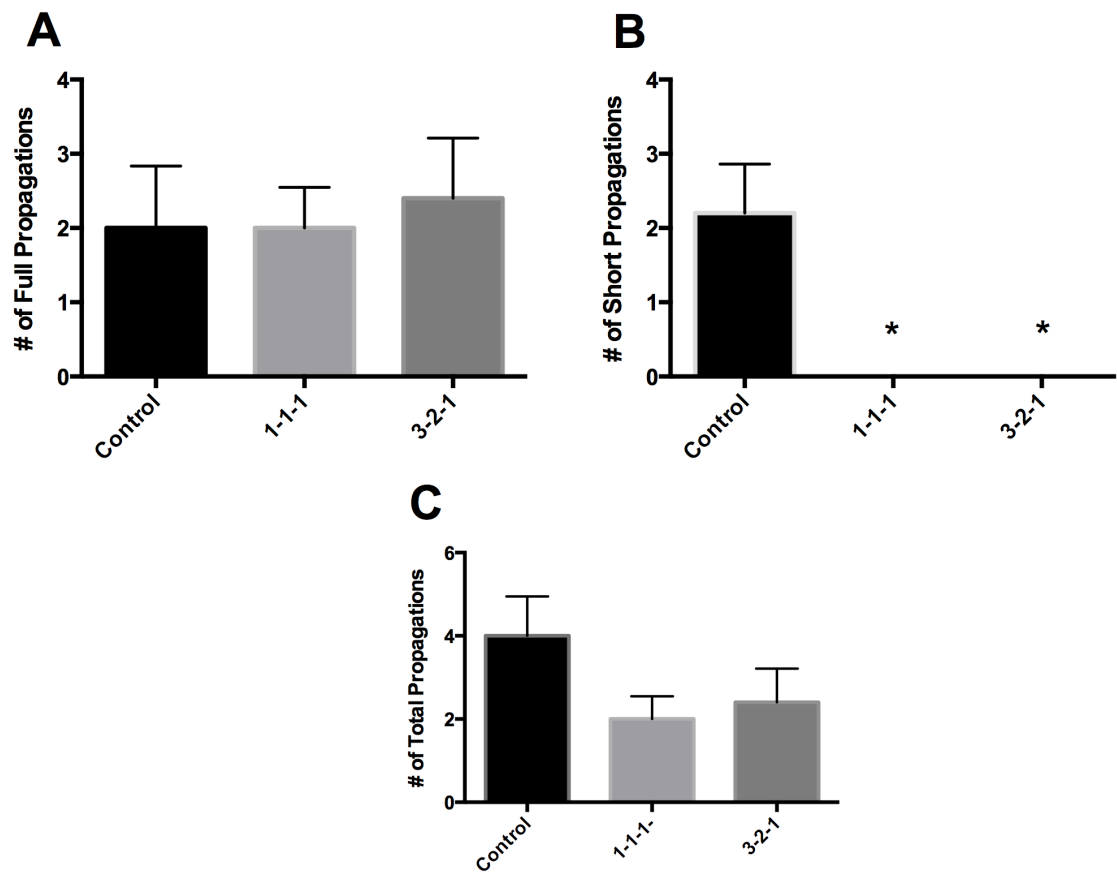


Figure 22. Effect of Mixture Order on Non-propagations in Proximal Colon

If an equimolar mixture of SCFAs was luminally perfused following control (n=2), it had a lasting inhibitory effect on non-propagating contractions. However, if a physiological mixture of SCFAs was perfused following control (n=3), there was no significant effect on non-propagations. (Mean \pm SEM, $p < 0.05$)

Figure 22. Effect of Mixture Order on Non-propagations in Proximal Colon

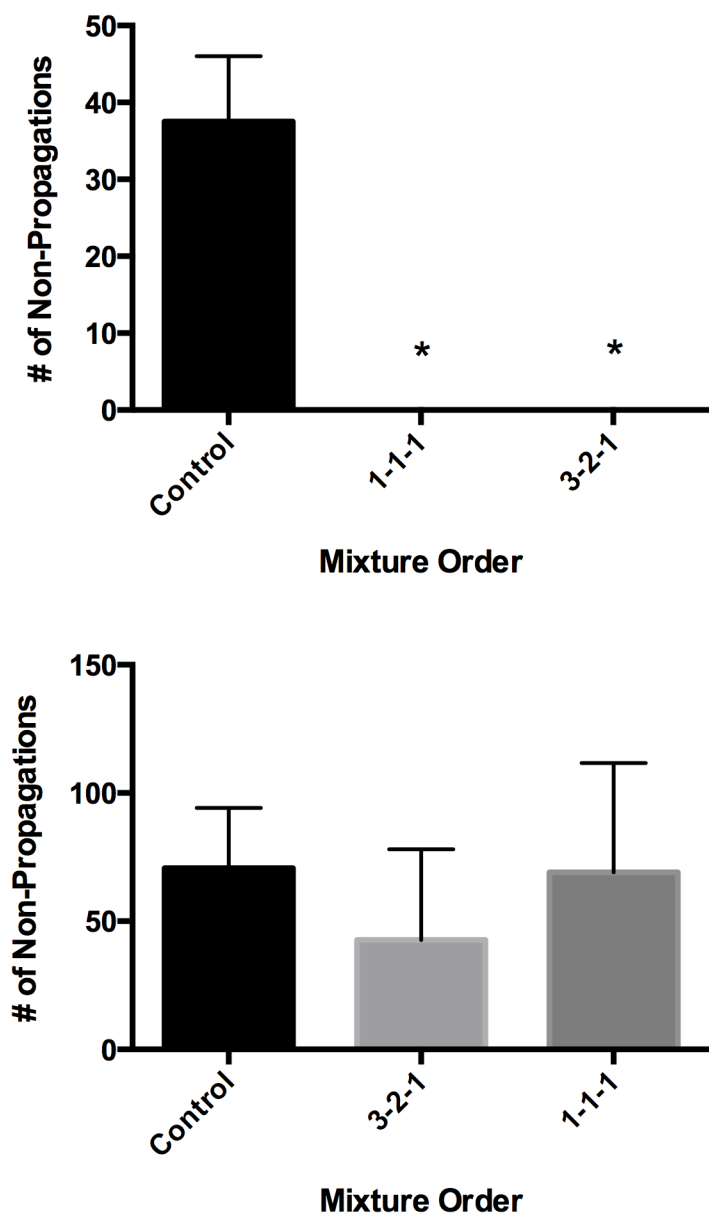
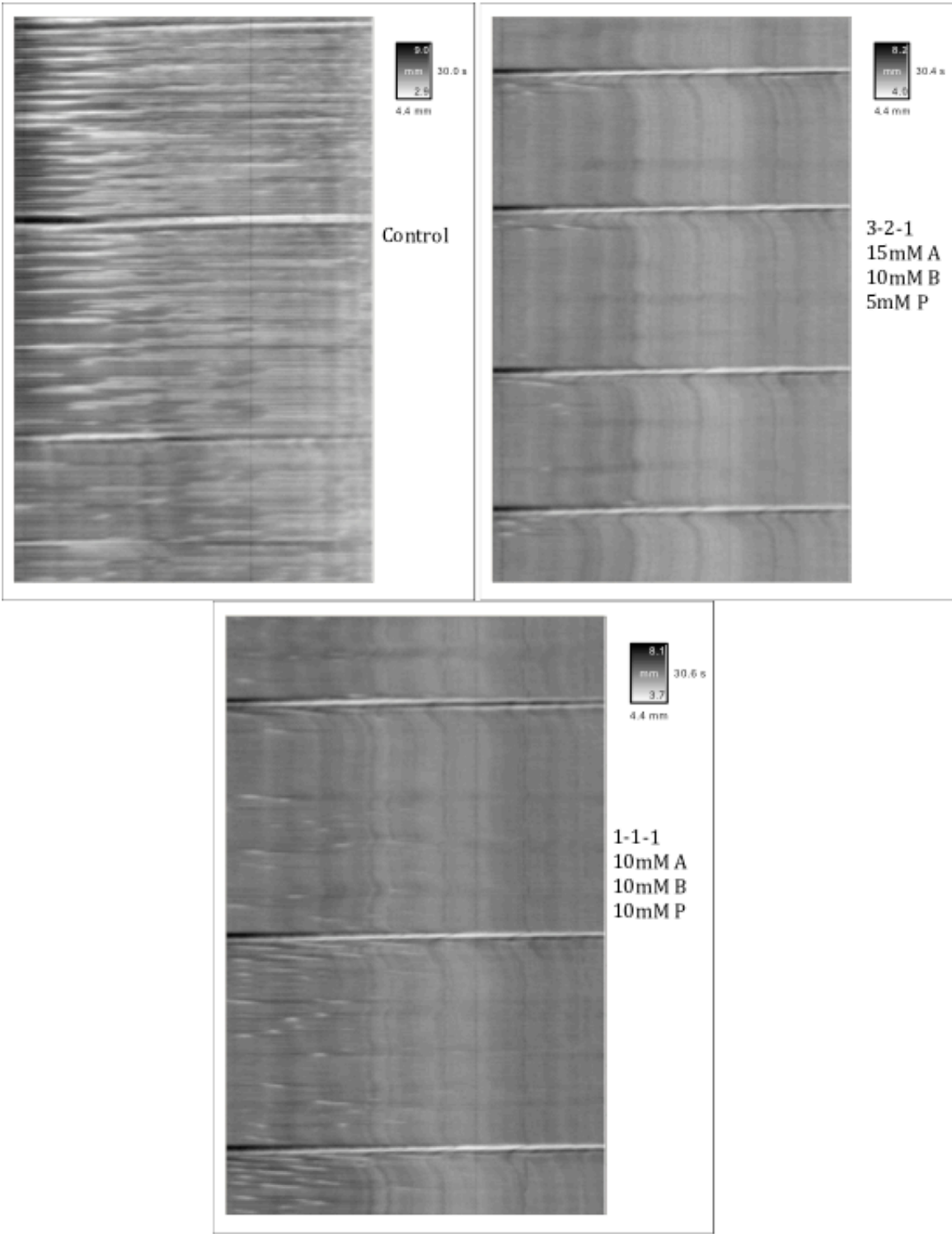


Figure 23. Effect of SCFA mixtures on Overall Motility in Proximal Colon

Following perfusion of SCFA mixtures with a total concentration of 30mM, an overall decrease in motility was observed. In trials which had the physiological first (pictured) non-propagations still occurred, whereas non-propagations were abolished with an initial perfusion of an equimolar mixture.

Figure 23. Effect of SCFA mixtures on overall motility in proximal colon



4. Discussion

The series of experiments demonstrated that individual SCFAs have differential effects when perfused through the lumen of the proximal and distal colon of the guinea pig. In particular, butyrate at physiological concentrations of 10 or 30mM increased the frequency of propulsive contractions in the proximal colon and increased the velocity of artificial fecal pellets in the distal colon. Propionate at the same concentration decreased frequency of propulsive contractions in the proximal colon and decreased velocity of the artificial fecal pellets in the distal colon. Acetate showed a slight decreased in propulsive contractions in the proximal colon as well as pellet velocity in the distal colon. At equimolar and physiological mixtures, SCFAs showed decreased overall motility in the proximal colon.

The effects observed throughout this study demonstrate differential effects that result from the presence of SCFAs in the lumen. Because sodium salts of acetate, propionate and butyrate were used, there was no shift in pH upon solvation of the SCFAs. However, an increase of 0.1M sodium was introduced to the solution and lumen at maximal doses of any SCFA solution. Therefore, control experiments were performed using standard Krebs buffer with 0.1M additional NaCl. This showed no difference in the motility of the proximal colon. The result of this control is further enhanced by the opposite effects observed by propionate and butyrate at the same concentration.

In the ileum of many species, the presence of SCFAs result in strong caudal propagations. (59, 60, 61) The bacteria of the microbiome reside in the colon and cecum and receive undigested material that exits the ileum. Leakage of the cecocolonic contents

back into the ileum would introduce bacteria to the small intestine. Small intestinal bacterial overgrowth (SIBO) is thought to contribute to IBS, though evidence from case to case is contradictory (62). It is likely that the contractile effect of SCFAs in the distal ileum are preventative to small intestinal colonization by colonic bacteria.

In the cecum and colon, however, the microbiome is continuously present. Indeed, the SCFAs are the primary nutrient of colonocytes, and also play an osmotic role in water and ion transport. As contents are moved from the cecum to distal colon, they become more solid and are formed into feces to be excreted. The proximal colon is the primary site of water and ion absorption. Therefore, the movement of luminal contents should allow enough time for fermentation, and thus nutrient absorption and water and ion transport to occur. This can explain the slowing of overall motility with acetate, propionate, and both mixture ratios. It is also the reasons that pellets were used in the distal colon and fluid was used in the proximal colon.

The overall decrease in motility following perfusion of the mixtures agrees with previous studies performed by Cherbut (19) which showed decreased frequency of spike bursts of muscle in rat colon following infusion of a physiological SCFA mixture. This decrease in spike bursts occurred in conjunction with an intravenous increase in PYY. PYY has been colocalized with enteroendocrine cells containing FFA2 (35), and thus gives a possible mechanism for SCFA induced decreases in motility. However, Cherbut also found that propionate and butyrate both decreased spike bursts, while butyrate increased the progression of colonic transit markers. This finding implies that spike bursts do not completely correlate to colonic transit.

PYY is a neuroendocrine mediator that slows gastric emptying and promotes digestion and nutrient absorption in the small intestine. It is present throughout the GI tract in enteroendocrine cells, but experiences its largest release following nutrients entering the ileum and chyme exiting into the colon. These events result in the mechanism of the ileal and colonic brakes and signal the end of a meal throughout the GI tract. (36) All SCFAs stimulate the release of PYY, as shown by Cherbut.

PYY is similar to neuropeptide Y (NPY), another tyrosine containing peptide. These peptides, as well as pancreatic polypeptide (PP) are expressed by cell systems throughout the gut-brain axis and mediate the communication either exclusively within the GI tract (PYY and PP) or throughout the axis (NPY). (57) PYY and NPY both activate a family of receptors Y_{1-4} which are expressed throughout the GI epithelium as well as on enteric neurons. Y_2 , for example, is expressed primarily on intrinsic afferent neurons and secretomotor neurons within the submucous and myenteric plexus (36). In particular, Y_2 has been colocalized on VIP containing motor neurons. This provides a possible explanation for the inhibitory effect seen with SCFA infusion in the colon.

Combining this background knowledge with the present results, it seems possible that propionate stimulates the greatest release of PYY and results in the overall inhibition of colonic motility. Propionate also displays the greatest potency to FFA2/3 (34, 35). This could further explain how propionate could possibly facilitate the greatest PYY release.

Soret (22) demonstrated that contraction of isolated circular muscle strips from the rat colon is augmented by butyrate when stimulated through electrical-field stimulation (EFS), and is inhibited by atropine. This effect is not seen when carbachol is used in place

of EFS, implying that butyrate influences the behavior of enteric neurons that are responsible for initiating contraction of the smooth muscle, rather than any direct effect on smooth muscle cells. This finding introduces the possibility that luminal perfusion of butyrate may be affecting enteric neurons that lie beyond the mucosa. It is possible that following excision, the mucosa of the guinea pig begins to die and allows luminal contents to pass into direct contact with the submucosal plexus. However, almost no mucosa was observed in the outflow bath following the experiments. Histological views of colonic cross-sections before and after the experiments would better inform this possibility.

Acetate, propionate, and butyrate not only bind to FFA2 and FFA3, but are transported into colonocytes via monocarboxylate transporters (MCT1), some of which are sodium-coupled (SMCT1). (63) Recently, other MCTs have been attributed to intestinal epithelial transport. (64) MCTs have been shown in various cell types within the central nervous system. But this remains to be shown on enteric neurons (22). Soret's results for butyrate on contraction of circular muscle show that the effect is actually occurring through the enteric neurons on the circular muscle cells. This provides a possible explanation for butyrate's opposing effect to that of acetate and propionate on propagations and non-propagations. Additionally, this provides a possible explanation for the lack of disappearance of non-propagations in the physiological mixture. It is possible that butyrate increases release of transmitters from motoneurons closely associated with smooth muscle cells in the circular layer, while PYY release is increased by propionate and binds to receptors on the interneurons within the reflex.

In the physiological mixture, in which propionate is at its lowest concentration, it only partially blocks the local contractions (non-propagations) but is sufficient to decrease propagations which require coordination throughout the segment of tissue to initiate propagations.

Grider (21) has previously demonstrated that acetate, propionate and butyrate all increase the ascending contraction and descending relaxation of the peristaltic reflex in a three-chambered flat-sheet preparation of rat distal colon. The response was dose dependent increasing from 5-100mM. Release of 5-HT and CGRP were also increased several-fold following mucosal application of SCFAs. This increase in the peristaltic reflex is consistent with the present findings of butyrate but not for propionate or acetate. Colocalization of FFA2 or FFA3 with 5-HT has not been found in the mucosa of the GI tract. (35) However, FFA2 has been colocalized with 5-HT containing mast cells that reside just beneath the mucosal layer. (35, 32) It is possible that these mast cells are response for the release of 5-HT and thereby facilitate release of CGRP, which initiates the peristaltic reflex. Additionally, 5-HT containing mast cells have only been observed through immunoreactivity staining in the submucosa of mice. 5-HT containing mast cells have not been observed in the submucosa of humans (44). It is therefore possible that such differences exist between rodent species as well and thus explain the differences observed between rats and the guinea pigs used in the present study.

To determine the downstream effects of SCFAs on colonic motility, several studies could be performed. Block of FFA2/3 either specifically or individually is at present not possible due to a lack of antagonists, however, pharmacological studies are showing

progress and it is likely several will be commercially available in the coming years (34, 35) Luminal perfusion of intracellular inhibitors downstream of FFA2/3 would be possible, but would interfere with too many accessory cellular processes to yield significant results. Luminal perfusion of antagonists for 5-HT receptors just beyond the mucosa could determine if the increases observed with butyrate are mediated through 5-HT initiation of the peristaltic reflex. The same experiment could also be performed with a Y2 receptor antagonist, which would block any effect that PYY release may have on colonic motility. Use of other species following the same experiments could also aid in determining the absolute effect of SCFAs on colonic motility.

In summary, the present study demonstrated that individual SCFAs, which are the result of bacterial fermentation, have differential effects on contractile motility of the proximal and distal colon of the guinea pig. These effects do not differ from the proximal to distal colon, but do differ with SCFA chain length.

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Literature Cited

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