Neurogenic and Myogenic Properties of Pan-Colonic Motor Patterns and Their Spatiotemporal Organization in Rats

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Abstract

Background and Aims: Better understanding of intrinsic control mechanisms of colonic motility will lead to better treatment options for colonic dysmotility. The aim was to investigate neurogenic and myogenic control mechanisms underlying pan-colonic motor patterns.

Methods: Analysis of in vitro video recordings of whole rat colon motility was used to explore motor patterns and their spatiotemporal organizations and to identify mechanisms of neurogenic and myogenic control using pharmacological tools.

Results: Study of the pan-colonic spatiotemporal organization of motor patterns revealed: fluid-induced or spontaneous rhythmic propulsive long distance contractions (LDCs, 0.4–1.5/min, involving the whole colon), rhythmic propulsive motor complexes (RPMCs) (0.8–2.5/min, dominant in distal colon), ripples (10–14/min, dominant in proximal colon), segmentation and retrograde contractions (0.1–0.8/min, prominent in distal and mid colon). Spontaneous rhythmic LDCs were the dominant pattern, blocked by tetrodotoxin, lidocaine or blockers of cholinergic, nitrergic or serotoninergic pathways. Change from propulsion to segmentation and distal retrograde contractions was most prominent after blocking 5-HT₃ receptors. In the presence of all neural blockers, carbachol consistently evoked rhythmic LDC-like propulsive contractions in the same frequency range as the LDCs, indicating the existence of myogenic mechanisms of initiation and propulsion.

Conclusions: Neurogenic and myogenic control systems orchestrate distinct and variable motor patterns at different regions of the pan-colon. Cholinergic, nitrergic and serotoninergic pathways are essential for rhythmic LDCs to develop. Rhythmic motor patterns in presence of neural blockade indicate the involvement of myogenic control systems and suggest a role for the networks of interstitial cells of Cajal as pacemakers.

Introduction

Transit, absorption of nutrients, salts, vitamins and water; storage, stool shaping and excretion are major functions of the colon that may involve specialized colonic motor functions, which are not fully understood. A more comprehensive insight into colonic motor patterns including propulsive and non-propulsive activities and their control mechanisms is needed to identify possible biomarkers of colonic motility disorders. Although many in vivo and in vitro studies have shed light on mechanisms of colonic motor activity, high-resolution techniques are markedly increasing our ability to study essential details of motor patterns. Using such techniques, cooperation between neurally-induced pacemaker activity by interstitial cells of Cajal (ICC) and enteric neural programs were hypothesized to control colonic propulsive motor patterns in rats [1] and mice [2], suggesting that division of motor activities or motor dysfunction into exclusively neurogenic or exclusively myogenic may not reflect the reality of gut motility control. The present study pursues further insight into the integration of neurogenic and myogenic control mechanisms of propulsive and non-propulsive motor activities. Patients with slow transit constipation showed a markedly abnormal colonic motor pattern with paucity of propagating pressure waves in the mid colon and increase in frequency of retrograde propagating sequences in the proximal colon, suggesting the importance of antegrade long distance contractions in normal colonic transit [3].
which is the focus of the present study. Different regions of the colon have different functions and therefore may show different dominant motor patterns under physiological and pathophysiological conditions. These patterns, their control mechanisms and in particular the interaction of these patterns can only be observed in pan-colonic studies hence we investigated the spatiotemporal organization in the whole colon in vitro. The focus of the present study was the investigation of rhythmic pan-colonic propulsive motor patterns, before and after nerve blockade to explore the potential roles of myogenic control systems including ICC and the enteric nervous system.

**Materials and Methods**

The whole colon was examined from 55 adult male Sprague-Dawley rats weighing 150–300 g. Animals were killed by cervical dislocation. The entire colon was removed and placed in gassed (3% CO2 and 95% O2 (v/v)) Krebs solution (pH 7.3–7.4) at 37°C. Krebs solution consisted of (mM) NaCl 118.1, KCl 4.8, NaHCO3 25, NaH2PO4 1.3, MgCl2 6 H2O 1.2, glucose 12.2 and CaCl2 2.5. The contents of the colon were gently flushed out using warmed Krebs solution and the external connective tissue was removed. The distal and proximal ends were cannulated and fixed to the beginning of the experiment was 5 cm H2O. Propulsive contractions were identified by the rising fluid level in the narrow container. After an LDC was completed, fluid flowed back into the whole colon; the wave of relaxation started in the most distal part of the colon and propagated in oral direction. The whole contracted colon; the wave of relaxation started in the most proximal colon which was followed by a relaxation phase lasting 1–5 s of the contraction. The contraction was preceded by the relaxation phase lasting 8.8 ± 3.2 seconds. Krebs solution consisted of (mM) NaCl 137.1, KCl 2.7, NaH2PO4 10, KH2PO4 2.

The following drugs were used at the final bath concentrations indicated: 100 μM indocaine (Haalu Pharmaceutical Co. Ltd., Shandong, China) and 0.2 μM tetrodotoxin (TTX; Baoman Biochemistry Co. Ltd., Shanghai, China) were used to block neural activity. 200 μM nitro-L-arginine (L-NA; from Aladdin Chemistry Co. Ltd., Shanghai, China) was used to inhibit nitric oxide synthesis. 5-HT3 antagonist granisetron (NingBo Team Pharm Co., Ltd., China) was used to block 5-HT3 receptors. 2 μM atropine (from Aladdin Chemistry Co. Ltd., Shanghai, China) was used to block muscarinic acetylcholine receptors. 2 μM betahanechol (3B Scientific Corporation, Libertyville, Illinois, USA) or carbachol 2 μM (Shandong Chia TaiFreda Pharmaceutical Group) was used to evoke activity after nerve blockade. Krebs reagents were purchased from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China.

**Results**

We observed distinct colonic motor patterns in the fluid filled whole rat colon: long distance contractions (LDCs), interrupted LDCs, tandem contractions, rhythmic propulsive motor complexes (RPMCs), propagating ripples, segmentation and retrograde contractions. Their generation was not solely myogenic or neurogenic. Several of these motor patterns occurred predominantly in one region of the colon.

**Long Distance Contractions (LDCs)**

The LDC had 2 components, a contraction phase lasting ~30 s which was followed by a relaxation phase lasting ~10 s (Figure 1, Table 1, Video S1). The LDC started in the most proximal colon and propagated most often to the distal end but covered at least 2/3 of the colon (Table 1); the forceful circumferential contraction moved all the intraluminal content in anal direction, out of the colon into the outflow reservoir. The contraction was preceded by a relaxation (white band in spatiotemporal map) starting 1/4–1/3 down the colon. The LDC was not a ring contraction since the colon remained contracted following the propagating front of the contraction. The contraction was followed by the relaxation phase that was characterized by a complete and sudden relaxation of the whole contracted colon; the wave of relaxation started in the most distal part of the colon and propagated in oral direction. The intraluminal liquid content would flow back into the whole colon in our standard open outflow experimental setup. The content would also flow back when the outlet was obstructed. In spatiotemporal maps, the contraction phase of the LDC showed as a black elongated triangle (Figure 1). LDCs occurred spontaneously in a rhythmic manner at 0.64±0.17/min. In a quiescent colon, rhythmic LDCs could invariably be induced by fluid infusion into the proximal colon, occurring within 1–3 s of the start of infusion. Fluid infusion-induced LDCs had similar characteristics as the spontaneous LDCs (Figure 1a, Table 1, Video S1).

An “interrupted LDC” developed when the relaxation that started to precede the contraction, transiently abolished the
contraction in the middle of the colon with a relaxation that spanned 1.9 ± 1.1 cm and lasted for ~ 10 s (Table 1) whereupon the contraction started again and proceeded to the distal colon (Figure 1b, Table 1, Video S2).

The “tandem contraction” was a distinct motor pattern seen in all colons (Figure 1c, Table 1, Video S3). The pattern either started with a slowly developing propulsive contraction in the proximal colon whereupon a second contraction started to develop after 8.9 ± 6.4 s in the mid or distal colon, or the pattern started with the distal contraction followed by the proximal contraction (interval 12.7 ± 7.0 s). Both proximal and distal contractions propagated simultaneously in anal direction. Invariably, a relaxation developed anal to the proximal contraction and this contraction ended in the middle of the colon where the second contraction was ongoing. The content of the proximal colon entered the relaxation part of the colon and then flowed back since the distal contraction prevented it from moving to the distal colon.

RPMCs and LDCs

Starting from the mid or distal colon, RPMCs occurred with variable frequencies of 0.5 to 2/min (average 1.6 ± 0.4/min, n = 8) and variable velocities (4.3 to 9.2 cm/min, average 6.8 ± 1.5 cm/min, n = 8) (Figure 1d, video S4). They propelled the content into anal direction with amplitudes as high as that of spontaneous LDCs (average lumen diameter reduction 32 ± 8% vs 41 ± 12%, p = 0.218). The RPMCs most often did not have a relaxation preceding the propagating contraction and there was no relaxation phase upon termination of the contraction. RPMCs were seen to be propagating ring contractions without the sustained component of the LDC. Three to 8 RPMCs often occurred in between 2 LDCs (Figure 1d) in the distal colon but could also occur in the absence of LDCs; they were often abolished by TTX, identifying them as neurogenic.

Propagating Ripples and LDCs

Ripples are superficial ring contractions of the circular muscle that occur at a distinct frequency, similar to the frequency of the dominant slow wave and quite constant within a preparation. They have been described in the guinea pig colon [4,5] and in the rat colon [1].

In the present study, propagating ripples were observed as rhythmic, high frequency (ranging from 6–12/min; 9.5 ± 0.5/min, n = 30) superficial contractions. Diameter changes were of low amplitude at 0.07 ± 0.02 cm (n = 30) in most parts of the colon during the whole recording time. They originated most often in the proximal colon, propagating antegrade, retrograde or in both directions (Figure 1d). LDCs were always followed by a period of high excitation in the proximal colon, as reflected by ripples at relatively high amplitude, propagating most often retrograde but sometimes antegrade for a period of 30–60 seconds (Figures 1E, 2A). The diameter changes were 0.08 ± 0.01 cm after LDCs vs. 0.03 ± 0.03 cm preceding LDCs (p < 0.001).

Sustained Narrow Bands of Constriction and The Initiation of Rhythmic Propulsive Contractions

A sustained distention, preceded and followed by narrow bands of sustained contractions, occurred spontaneously (n = 2) for the duration of the experiment (Figure 2a,b). An LDC either stopped at the preceding contraction (Figure 2b) or continued at the distal...
Table 1. Characteristics of the propulsive colonic motor patterns.

| Presence of nerve blockade (TTX or lidocaine) plus spontaneous (n = 26) | Induced (n = 13) | Interrupted (n = 11) |
|---|---|---|
| **Characteristics** | **Spontaneous** | **Induced** | **Interrupted** |
| **In presence of nerve blockade (TTX or lidocaine) plus spontaneous activity** | | | |
| | | | |
| **Spontaneous activity** | | | |
| | | | |
| **Contraction duration (s)** | 32.8 ± 6.0 | 13.2 ± 3.8 | 21.6 ± 10.2 |
| | | | |
| **Relaxation phase duration (s)** | 10.3 ± 3.9 | 10.2 ± 2.6 | 9.9 ± 2.7 |
| | | | |
| **Frequency (cpm)** | 0.65 ± 0.31 | 0.31 ± 0.43 | 0.50 ± 0.01 |
| | | | |
| **Velocity (mm/s)** | 3.5 ± 1.5 | 1.5 ± 0.9 | 5.3 ± 2.6 |
| | | | |
| **Diameter change (cm)** | 0.41 ± 0.12 | 0.42 ± 0.12 | 0.43 ± 0.11 |
| | | | |
| Mean values ± S.D. The length of the colon was 13.8 ± 1.8 cm; the colon diameter was 0.83 ± 0.17 cm in both 65 ± 0.01). * = p < 0.05; ** = p < 0.001 comparing LDC-like activity after TTX or lidocaine with spontaneous LDCs before nerve blockade. NA = not applicable. n = number of animals.

**Effects of Inhibition of Neural Pathways**

TTX (1 µM) and lidocaine (0.1 mM) completely inhibited LDCs. Fluid infusion-induced LDCs did not develop in the presence of TTX and spontaneous LDCs reduced in frequency until they were abolished after 5–30 min (n = 10, Figures 3b,d). In the presence of TTX, rhythmic contractile activity in the proximal colon most often remained, appearing similar to the starting part of the LDCs with shorter length (3.1 ± 0.9 cm), lower change in diameter (0.12 ± 0.05 cm) and shorter duration (~ 22 s) (Table 1). The frequency varied from 0.6 to 2/min (average 1.1 ± 0.6/min). The effect of lidocaine (n = 6) was similar to that of TTX with rhythmic proximal contractions remaining at variable frequencies up to 6/min (Figure 4b).

In the presence of TTX or lidocaine, carbachol (2 µM) or bethanechol (2 µM) induced strong rhythmic contractile activity (n = 8, Figures 3e, 4c, Table 1). This activity was similar to LDCs and is here described as LDC-like. The amplitude was higher, the relaxation phase was short lasting, the propagation velocity in the mid and distal colon was slower, the duration was longer and there was no increased ripple activity after the contraction (Table 1). The LDC-like activity was almost always associated with retrograde contractions in the distal colon (Figures 3e, 4c, Table 1).

Atropine (2 µM) abolished LDCs evoked by fluid-induced distention (Figure 5b, n = 6). Spontaneous LDCs were inhibited: 30 min after addition of atropine their frequency was decreased from 0.48 ± 0.11 to 0.18 ± 0.11/min (p < 0.01). After 30 min, in all experiments, rhythmic activity remained in the proximal colon and appeared to be the start of LDCs but did not propagate after 4.2 ± 0.6 cm. The proximal contractions in the presence of atropine occurred at 0.32 ± 0.10/min.

L-NNA (0.2 mM) abolished the LDCs (n = 5, Figure 5d). After abolishment of the LDCs, the proximal colon activity was dominated by strong ripple activity at 11.2 ± 0.4/min, propagating over a length of 7.2 ± 1.4 cm. The direction of ripple propagation changed frequently. The distal colon activity was dominated by RPMCs starting in the mid colon, occurring rhythmically at high frequency of 6.4 ± 0.9/min and velocity of 8.8 ± 3.5 cm/min. The high frequency activity in the distal colon was not observed when both atropine and L-NNA were present (n = 6). Ripples remained prominent in the presence of both atropine and L-NNA at 12.0 ± 1.4/min, propagating over a length of 6.0 ± 3.5 cm. The direction of propagation changed frequently.

Granisetron (3.8 µM, Figure 6, n = 8) abolished LDCs in 10–20 min. In the presence of granisetron, segmentation activity was prominent in mid and distal colon (Fig. 6b) but in 2 of 8 experiments it was preceded by a 3–5 min period of complete absence of activity (Figure 6c). After addition of bethanechol in the presence of granisetron, rhythmic LDC-like pan-colonic propulsive contractions and concomitant distal retrograde contractions developed (Figure 6d, Table 2). In some experiments, the retrograde contractions became dominant (Figure 6e) and LDC-like activity did not develop.

**Segmentation**

RPMCs were observed to “break up” into several isolated contractions (~ 0.5 cm long) such that several (2–5) short-lasting rings of contraction occurred at the same time dividing the colon into segments by contractions that were non-propulsive or propulsive over a very short distance (Figure 5b). Content moved back and forth. Periods of segmentation could occur at any time in the absence and presence of nerve blockade. Segmentation
became prominent in the presence of the 5-HT<sub>3</sub> antagonist granisetron (3.8 μM; Figure 6b).

Pan-colonic Spatiotemporal Organization of Motor Patterns

The pan-colonic spatiotemporal organization showed rhythmic recurring patterns of motor activity: LDCs followed by ripples or a group of RPMCs followed by LDCs. Importantly, distinct patterns occurred in proximal, mid and distal colon. The LDCs were propulsive throughout the whole colon. The proximal colon, in addition, was dominated by retrograde propagating ripples, and the distal colon by RPMCs propagating in anal direction. 269 periods of 3 min duration were analyzed. Under normal conditions, spontaneous LDCs were associated with RPMCs in the mid and distal colon, 56% of the time (151/269). A pattern of 2–5 RPMCs followed the LDC facilitating clearance of the distal colon (Figure 1d). This pattern would repeat itself in a very similar manner after the subsequent LDCs. A segmentation pattern followed the LDC 30% of the time (75/269) or no other patterns were obvious (43/269, 16%). Ripples almost always followed the LDCs with enhanced diameter changes (Figures 1a,b) and ripples could be seen superimposed on the LDCs in the proximal colon (Figures 4b, 5b).

Discussion

In recent years, studies on propulsive contractions in the colon of various species have often grouped them together and named them colonic migrating motor complexes (CMMCs) [4,6,7], giant contractions [8], haustral contractions [9], mass peristalsis [9] or “rhythmic propulsive motor complexes” [1]. We report here that distinctly different propulsive motor patterns occurred in the fluid filled rat colon which could not be characterized by a single term. Three dominant colonic motor patterns occurred. Antegrade propagating long distance contractions (LDCs) characterized by a sustained component and a retrograde propagating relaxation phase occurring at a frequency of 0.3–2/min. Antegrade propagating rhythmic propulsive motor complexes [1] (RPMCs) which were ring contractions without a sustained component and propagating with highly variable speeds at 0.3–6/min. In addition, ripples [1,4,10] occurred at a constant frequency of 6–10/min; ripples were of low amplitude and often changed direction of propagation, suggesting that they served primarily absorption.
The pan-colonic spatiotemporal organization was as follows: LDCs, interrupted LDCs and tandem contractions started in the very proximal colon and ended in the distal colon. Retrograde propagating ripples were prominent in the proximal colon and almost always followed LDCs. RPMCs almost always occurred simultaneously with LDCs in the distal colon. LDCs and RPMCs were abolished by TTX, atropine and 5-HT3 antagonists, indicating their neurogenic nature. Most recent studies suggest the enteric nervous system to be responsible for the rhythmicity [4]. However in the presence of TTX, very similar motor patterns were evoked by muscarinic receptor stimulation indicating that myogenic control systems exist that guide rhythmic initiation and propagation of propulsive motor patterns.

The present study was performed on fluid-filled colons. Pellets inhabit the mid and distal colon most of the time and the influence of the motor patterns on pellets needs further investigation, as does the influence of pellets on the motor patterns. It was recently noted that pellets make rhythmic propagating motor activity more regular and increase the frequency of the contractions [11]. Are the LDCs similar to CMMCs reported in the literature? CMMCs were almost always studied in flat sheet preparations where contractions are monitored at 3–4 sites. In such preparations it is difficult to distinguish between the various motor patterns identified in the present study where diameter changes are measured all along the colon. Second, the ENS is disrupted, in particular the circumferentially-oriented network of AH neurons [12]. For example, it was reported that the majority of CMMCs start in the mid colon [13] or seen to travel retrograde [11]. Since LDCs propagated antegrade and always start in the proximal colon, it would classify CMMCs as RPMCs. Another example is the effect of blockade of nitric oxide synthesis. It was reported that L-NNA increased the frequency of CMMCs [14] but we report here that L-NNA abolishes LDCs and replaces them with high frequency contractions with features very different from LDCs. It is therefore likely that the flat sheet preparation alters the ENS circuitry sufficiently to markedly affect the features of colonic motor patterns.

Myogenic Pacemakers

The colonic rhythmic ring contractions with the most constant frequency were the ripples. Ripples persist in the presence of TTX. Their frequency is the same as that of the omnipresent slow wave activity of the rat colon, which is generated by the ICC associated with the submuscular plexus (ICC-SMP) [1,15]. The ripples often propagate retrograde but are seen to change direction frequently. They are always of low amplitude hence they are not a force in propulsion of colonic content and likely promote absorption, consistent with the interpretation in other studies [6]. Both LDCs and RPMCs occur in a rhythmic manner, the rhythmicity is usually stable within a preparation under certain conditions but it is quite variable when conditions change and variable comparing different preparations. Most often the activity is between 0.3 and 2 cycles/min. Although both motor patterns can be abolished by TTX, this does not prove that the ENS...
The presence of TTX suggests that myogenic activities similar to both motor patterns can be evoked pharmacologically, indicating a myogenic pacemaker present under all conditions. LDC-like contractions in the presence of nerve block imply myogenic mechanisms of initiation, rhythmicity, propagation, sustained contraction, and sudden complete relaxation are present. Slow wave activities can orchestrate all these properties of contraction. Observations in mouse colon support this, showing slow depolarizations during low frequency propagating contractions. The laboratories of Jimenez and Takaki have provided evidence that ICC associated with the myenteric plexus (ICC-MP) have a low frequency pacemaker, driving contractions from 0.3 to 2 cycles per minute. The ICC-MP may therefore drive all characterised 'neurogenic' propulsive activities since rhythmic motor activity persists after mucosal removal. Serotonergic interneurons may be essential for rhythmic LDCs, as granisetron abolishes the propulsive LDC and promotes segmental contraction. The 5-HT3-antagonist alosetron reduced diarrhea in some patients but was effective for relieving diarrhea in limited success. Although consistent with clinical studies, granisetron abolished the propulsive LDC, inhibiting sigmoid motility, delaying colonic transit, and increasing stool consistency in IBS patients. The 5-HT3-antagonist alosetron's effects indicate serotonin's role in motor dysfunction and genetic variation amongst patients.
Cholinergic motor neurons play a critical role in the generation of LDCs and RPMCs since atropine abolished these contractions. Muscarinic acetylcholine receptors are found on smooth muscle cells as well as ICC [27,28]. Since atropine reduced the frequency of LDCs it is likely that the ICC-MP pacemaker frequency is regulated by muscarinic acetylcholine receptor activation on ICC-MP. Consistent with this is the abundant innervation of ICC-MP by cholinergic nerves [1]. Also consistent is the abnormal colonic motor functions in Hirschsprung’s disease where cholinergic innervation is compromised [29] and the reduced amplitude of mass movement in response to hexamethonium and atropine in vivo [30].

In the presence of TTX and bethanechol retrograde contractions starting from the distal colon are always present. Without neural blockade, the motor complexes in the distal colon are predominantly antegrade propagating; this suggests that under these conditions a distal pacemaker is inhibited by neural activity, further emphasizing that the dominance of pacemakers in specific regions of the colon is modulated by the ENS.

Enteric neurons other than nitricergic, cholinergic and serotonergic nerves likely play a role in the orchestration of propulsive activities. A prominent inhibitory component in the rat colon is the purinergic innervation [33]. Another candidate for excitatory neurotransmission is substance P [34]. Future studies will reveal more details of the neuronal programs that govern the various motor patterns.

**Sustained Rings of Constriction**

The present study shows that a sustained distention/contraction triggers RPMCs. An LDC can proceed distal to the distention/contraction or it can be halted. Hence, the enteric neural programs are intricate neural circuitries of sensory and motor neurons that are sequentially activated to create a complex motor pattern with feedback from content or the contractile state of the colon. This contrasts with a simple reflex where a stimulus evokes ascending contraction and descending inhibition [35]. Sustained rings of constriction occurred in 2/55 preparations; they were present for the entire duration of the experiments. Preliminary experiments showed that such constrictions were common in...
colons inflamed by TNBS, with as prominent characteristic the initiation of distal RPMCs (Chen and Huizinga, unpublished data). All constrictions were in between mid and distal colon. The constriction has resemblance to Cannon’s ring or Cannon’s sphincter noted in the human colon which is a transient but relatively long lasting constriction occurring at the mid transverse colon, from which contractions can be initiated. Theories as to the origin of sustained constriction at that site are that Cannon’s sphincter occurs where there is overlap of the superior and inferior mesenteric nerve plexuses [36]; also, a “neuromuscular imbalance” is proposed [37]. There is no structural evidence of

![Figure 6. Effect of the 5-HT₃ antagonist granisetron.](image)

Table 2. Bethanechol-induced motor patterns in the presence of granisetron.

|                      | LDCs before granisetron (n = 8) | In the presence of granisetron and bethanechol |
|----------------------|---------------------------------|-----------------------------------------------|
|                      | LDC-like motor pattern          | Retrograde contractions                       |
| Propagation length (cm) | 10.42±1.89                      | 9.75±1.73                                      | 3.10±1.04          |
| Contraction duration (s) | 31.33±9.42                      | 96.25±12.69**                                 | 33.67±8.14         |
| Relaxation phase duration (s) | 12.00±3.46                      | 23.00±3.16*                                   | 17.67±7.51         |
| Frequency (cpm)         | 0.52±0.12                       | 0.44±0.13                                     | 0.59±0.24          |
| Velocity (mm/s)         | 3.7±1.7                         | 1.00±0.20*                                    | 1±0.5              |
| Relaxation phase velocity (mm/s) | 9.4±3.3                      | 4.30±0.80*                                    | 2.1±1.5            |
| Diameter change (cm)    | 0.42±0.10                       | 0.37±0.04                                     | 0.41±0.17          |

Mean values ± S.D. The length of the colon was 13.8±1.8 cm; the colon diameter was 0.83±0.17 cm (n = 55). * = p<0.05; ** = p<0.001 comparing LDC-like activity after granisetron and bethanechol with spontaneous LDCs before granisetron.

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increased density of ICC pacemaker cells in the constricted region in the rat (Wang and Huizinga, unpublished observations).

**Comparison with Human Motor Activity**

Is rat colon motor pattern information relevant for the human colon? In contrast to the rat colon, the human colon longitudinal muscle is divided into taeniae and the human colon does not create highly regularly shaped pellets. However, it is not known whether or not this indicates differences in basic motor patterns. There are many similarities. The ICC composition and structure is very similar. Networks of ICC are found in the myenteric and submucosal plexus areas and intramuscular ICC are abundant [38–40]. One difference is that the human colon has more septa and hence more ICC-IM associated with septa. At this moment there is no reason to assume that the enteric neuronal circuitry is very different.

The electrical activities recorded from human, dog, mouse and rat colon musculature reflecting properties of ICC pacemaker cells and the musculature have similar features [15,18,41–44]. All have a slow wave type oscillation, which is most prominent near the submucosal border of the circular muscle and hence likely associated with ICC-SMP. And all have low frequency slow depolarizations near the myenteric plexus with superimposed high intensity spiking and associated forceful contractile activity. In the human, in this area also high frequency oscillations (called membrane potential oscillations (MPOs) by some groups) are recorded but they often occur in bursts and then again show slow transient membrane depolarizations with superimposed oscillations and/or action potentials [42]. The slow depolarizations are most likely originating from ICC-MP [15,18].

Correlation of the motor patterns described in the present study with in vivo motor patterns in humans will require in vivo high-resolution manometry in humans which is still in its infancy, but we do know that rhythmic propulsive motor activity is common in humans [45]. Slow transit constipation patients show a paucity of propagating pressure waves and an increase in frequency of retrograde propagating sequences [3]. The present study suggests that a paucity of propagating contractions can indicate a reduction in one of many critical components of enteric neural control, including a reduction in 5-HT₃ receptor mediated activity. In addition, an abnormal pacemaker network can hinder the initiation of propulsive activities [46,47], which has been demonstrated in slow transit constipation [48].

In conclusion, high-resolution spatiotemporal mapping of pan-colonic motility reveals distinct motor patterns in proximal, mid and distal colon that may serve simultaneously different functions of the colon such as transit, storage, absorption, feces shaping and excretion. Serotonergic, cholinergic and nitrergic circuits are all essential components of the enteric nervous programs that generate propulsive and non-propulsive (promoting absorption) motor activities likely in concert with pacemaker activities from ICC-DMP and ICC-MP.

**Supporting Information**

**Video S1** Induced and spontaneous LDCs. Two LDCs are shown, the first one occurred in response to liquid infusion, the second one occurred spontaneously. See boxed area in figure 1a. The colon section in the movie was 7.5 cm long, not visible are the proximal 1.5 cm and the distal 1.5 cm of the colon. The video shows in real time.

**Video S2** The interrupted LDC. One interrupted LDC is shown. See boxed area in figure 1b. The colon section in the movie was 7.5 cm long, not visible are the proximal 1.5 cm and the distal 1.5 cm of the colon. The video shows in real time.

**Video S3** The tandem contraction. One tandem contraction is shown. See boxed area in figure 1c. The colon section in the movie was 7.5 cm long, not visible are the proximal 1.5 cm and the distal 1.5 cm of the colon. The video shows in real time.

**Video S4** Rhythmic propulsive motor complexes (RPMCs). RPMCs are shown followed by a spontaneous LDC. See boxed area in Figure 1d The colon visible in the movie is 9.1 cm long. Not visible are 1.3 cm at the proximal end and 1.4 cm at the distal end that were used for anchoring colon on in and outflow tubes. The video shows in real time.

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**Author Contributions**

Conceived and designed the experiments: JHC JDH. Performed the experiments: JHC QZ YY KL H. Liao LJ LH XD SC SY QG XY JDH. Analyzed the data: JHC QZ YY KL JDH. Contributed reagents/materials/analysis tools: XH H. Luo. Wrote the paper: JHC QZ JDH.

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