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Haustral boundary contractions in the proximal 3-taeniated rabbit colon

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Chen J-H, Yang Z, Yu Y, Huizinga JD. Haustral boundary contractions in the proximal 3-taeniated rabbit colon. *Am J Physiol Gastrointest Liver Physiol* 310: G181–G192, 2016. First published December 3, 2015; doi:10.1152/ajpgi.00171.2015.—The rabbit proximal colon is similar in structure to the human colon. Our objective was to study interactions of different rhythmic motor patterns focusing on haustral boundary contractions, which create the haustra, using spatiotemporal mapping of video recordings. Haustral boundary contractions were seen as highly rhythmic circumferential ring contractions that propagated slowly across the proximal colon, preferentially but not exclusively in the anal direction, at ~0.5 cycles per minute; they were abolished by nerve conduction blockers. When multiple haustral boundary contractions propagated in the opposite direction, they annihilated each other upon encounter. Ripples, myogenic propagating ring contractions at ~9 cycles per min, induced folding and unfolding of haustral muscle folds, creating an anarchic appearance of contractile activity, with different patterns in the three intertaenial regions. Two features of ripple activity were prominent: frequent changes in propagation direction and the occurrence of dislocations showing a frequency gradient with the highest intrinsic frequency in the distal colon. The haustral boundary contractions showed an on/off/on/off pattern at the ripple frequency, and the contraction amplitude at any point of the colon showed waxing and waning. The haustral boundary contractions are therefore shaped by interaction of two pacemaker activities hypothesized to occur through phase-amplitude coupling of pacemaker activities from interstitial cells of Cajal of the myenteric plexus and of the submuscular plexus. Video evidence shows the unique role haustral folds play in shaping contractile activity within the haustra. Muscarinic agents not only enhance the force of contraction, they can eliminate one and at the same time induce another neurally dependent motor pattern.

colon; haustra; ICC; motility; rabbit

THE COLONS OF THE RAT, MOUSE, cat, and dog have been extensively studied, yet they differ in an important aspect from the human colon: their longitudinal muscle is not organized in taenia. The rabbit 3-taeniated proximal colon does resemble the human colon remarkably. Bayliss and Starling (4) carried out one of the first studies on the rabbit colon; interestingly, this study describes the typical neural reflex: a pellet (or balloon) slowly moves anally by a propulsive contraction initiated by the pellet (or balloon) distention [neurally mediated peristalsis, (14)], and it describes the situation in which the balloon is prevented from expulsion and then rhythmic propulsive contractions pass down over it [a motor pattern evoked by interaction of myogenic and neurogenic mechanisms (9)].

Although haustra are a key feature of the human and rabbit colon, little is known about the motor patterns associated with this structure. In fact, what a haustrum actually is, is ambiguously defined. In general, haustra are defined by mucosal or muscle folds, or by circular muscle contractions. Radiologists may see haustra defined by muscle wall foldings: "The haustra or haustral sacculations are outpouchings of the bowel wall between the taeniae; they are caused by the relative shortness of the taeniae, about one-sixth shorter than the length of the bowel wall" (32). Or: "Haustra coli are sacculations between the taenia and are separated by crescent-shaped folds called plicae semilunares" (10). On the other hand, haustra are seen to be formed by "irregularly spaced circumferential constrictions . . . caused by sustained contractions of the circular muscle" (11), that move slowly (5), or "the circular muscle layer is contracted intermittently to divide the colon into functional segments known as haustra" (2). Important insight into rabbit colon motility has been provided by Ehrlein (17), through his X-ray movies, and recently by Lentle et al. (37) and Dinning et al. (13) through spatiotemporal mapping. On the basis of spatiotemporal maps of video recordings, Lentle et al. defined the haustra as created by rhythmic propagating circular muscle contractions, 15 mm apart, whereas Dinning et al. saw them created by stationary folds, 3–4 mm apart. Interestingly, Hawkins and Hardy (24) wrote in 1950 about these two definitions and concluded that the contractions can only be confidently observed in live motility recordings. The two definitions come down to seeing haustra as passively formed by contracted taenia or actively formed by circumferential muscle contractions.

The objective of the present study was to present a characterization of the haustral boundary contractions in the proximal 3-taeniated colon by using videos, spatiotemporal maps, and amplitude profiles to resolve the disparate characterizations in previous studies, to study the interactions between the different motor patterns, and to increase our understanding of the influence of the intertaenial muscle folds on motor patterns, and lastly, to formulate hypotheses as to how various pacemaker activities might interact with each other and the enteric nervous system to generate the motor patterns observed.

MATERIALS AND METHODS

All experiments were approved by the animal ethics committee at Renmin Hospital related to a grant from the National Natural Science Foundation of China (NSFC), no. 81170249 to J.-H. Chen. In all experiments, the 3-taeniated proximal colon was removed from male or female adult New Zealand white rabbits of 1.8–2.5 kg body wt, anesthetized with 3% pentobarbital sodium injected into a marginal ear vein. After a midline incision, the whole proximal colon was removed and placed in warmed (37°C) and continuously oxygenated (5% CO_2 and 95% O_2) Krebs solution (pH ~7.3–7.4), which consisted of (in mM) 118.1 NaCl, 4.8 KCl, 25 $NaHCO_3$, 1.3 NaH_2PO_4, 1.2 $MgCl_2 \cdot 6H_2O$, 12.2 glucose, and 2.5 $CaCl_2$. The rabbit was euthanized thereafter by injecting air into the ear vein. We gently

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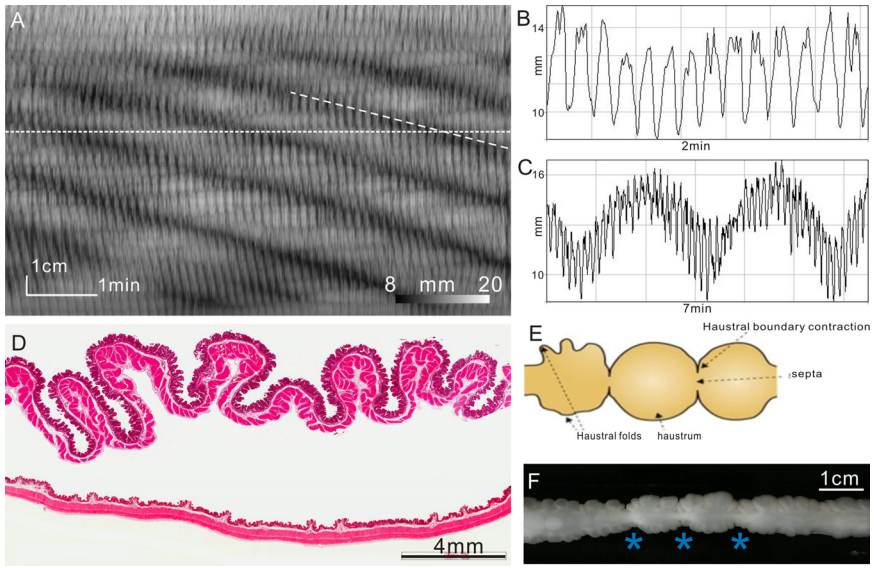

Fig. 1. Haustral boundary contractions. *A*: spatiotemporal D-map (quantifying diameter changes over time) of the 3-taeniated proximal colon prominently showing "haustral boundary contractions," propagating slowly at a frequency of 0.5 cpm from proximal (top) to distal (bottom). A haustral boundary contraction shows rhythmic increases and decreases of its amplitude (an on/off/on/off pattern, long dashed line in *A* and *B*) at the frequency of the ripples (the much faster propagating low-amplitude contractions at ~8 cpm). The haustral boundary contractions (*E*, blue stars in *F*) create the haustra (*E*, Supplemental Movie S1). *B*: amplitude profile along a wave of a haustral boundary contraction showing the on/off/on/off pattern at the ripple frequency along dashed line in *A*. *C*: amplitude profile at 1 point along the colon over time (dotted horizontal white line in *A*) showing the rhythmic baseline increase associated with the haustral boundary contractions and the superimposed phasic contractions at the ripple frequency. Note the waxing and waning appearance of the phasic contractions. *D*, top: haustral folds (folds of the entire intestinal wall) in the intertaenial domains in the proximal colon; circular muscle bundles do not have longitudinal muscle attached. Bottom: circular and longitudinal muscle cut lengthwise along a taenia. Note that the density of the circular muscle cells is higher in the area attached to the taenia compared with the intertaenial region. *E*: outline of haustral boundaries and haustral folds. Modified from Ref. 5 with permission from Elsevier. *F*: outline of the proximal rabbit colon, taken from Supplemental Movie S1. Stars depict indentations caused by the haustral boundary contractions. Indentations in between the stars are propagating ripple contractions that are shaped by haustral folds, which rhythmically fold and bulge out.

flushed out colon contents with warmed Krebs solution and removed external connective tissue. The colon was cannulated and fixed to the bottom of the organ bath. The proximal inflow tube (inner diameter 3 mm, outer diameter 4 mm) was connected to a 50-ml syringe, placed ~15 cm above the level of the colon; syringe and colon were filled with phosphate buffer solution (PBS). PBS consisted of (in mM) 137.1 NaCl, 2.7 KCl, 10 Na_2HPO_4, 2 KH_2PO_4. The distal outflow tube (inner diameter 5, outer diameter 6 mm) was positioned in a narrow upright 50-ml graduated cylinder filled with PBS so that the outflow could be measured for each occurring motor pattern. Since the experiments were conducted in a closed system, each outflow returned into the colon upon cessation of the propagating contraction. The height of the fluid level in the graduated cylinder determined the intraluminal pressure and the standard intraluminal pressure at the beginning was 4.5 cmH_2O. The colon was left to equilibrate for 20–30 min. A Microsoft video camera was mounted above the preparation to record motility during the entire experiment.

Drugs used were tetrodotoxin (TTX; Baoman Biochemistry, Shanghai, China), bethanechol (3B Scientific, Libertyville, IL), and neostigmine (Selleck Chemicals, Houston, TX). Krebs and PBS reagents were purchased from Sinopharm Chemical Reagent, Shanghai, China.

Data acquisition occurred through a Microsoft camera using Microsoft Lifecam software. Video recordings were analyzed with Image J aided by plugins written by Dr. Sean Parsons. Two types of spatiotemporal maps were created: D-maps quantifying diameter changes over time (created by circular muscle contraction) and upper and lower R-maps representing radial changes: changes of the dis-

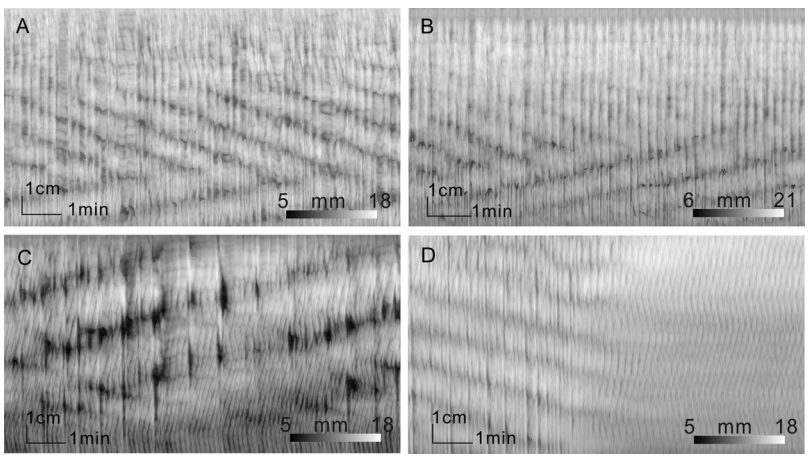

Fig. 2. Characteristics of the haustral boundary contractions *A*: when antegrade and retrograde propagating haustral boundary contractions collide, they are annihilated. *B*: rhythmic antegrade propagating haustral boundary contractions terminate when colliding with, or approaching a retrograde wave of contraction. *C*: strong retrograde haustral boundary contractions were observed in the presence of bethanechol. *D*: haustral boundary contractions terminate when nerve conduction is blocked by TTX (0.5×10^{-6} M) at arrow. After nerve conduction block, the ripple activity is dominant.

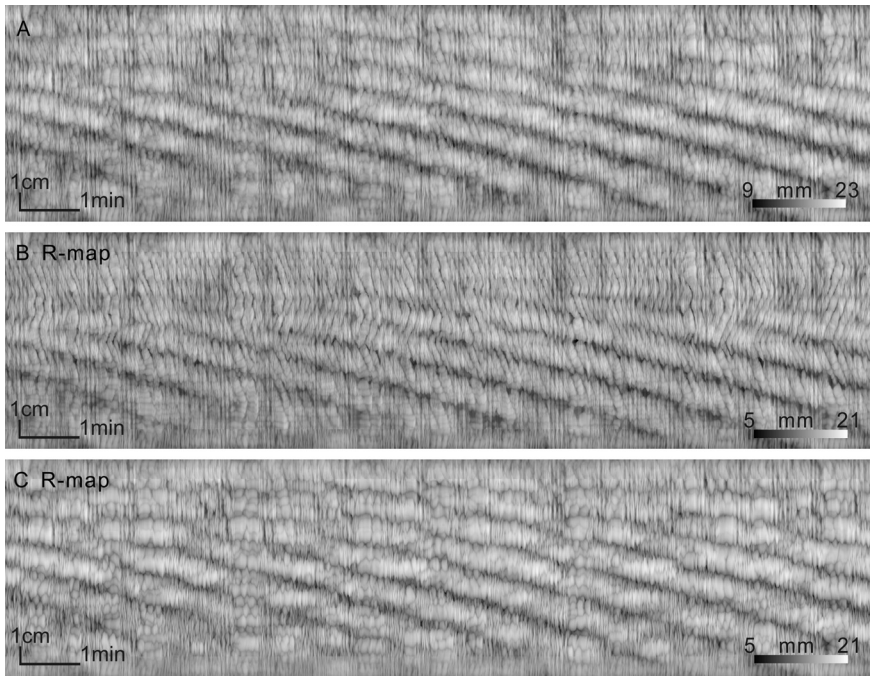

Fig. 3. Haustral boundary contractions: interaction with clustered fast-propagating contractions. *A*: D-maps are sometimes difficult to interpret because the circular muscle at both sides of the taenia can behave quite independently; part of map is shown in Supplemental Movie S2. No TTX is present. *B*: R-map (spatiotemporal map representing radial changes over time) of only the top (with reference to Supplemental Movie S2) intertaenial domain of the colon. The ripples show a zigzagging behavior; that is, their direction of propagation changed frequently. This is interspersed with clusters of fast-propagating contractions. *C*: R-map of only the bottom (with reference to Supplemental Movie S2) intertaenial domain of the colon, showing strong rhythmic outbulging of the haustral folds when a ripple is traversing the colon. The map shows 3 motor patterns: the haustral boundary contractions, ripple activity "deformed" by strong "activity" of the haustral folds, and clusters of fast-propagating contractions.

tance between the top or bottom colon boundary (in the supplemental movies; Supplemental Material for this article is available online at the Journal website) with respect to the center of the colon. In this way, motility of each intertaenial region was evaluated independently. This was done because the three intertaenial regions of the rabbit colon can show markedly different activities, occurring simultaneously. Maps are D-maps unless otherwise indicated. Black represents contractions. Colon width (coded as image intensity, black to white) is calculated at each point along the colon's length (image *y*-axis), for each video frame (image *x*-axis). Changes in diameter were quantified after distance calibration, by dots at the bottom of the organ bath, which were separated by exactly 1 cm (n = number of animals; values are expressed as average ± SD).

Modeling of phase-amplitude coupling. The haustral boundary contractions were created using a model of phase-amplitude coupling as outlined in a recent paper (30). The phase-amplitude function was created by using the following parameters: base (arbitrary value) 2; peak height (arbitrary value) 5; peak center 0.5 radians/2pi; peak width 0.12 radians/2pi. Other parameters are shown in the text and figure legend.

RESULTS

Haustral boundary contractions. Slowly propagating circular muscle ring contractions were a prominent motor pattern of the rabbit proximal 3-taeniated colon, dividing the colon into pockets or haustra (Figs. 1–4) (Supplemental Movies S1 and S2). These rhythmic contractions are referred to here as "haustral boundary contractions" after Lentle et al. (37), who refers to them as either haustral progressions or haustral boundary contractions. The frequency of the haustral boundary contractions was 0.52 ± 0.05 cpm (n = 16), very constant within a preparation and remarkably similar comparing colons from different rabbits. The haustral boundary contractions moved slowly, predominantly in the anal direction at 0.013 ± 0.004 cm/s (n = 16), regularly spaced at 1.63 ± 0.25 cm. Although by far the dominant direction of propagation was antegrade, retrograde propagation was observed, and, interestingly, when two contraction waves propagating in opposite direction were seen to collide (n = 4), one or both contractions terminated (Fig. 2, *A* and *B*). Strong retrograde haustral boundary contractions were observed in the presence of bethanechol (n = 7) (Fig. 2*C*). Haustral boundary

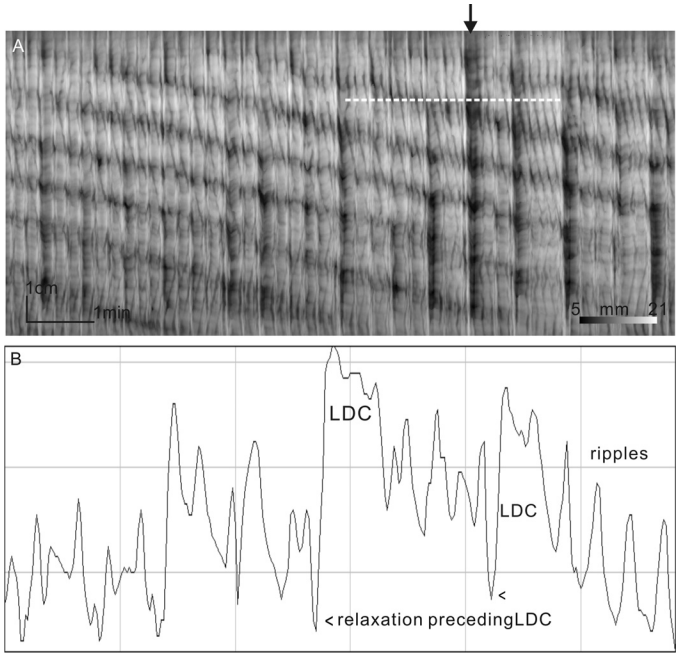

Fig. 4. Haustral boundary contractions interacting with long-distance contractions (LDCs). *A*: haustral boundary contractions with an LDC at arrow and elsewhere. LDCs are characterized by their triangular shape, they are not ring contractions: the colon remains contracted for 12–20 s while the contraction front is propagating anally (see Ref. 9). *B*: amplitude profile along the white dotted line in *A*, showing ripples and LDCs including the relaxation or dilatation that typically precedes an LDC.

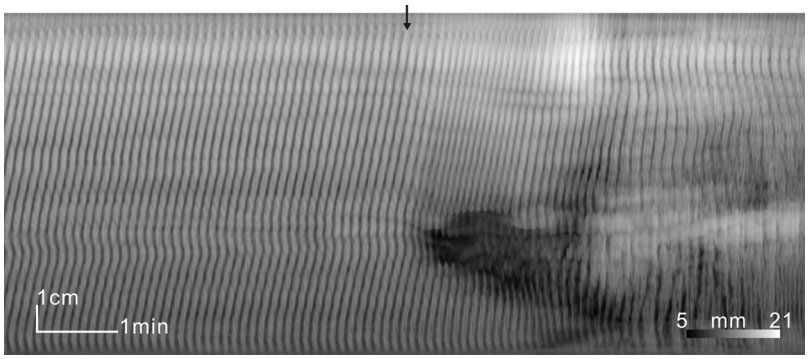

Fig. 5. Ripples increase in frequency in response to muscarinic stimulation. Ripple activity at 8 cpm increases in frequency to 13 cpm after addition of bethanechol (10^{-6} M) at arrow.

contractions were inhibited by the nerve conduction blocker TTX (0.5×10^{-6} M; $n = 7$; Fig. 2*D*).

The haustral boundary contractions were always interacting with another motor pattern, the ripples. Ripples were highly rhythmic ring contractions of circular muscle with a variable direction of propagation. They were not inhibited by TTX ($n = 7$). The consequence of the interaction between ripples and haustral boundary contractions was an on/off/on/off pattern of contraction of the haustral boundaries. Figure 1, *B* and *C*, shows amplitude profiles that illustrate the rhythmic on/off patterns at the ripple frequency. The amplitude profile at one point along the colon over time (Fig. 1*C*) shows that the haustral boundary contraction is a strong rhythmic increase in baseline contraction with superimposed phasic contractions at the ripple frequency. The duration of the baseline contraction was 100 ± 22 s, and the duration of the superimposed phasic contractions at half maximal amplitude was 8.7 ± 1.3 s. The ripples at the crest of the haustral boundary contraction were of higher amplitude compared with the ripples in between the haustral boundaries; hence the amplitude profile has a strong waxing and waning appearance.

Haustral boundary contractions also interacted with fast-propagating contractions (Fig. 3) in a manner similar to their interaction with ripples ($n = 10$).

The ripples. The ripples were propagating ring contractions of the circular muscle and were the dominant motor pattern after nerve conduction block by TTX but were also identified in most maps without nerve conduction block. The ripple frequency was 7.8 ± 1.4 cpm ($n = 9$). The propagation velocity was 0.24 ± 0.18 cm/s but varied considerably over time and along the colon. Retrograde propagation velocity was 0.16 ± 0.03 mm/s ($n = 9$). Ripples did occasionally propagate smoothly from the oral to the anal end of the preparation or vice versa, but the pattern was most often chaotic. Because of the haustral folds, the contractions appeared disorganized on a spatiotemporal map (Figs. 5–8), since the folds disappeared [the folds flapped open (Supplemental Movies S3–S5)] and appeared (folded) again. In addition to this, the apparent direction of propagation often switched, contributing to seemingly chaotic motor patterns.

Dislocations, local changes in ripple frequency caused by a terminating ripple, were seen in Figs. 9–11 with the highest frequency occurring distally. Hence going from distal to proximal, the frequency dropped by one at a dislocation.

The ripple frequency was subject to excitation. Bethanechol (2×10^{-6} M) increased the frequency of ripples from as low as 7 to as high as 15 cpm (Fig. 5).

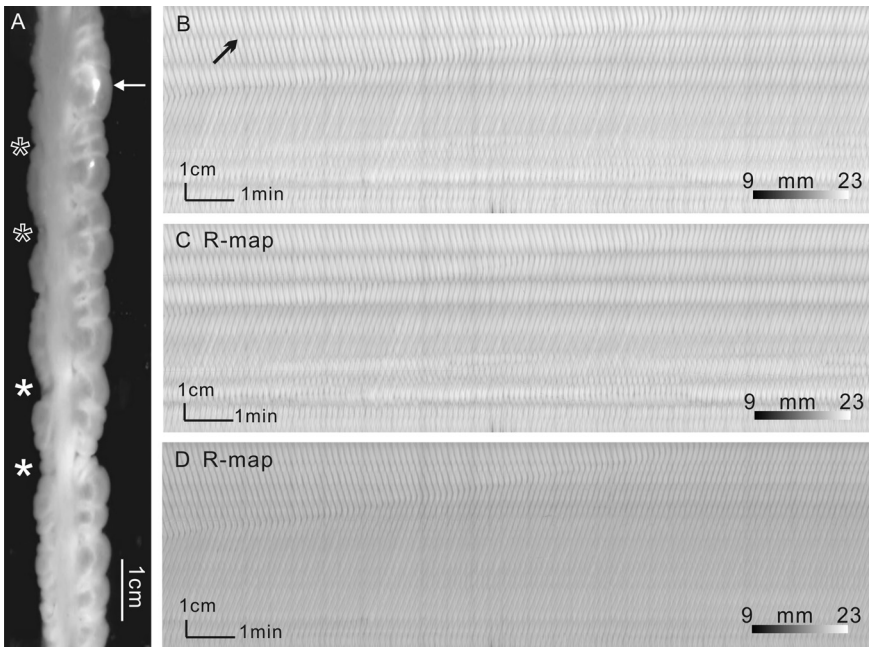

Fig. 6. Asynchronous circular muscle activity: haustral folds and ripple contractions. *A*: the colon profile shows markedly different haustral fold structures in the 2 intertaenial domains, taken from Supplemental Movie S3. The supplemental movie shows an example of annihilating ripple contractions (open stars indicate haustral boundaries that propagate antegrade, solid stars indicate haustral boundaries that propagate retrograde) and of collapsing and refolding of haustral folds (white arrow). *B*: D-map. Black arrow identifies a position where continuously collapsing and refolding of haustral folds are present, seen in Supplemental Movie S3 at the white arrow in *A*. *C*: R-map of top (*right* side in *A*) intertaenial domain of *A*, dominated by folding and collapsing haustral folds. *D*: R-map of bottom (*left* side in *A*) intertaenial domain of *A*, dominated by antegrade and retrograde ripple contractions that annihilate when they collide.

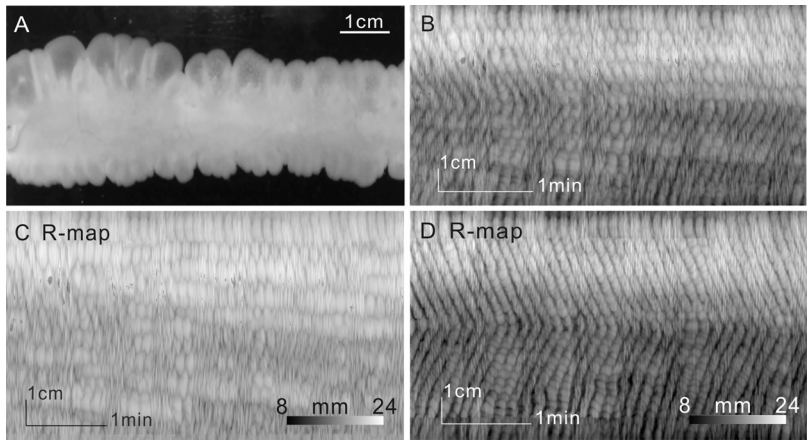

Fig. 7. Asynchronous circular muscle activity: the origin of apparent fast contractions. *A*: colon profile from Supplemental Movie S4. *B*: D-map. Ripples appear erratic and apparent high-frequency activity is seen; because the top and bottom circular muscle activity showed markedly different activity, a D-map will not give an accurate view and R-maps were created. *C*: R-map of top half of *A*. The ripple frequency is easily distinguished in the map but clear propagation is not; the ripple activity resembles a hexagon network. This is caused by folding and unfolding of the haustral folds. Rhythmic increase in excitation is seen as apparent high-frequency activity, but this is in fact the very rapid folding and unfolding of the haustral folds, clearly identifiable in Supplemental Movie S4. The unfoldings of the haustral folds increase the colon diameter and hence appear whiter in the R-map; therefore, the near vertical stripes in the map are not fast-propagation contractions but rapid unfolding of the haustral folds. *D*: R-map of bottom half of *A*. Antegrade and retrograde propagating ripple contractions dominate the map, coming together and annihilating in the middle of the map. The activity of the haustral folds gives the map a corncob like appearance. About 2 times per minute the map turns darker, the colon becomes somewhat contracted, and increased activity occurs, the haustral folds quickly fold and unfold giving the appearance of fast-propagating contractions. Since they occur over the length of the colon, they can be confused with fast-propagating contractions but they are a consequence of ripples whipping up the haustral folds.

The haustral folds. In the three regions between the taenia, the entire colon wall is folded in regular folds as shown in Figs. 1*D* and 8. The fold pattern was different in the three regions. The folds were semipermanent; that is, they came and went but always returned to their original position. This was deduced from visual observation of the movies; their "signature" in maps when they were not active showed as horizontal lines (e.g., Fig. 10*B*).

Fast-propagating contractions. Fast-propagating contractions (5.8 ± 1.6 cm/s) were ring contractions of narrow width (~3 mm, $n = 10$) of the circular muscle that occurred at variable frequencies (5–21 cpm) (Fig. 10). In the presence of bethanechol, clusters of fast-propagating contractions were of long duration, 30–60 s (Fig. 10, *C* and *D*). The fast-propagating contractions, similar to the ripples, were shaped by haustral fold activity; they were not smooth ring contractions but showed variable diameter changes due to the folding and unfolding of the haustral folds.

Fast-propagating contractions often occurred in clusters, the cluster frequency ranging from 0.6 cpm to 2.1 cpm (1.44 ± 0.31 cpm) ($n = 10$). A cluster was propulsive, moving 1–3 ml PBS out of the colon segment. The frequency of fast contractions within the clusters was 15.8 ± 2.1 cpm. Clusters of fast contractions were inhibited by TTX ($n = 10$) but were prominent in the presence of TTX when bethanechol was added (Fig. 10).

LDCs. The long-distance contraction (LDC) was the most forceful, high-amplitude propulsive motor pattern, associated with 3–5 ml outflow of content from the 12-cm segments (Figs. 11 and 12), identical in appearance to the LDC of the rat colon (9, 33, 34). In spatiotemporal maps, the rabbit colon LDCs were triangular in shape, widest at the proximal end, and

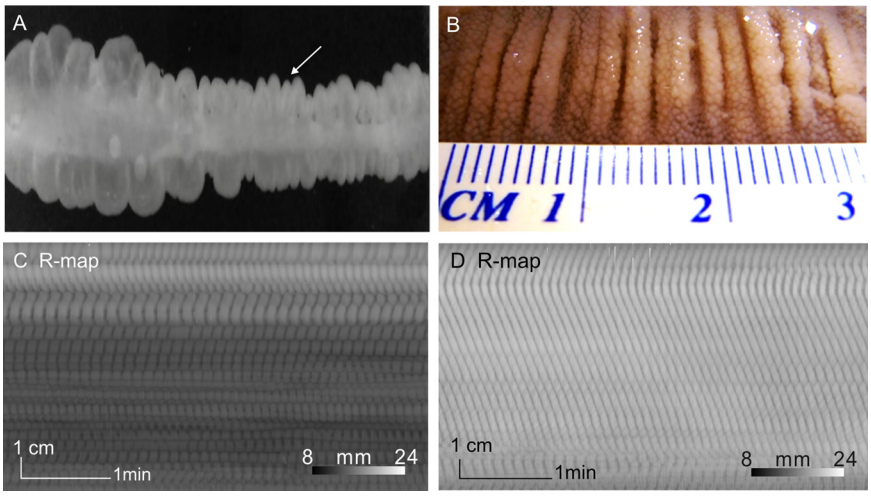

Fig. 8. Asynchronous circular muscle activity: the haustral folds. *A*: the colon profile shows haustral folds on top and ripple contractions at the bottom. This identification relies on observing the motor activity in Supplemental Movie S5. The arrow is pointed to the haustral folds, shown from the luminal side in *B*. *B*: intraluminal haustral folds of the proximal colon. *C*: R-map of the top part of the colon showing haustral fold activity; the map appears to show different frequencies of ripple activity. Our interpretation is as follows: the slow waves underlying the ripples (as seen in the bottom half) traverse the top half as well. However, haustral folds are folding and unfolding when the ripple traverses them, and the density of haustral folds is irregular. The folding and unfolding gives the appearance of very short propagating diameter changes in both directions. *D*: R-map of the bottom half of *A*, regular ripple activity propagates predominantly in the anal direction, very obviously notable in Supplemental Movie S5.

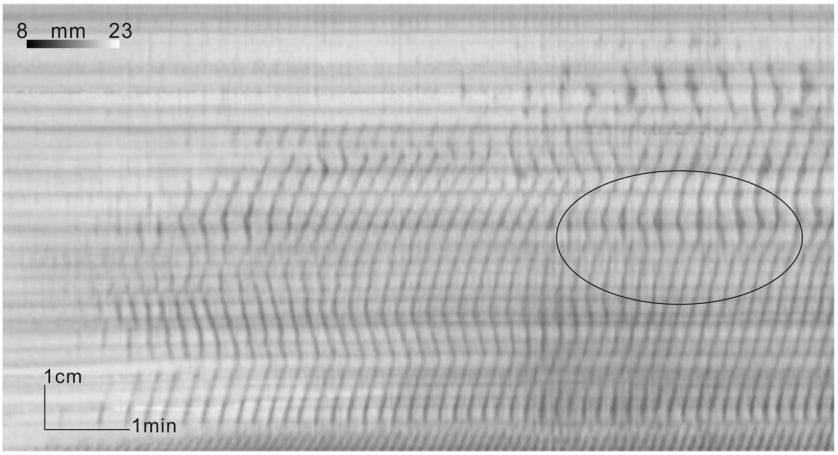

Fig. 9. Terminating ripples, interpreted as signifying dislocations of slow-wave activity in the network of interstitial cells of Cajal of the submuscular plexus (ICC-SMP) pacemaker network. Within the oval, dislocations are observed. Propagating contractions terminate. The highest frequency of the ripples is seen to be in the distal part. A coupled oscillator system with a frequency gradient is assumed to be underlying the ripple motor pattern. At a certain point, the coupling is not strong enough to overcome the difference in intrinsic frequency and dislocations occur (45).

preceded by a strong relaxation or dilatation. The LDCs were typified by the fact that the section of the colon studied was contracted almost along its entire length for several seconds (measured at the proximal end: 20 ± 6 s); it was never a ring contraction similar to most other motor activities. LDCs did not hinder the progression of the haustral boundary contractions when they occurred at the same time (Figs. 4 and 11). However, upon stimulation of the tissue by bethanechol, haustral boundary contractions were most often replaced by LDC activity (Fig. 12). When the stimulation was less forceful, such as with neostigmine, LDC activity appeared in conjunction with haustral boundary contractions (Fig. 12).

Phase-amplitude coupling. The parameters of a typical experiment were used in a model of phase-amplitude coupling, described previously (30). The haustral boundary activity was modeled as a sine wave with a frequency of 0.45 cpm and a velocity of 0.015 cm/s. The ripple pacemaker was modeled as a sign wave at 9 cpm and a velocity of 8 cm/s (Fig. 13). In the model, the amplitude of the ripple pacemaker is modified by the phase of the haustral boundary pacemaker, and this mod-

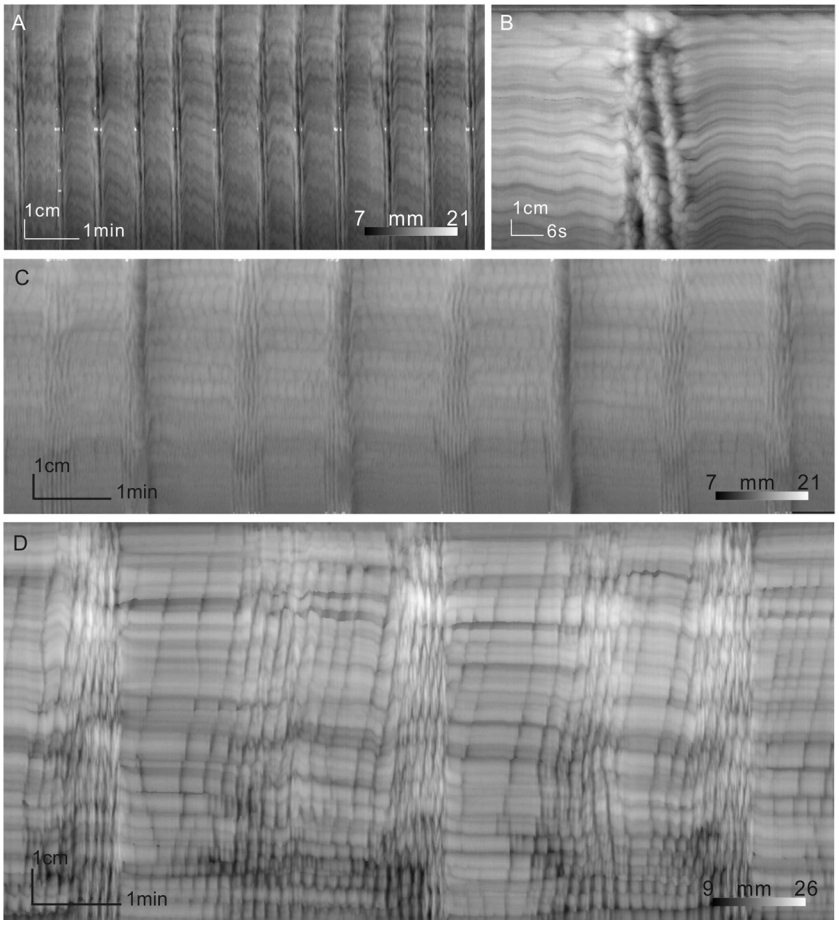

Fig. 10. Fast-propagating contractions. *A* and *B*: spontaneous fast-propagating contractions at a frequency of 15 cpm, appearing in clusters, at a cluster frequency of 1.5 cpm. The periods of excitation (the clusters) alternate with periods of inhibition where no ripples are seen and the haustral folds are quiet. The markings of the haustral folds (horizontal wiggle lines) show clearly in *B*. *C*: clusters of fast-propagating contractions in the presence of bethanechol and TTX. Ripples are seen in between the clusters. *D*: clustered activity in the presence of bethanechol and TTX. The clusters show either ripples at increased frequency or fast-propagating contractions. Dislocations are seen in the bottom part of the figure. Part of map corresponds to Supplemental Movie S6.

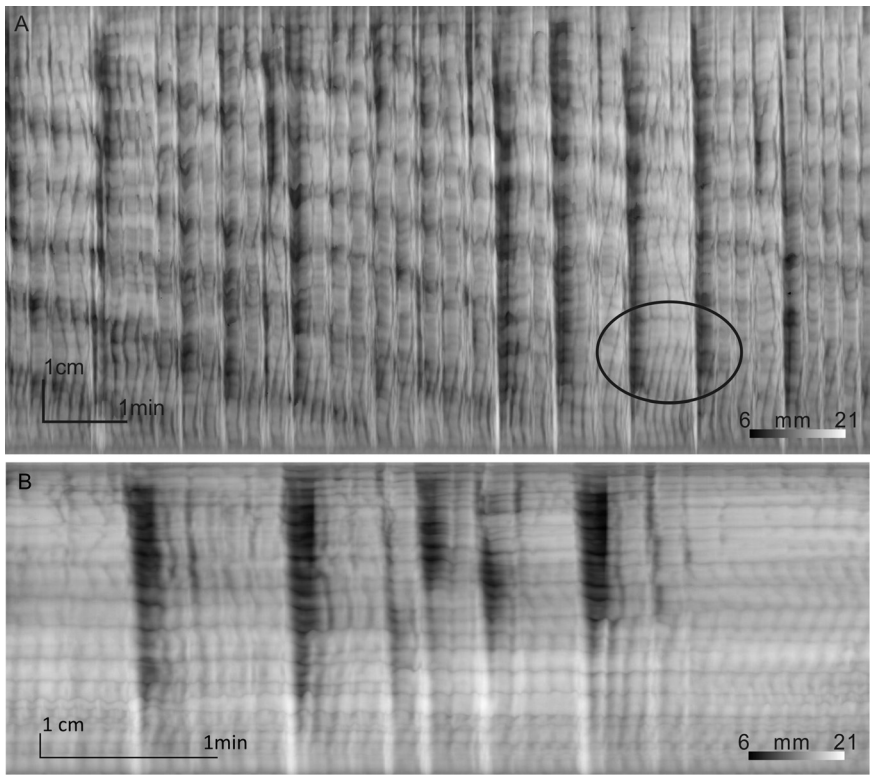

Fig. 11. Spontaneous LDCs and haustral boundary contractions. *A*: activity showing LDCs, haustral boundary contractions, and ripples and fast-propagating contractions. Dislocations are seen in the oval, showing the highest ripple frequency to be distal. *B*: a different colon from *A*. Typical triangle-shaped LDCs (see Ref. 9), preceded by relaxations.

ified activity is summed with the haustral boundary pacemaker to obtain the predicted activity occurring in the musculature. The similarity with the actual haustral boundary contraction motor pattern is striking (Fig. 13).

DISCUSSION

The haustral boundary contractions and the ripples. The haustral boundary contractions in the rabbit colon were seen as very slowly propagating ring contractions of the circular muscle (~0.1 mm/s); they were blocked by TTX, hence the enteric nerves play a critical part in their generation. They are governed by a remarkably constant pacemaker at ~0.5 cpm. The ongoing contractions are ~1.6 cm apart and divide the colon into pockets, defining them as haustra. The haustral boundary contractions and the ripples interact with each other so that the haustral boundary contractions show a contraction-relaxation cycle at the ripple frequency. When haustral boundary contractions propagate in opposite directions, they annihilate when colliding. Annihilation takes place when the propagating events occur within the same physical structure and are followed by a refractory period, as happens with nerve action potentials, slow waves, and forest fires. Annihilation is well known to take place with motor patterns that are driven by an interstitial cells of Cajal (ICC) network (35, 42, 45).

The ripples are rhythmic, ~8 cpm, slow-wave-driven propagating ring contractions of the circular muscle, independent of the enteric nervous system; the ripples traverse over the proximal colon in variable directions at ~0.24 cm/s (13, 37). Ripples occur at the dominant slow-wave frequency, as recorded in the rabbit colon (7, 46, 56). Lentle et al. (37) showed that the ripples are a unique circular muscle activity, since direct measurement of the longitudinal muscle movements did not show an equivalent activity. Hence they suggested that the pacemaker of the ripples is likely originating in the ICC of the submuscular plexus (ICC-SMP), consistent with other animal models where ICC-SMP are the generators of the omnipresent colonic slow-wave activity (38, 41, 47, 49, 55) driving circular muscle activity.

When two ripples collided with each other, or when two haustral boundary contractions collided with each other, they were annihilated. These were also the two motor patterns with a remarkably constant frequency. Encounters between haustral boundary contractions and ripples did not cause annihilation, suggesting them to be originating in separate structures. The manner of interaction of the two pacemakers that are involved in haustral boundary contractions demand extensive research. In the intestine we provided evidence (28) that a stimulus-dependent low-frequency pacemaker interacted with a higher frequency myogenic pacemaker to create the classical segmentation motor pattern originally described by Cannon (8) through phase-amplitude coupling, although different mechanisms have also been suggested (31). In the present study, when we tested the hypothesis that the slowly propagating haustral pacemaker activity interacted with the much faster myogenic pacemaker (creating the ripples) through a model of phase-amplitude coupling, a stunning resemblance to the haustral boundary contraction motor pattern emerged (Fig. 13). Therefore, in line with the organization of the pacemakers in the small intestine, we propose that the haustral boundary pacemaker activity comes from the network of ICC of the myenteric plexus (ICC-MP), although Lentle et al. (37) proposed the ICC-SMP. Can the ICC be involved in the generation of the rhythmicity of the haustral boundary contractions when the motor pattern is abolished by TTX? Within all ICC net-

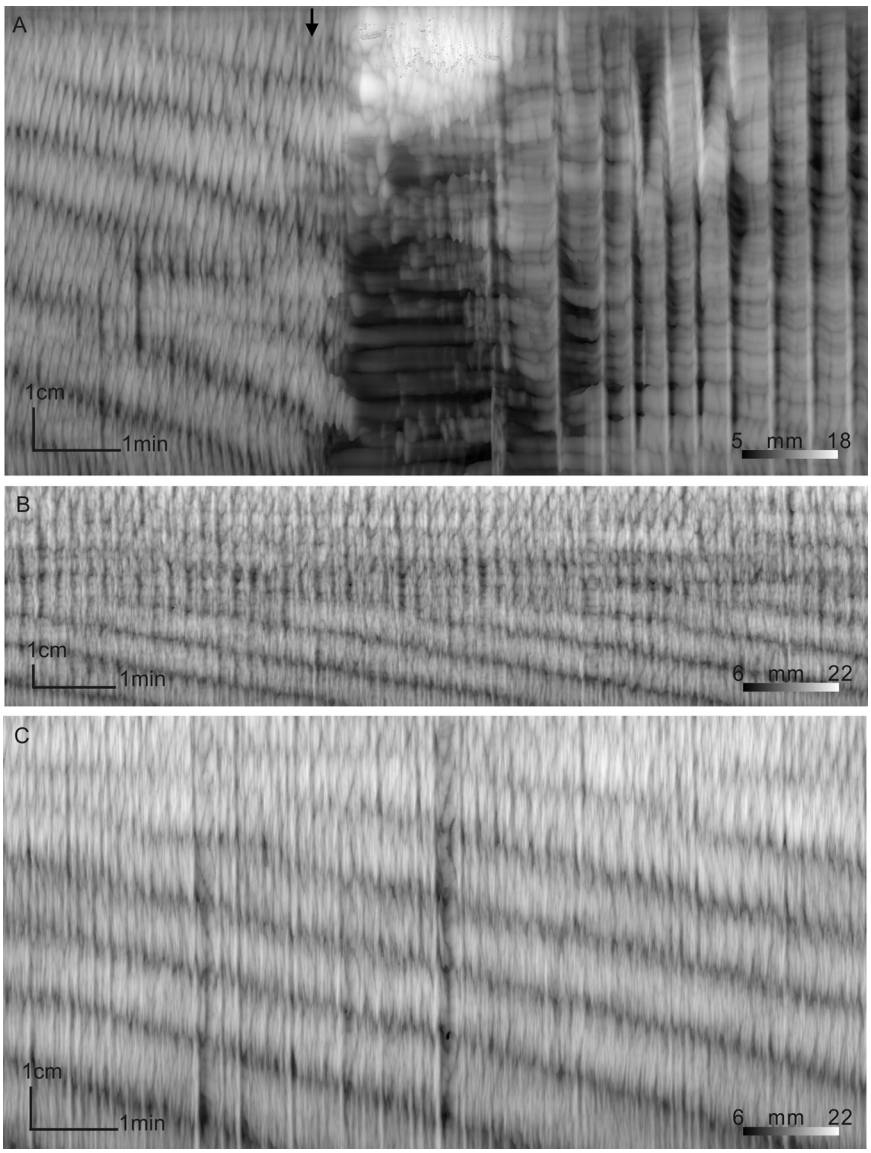

Fig. 12. Effects of cholinergic agonists on haustral boundary contractions. *A*: bethanechol 10^{-6} M (at arrow) changes haustral boundary contractions into LDCs. *B*: control activity before neostigmine stimulation. *C*: increase in endogenous acetylcholine by adding neostigmine 10^{-6} M increases amplitude of haustral contractile activity and makes it originate more proximal, LDCs are also induced.

works, the ICC-MP, the ICC-SMP, and the intramuscular ICC, each ICC is innervated by multiple enteric neurons and many neurotransmitter receptors are identified on ICC (19, 52, 57, 61). This structural arrangement led Ramon y Cajal to propose them to be part of the neural network (50). Some neural pathways may go to the musculature exclusively via ICC (23, 60). Hence ICC are embedded into the enteric neural circuitry. Although omnipresent myogenic pacemaker activity originating in ICC is well known to exist in the stomach (20, 26, 44), the small intestine (12, 27, 36, 39), and the colon (1, 16, 55), stimulus-dependent pacemaker activity has also been documented, for example in the stomach (25, 63), guinea pig small intestine (15), and esophagus (48, 59); hence ICC pacemaker activity can be dependent on activity from the enteric or central nervous system. In the mouse colon, direct evidence was provided that enteric excitatory nerves can synchronize calcium transients in ICC-MP to provide pacemaker activity to colonic migrating motor complexes (3). The gap junction contacts between ICC and smooth muscle cells make the ICC ideal to convey important information from the nerves to smooth muscle cells. Even in ICC with stimulus-independent slow-wave activity, the enteric nerves can modulate the pacemaker frequency (22, 64). Hence, if the haustral boundary contractions have an ICC network as the origin of their rhythmicity, and if this pacemaker activity is dependent on neural excitation of the ICC, then the pacemaker activity will be abolished by TTX.

With every experiment, each motor pattern was evaluated for its propulsive nature (see MATERIALS AND METHODS) and haustral boundary contractions were never propulsive. In the rabbit, the haustra are important for pellet formation and pellet transport (17, 18); the present study shows that it is likely its on/off pattern that causes propulsion of fluid to be ineffective.

The ripples and the haustral folds. Ripple contractions appear anarchic because of the muscle folds. When the depolarization-hyperpolarization cycle (the slow wave underlying the ripples) and hence the contraction-relaxation cycle enters a fold, the fold or two folds at the same time collapse and bulge out and return again to their original position when the depolarization wave subsides. It results in an apparent chaotic motor pattern, perfect for mixing and absorption (6, 53, 54). We hypoth-

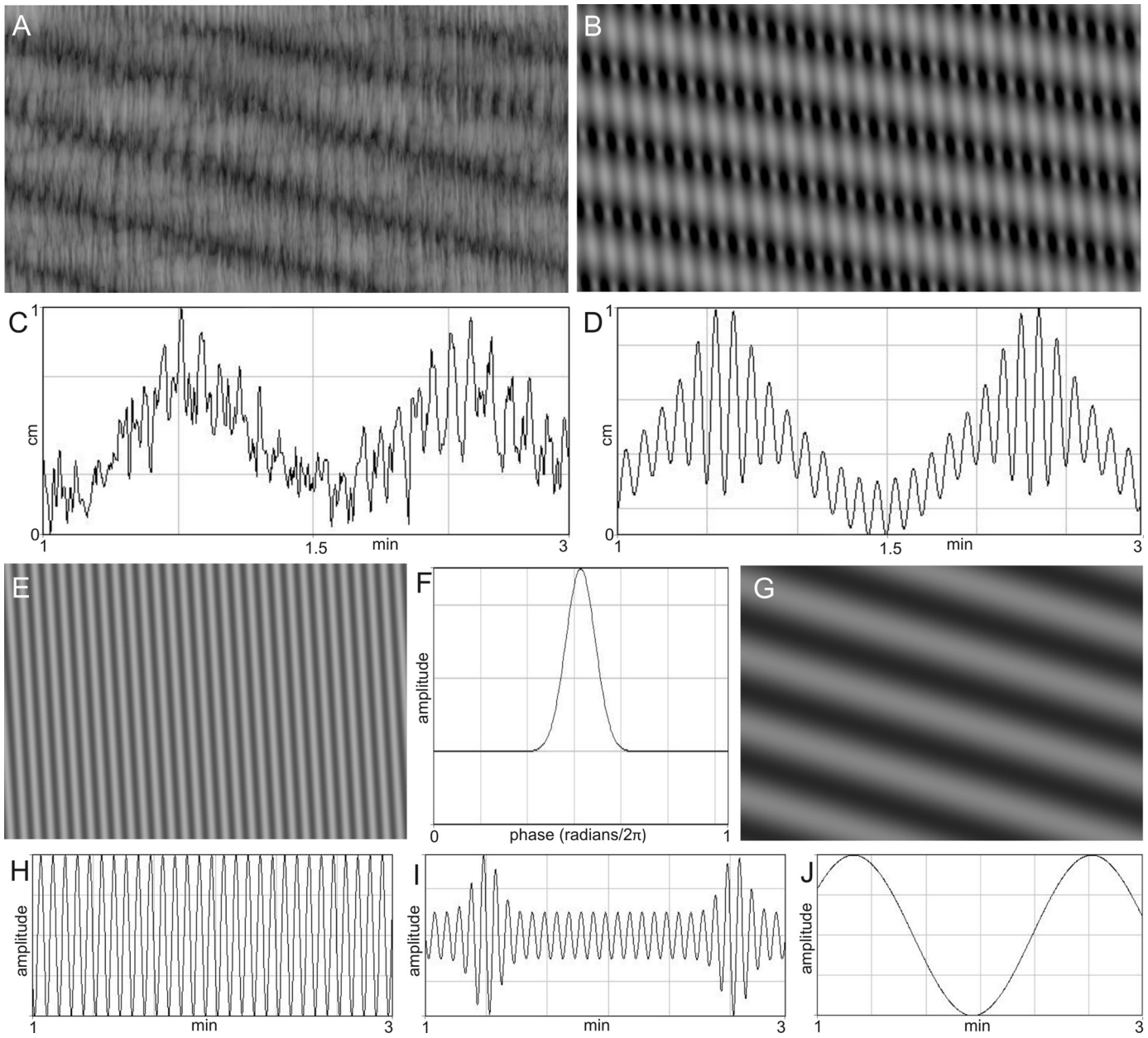

Fig. 13. Phase-amplitude coupling and the haustral boundary contractions. The haustral boundary contractions are in part shaped by the interaction of 2 pacemaker activities underlying its 2 rhythms, i.e., the frequency of ~0.5 cpm and the on/off pattern at the ripple frequency of ~8 cpm, no matter the origin of the rhythm generator. Here the output of modeling of this interaction is demonstrated according to the model outlined in Ref. 30. *A*: spatiotemporal map of haustral boundary contractions. A map was created from a 6-cm section of the proximal colon over a period of 3.3 min. *B*: spatiotemporal map of the underlying pacemaker activities where the phase of the low-frequency component (the haustral contraction rhythm) interacted with the amplitude of the high-frequency component (the ripple rhythm). *C*: an amplitude-over-time profile of the motility recording in *A*. *D*: an amplitude-over-time profile of the model output seen in *B*. *E*: the ripple pacemaker activity used in the model. *F*: the phase-amplitude function used to make the phase of the low-frequency component interact with the amplitude of the ripple pacemaker. *G*: the haustral boundary pacemaker activity used in the model. *H*: amplitude-over-time profile of the ripple pacemaker. Amplitude value is arbitrary. *I*: amplitude-over-time profile of the ripple activity after modulation by the haustral boundary pacemaker. *J*: amplitude-over-time profile of the haustral boundary pacemaker. Note that, to create the spatiotemporal map of the combined pacemaker, activities shown in *B*, *I*, and *J* are combined, which creates *D*.

esize that the slow wave propagates as a ring depolarization; in one intertaenial domain this may lead to a simple propulsive circular muscle activity when haustral folds are relatively undisturbed and at the same time the opposing intertaenial domain can show dramatically active haustral fold activity that overshadows the propulsive nature of the basic slow wave driven activity and causes effectively nonpropulsive motor activity, as seen in Figs. 6–8 and associated movies. This interpretation negates the need to hypothesize that the asynchrony of intertaenial ripple activity is caused by asynchronous circumferential slow-wave activity. Recognition of asynchronous intertaenial activity is of critical importance for the interpretation of D-maps since two motor patterns may appear to be occurring simultaneous on a D-map whereas they clearly occur separately in the adjacent intertaenial regions as can be deduced from R-maps.

The pacemaker network underlying the ripples shows dislocations, shown here in Figs. 9, 11, and 12. Another example of dislocations in the rabbit colon, noted as a "chaotic pattern of initiation," is seen in Fig. 7 from a study by Dinning et al. (13). Dislocations are seen at the end of a frequency plateau where a wave is lost when the coupling is not strong enough to overcome the frequency difference at that point (45). In the present study, the distal side of the plateau always had a higher frequency, indicating that the highest intrinsic frequency is in the distal colon, which was also noted in previous studies (51, 56, 58). The ripple maps also show frequently changing direction of propagation, which can be explained by the emergence of multiple pacemakers (43) or it may suggest limited coupling between weakly coupled oscillators (45).

Fast-propagating contractions. Fast-propagating contractions were evident in many maps; they were narrow-width ring contractions at a high frequency (10–25 cpm) that propagated at ~6 cm/s, usually in antegrade direction, often appearing near simultaneous along the colon. They appeared distinct from ripples; ripples and fast-propagating contractions were seen in many maps to alternate with sharp differences in frequency and propagation velocity although, overall, their frequency range overlapped. We noted that the fast-propagating contractions usually occurred in clusters and were associated with propulsion of content; the clusters clearly represented a transient increase in excitation, associated with an increase in the amplitude of contractions and outflow. In the present study and in the study by Lentle et al. (37), the clustering of fast-propagating contractions was often observed in the presence of TTX, indicating that the pacemaker behind the clusters is nonneural.

LDCs. The colonic migrating motor complexes (3, 21) are comprised of at least two distinct motor patterns as identified in the rat colon: LDCs and rhythmic propagating motor complexes (9, 33, 34). The LDCs are a very distinct motor pattern; the contraction is triangular in shape in spatiotemporal maps because the proximal part remains contracted while the wave front is progressing anally. In the rabbit, the LDC is the strongest propulsive contraction. Under the conditions of the experiments shown here, the enteric nervous system was essential for their development as they were blocked by TTX. The LDCs can be strongly rhythmic; hence a neural or ICC pacemaker is likely associated with it. In the rat colon the ICC-MP were proposed as LDC pacemakers (9, 29, 40, 47, 62). In the mouse colon, excitatory nerves synchronize calcium transients in ICC-MP to provide pacemaker activity to colonic migrating motor complexes (3), a term that incorporates the LDCs as reported here (9). Can an ICC network provide pacemaker activity to more that one motor pattern to which different neuronal programs make an essential contribution? If ICC generate rhythmic depolarizations that are transmitted to the musculature on an ongoing basis, then any neural program that excites the musculature will work in concert with this basic activity of the musculature. ICC pacemaker activity may create "tracks" that are used by whatever other stimulus is used to excite or program the musculature.

In summary, the rabbit colon forms haustra that are caused by rhythmically contracting, slowly propagating circular ring contractions. The haustral boundary contractions are dependent on activity from the enteric nervous system. They exhibit an on/off/on/off pattern at the ripple frequency. The haustral boundary contractions are therefore shaped by interaction of two pacemaker activities; we hypothesize this to occur through phase-amplitude coupling of pacemaker activities from ICC-MP and ICC-SMP. Video evidence shows the unique role that haustral folds are playing in shaping contractile activity within the haustra. Muscarinic agents not only enhance the force of contraction, they can eliminate one and at the same time induce another neurally dependent motor pattern.

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SUPPLEMENTAL MATERIAL

Movie S1 = Associated with Figure 1
Movie S2 = Associated with Figure 3
Movie S3 = Associated with Figure 6
Movie S4 = Associated with Figure 7
Movie S5 = Associated with Figure 8
Movie S6 = Associated with Figure 10*D*

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

J.-H.C., Z.Y., and J.D.H. conception and design of research; J.-H.C., Z.Y., Y.Y., and J.D.H. analyzed data; J.-H.C., Z.Y., and J.D.H. interpreted results of experiments; J.-H.C., Z.Y., and J.D.H. edited and revised manuscript; J.-H.C., Z.Y., Y.Y., and J.D.H. approved final version of manuscript; Z.Y. and Y.Y. performed experiments; Z.Y. and J.D.H. prepared figures; J.D.H. drafted manuscript.

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