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Organization of Vagal Afferents in Pylorus: Mechanoreceptors Arrayed for High Sensitivity and Fine Spatial Resolution?

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Abstract

The pylorus is innervated by vagal mechanoreceptors that project to gastrointestinal smooth muscle, but the distributions and specializations of vagal endings in the sphincter have not been fully characterized. To evaluate their organization, the neural tracer dextran biotin was injected into the nodose ganglia of rats. Following tracer transport, animals were perfused, and their pylori and antra were prepared as whole mounts. Specimens were processed to permanently label the tracer, and subsets were counterstained with Cuproline blue or immunostained for c-Kit. Intramuscular arrays (IMAs) in the circular muscle comprised the principal vagal afferent innervation of the sphincter. These pyloric ring IMAs were densely distributed and evidenced a variety of structural specializations. Morphometric comparisons between the arbors innervating the pylorus and a corresponding sample of IMAs in the adjacent antral circular muscle highlighted that sphincter IMAs branched profusely, forming more than twice as many branches as did antral IMAs (means of 405 vs. 165, respectively), and condensed their numerous neurites into compact receptive fields (~48% of the area of antral IMAs) deep in the circular muscle (~6 μ m above the submucosa). Separate arbors of IMAs in the sphincter interdigitated and overlapped to form a 360° band of mechanoreceptors encircling the pyloric canal. The annulus of vagal IMA arbors, putative stretch receptors tightly intercalated in the sphincter ring and situated near the lumen of the pyloric canal, creates an architecture with the potential to generate gut reflexes on the basis of pyloric sensory maps of high sensitivity and fine spatial resolution.

Keywords

antrum; gastric emptying; gastroduodenal sphincter; interstitial cells of Cajal; nodose ganglion; pyloric canal; pyloric reflex

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Introduction

Both the fact that the pylorus plays crucial roles in gastrointestinal (GI) physiology and the corollary that the sphincter is affected in multiple GI pathophysiology are universally recognized (see reviews: Van Nueten et al., 1990; Keet, 1993; Mayer, 1994; Ramkumar and Schulze, 2005). The sphincter paces gastric emptying of food and fluids, in the form of chyme, to the intestines, where the material is broken down more completely and then absorbed. The sequencing entails complex motor choreographies programmed by reflexes and modulated by hormones. The reflexes retain ingested material in the stomach for gastric-phase digestion and establish the nutrient-reservoir operations of the organ, while concomitantly delivering chyme readied for additional digestion and absorption into the duodenum, to maintain a supply of nutrients to tissues. Simultaneously, the pylorus operates to create a barrier (or gate, as its etymology implies) that minimizes reflux of bile and intestinal enzymes. Furthermore, reciprocating cycles of gastric digestion, emptying, and nutrient delivery to the intestines generate neural and endocrine signals that establish patterns of feeding and satiety (Friedman and Stricker, 1976; McHugh and Moran, 1985; Malbert and Ruckebusch, 1989; Phillips and Powley, 1996; Powley and Phillips, 2004).

In early observations, the pyloric sphincter was generally assumed to operate bi-stably, cycling between normally closed and transiently open states. Modern monitoring technologies (pressure recordings, imaging techniques, fluid-mechanical analyses, etc.) have established, however, that the motor choreography is both more graded and more complex than initially appreciated (e.g., Dent, 1990; Ramkumar and Schulze, 2005). Paralleling the discoveries of the motor complexities, physiological analyses have established that the operation of the sphincter is controlled by multiple reflexes programmed by both intrinsic (Allescher et al., 1988; Bortoff, 1990) and extrinsic (Allescher et al., 1988; Allescher, 1990) circuits. Correspondingly, morphological studies have documented that the pylorus is heavily innervated (Cai and Gabella, 1984; Wang et al., 2007), receiving multiple afferent and efferent projections that have been identified and partially characterized (Daniel and Allescher, 1990; Elfvin and Lindh, 1982). Though such pathways have been established histologically and, in some cases, demonstrated physiologically, much about their structural organization and function remains unclear.

In the case of the vagal afferent innervation of the pylorus, which was the focus of the present experiment, it has been established that the nerve supplies both of its two common types of GI mechanoreceptors, namely intraganglionic laminar endings (or IGLEs) and intramuscular arrays (or IMAs), to the smooth muscle of the sphincter region (Kressel et al., 1994; Wang and Powley, 2000). These initial observations also suggest that the pyloric muscle is heavily innervated by IMAs. Though the vagal afferents to the pylorus must form the sensory arms of reflexes that presumably have key roles in the operation of the sphincter, relatively little is yet known about their regional organization and local morphological specializations within the muscle of the pylorus. This lack of a detailed characterization of the sensory endings within the pyloric ring has meant that these afferents are often ignored in GI physiology and gastroenterology, where conventional descriptions have skewed both experimental and therapeutic investigations toward a focus on motor-neuron and smooth-muscle mechanisms of the sphincter, with considerations of sensory mechanisms limited to

longer reflexes triggered in the antrum or duodenum. Arguably, this myopia has impeded development of a more comprehensive and adequate understanding of pyloric functions.

To redress some of the imbalance in information on the reflex circuitry of the pylorus, the present experiment was designed to more fully characterize and map the afferent endings that the vagus nerve supplies to the sphincter smooth muscle.

Materials and Methods

Animals

Male Sprague-Dawley rats (n = 62; Harlan, Indianapolis, IN, USA) two to three months of age with a mean ($\pm$ SEM) weight of 278 ± 5.7 g at the time of surgery and tracer injection were individually housed in an AAALAC-approved colony facility maintained at 22 – 24°C and on a 12:12 h light:dark schedule. Pelleted chow (Diet No. 5001; PMI Feeds, Inc., Brentwood, MO, USA) and filtered tap water were available ad libitum, except the evening prior to surgery, when food, but not water, was removed until post-surgery. All animal handling and husbandry procedures conformed to The NIH Guide for the Care and Use of Laboratory Animals (8th ed., The National Academic Press, Washington, D.C.) guidelines and were approved by the Purdue University Animal Care and Use Committee. Every effort was made to minimize any suffering and the number of animals used.

Neural Tracer Labeling

For tracer injections, animals were anesthetized with Isoflurane (Fluriso®; MWI, Boise, ID) after an overnight fast. Once anesthesia had been induced, Glycopyrrolate (0.2 mg mL^{-1} , s.c.; American Regent Inc., Shirley, NY, USA) was injected to reduce secretions.

With the animal in a supine position, the nodose ganglia were exposed with a midline incision of the skin of the ventral neck and blunt dissection of the overlying muscles. Different subsets of the animals then received bilateral (n = 38), left-only (n = 14), or right-only (n = 10) tracer injections into the nodose ganglia using a 35 ga NANOFIL needle with syringe (NF35BV; World Precision Instruments, Inc., Sarasota, FL). Per ganglion, each injection consisted of 1 μL of a lysine-fixable dextran biotin solution consisting of a 1:1 mixture of 3 K and 10 K MW dextrans in ultrapure water [final concentration: 15%, consisting of 7.5% D7135 (i.e. 3 K) and 7.5% D1956 (i.e. 10 K); Invitrogen, Carlsbad, CA, USA]. To facilitate the placement of the injections and monitoring their distribution, 0.01 mg of Fast Green FCF (Sigma-Aldrich, CO, USA) per 100 μL of solution was added to the tracer mixture. After injections, the skin was closed with interrupted sutures, and the animals were recovered on a circulating water heating pad prior to being returned to their homes cages. For analgesia, the animals received 0.01 mg kg^{-1} Buprenorphine subcutaneously (Buprenex®, Reckitt Benckiser Pharmaceuticals Inc., Richmond, VA, USA) prior to suturing and again the following morning.

Tissue Fixation and Dissection

Following a 14 d post-injection survival period for tracer transport, rats were given lethal doses of sodium pentobarbital (180 mg kg^{-1} , i.p.). When each animal was entirely

unresponsive to nociceptive pinching, it was perfused transcardially with physiological saline followed by 4% paraformaldehyde in 0.1 mol L⁻¹ phosphate-buffered saline (PBS, pH 7.4). An effort was made to fix the stomach tissues in normally full, albeit not distended, condition: Animals had food until the time of perfusion, and, to further insure that their stomachs were normally distended at the time of fixation, 10 mL of physiological saline at body temperature was infused slowly by catheter into the stomach as the vasculature was infused with saline for exsanguination.

After the perfusion, the entire stomach was excised by transecting the distal esophagus and the proximal duodenum (Fig. 1A). The organ was then opened with a longitudinal cut on the greater curvature and all chyme and contents were removed by gentle rinsing. From the complete gastric specimen, the pylorus and tissues of the lesser curvature including cardia, lower esophageal sphincter, and antrum were trimmed out as a single whole mount (see shaded area in Fig. 1A and 1D). To insure that the whole mount included, in its entirety, the thickened circular muscle ring that constitutes the sphincter (see Fig. 1B and 1C), care was taken to identify the sphincter ring and then to separate the duodenal bulb from the stomach. This cut was made just distal to where the sphincter ring of thickened circular muscle ended and duodenal tissue began (and which corresponds to the transition site where gastric mucosa is abruptly replaced by intestinal villi). The muscle layers of the whole mount were then isolated by removing the mucosa and submucosa from the specimen.

Staining

Tracer processing, immunohistochemistry and counterstaining of the whole mount were then done with the specimen free floating. Tracer processing consisted of treating the tissue with a hydrogen peroxide:methanol block (1:4) to quench endogenous peroxidase activity and then soaking the specimen for 3–5 days in PBST (PBS containing 0.5% Triton X-100 and 0.08% Na azide) to improve penetration through the muscle sheets. Specimens were then incubated for 1 hour in avidin-biotin-horseradish peroxidase complex (PK-6100; Vectastain Elite ABC Kit, Standard; Vector Laboratories Inc., Burlingame, CA, USA). Once reacted, the tissue was rinsed in PBS and reacted with diaminobenzidine and H₂O₂ for 5 – 6 min to yield a permanent gold-brown stain of labeled afferent neurites.

To maximize tissue transparency and the definition of the afferent label, one group of whole mount specimens (n = 38) was coverslipped without further staining (Powley and Phillips, 2005). Another subset of the specimens with vagal afferents labeled (n = 19), however, was counter-stained with Cuprolinic blue (17052; Polysciences, Inc., Warrington, PA) for enteric neurons, using a previously published protocol for the neuron-specific stain (Phillips et al., 2004; Walter et al., 2009). Similarly, a different subset of specimens (n = 5) with dextran-labeled vagal afferents was immunohistochemically processed for c-Kit (1:1000; AF-1356; R&D Systems, Inc., Minneapolis, MN) to label the interstitial cells of Cajal in the pyloric region with steel grey (Vector SG Peroxidase; SK-4700; Vector Laboratories, Inc.) as the substrate, using our previously published protocol (Powley et al., 2012).

Once the specimens had been processed with DAB to label dextran-labeled terminals and any counter- or immuno-staining had been performed, the whole mounts were rinsed and mounted serosa-side-up on gelatin-coated slides. The specimens were then air dried,

dehydrated with alcohols, cleared with xylenes, and coverslipped with D.P.X. (317616; Sigma-Aldrich, St. Louis, MO, USA) or Cytoseal XYL (8312-4; Richard-Allen Scientific, Kalamazoo, MI).

Afferent terminal inventories, morphometry, and image analysis and photography

Labeled vagal afferents to the pylorus were evaluated in a stepwise series of examinations. Whole mounts were first scanned in their entirety for labeled neurites. This initial evaluation employed brightfield and differential interference contrast (DIC) optics on Leica DMRE and DM5500 microscopes outfitted with long working distance objectives. Tracer-labeled fibers and their terminals were inventoried as to location and provisional type (IGLE, IMA, etc.).

In a second-stage evaluation, the fibers and terminals that had been initially identified were assessed for quality or density of the dextran labeling and for the completeness of the ending and the adequacy of the specimen for image analysis (i.e. site free of tears and dissection damage, lack of tissue folds or artifacts obscuring part of the neurite, etc.). From the pool of inventoried endings, those endings most adequately satisfying the several criteria for complete analysis were then mapped onto a normalized schematic of the pylorus and neighboring pyloric antrum (cf. Fig. 1F).

Finally, because the focus of the experiment was on the vagal afferent innervation of the pyloric smooth muscle and because the initial assessments indicated that the circular muscle ring that established the pylorus was near-exclusively innervated by intramuscular arrays (IMAs; see Results section), the vagal IMAs that best satisfied the imaging criteria just mentioned were digitized, reconstructed, and evaluated quantitatively on a NeuroLucida (MicroBrightfield Inc., Williston, VT, USA) workstation employing a Zeiss Axio Imager Z2 microscope equipped with DIC optics and long working distance objectives. In addition to IMAs in the pyloric sphincter circular muscle, a group of IMAs innervating the immediately neighboring distal antrum were similarly digitized to provide a comparison control group.

The digitization for both groups of IMAs consisted, more specifically, of tracing an individual parent neurite (typically traveling distally in a vagal fascicle in the lesser curvature) in three dimensions through the whole mount and then continuing to successively follow (also through the three dimensions of the specimen) all daughter branches of the ending as they repeatedly bifurcated, arborized and finally terminated. Employing the NeuroLucida program to digitize and trace the individual arbors concomitantly provided detailed morphometric profiles of the endings (branch orientations, number of branch points, branch segment lengths, etc.).

Single field-of-view photomicrographs were acquired digitally with a Spot Flex camera controlled with Spot Software (V4.7, Advanced Plus; Diagnostic Instruments, Sterling Heights, MI, USA). To maximize the depth of field of some of the images, Helicon Focus Pro X64 software (V5.3.7; HeliconSoft Ltd, Kharkov, Ukraine) images taken at different z-planes in the specimen were merged (i.e. focus stacking was performed).

For fibers and endings that had more extensive distributions in the x- and/or y-planes, multiple-field composites or mosaics of higher magnification views were collected and

merged using Surveyor with Turboscan software (V6.0.5.3; Objective Imaging, Cambridge, UK) on a Leica DM5500 workstation. Z-stacks of these extensive mosaics of IMA arbors were also compiled, exported to Photoshop CS5 (Adobe Systems, San Jose, CA, USA), and then used to trace complete afferent arbors (e.g., Figs. 3 and 5 below). Whereas Neurolucida tracings captured all of the morphometric information on terminals, the stick-figure facsimiles of endings rendered by the software failed to preserve the varicosities, dilations, lamellar plates, and other neurite details that could be preserved with the Photoshop tracings generated from the mosaic stacks of photomicrographs.

For figure production, Photoshop CS5 was used (a) adjust brightness, contrast and sharpness, (b) apply text and scale bars, and (c) organize the final figure layouts.

Statistics

Statistical comparisons, typically unpaired t-tests, were performed with GraphPad Prism (V6.02; Graphpad Software, Inc., San Diego, CA).

Results

The dextran labeling protocol, combined with the preparation of the smooth muscle wall as whole mounts, produced high definition Golgi-like profiles of random samples of the vagal peripheral fibers and terminals. These profiles made it practical to trace, reconstruct, and measure individual terminal fields in their entirety. Furthermore, the whole mount preparations clearly revealed the thickening of the circular muscle sheet that constitutes the pyloric sphincter, making it possible to distinguish the muscular ring (see Fig. 1F, bottom row) from the adjacent antrum and the more caudal duodenal bulb (which was removed). We use the terms *pyloric sphincter* and *pyloric ring* interchangeably to refer specifically to the thickened circular muscle ring (Fig. 1B and 1C, top row) at the transverse level typically considered the gastroduodenal valve or sphincter. Additionally both *pylorus* and *pyloric* are also employed more inclusively to describe the associated segment of overlying longitudinal muscle and the corresponding serosal sheet at the same frontal levels. In contrast, the two terms referring to the sphincter are not used to describe the tissues of the distal-most antrum or proximal-most duodenum.

As previously reported (e.g., Kressel et al., 1994; Wang and Powley, 2000), the vagal afferent innervation of the smooth muscle layers of the pylorus consisted of the two common vagal afferent terminals in GI smooth muscle, IGLEs (see Fig. 2) and IMAs (see Fig. 3). The two types of afferents can be considered in that order.

Intraganglionic Laminar Endings (IGLEs) in Pyloric Region

IGLEs in the muscle wall in the region of the pylorus (e.g., Fig. 2) appeared similar—if somewhat less numerous—to IGLEs that have been described previously (plates of lamellipodia-like telodendria associated with the myenteric ganglion boundaries with the smooth muscle sheets) in other regions of the stomach and did not evidence unusual morphological features that might signal region-specific specializations. At the sphincter level the longitudinal muscle sheet was attenuated or thinned out and somewhat patchy. As the longitudinal muscle layer diminished, the circular muscle layer thickened dramatically to

form the pyloric ring. Viewed from the serosal surface, an identifiable transitional region or “sulcus” defined the distal limit of the antrum and the proximal limit of the thickened pyloric ring (see Fig. 1F). Surveying the whole mounts progressively caudally from the antrum to the pylorus and on to the proximal duodenum indicated that the IGLEs and associated myenteric plexus had a typical “gastric” organization up to the transitional region, but they then became sparser at the level of the transition. In addition to the IGLEs in the region of the pylorus occurring in conjunction with the planar myenteric plexus at the longitudinal-circular muscle boundary, some of the pyloric IGLEs were found in conjunction with small groupings of myenteric ganglion cells embedded or folded deeper in the circular muscle wall. The planar or 2-dimensional distribution of the myenteric plexus and associated IGLEs then re-appeared in the duodenal bulb.

In general, given no increase—even a decrease—in the packing density of IGLEs and given the apparent lack of a structural specialization of the IGLEs at the level of the pylorus, the present survey focused on the vagal IMAs innervating the pyloric circular muscle.

Features of Intramuscular Arrays (IMAs) in Pyloric Sphincter

In contrast to the apparent sparseness of IGLEs in the immediate region of the pylorus, IMAs formed a dense, continuous annulus of terminals in the circular muscle of the pyloric ring. Those IMAs at the level of the sphincteric thickening of muscle also evidenced conspicuous specializations of their arbors (see below). Generally, though, the vagal IMAs innervating the pylorus (see Fig. 3) had the defining features of conventional IMAs that have been observed in other regions of the stomach (Fox et al., 2000; Phillips and Powley, 2000; Powley and Phillips, 2002), including forestomach (Berthoud and Powley, 1992; Berthoud and Neuhuber, 2000; Wang and Powley, 2000), corpus (Wang and Powley, 2000; Powley and Phillips, 2011), antrum (Powley et al., 2012), and lower esophageal sphincter (Powley et al., 2013). In brief, such gastric IMAs consist of parent afferents which terminate in arbors of parallel neurites embedded in a smooth muscle sheet and run parallel to the muscle fibers and network of interstitial cells of Cajal of the intramuscular type (ICC-IMs).

The afferent parent fibers of the pyloric IMAs coursed over the distal stomach wall, particularly within the lesser curvature, in small fascicles that separated from larger branches of the main gastric vagal bundles. As the parent fibers neared the pylorus, they separated from the fascicles to course to the region of the pyloric ring of thickened circular muscle wall and then began to arborize, within the ring, in a relatively stereotypical pattern. The parent fibers branched, dividing into daughter neurites that ran between, and parallel to, circular muscle fibers. These daughter fibers then bifurcated repeatedly, forming shorter bridging and forking fibers that ran obliquely to the muscle fiber axis before bifurcating to produce longer daughter fibers that again coursed between and parallel to the muscle fibers (Fig. 3A and B).

As observed in other regions of the stomach, the branches and bridging fibers of the IMA arbors also appeared to parallel and make contacts (by light microscopic criteria: 1000X evaluation at single focal plane, etc.) with ICC-IMs in the circular muscle (Fig. 4; particularly Fig. 4C). This pattern of longer neurites that paralleled neighboring muscle

fibers and shorter bridging elements that spanned from muscle fiber to muscle fiber, contacting or using ICC-IMs for guidance, generated the characteristic arbors of IMAs.

Though pyloric IMA arbors evidenced the normative qualitative features described for IMAs throughout the stomach, closer examinations as well as morphometry (see below) indicated that these endings in the pyloric circular muscle elaborated multiple distinctive structural adaptations that specialize the endings to innervate the sphincter ring. Some of the specializations became apparent when the IMAs in the pylorus (e.g., Fig. 3) were directly compared with those IMAs in antral circular muscle (e.g., Fig. 5). This pylorus vs. antrum comparison was particularly appropriate and instructive because (a) the antral IMAs were located closely adjacent to the pylorus in the neighboring tissues of the stomach, and (b) other experiments have found that antral IMAs are representative of those found in other regions of the stomach. One modification of the IMAs innervating the sphincter consisted of the formation of exceptionally heavily branched arbors, and a second adaptation consisted of the neurite branches aggregating as unusually compact terminal arbors. The resulting dense arrays of relatively short branches presumably adapted the endings to innervate the large number of muscle fibers that occurred in pyloric circular muscle. It should be noted that to make the dense array of the pyloric IMA in Fig. 3 legible, the tracing had to be scaled at a higher magnification than the antral IMA in Fig. 5. The extent of how dramatically condensed the pyloric IMAs were, when compared to antral IMAs, can be more clearly appreciated in Fig. 6 (left column), which displays the tracings of the pyloric and antral IMAs illustrated in Figs. 3 and 5, at the same scale or magnification. The pattern of short, frequently branching neurites caused the pyloric IMAs to concentrate their short branches into tightly localized “receptive fields.” [For the present morphological analyses, we assumed that a receptive field corresponded to the planar area occupied by an arbor within and parallel to the smooth muscle sheet. This area was measured with the Neurolucida “convex hull” algorithm—see below.]

Even within the sphincter smooth muscle ring, pyloric IMAs tended to distribute selectively. In the case of a majority (41 of 53) of the measured pyloric IMAs, the parent fiber coursed into the circular muscle ring and continued both caudally several hundred microns and deep towards the submucosal side of the circular muscle sheet before beginning to arborize into an array of closely spaced neurites, similar to the IMA illustrated in Fig. 3, that was concentrated in the more aboral half of the pyloric ring.

Though the majority of the pyloric IMAs consolidated their neurites into these tight bands of branches deeper in the distal part of the ring, a minority (12 of 53) of the cases distributed their branches less compactly. In this variant pattern, the parent fiber of the IMAs began giving off small numbers of collateral neurites among the circular muscle fibers as soon as it entered the sphincter ring. Even these less compactly arborized IMAs tended to issue only a limited number of collaterals into the more oral and more superficial parts of the sphincter ring and then to continue distally to branch extensively and form a more coherent and tight array of neurites in the distal half of the sphincter muscle band. Among the 12 out of the total of 53 pyloric IMAs that exhibited these more oral collaterals, however, considerable variability was observed in terms of how many of the “early” or “oral” collaterals were

issued into more rostral part of the sphincter (see Fig. 7; see also the IMA designated with green in Fig. 8).

Not only were the neurites within the individual arbors densely aggregated, the separate neuronal arbors were also tightly packed and extensively interdigitated with other IMAs in the pylorus. These observations emerged in part because our nodose ganglion injections yielded an unexpectedly large number of pyloric IMAs per injection. Based on the labeling rates of several other earlier surveys of other gastric regions (e.g., Powley and Phillips, 2005, 2011; Powley et al., 2012, 2013), as well as unpublished observations, we initially employed bilateral nodose injections that we had extrapolated would achieve an appropriate yield of well labeled but also well separated (i.e. non-overlapped) terminals to optimize tracing and morphometry. After employing the bilateral-injection strategy in the initial phase of the present experiment, however, it became apparent that the injection parameters, which we had previously used routinely for other gastric surveys, labeled so many IMAs in the pyloric ring that neurite arbor interdigitations and neurite overlap were so frequent that it was difficult to reliably obtain a high enough percentage of isolated or largely non-overlapping IMA terminals for morphometry in the sphincter smooth muscle. From the inventories of endings in the bilateral-injection cases it was clear that pyloric IMAs were not only numerous, but also that pyloric IMAs were tightly arrayed with considerable interdigitation. Indeed, it became clear that IMAs in the pylorus were heavily interdigitated end-to-end (Fig. 8), creating a continuous, even interlocking, band or chain of IMA arbors completely encircling the sphincter canal. The sample of tissue from the bilateral injections also indicated that the separate IMAs innervating the sphincter were not only intertwined end-on-end, they were also tightly situated side-to-side throughout the thickness of the circular muscle ring. Something of the manner in which the IMAs braided into an annulus can be appreciated in the summary map of Fig. 9 where the sample of IMAs (in this case, from multiple animals) that were evaluated morphometrically is displayed on a schematic pyloric ring.

For the later series of injections in the experiment, in order to increase the yield of isolated endings and thus the percentage of the labeled fibers that could be more readily digitized for morphometry, we elected to inject each of the remaining animals only unilaterally. The two series of unilateral injections introduced for the later phase of the experiment did yield a higher percentage of the isolated arbors we needed to facilitate complete morphometry and tracings, but the series also revealed another feature of the pyloric IMA organization. Basically the entire circumference of the pyloric sphincter is innervated by each of the nodose ganglion: In contrast to most of the stomach which is innervated almost exclusively by the ipsilateral nodose ganglion, the complete circumference of the pyloric ring receives innervation from both the left and right nodose.

Morphometry of Pyloric IMAs

Contrasting the sample of digitized IMAs of the pyloric circular muscle ($n = 53$) with a sample of the IMAs innervating immediately neighboring antral circular muscle ($n = 22$) provided a quantitative picture that amplified and extended the general impressions of the pyloric IMA specializations provided by a comparison of Figs. 3, 5, and 6: Presumably to

heavily innervate the pyloric muscle fibers, the sphincter IMA arbors elaborated more than twice the number of branches found in antral IMA arbors (405 ± 41.2 branches vs. 165 ± 13.9 branches; $p = 0.0004$). The pyloric IMA endings produced their elaborate arbors by bifurcating an average of more than twice as many times as did antral IMA arbors (201.8 ± 20.6 vs. 81.7 ± 6.9 ; $p = 0.0004$). And, additionally, pyloric IMAs also branched to significantly higher-order bifurcations than did antral IMAs (highest branch order of 25.4 ± 1.2 vs. 19.0 ± 1.1 ; $p = 0.002$).

Morphometric comparisons between the pyloric IMAs and the antral circular muscle IMAs also delineated how the pyloric IMAs were specialized structurally to generate their exceptional number of branches that bifurcated more frequently. Instructively, in terms of potential neural mechanisms regulating the size of nodose afferent arbors, both the pyloric IMA arbors and their counterpart antral IMAs appeared constrained by a limited cumulative length of neurites that they (i.e. presumably their somata) would support. This implication follows from the observation that when the distinctive pyloric IMAs were compared to the counterpart IMAs innervating the circular muscle of the neighboring antrum, both variants of afferents issued arbors with comparable cumulative or sum total length of all branches (mean sum total pyloric tree lengths of $22,052 \mu\text{m} \pm 1,965 \mu\text{m}$ vs. sum total antral arbors of $21,088 \mu\text{m} \pm 1,748 \mu\text{m}$; $p = 0.77$, n.s.). The pyloric IMAs achieved the same overall sum total length of their neurites in the face of many more neurites (compared to the antral circular muscle IMAs) by issuing much shorter branches ($66.1 \mu\text{m} \pm 4.4 \mu\text{m}$ vs. $138.7 \mu\text{m} \pm 9.5 \mu\text{m}$; $p < 0.0001$).

The shorter and more frequently branched elements of the pyloric IMAs also generated substantially more compact arbors (see Fig 6; also Figs. 7 and 8). In their longest dimension (i.e. parallel to circular muscle fibers), these sphincter IMAs were on average only roughly half the length of the antral IMA arbors ($1,796 \mu\text{m} \pm 99.9 \mu\text{m}$ vs. $3,321 \mu\text{m} \pm 207.6 \mu\text{m}$; $p < 0.0001$). In contrast to this major axis paralleling circular muscle, the other two primary axes of the pyloric arbors (i.e. a “longitudinal” axis paralleling longitudinal muscle fibers, or at right angles to the primary circular muscle axis, and a “z-axis” or axis perpendicular to the serosal and mucosal surfaces) were maintained comparable to the same axes in the antral circular muscle IMA arbors. Respectively, the longitudinal axis dimensions were $367.1 \mu\text{m} \pm 34.2 \mu\text{m}$ vs. $403.8 \mu\text{m} \pm 30.4 \mu\text{m}$ ($p = 0.52$, n.s.), and the z-axis or radial dimensions were $24.9 \mu\text{m} \pm 1.3 \mu\text{m}$ vs. $27.3 \mu\text{m} \pm 1.6 \mu\text{m}$ ($p = 0.28$; n.s.).

Overall, the net effect of the branching pattern specializations of the pyloric IMAs [i.e. increased branch number, decreased branch length, and dramatically decreased overall arbor length, without changes in either cumulative neurite length or axial (i.e. longitudinal muscle direction) arbor dimension] was to substantially reduce the receptive field size (see Fig. 6). For example, if the 2-dimensional receptive field areas are computed (Neurolucida convex hull algorithm) from a perspective perpendicular to the serosa and muscle sheets, pyloric IMAs had receptive field values less than half (~48%) those of antral circular muscle IMAs ($7,064 \times 100 \mu\text{m}^2 \pm 892 \times 100 \mu\text{m}^2$ vs. $14,848 \times 100 \mu\text{m}^2 \pm 1,551 \times 100 \mu\text{m}^2$; $p < 0.0001$; see Fig. 6A).

The arbors of the pyloric IMAs were situated deep in the circular muscle layer, four times closer to the submucosa ($6.2 \pm 1.0 \mu\text{m}$) than to the serosa ($26.8 \pm 1.7 \mu\text{m}$). With their proximity to the pyloric canal, the individual pyloric ring IMAs subtended an average of $53.6 \pm 3.0^\circ$ of sphincter circumference.

Discussion

Gastroenterology has established much about the varied roles of the pylorus in both healthy and disordered gastrointestinal functions (cf. Van Nueten et al., 1990; Keet, 1993; Ramkumar and Schulze, 2005; Rayner et al., 2012). With this information has come the recognition that the sphincter executes complex, dynamic motor programs that orchestrate transpyloric movements of chyme and materials. Such programs include participation not simply in emptying, but also, for example, in gastric sieving, antral pumping and systole, as well as straining, decanting, and nozzle effects that create flow vortices and mixing patterns as chyme enters the duodenal bulb (Van Nueten et al., 1990; Ramkumar and Schultze, 2005).

Pyloric motor programs incorporate feedforward reflexes elicited by stimulation of afferents in the corpus and antrum (e.g., Dent, 1990; Mayer, 1994; Anvari et al., 1995; Rayner et al., 2012) and feedback reflexes elicited by activation of sensory receptors in the duodenum (Heddl et al., 1989; Dent, 1990; Fraser et al., 1992; Rayner et al., 2012). Analyses of antropyloric and duodeno-pyloric reflexes have proven practical because it is readily possible to monitor sphincter activity while experimentally stimulating either the corpus-antrum or the duodenum.

In contrast, there is a paucity of information on more local or “pyloro-pyloric” reflexes (and other reflexes with afferent vagal arms originating in sphincter tissue per se) that may contribute to pyloric functioning, perhaps because simultaneously stimulating and recording smooth muscle motor activity at the same site is more challenging for physiological experimentation. In addition, making physiological analyses still more problematic, very little single-unit electrophysiology has been focused on the afferent projections to the pylorus. Even the few electrophysiological reports that have considered “pyloric” sensory fibers (e.g., Bitar et al., 1974; Cottrell and Iggo, 1984; Malbert and Leitner, 1993) have examined neural units that respond to stimulation of the general antro-pyloro-duodenal region as representative of the pyloric sphincter proper. Since such studies recording from afferents have generally employed large and nonspecific stimuli (e.g., inflation of the antrum or duodenal bulb) and have not typically mapped the receptive fields of the units evaluated, it is not at all clear that the experiments effectively sampled the vagal afferents innervating the sphincter itself.

The lack of physiological and electrophysiological information about the sensory innervation of the sphincter ring seems to be compounded even further by the fact that a similar paucity of detail is available on the structural features of the afferents innervating the pylorus. Arguably, as long as there is such a dearth of physiological, electrophysiological and morphological detail on the sensory innervation of the sphincter muscle, it will remain

impractical to design sensitive methodologies to understand sphincter mechanisms and to develop an adequate physiology of pyloric afferents.

In recognition of the limited information available, the present experiment was designed to address the poverty of structural information on the vagal afferents innervating the pyloric smooth muscle wall. The present results indicate that the thickened ring of circular muscle creating the sphincter is exceptionally heavily innervated by a band of vagal mechanoreceptors, specifically intramuscular arrays (IMAs), with neurites disposed to detect mechanical information from the pyloric canal with both high sensitivity and fine spatial resolution. The structural features and functional implications of this band of IMAs in the pyloric circular muscle ring should be considered in more detail. First, though, a few inferences about the pattern of vagal intraganglionic laminar endings (IGLEs) in the pyloric area should be considered.

Pyloric IGLEs: Structure and Function

Though the present experiment focused on IMAs, the nodose injections also labeled simultaneously a sampling of IGLEs, and we examined those IGLEs in the immediate region of the pylorus. A detailed analysis of these endings was not undertaken, however, because the endings did not evidence any modifications of features that would suggest they were particularly specialized and concentrated in the pyloric area. In fact, the packing density of IGLEs appeared to become somewhat attenuated, as did the ganglia of the myenteric plexus, at the gastroduodenal junction. The pattern appears consistent with the earlier observations that there is a paucity of pacemaking ICCs of the Auerbach's plexus type in the pyloric region (i.e. ICC-AP; Wang et al., 2005; 2007) and that the lack of ICC-APs could be responsible for a corresponding interruption in the propagation of gastric peristaltic slow waves from antrum to duodenum (Wang et al., 2005; 2007).

On the other hand, the paucity of IGLEs at the transverse level of the sphincter and their lack of any clear specializations suggested that they were unlikely to be principal vagal mechanoreceptive afferents crucially involved in the active programming of reflex programs initiated from the sphincter muscle.

Pyloric IMAs: Structural Features

In contrast to the relative poverty of IGLEs in the pyloric region, IMAs appeared to substantially increase in packing density in the pylorus (compared to neighboring antrum) and also to evidence structural modifications that suggest their arbors establish key receptor structures involved in transducing and relaying pyloric mechanoreceptive information to the brainstem and into reflex arcs programming pyloric coordination.

A conspicuous feature of the pyloric IMAs in the present morphometric assessment is that these sphincter afferents have structural modifications compared to other gastric IMAs such as those innervating the antrum. The individual IMAs in the sphincter established unusually small receptive fields composed of arrays of densely packed branches. The relatively small size of the pyloric arbors can be appreciated by the side-by-side comparison with an antral IMA arbor in Fig. 6, and the density of the branching pattern can also be seen in Figs. 3, 7, and 8. Alternatively the comparisons can be gauged more quantitatively in bar graphs in Fig.

6. As is illustrated in Fig. 6, when their antral counterparts are used for a comparative baseline, the IMAs innervating the sphincter circular muscle issued more than twice the number of branches and packed them into approximately one-half as much receptive field area.

Not only were the branches within the individual arbors particularly densely packed, the separate receptive fields formed by the individual arrays were also tightly packed and interdigitated. As illustrated in Fig. 8, for example, end-to-end, the arbors overlapped, with considerable interdigitation of the higher order branches of the arbors. Furthermore, scanning longitudinally, at right angles to the pyloric smooth muscle ring, neighboring IMAs arbors are packed tightly side-by-side as well.

In addition, the arbors of the individual IMAs were distributed deep in the circular muscle sheet and thus formed their aggregate annulus just superficial to the pyloric canal. Taken together, the close circumferential and longitudinal packing of the IMAs innervating the pylorus create a belt of mechanoreceptors located deep in the pyloric circular muscle.

Pyloric IMAs: Functions

The dearth of available physiological and electrophysiological experiments designed to characterize the specific afferents innervating the circular muscle that constitutes the pyloric sphincter makes definitive conclusions about functions of the afferents impractical. In the absence of such information, however, some working inferences can be drawn from comparing the present structural observations with experiments involving the neighboring antral and duodenal regions as well as from other experimentation in other sensory systems.

Though vagal IMAs have only been recognized and analyzed in the last two decades, investigations of the endings in other regions of the stomach indicate that they are mechanoreceptors that respond to changes in stretch (and/or perhaps tension) of the muscle sheet they innervate (Phillips and Powley, 2000; Zagorodnyuk et al., 2001; Powley and Phillips, 2002; Zagorodnyuk et al., 2003). More particularly, a number of structural and functional considerations also suggest that IMAs likely operate as stretch receptors (see Phillips and Powley, 2000; Powley and Phillips, 2011; Powley et al., 2012, 2013), whereas, in contrast, IGLEs function as tension receptors (Zagorodnyuk et al., 2001, 2003).

Consistent with the stretch-receptor idea, IMA arbors appear to form complexes, and have appositions, with both the networks of interstitial cells of Cajal of the intramuscular type (i.e. ICC-IMs; also see Fig. 4) and the smooth muscle fibers in the muscle sheets (cf. Powley et al., 2008). Additionally, both the distributions of IMAs within GI tissues (parallel to smooth muscle fibers, etc.) and regions (concentrated in sphincters and stretch-sensitive sites such as the forestomach) suggest length- or stretch-sensitive operation. Furthermore, as mentioned, the limited physiological observations that have been associated with IMAs to date are consistent with the conclusion that they operate as functional analogues of the muscle spindle stretch receptors innervating striated muscle (Phillips and Powley, 2000; Powley and Phillips, 2002, 2011). And, most particularly, the pyloric IMA appositions with ICC-IMs are consonant with the muscle-spindle analogy insofar as pyloric ICC-IMs have

been shown to respond to stretch (Won et al., 2005) and therefore may cooperate in the transduction of stretch stimuli.

The pyloric ring IMA organization evaluated in the present experiment is also consistent with the stretch-receptor conclusion, and the architecture reinforces the argument that IMAs function to gauge muscle length or stretch. In forming a sleeve of mechanoreceptors so close to, and encircling, the pyloric canal, the arrays seem distributed to continuously monitor whether, and to what degree, the sphincter is closed or open. For such an annulus of IMAs, situated in the sphincter circumference, any small changes in pyloric canal diameter would, in effect, be amplified by Pi (π) and should readily stretch the afferents. With the IMAs individually spanning limited degrees ($\sim 54^\circ$) of the circumference of the sphincter, the band of discrete arbors would, presumably, even capture the information needed to pinpoint local or asymmetrical distortions of the canal that might be produced by emptying of solids in inhomogeneous chyme. In keeping with the argument that the putative stretch/length detection function of IMAs seems most plausible for a mechanoreceptor map of the pylorus, it is also worth noting that vagal IMAs constitute the principal innervation of the clasp and sling muscle fibers that form the lower esophageal sphincter (Powley et al, 2013).

The considerable overlapping and intertwining of neighboring pyloric IMAs has implications for the sensitivity of the afferent map. With only a relatively modest number of structural analyses of visceral afferents in the gut yet available, the functional implications of high packing density of receptors has not been formally addressed for the vagal innervation of the GI tract, but such implications have been modeled and evaluated in other sensory systems, most notably the retina. The overlap or “coverage factor” of the “tiling” that occurs in the ganglion cell mosaics of the retina, for example, has been explored by Balasubramanian and Sterling (2009), and these investigators have demonstrated mathematically that sensory sensitivity and acuity are maximized by overlapping receptive fields. Similarly, Brown and colleagues (2004) have calculated that the same receptive field principles operate in the case of cutaneous mechanoreceptive fields. If such conclusions can be extrapolated from first-order retinal ganglion cells and cutaneous mechanoreceptors to first-order nodose afferents, then the IMAs forming the high-density mechanoreceptor ring in the pylorus should generate a high resolution and sensitive representation of the mechanical dynamics that play in the pyloric canal.

As discussed, the individual arbors of the pyloric IMAs were considerably shorter on their long axis (the vector parallel to the circular muscle fibers) and had substantially smaller innervation areas or “receptive fields” than did the IMAs innervating antral circular muscle. While the receptive fields of the IMA were significantly smaller, they were also significantly more densely arborizing with the twofold, or more, larger numbers of branches densely distributed within their more limited areas. In other sensory modalities, small and compact receptive fields are correlated with high spatial acuity, and the principle implies that the densely packed pyloric IMAs with their short and compact arbors are organized in a pattern that should yield high spatial resolution of mechanical signals originating within the pyloric canal or sphincter wall (e.g., Brown et al., 2004; Cody et al., 2010).

Another detail of pyloric IMA structure with possible functional implications is the fact that the IMAs in the sphincter were situated deep (or close to the submucosal border wall) in the circular muscle sheet. Specifically, the deeper neurites of individual arbors were distributed roughly 6 microns from the submucosal boundary, whereas the superficial neurites of the arbors were rough 27 microns below the serosa-muscle interface. One implication of this location is that the pyloric IMAs, being situated at the mucosal face of the circular muscle sheet, seem to be arrayed so as to be especially sensitive to tissue displacements or movements originating from stimuli within the lumen of the pyloric canal. As putative length or stretch receptors (discussed above), the pyloric IMAs are well positioned so that even minute changes in the diameter of the pyloric canal or displacements occasioned by the passage of boluses of chyme or material that changed the circumference of the pyloric ring by even a few minutes of arc might be transduced by the IMA arbors.

A second implication of the IMAs in the pylorus extending into the deeper layers of the circular muscle sheet is that their location coincides with the distribution of ICCs of the intramuscular type (ICC-IMs). IMAs throughout gastric smooth muscle are associated with the network of ICC-IMs distributed within the muscle layers (Berthoud and Powley, 1992; Powley et al. 2008, Powley and Phillips, 2011), and the present results indicate that this common pattern of vagal IMAs arbors intertwining with ICC-IMs in the circular muscle holds for the specialized IMAs found in the pylorus. Furthermore, the location of the pyloric IMAs in the deeper layers of the circular muscle would seem to be in good registration with the observations of Wang and coworkers (Wang et al., 2007) indicating that, in the pylorus, ICC-IMs are particularly densely distributed in deep circular muscle near the boundary with the submucosa. The tightly woven ring of IMA neurites that we observed within the pyloric circular muscle forming the sphincter also seems particularly consistent with Wang and coworkers' (2007) complementary observation that the same inner layers of the circular muscle sheet also have an unusually high concentration of neurites, some of them having junctions with ICC-IMs. Wang and colleagues inferred that the neurites were intrinsic fibers, though their observations do not rule out the possibility that some of the neurites they observed could have been processes of vagal IMAs.

A third implication of the distribution of the vagal IMAs deep in the pyloric circular muscle and heavily concentrated in the more distal portion of the pyloric ring is that the distribution of the mechanoreceptors appears to be strikingly co-extensive with available maps of CCK receptors in the pylorus (e.g., Smith et al., 1984; Curry et al., 1995). For example, Smith and colleagues (1984) demonstrated that CCK binding is localized to the distal pyloric sphincter, whereas the "pyloric antrum," the "pyloric duodenum," and even the "proximal pyloric sphincter" evidence little specific binding activity of the hormone. Mapping experiments have also established that CCK receptors are found in vagal afferent fibers (Moran et al., 1987) as well as in pyloric smooth muscle and associated ICC-IMs (Patterson et al., 2001), and furthermore, both electrophysiological and physiological experiments have indicated that the sensitivity of load-sensitive vagal afferents innervating the stomach, including the distal stomach, is modulated by circulating CCK (Murphy et al., 1987; Schwartz and Moran, 1996; Schwartz, 2000; Friedenberg et al., 2008). Taken together, the various experiments suggest that the terminal arbors of the pyloric IMAs that we have characterized in the present experiment, given that their distribution is coextensive with the distribution of CCK

receptors, are modulated directly by, or are influenced indirectly through local smooth muscle by, cholecystokinin. Presumably the sensitivity of the IMAs to stretch and distortion originating by signals originating in the lumen of the pylorus and interacting with the degree of contraction maintained in the pyloric smooth muscle is tuned by the intestinal hormone CCK. Though fewer observations are available for other hormones involved in GI physiology, evidence suggests that, for example, secretin (e.g., Fisher et al., 1973), ghrelin (e.g., Ariga et al., 2008), and C-type natriuretic peptide (e.g., Sogawa et al., 2013) may similarly modulate the sensitivity of the pyloric IMAs.

Conclusions

Though the sensory programming and coordination of pyloric control of gastric emptying and GI transit have traditionally been examined in terms of antro-pyloric and duodeno-pyloric reflexes, the present observations establish that there is an extensive population of vagal intramuscular arrays in the circular muscle that forms the sphincter ring. Additionally, the present investigation documents several structural specializations of the IMAs located in the pylorus that, in the aggregate, suggest these vagal afferents establish a reticulated sensory annulus around the sphincter canal that should be capable of reporting pyloric events and processes with high sensitivity and fine spatial resolution. Taken together, these morphological results suggest that, in addition to antro-pyloric and duodeno-pyloric reflexes, pyloro-pyloric reflexes and other motor responses initiated by the sphincter vagal afferents may also make critical contributions to the motor programs of the pylorus.

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Figure 1.

Preparation of the whole mounts and the region sampled. Top row: A: A dorsal view of the stomach illustrating the tissue trimmed for whole mounts (stippled area between dashed lines) and progressively higher magnification sagittal views (panels B and C) of the thickening of the circular muscle sheet constituting the sphincter ring. Middle row: D: a view of the stomach opened on the greater curvature and illustrating the region of the whole mount tissue (stippled area again) from a serosa-side perspective. E: a whole mount specimen that has had the mucosa and submucosa removed and has been prepared for processing. Bottom row: F: An enlarged view of the whole mount region that was sampled for vagal terminals. The conspicuous thickening of the pyloric ring is cupped at the bottom center; the remainder of the region consists basically of the antral wall in which circular muscle afferents were sampled. For orientation, the previously described principal bands of sling muscles associated with the lower esophageal sphincter (Powley et al., 2012) are shown running vertically on either side of the midline.

Figure 2.

Vagal IGLEs and their parent neurites in the muscularis externa of the pyloric sphincter labeled with dextran biotin. Panel A: A labeled vagal afferent forming a typical IGLE between the longitudinal and circular muscle layers from a whole mount prepared without counterstain. Panels B, C and D: Three views illustrating the variety of IGLE profiles seen in the pyloric sphincter region. In the three panels, slightly out-of-focus and lightly Cuprolinic-blue-stained myenteric ganglion neurons can be seen in order to appreciate the typical IGLE-myenteric-ganglion architecture. The arrows in Panels B, C and D designate, in each panel, one of the faint or very lightly Cuprolinic blue-stained myenteric neurons. With the Cuprolinic blue protocol, the somatic cytoplasm and the nucleolus are stained whereas the nucleoplasm is unstained. The scale bar in Panel D is 20 μm and applies to all panels.

Figure 3.

A vagal IMA innervating the circular muscle of the pylorus. The black and white tracing is a Photoshop tracing, obtained from a z-stack of high magnification mosaic photomicrographs, that illustrates a vagal parent neurite crossing the distal antral wall (at roughly 3 o'clock) and then arborizing extensively into an intramuscular array of IMA in the (unstained) circular muscle of sphincter. Panels A and B: All-in-focus composites of z-stacks of photomicrographs illustrating the dense arborization of the traced pyloric IMA at two sites designated with corresponding letters in the tracing. Images are from a whole mount without counterstaining or immunostaining. Scale bar in Panel B = 20 μm for both windows A and B.

Figure 4.

Vagal IMAs in pyloric sphincter form contacts with interstitial cells of Cajal of the intramuscular type (ICC-IMs). Panels A through F illustrate examples of apparent IMA neurite (brown stained fibers) contacts with ICC-IMs (blue-grey cells stained immunohistochemically with Steel Grey chromogen). Arrowheads in figures illustrate representative sites where neither thorough visual examination nor photomicrography, both at 1000X, could detect any separation in the x-, y- or z-planes between IMA fibers and ICC-IM cells. Panels A, B, and C (Panel C is a single-plane-of-focus image or optical plane extracted from the all-in-focus z-stack composite image illustrated in Panel B; see Methods) illustrate examples in which IMA fibers form presumptive contacts with the proximal processes of ICC-IMs. Panels D, E, and F illustrate cases in which IMAs form presumptive contacts with somata of ICC-IMs. Images are all-in-focus composite photomicrographs of z-stacks, except for Panel C which is a single plane of focus. Scale bar in Panel F = 10 μ m.

Figure 5.

A vagal IMA innervating the circular muscle of the distal antrum. The black and white tracing is a Photoshop tracing, obtained from a z-stack of high magnification mosaic photomicrography, that illustrates a vagal parent neurite traveling distally in the antral wall (at roughly 3 o'clock) that then arborizes into the IMA terminal in the circular muscle of the antrum. Panels A and B: All-in-focus composites of z-stacks of photomicrographs illustrating the arborization of the traced IMA at two sites designated with corresponding letters in the tracing. Images are from a whole mount without counterstaining or immunostaining. Scale bar in window B = 20 μm for both panels A and B.

Figure 6.

IMAs innervating the pyloric sphincter had much smaller receptive fields and denser arbors than the corresponding IMAs innervating the circular muscle of the adjacent antrum. The differences in field size and density are illustrated in the left column, in which the pyloric IMA from Fig. 3 (i.e. the considerably shorter IMA on the right side of the pair) and the antral IMA from Fig. 5 (i.e. the longer IMA on the left side of the pair) are represented on a common scale (scale bar is below pyloric IMA). Panels A, B, C, and D illustrate the differences in receptive field size (see Panel A) and shape (see Panels B, C and D) between the pylorus and the antrum. If the receptive field is calculated as a 2-dimensional area (Panel A), the differing lengths (the axis parallel to the circular muscle fibers) of the pyloric and antral arbors (Panel B) account for the dramatically different receptive field sizes, since the arbors from the two regions have comparable widths (the axial or longitudinal muscle axis; Panel C) and comparable z-axis extent depths (serosa-to-mucosa axis) extent. In Panels A and B, the asterisks indicate that the pyloric and antral IMAs differed significantly ($p < 0.0001$) in, respectively, receptive field area and arbor length.

Figure 7.

Variations in pyloric IMA arbors. Though pyloric IMAs typically distributed their entire arbors in the deeper and more distal circular muscle ring forming the sphincter, some IMAs also distributed neurites more superficially and/or more proximally in the sphincter muscle. Panel A: Most commonly (41 of the 53 cases), pyloric IMA afferents distributed their neurites in single, coherent arbors. Panels B and C: A minority (12 of the 53 cases) of IMAs issued secondary collaterals into the wall before ending in a principal arbor. IMAs in this minority varied in the extent and number of early collaterals from a few short spurs (Panel B) to relatively extensive secondary arbors (Panel C).

Figure 8.

IMAs innervating the pyloric sphincter were densely packed with interdigitating arbors. Column on left: Photoshop tracings of three neighboring IMAs, designated in different colors, illustrate that the arbors interdigitate end-to-end to form a seamless annulus (that continues a full 360° to encircle the pyloric canal). The bowing or arcing of the three profiles follows the circumference of the pyloric ring. Column on right: An enlargement of much of the middle (red) IMA, corresponding to the region between the two arrows in the lower power image, in which the intermingling of the other two (green and black) IMAs can be seen more clearly. In this enlargement, the opacity of the red ending has been reduced to 40% to make the intertwining neurites easier to see.

Figure 9.

The distribution of pyloric IMA arbors evaluated morphometrically in the present series are represented schematically on the pyloric-antral whole mount illustration from Fig. 1F. The IMA arbors—and thus presumably the receptive fields of the afferents—surveyed are represented as shaded rectangular areas with a length (circular muscle orientation) equal to the average length of the arbors sampled and, similarly, a width equal to the average width of the arbor sample. For each of the schematic receptive fields, the rectangular symbol is positioned in the whole mount schematic so that the center of the rectangle sits at the coordinate site corresponding to the location of a particular IMA sampled. In this case, the IMA sample comes from several different animals, but the composite distribution suggests the way in which pyloric IMA arbors had overlapping receptive fields as they collectively formed a sensory annulus in the sphincter ring.