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Hans-Rudolf Berthoud · Laurel M. Patterson
Friederike Neumann · Winfried L. Neuhuber

Distribution and structure of vagal afferent intraganglionic laminar endings (IGLEs) in the rat gastrointestinal tract

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Abstract Intraganglionic laminar endings (IGLEs) are special terminal structures of vagal afferent fibers and have been demonstrated in the myenteric plexus of esophagus and stomach. In order to quantitatively map their presence and distribution over the entire gastrointestinal tract, including the small and large intestines, vagal afferents were anterogradely labeled in vivo by microinjections of the fluorescent carbocyanine dye DiI into the left or right nodose ganglion of adult male rats. In the most successfully labeled cases the highest density of IGLEs was found in the stomach, with about half to one-third of the myenteric ganglia receiving at least one IGLE. The proportion of myenteric ganglia innervated by IGLEs decreased in the small intestine; however, because of its large surface area this gut segment was estimated to contain the highest total number of IGLEs. Both the cecum and colon also contained significant numbers of IGLEs. In the stomach, this vagal afferent innervation by IGLEs was more or less lateralized, with less than 20% of labeled IGLEs found on the contralateral side with respect to the injection. The left/ventral vagus contributed a larger proportion of IGLEs to the proximal duodenum, while the right/dorsal vagus contributed a larger proportion of IGLEs to the distal duodenum and jejunum. Laser scanning confocal microscopy on select specimens revealed further structural details. The parent axon typically formed two or more branches that flanked the ganglia laterally, and in turn produced numerous highly arborizing laminar terminal branches that covered one or both flat sides of the ganglion in a dome-like fashion. The similar distribution patterns and structural details suggest a uniform function for the IGLEs throughout the gastrointestinal tract, but there is as yet no clear proof for any of the hypothesized roles as specialized mechanosensors or local effector terminals.

Key words Vagal afferents · Visceral afferents · Visceral sensation · Myenteric plexus · Enteric nervous system

H.-R. Berthoud (✉) · L.M. Patterson
Pennington Biomedical Research Center,
Louisiana State University, 6400 Perkins Road,
Baton Rouge, LA 70808, USA
Tel.: (504) 763 2688; Fax: (504) 763 3030;
e-mail: berthohr @ MHS.PBRC.EDU

F. Neumann · W.L. Neuhuber
Anatomisches Institut, Universität Erlangen-Nürnberg,
Erlangen, Germany

Introduction

Afferent nerve endings with flattened branches in contact with the inner surface of the connective tissue capsule enveloping the myenteric ganglia of dog esophagus were first recognized by Nonidez (1946). The term "intraganglionic laminar ending" (IGLE) was coined later by Rodrigo et al. (1975). Using non-specific silver staining methods, these investigators further characterized the terminals and established their vagal afferent origin on the basis of an absence of staining following infra-nodose but not supra-nodose vagotomies (Rodrigo et al. 1982). However, it was not until relatively recently that vagal afferent fibers were directly and selectively labeled by injecting either tritiated leucine (Sato and Koyano 1987), wheat germ agglutinin-conjugated horseradish peroxidase (Clerc and Condamin 1987; Neuhuber 1987), or DiI (Berthoud and Powley 1992; Berthoud and Neuhuber 1994) into the nodose ganglia of rats and cats. Labeling of vagal afferent fibers with the carbocyanine dye DiI was particularly useful because it can be analyzed by conventional and confocal epifluorescence microscopy without histochemical processing in whole-mounted muscularis externa peels. Using this method, it soon became clear that IGLEs are not limited to the esophagus in the dog, but are also found in the stomach (Berthoud and Powley 1992), duodenum (Berthoud et al. 1995), and throughout the small and large intestines (Berthoud and Neuhuber 1994).

At present, it is not clear what functional role(s) these very distinctive afferent nerve terminals might serve. Because of their intimate anatomical relationship with the connective tissue layer that separates the myenteric gan-

glia from the overlying longitudinal muscle, it has been proposed that IGLEs may serve as mechanoreceptors detecting shearing forces between the two smooth muscle layers (Neuhuber and Clerc 1990; Neuhuber et al. 1995). This hypothetical role seems particularly plausible for the esophagus and the intestines, where little evidence for specialized vagal tension receptors in the muscle layers has been found (Neuhuber 1987 and unpublished observations). However, in the stomach, where many vagal afferent endings (intramuscular arrays) are found in both the longitudinal and circular muscle layers (Berthoud and Powley 1992; Kressel et al. 1994), there seems to be no need for additional mechanosensors in the myenteric plexus. The presence of small clear and large dense core vesicles in HRP-labeled IGLEs in rat esophagus additionally suggested that these terminals might also release transmitters or modulators onto the contacted myenteric ganglion neurons (Neuhuber 1987). Alternative functional roles may include the detection of the neurochemical environment of, or electrical events in the ganglia, as originally suggested by Kolossow and Milochin (1963).

To prove or disprove any of these hypotheses, direct functional manipulation of IGLEs will ultimately be necessary. However, a thorough analysis of distribution patterns and structural details in the various gut segments will provide the necessary data base for such functional studies. It is quite possible that IGLEs serve different functions in different gut segments. In the present study we quantitatively describe the distribution and local density, as well as additional structural details of IGLEs throughout the gut.

Materials and methods

Animals

Adult, male Sprague-Dawley (n=22; Harlan Industries, Indianapolis, Ind.) and Wistar rats (n=9; ZUR:SIV strain, Institute for Laboratory Animal Science, University of Zurich), weighing 150–300 g were held under standard laboratory conditions (12/12 h L:D cycle, 23 ± 3°C) with food and water available at all times except the night before surgery.

All experimental protocols used were approved by the Pennington Biomedical Research Center Institutional Animal Care and Use Committee.

Labeling of vagal afferents and enteric neurons

Animals were anesthetized with either pentobarbital sodium (60 mg/kg, i.p.) or a mixture of Sedalande (Delalande, 10 mg/kg) and Fentanyl (Janssen, 0.2 mg/kg, i.m.) and the additional administration of Valium (Roche, 2.5 mg/kg, i.m.). When all reflexes were absent, the left (n=21) or right (n=10) nodose ganglion was exposed, and 0.1–1.0 µl of DiI (1,1'-dioleyl-3,3,3',3'-tetramethylindocarbocyanine methanosulfonate, 25 mg/ml in methanol, Molecular Probes, Eugene, Ore.) was injected, using a Hamilton µl syringe. The wound was closed, and, if needed, postoperative care included antibiotics and/or analgesics. Four to seven weeks were allowed for uptake and transport of the dye. Controls included animals with prior supranodose vagotomy (n=2) and with ipsilateral cervical (infranodose) vagotomy (n=2), immediately following the dye injection.

In order to produce a counterstain of all enteric neurons in the myenteric plexus, Fluorogold (2 mg/rat, in 1.2 ml of sterile saline, Fluorochrome, Engelwood, Colo.) was injected i.p. in over-night food deprived rats 5 days before sacrifice. For details of this method see Powley and Berthoud (1991).

Tissue processing

Following a lethal dose of pentobarbital sodium (120 mg/kg, i.p.), when all reflexes were absent, animals were perfused transcardially with 300 ml of cold, heparinized (20 units/ml) saline, followed by 500–800 ml of phosphate-buffered 4% formalin (pH 7.4). The entire gastrointestinal tract was then excised, slit near the mesentery attachment, rinsed thoroughly and stored in the same fixative at 4°C in the refrigerator. Whole-mounts of the muscularis externa from specific locations were prepared as follows. Pieces of gut wall were cut with fine scissors parallel to the circular muscle fibers. In the stomach, the pieces had a trapezoid shape with the shorter parallel side of ~5 mm from near the cardia, and the longer parallel side measuring ~10–15 mm from the greater curvature. In the small intestine and colon, pieces 5 mm wide and as long as the circumference were cut parallel to the circular muscle fibers.

The tissue specimens were then transferred into a soft black rubber dish containing PBS, and, serosa down, the mucosa was first carefully scraped off using the tip of a no. 10 scalpel blade. The submucosa was then loosened along the narrow side of the piece, and peeled from the underlying muscle layers, with a no. 7 pair of curved jewelers forceps, while holding down the latter with a second pair of forceps. In order to avoid tearing the muscle layers, it is important that the direction of peeling is always in parallel to the circular muscle fibers and that the angle between the direction of pull and the horizontally placed piece of tissue is as small as possible. The peels were rinsed in PBS and transferred into 70% glycerol in PBS and then 100% glycerol. They were finally mounted and coverslipped in 100% glycerol to which 5% n-Propylgallate had been added as an antifade reagent.

Quantitative analysis and sampling strategy

All specimens were inspected with a Zeiss Axiophot epifluorescence microscope using filter combinations no. 02 (UV excitation) for visualization of Fluorogold, and no. 15 (green excitation), for DiI, or a Leica Aristoplan microscope using filter combinations A and N 2.1, respectively. Criteria for counting myenteric ganglia and IGLEs were as follows. The Fluorogold-labeled myenteric ganglia were first visualized through the appropriate filter combination. If any group of neurons was either separated from another group by three or more average neuron diameters, or there was only one row of more than three neurons connecting it with another group, it was considered an individual ganglion. In the case of single neurons, they had to be separated by five or more neuron diameters from any neighboring ganglion (or single neuron) to be counted as separate ganglia.

Typically, individual or small bundles of DiI-labeled axons could be followed as they coursed through the myenteric plexus, giving off numerous collaterals that formed IGLEs in several ganglia. In any given ganglion, there could be more than one patch (IGLE) of arborizing terminal branches. Independent of its size, each patch was counted as one IGLE if it was separated from the next patch by at least two diameters of the average myenteric neuron.

To obtain density scores (number of IGLEs per cm^2 of gut wall) and to extrapolate from the sampled area to the total gut segment, surface areas were measured as follows. The total surface areas of fundus, corpus and antrum for each rat were obtained by drawing an outline of the stomach and the limiting ridge on a transparent acetate film containing an orthogonal millimeter grid. The number of square millimeters was counted for the total stomach and the fundus surface area. The surface area of the antrum was assumed to be 10% of the total stomach surface, while the corpus surface area was calculated as the difference between total

area minus fundus minus antrum. Because of the different degree of distention, these numbers varied considerably between animals. For each animal, two peels each from fundus and corpus, and one peel from antrum were analyzed. The surface area of these peels was measured as above and expressed as a percentage of the total surface area of the corresponding stomach segment. The average percentages counted were: 40 ± 4% for fundus, 34 ± 5% for corpus, and 51 ± 7% for antrum.

For the duodenum, every other centimeter (50%, 10 peels) was counted. For jejunum and ileum, the anal-most 2-cm of every 10-cm segment (20%, 24–32 peels) were counted. For the cecum, approximately 20%, and for each segment of the colon (ascending, transverse and descending), approximately 30% was peeled and counted.

To assess a complementary aspect of innervation density, the percentage of myenteric plexus ganglia innervated by at least one IGLE was determined in a separate set of three animals with left nodose ganglion injections. Muscularis externa peels from both the ventral and dorsal sides of the three stomach areas included more than 70% of the total surface area. In the duodenum, the sampled area included the middle and distal portion, and both ventral and dorsal sides. In the jejunum, 16–18 consecutive peels from the mid-portion (~10% of total jejunum), and in the ileum, 14–20 consecutive peels (~60% of total ileum), were analyzed. The sample from the ileo-cecal junction comprised peels from within 1 cm of the junction. In the cecum, at least 50% and in the colon, at least 50% of the ascending and transverse portions were analyzed.

Structural analysis with confocal microscopy

Select specimens and IGLEs were further analyzed using optical sectioning by means of a laser scanning confocal microscope (Zeiss LSM 310). Single optical sections or extended focus images of up to ten optical sections, 0.5–2.0 μm apart, were obtained. In some cases, red/green stereo pairs were created and viewed with special glasses to appreciate the three-dimensional aspect of the structures. Finally, some images were further processed using brightness/contrast adjustments and filtering algorithms. Final images were then printed on a digital printer (Codonics), or photographed from a high resolution flat screen (Focus Graphics).

Results

The dye injections were variably successful in labeling a large percentage of all vagal afferent fibers projecting to the abdomen. Since there is no good procedure to independently verify the completeness of DiI-labeling other than by counting the labeled fibers and terminals themselves, the results reported here are based on those injections that resulted in the highest numbers of IGLEs in a given gut segment. We assume that in these “best cases” more than 80% of the vagal afferent fibers were successfully labeled. Based on initial counts in the gastric fundus, of the 27 experimental rats with left or right nodose ganglion injections, 10 were considered as highly successful, 10 as moderately successful, and 7 as poor. Complete analysis was only carried out in the 10 animals with highly successful injections. In addition, it has to be kept in mind that the injections were unilateral, and while for the stomach both the ipsilateral and contralateral sides were inspected separately, for the rest of the gut the entire circumference at a given level was inspected.

As can be seen from Table 1, the density of IGLEs was highest in the stomach and proximal duodenum and then gradually decreased in the aboral direction. Almost

Table 1 Density and distribution of IGLEs in the rat gastrointestinal tract

	Right nodose ganglion injection		Left nodose ganglion injection			
	Density[a] IGLEs/cm²	Extrapolated number[b]	Density IGLEs/cm²	Extrapolated number	Percentage of innervated ganglia[c]	
					Ventral	Dorsal
Stomach						
Fundus	199	913	92	628	46%	12%
Corpus	157	1227	124	678	31%	3%
Antrum	134	202	97	133	25%	17%
Duodenum					Bilateral	
Proximal	28	207	125	936		
Distal	116	486	30	107	6.6%	
Jejunum						
Proximal	53	2246	15	578	1.9%	
Distal	32	822	11	445		
Ileum	15	216	8	106	0.7%	
Ileo-cecal junction		n.d.			3.1%	
Cecum	6	195	12	360	1.6%	
Colon	23	633	7	273	1.0%	

[a] Based on animal with the highest number (most successful unilateral injection) selected from four animals with right nodose ganglion injections and six animals with left injections. Note that this measure does not take into consideration the different extent of gastric and intestinal distention

[b] Based on density and on measurement of sampled and total surface for stomach, and on sampled and average surface of small and large intestinal segments

[c] Based on animal with highest percentage score selected from three animals with left nodose ganglion injections

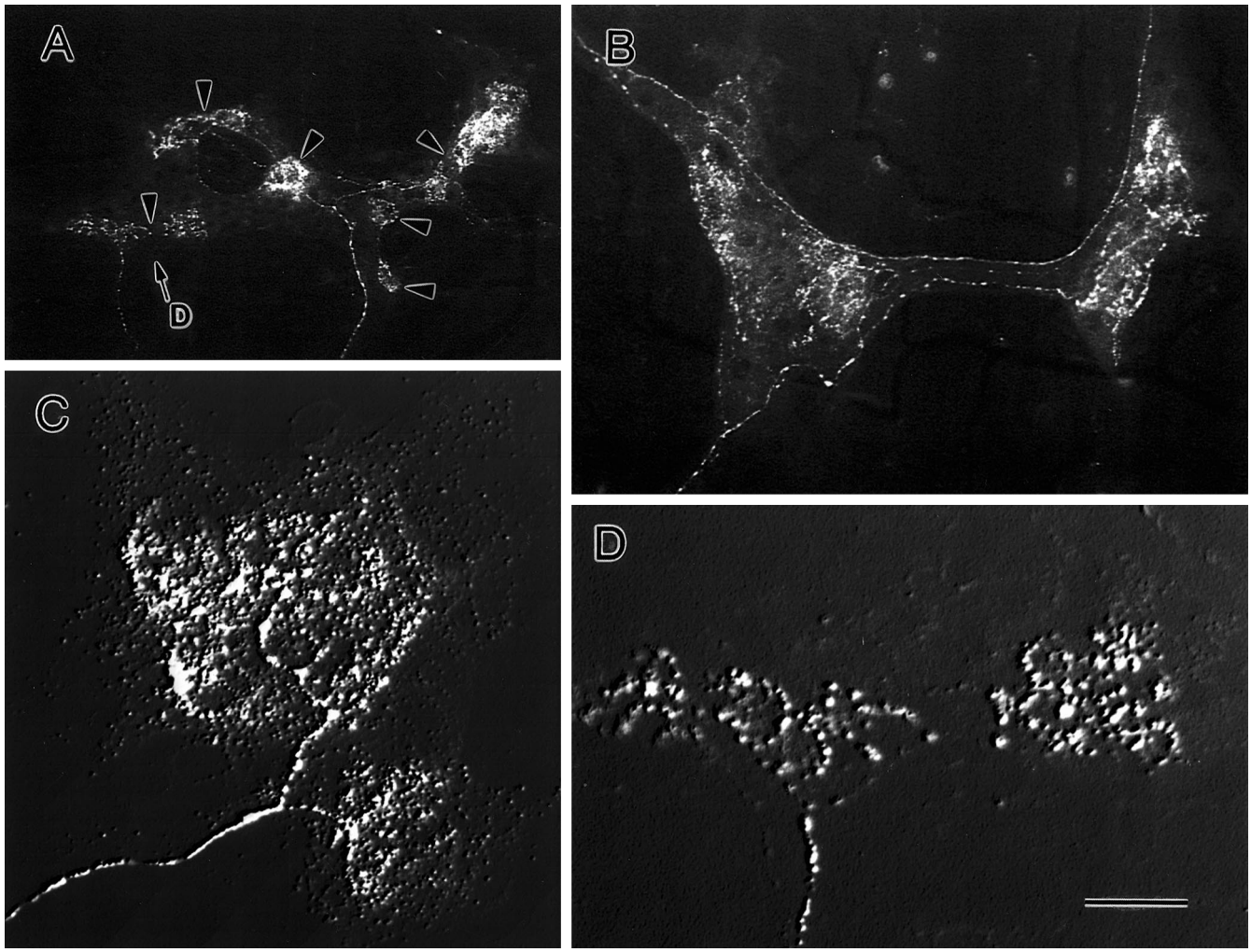

Fig. 1A–D DiI-labeled intraganglionic laminar endings (IGLEs) and vagal afferent fibers (*bright white*) in myenteric plexus of rat stomach. Laser-scanning confocal microscope images of whole mounted muscularis externa peels. **A** Several IGLEs covering different parts of a large myenteric ganglion in gastric fundus (neurons can be recognized by faintly greyish cytoplasm and dark nuclei). According to criteria described in the methods, a total number of six IGLEs can be distinguished (*arrowheads*). Note that there are at least two separate labeled axons entering the ganglion. The *arrow* points to the IGLE shown at higher magnification in **D**. **B** Three IGLEs in two ganglia of the gastric corpus. **C, D** Higher magnification extended focus images (five optical sections, 1 μm apart) showing the profusely arborizing laminar structure of IGLEs in the corpus **(C)** and fundus **(D)**. *Bar* in **D** represents **A** 200 μm; **B** 100 μm; **C** 75 μm; **D** 50 μm

half of the myenteric ganglia in the fundic stomach contained at least one IGLE (Table 1), while only a small percentage of the ganglia was innervated in the jejunum and further aboral. However, because of the large surface area of the jejunum, the extrapolated number of IGLEs in this gut segment was even higher than for the stomach. The best right nodose ganglion injection resulted in higher densities and extrapolated numbers of IGLEs in all gut segments, with the exception of the proximal duodenum and the cecum.

Stomach

In the fundic part of the stomach the best right nodose ganglion injection resulted in a slightly higher extrapolated number of labeled IGLEs on the dorsal side than the best left nodose ganglion injection did on the ventral side (Table 1, Fig. 1). From the separate analysis assessing the percentage of innervated ganglia following left nodose ganglion injection, it can be seen that almost half of the ganglia on the ventral side but only 12% on the dorsal side received at least one IGLE. Assuming that about the same proportion of ganglia on the ventral side also receive IGLEs from the right nodose ganglion, the complete bilateral innervation of both sides would amount to at least 1,710 IGLEs [(913+628)+11%].

In the gastric corpus, the best right injection resulted in almost twice as many labeled IGLEs on the dorsal side than left injection did on the ventral side (Table 1, Fig. 1). IGLE innervation of the corpus was almost completely lateralized, with about every third ganglion being innervated by at least one IGLE on the ipsilateral side and only 3% of innervated ganglia on the contralateral side. The total estimated number on both sides of the corpus amounted to at least 1,962 IGLEs.

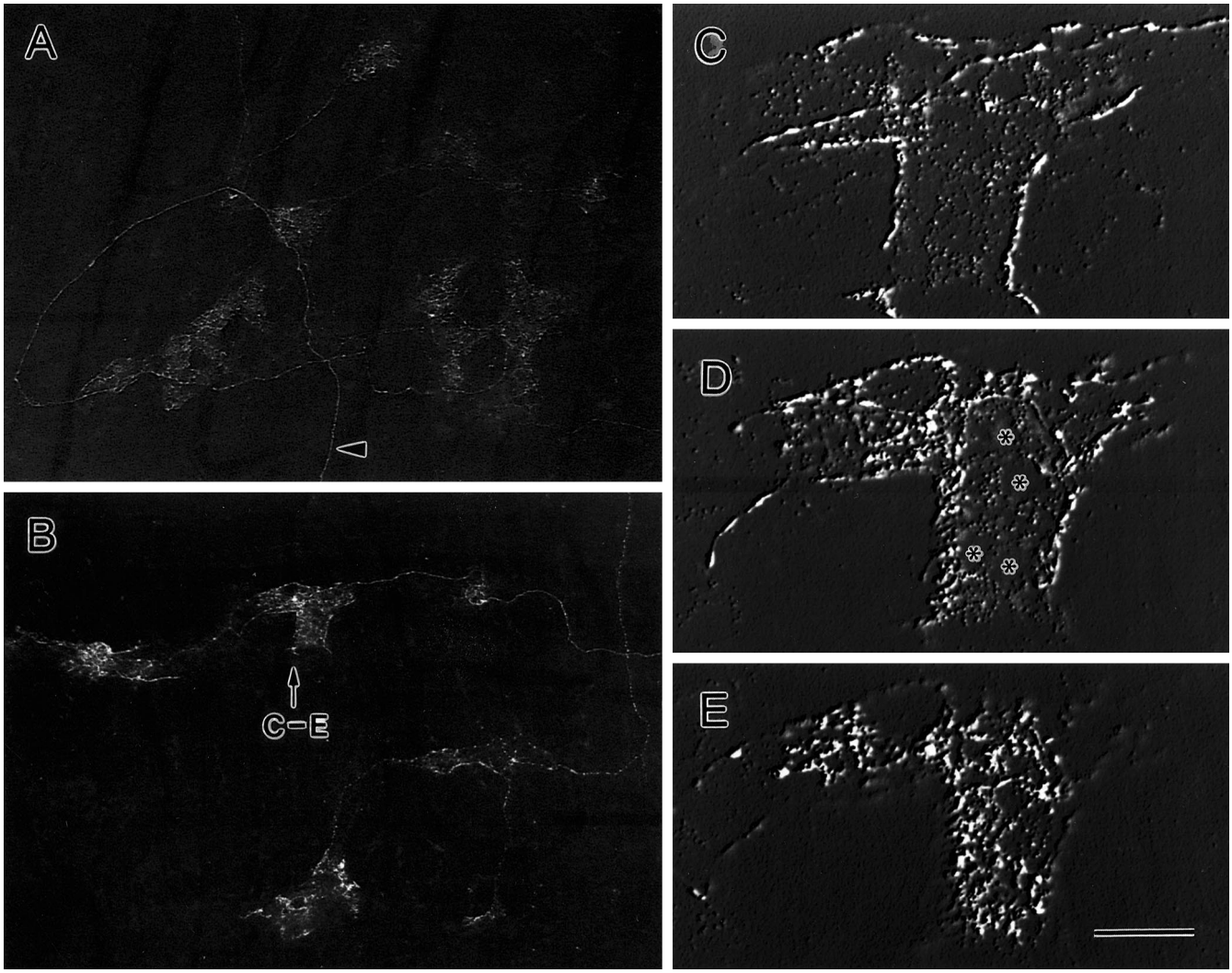

Fig. 2A–E DiI-labeled IGLEs in myenteric plexus of rat duodenum. **A, B** Clusters of numerous IGLEs at low magnification. Note axon in **A** (*arrowhead*) forming complete circle. **C–E** Single optical sections, 3 μm apart through IGLE indicated in **B** (*arrow*). Section in **C** is near circular muscle layer and shows labeled axons flanking ganglion. Section in **D** is at level of myenteric neurons (*asterisks*). Section in **E** is near border with longitudinal muscle layer. *Bar* in **E** represents **A, B** 400 μm; **C–E** 100 μm

In the pyloric antrum, the total number of IGLEs was considerably smaller because of a much smaller surface area. Lateralization was much less pronounced, with about every fourth ganglion being innervated by at least one IGLE on the ventral (ipsilateral) and about every sixth ganglion on the dorsal (contralateral) side (Table 1).

Small intestine

Examples of DiI-labeled IGLEs in the duodenal myenteric plexus are shown in Fig. 2. In the duodenum, the best left injection labeled primarily IGLEs in the proximal duodenum, while the best right injection labeled primarily IGLEs in the distal part (Table 1). This differential labeling from the left and right nodose ganglion was also observed in the less completely labeled cases. In the best left injection, 6.6% of the myenteric ganglia across the entire duodenum received innervation by at least one IGLE, and assuming a similar contribution from the right nodose ganglion, the real percentage of ganglia innervated must be close to 15%. Furthermore, although not measured in this study, this percentage is likely to be considerably higher in the proximal duodenum. There was poor lateralization in the duodenum, since for any (unilateral) injection, IGLEs were not confined to the ipsilateral side of the duodenal wall, but rather were found in equal numbers on both sides. However, most labeled IGLEs were concentrated near the mesentery attachment and rarely seen near the antimesentery pole (Fig. 2).

Examples of labeled IGLEs in the jejunum and ileum are shown in Fig. 3. As was the case for the distal duodenum, the best right nodose ganglion injection resulted in higher numbers of labeled IGLEs than the best left injection throughout the length of jejunum and ileum (Table 1). More than three times as many IGLEs were labeled in the first 30 cm of the jejunum in the best right

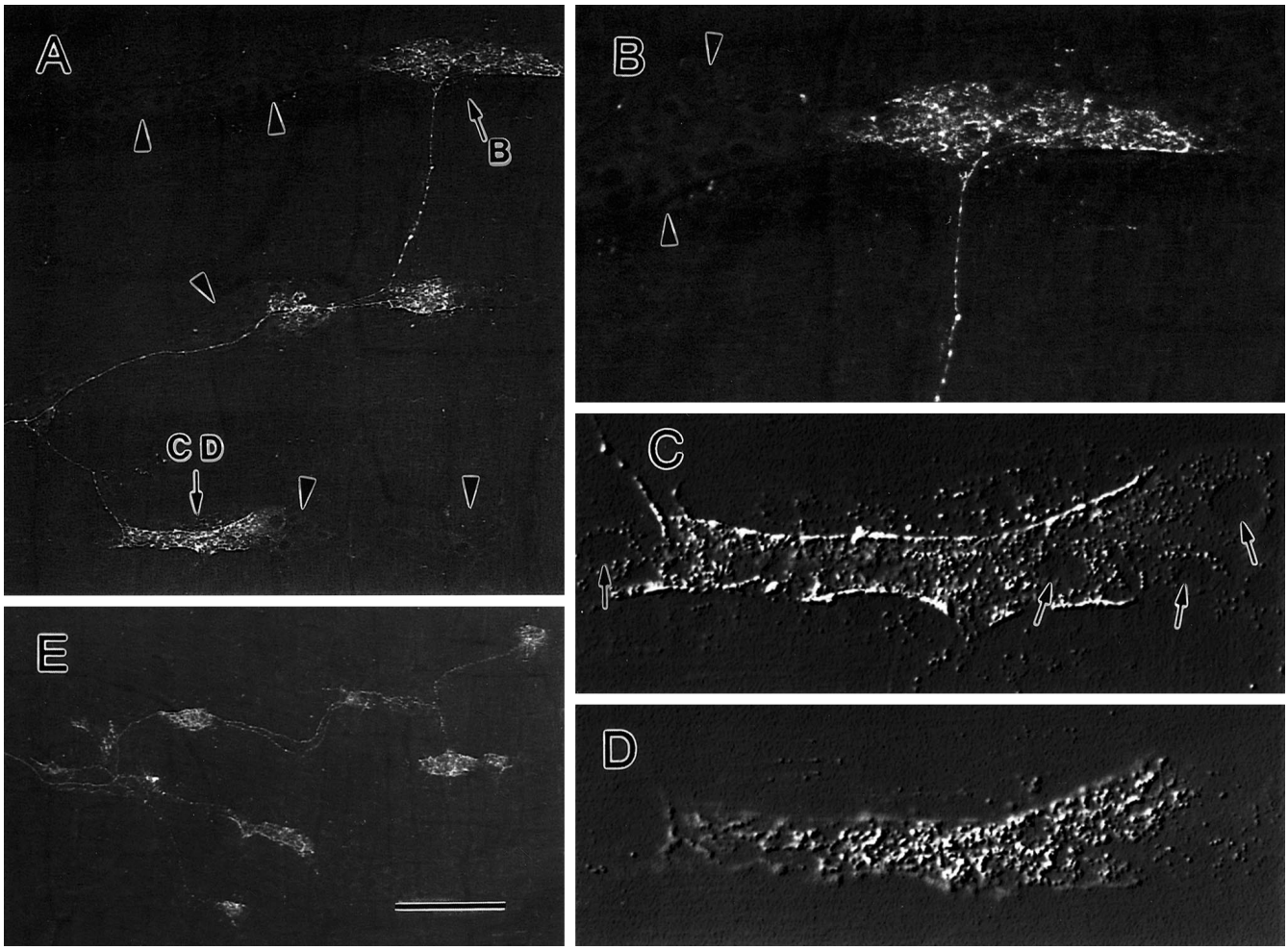

Fig. 3A–E DiI-labeled IGLEs in myenteric plexus of rat jejunum **(A–D)** and ileum **(E)**. **A** Single labeled axon producing four IGLEs in three rows of ganglia in proximal jejunum (J20). Note that portions of ganglia not covered by IGLEs can be recognized by the faintly greyish cytoplasm and dark nuclei of neurons (*arrowheads*). **B–D** Higher magnification of individual IGLEs seen in **A** (*arrows*). In **C** and **D**, thin single optical sections through same IGLE at two different horizontal levels are shown. At the level of the enteric neurons [recognizable by the dark empty spaces of the nuclei (*arrows*)] in **C**, the varicose axons are flanking the ganglion laterally, while at a level just above the neurons in **D**, the laminar endings lie on top of the neurons. **E** Family of six IGLEs in myenteric plexus of ileum, originating from single afferent vagal fiber. *Bar* in **E** represents **A** 350 µm; **B** 180 µm; **C, D** 90 µm; **E** 400 µm

injected animal than in the best left injected animal. The real (bilateral) percentage of innervated ganglia is therefore likely to be considerably higher than the 1.7% and 0.7% for jejunum and ileum, respectively, obtained with the best unilateral left injection. As was the case in the duodenum, most IGLEs were closer to the mesentery attachment than the antimesentery pole. Typically, a single labeled fiber left a small nerve that ran parallel to and near the mesentery attachment, and entered the myenteric plexus, turning more or less in the direction of the circular muscle. Then, usually within less than half the distance to the antimesentery pole, the single axon produced several collaterals that each ended in an IGLE (Fig. 3).

Based on the results from the best left injection, a slightly higher percentage of ganglia was innervated by IGLEs near the ileocecal junction than in the jejunum and ileum (Table 1).

Large intestine

Many IGLEs were also found in the myenteric plexus of the cecum and particularly the ascending and transverse colon (Table 1, Fig. 4). While in the cecum, the best left injection labeled more IGLEs than the best right nodose ganglion injection, the reverse was the case in the colon. Other characteristics of the local distribution pattern were similar to those in the small intestine.

Structural details

There was a large variability in the size of individual IGLEs both within and between the different gut segments. Some IGLEs covered only one enteric neuron, while others covered entire large ganglia consisting of 30 or more neurons. However, the basic structure was quite similar,

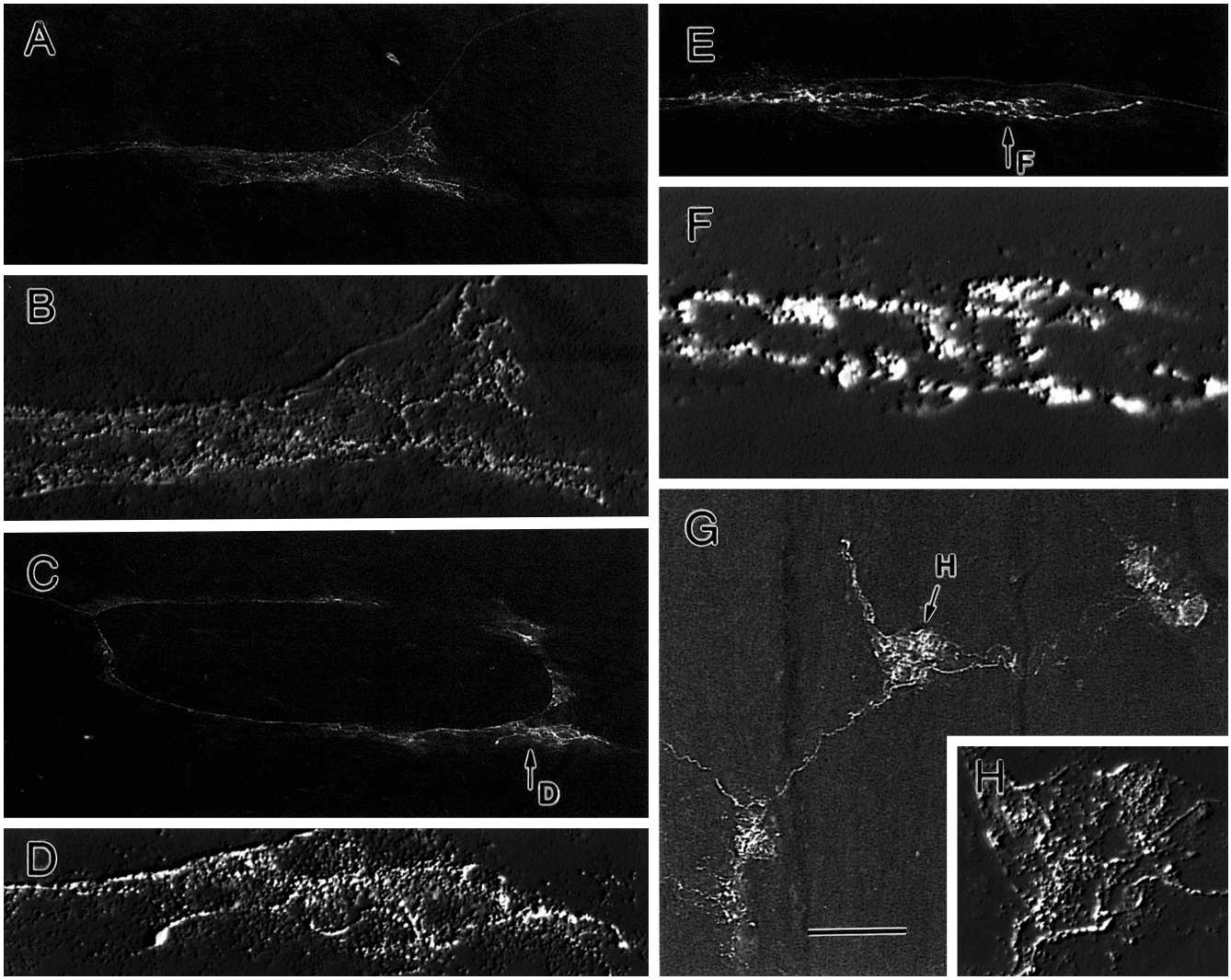

Fig. 4 DiI-labeled IGLEs in myenteric plexus of rat cecum **(A–F)** and colon **(G, H)**. **A,B** Vagal afferent axon forming IGLE in body of cecum. In the higher power extended focus (five optical sections, 1 μm apart) image (**B**), myenteric neurons can also be seen with faintly greyish cytoplasm and dark nuclei. **C** Circular labeled axon forming several IGLEs, one of them (*arrow*) shown in detail in **D**. **E** Spindle-shaped myenteric ganglion and IGLE in appendix of cecum. Note highly varicose laminar structure (*arrow*) shown at higher power in **F**. **G** Group of three IGLEs in transverse colon, one of them (*arrow*) shown at higher power in **H**. *Bar* in **G** represents **A, C** 350 μm; **E, G** 275 μm; **B, D, H** 90 μm; **F** 35 μm

no matter whether small or large, or in what gut segment. The basic structural elements consisted of: (1) a relatively large diameter parent axon that often bifurcated at the entry to a ganglion, the two branches flanking the perimeter of the ganglion; (2) fine branches leaving the parent axon(s) in a more or less vertical (in relation to the horizontal plane of the plexus) direction; (3) profusely arborizing laminar endings that covered the neurons of the ganglia on either one or both sides, in close contact to the surrounding connective tissue capsule (Figs. 2 C–E, 3 C,D).

Discussion

Vagal afferent IGLEs are distributed throughout the myenteric plexus of esophagus and gastrointestinal tract, and represent the most prominent terminal structures of vagal afferent fibers. Although the density of such structures gradually decreases from oral to aboral, the absolute number in the jejunum is higher than in the entire stomach. There are few structural differences between IGLEs in different gut segments, suggesting that they may serve a common function.

Methodological considerations

The raw counts and extrapolated numbers of IGLEs were based on the animal with the highest count for a given gut segment, rather than group means. Considering some of the methodological shortcomings, these numbers can at best be estimates. One of the problems is completeness of labeling and the lack of an independent validation method. Using the same injection technique and volume of tracer can result in widely disparate labeling. The main reason for this poor reproducibility is likely to be

the toxicity of the 100% methanol or ethanol vehicle. Very small injection volumes would tend to produce less cell damage; however, they might result in incomplete labeling. There is only a weak viscerotopic organization within the nodose ganglia, with a tendency of the distal gut to be represented more caudally and the upper portions more rostrally (Altschuler et al. 1989). Because of this considerable overlap, a successful injection usually labels a high percentage of fibers everywhere, and a poor injection labels few fibers throughout the gut.

Unfortunately, there is no procedure to verify the success of an injection, other than assessing peripheral labeling of terminals. Because of the very high fluorescence in the labeled ganglia, it is not possible to count the proportion of labeled perikarya. An assessment of the density or intensity of labeled central processes in the nucleus of the solitary tract can also provide only a relative measure of success, and would never reveal the proportion of labeled afferents. Therefore, selecting the animal with the highest count for a given area seems to provide an estimate that comes closest to the real number of vagal afferents.

Another potential source of error is in the criteria used to classify a terminal structure as IGLE and distinguish it from a neighboring one. Since, at present, we lack any criteria of what constitutes a functional unit, we used strictly structural criteria. Changing these criteria only slightly would clearly change the numbers counted.

Functional implications

The widespread distribution of IGLEs in the myenteric plexus documented in the present study is truly intriguing from a functional perspective. What are these structures doing there? Do they have a chemo- or mechanoreceptive function, a local effector function, or both? There is no direct evidence for any of these possibilities.

Maybe the strongest argument can be made for a mechanoreceptive function, as originally proposed by one of us (Neuhuber 1987). In the esophagus, very few vagal afferent endings can be found in the striated muscle layer, and since vagal afferents respond strongly to esophageal distension (Andrew 1956; Andrews and Lang 1982; Clerc and Mei 1983; Sengupta et al. 1989), it is reasonable to implicate the prominent IGLEs in this function. Except for the fundic stomach, intramuscular vagal endings are quite rare throughout the small and large intestines, and the same argument could be made as above for the esophagus. Recent ultrastructural and histochemical studies lend further, albeit indirect, support for a mechanoreceptive role of at least esophageal IGLEs. The laminar or filamentous endings of IGLEs are located mainly on the surface of myenteric ganglia immediately beneath the basal lamina, and they interdigitate with fine glial processes co-staining for both S-100 protein and vinculin, possibly providing a mechanical link to the periganglionar connective tissue matrix (Neuhuber et al. 1995). Furthermore, esophageal IGLEs stain for both calretinin and calbindin (Neuhuber et al. 1995), two markers that are often found in rapidly adapting mechanosensors (Duc et al. 1993).

A chemoreceptive function, however, cannot be ruled out. The question is what chemicals may be detected? Since the majority of the laminar endings are on the surface of the ganglia and are therefore not in perfect register with the neuropil surrounding the lateral spaces between neurons, they may not receive many direct axonal inputs. It seems more likely that certain substances such as neuropeptides or 5-HT may act on IGLEs in a paracrine fashion. Direct proof of such hypotheses will be extremely difficult to come by, because it would require an in vitro preparation with recording from identified (pre-labeled) vagal fibers that terminate in an IGLE.

There is also some evidence that IGLEs can have local effector functions by releasing neurotransmitters and/or neuropeptides. Ultrastructural analysis of esophageal IGLEs revealed the presence of small clear and some large granular vesicles, numerous mitochondria, and synaptic thickenings near enteric neurons (Neuhuber et al. 1995). It is now widely accepted that dorsal root ganglion afferent neurons produce multiple axon collaterals with some of them releasing neuropeptides such as substance P and calcitonin gene-related peptide as part of local axon reflexes (Holzer 1994). In analogy to this system, it is conceivable that vagal afferents can function in a similar manner. We have shown that in the gastric fundus individual vagal neurons produce axon collaterals terminating both in IGLEs and in intramuscular arrays (Berthoud and Powley 1992), thus providing the necessary pathway for axon reflexes. It is also likely that collaterals to the mucosa may participate in such reflexes; however, this speculation awaits anatomical confirmation.

In the gastric fundus, we have started to look at what types of enteric neurons are in closest contact with IGLEs (Berthoud 1995). One type of enteric neurons that received frequent contacts were NADPH-diaphorase positive, nitrergic neurons. Since most of these neurons project almost exclusively to the circular muscle layer and can be considered inhibitory motor neurons, we have suggested an axon reflex originating at intramuscular tension receptors and ending with IGLE-mediated activation of relaxatory nitrergic motor neurons (Berthoud 1995). It remains to be determined if this relationship is preferentially with nitrergic neurons or also involves other types of enteric neurons, e.g., excitatory cholinergic ones.

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References

Altschuler SM, Bao X, Bieger D, Hopkins DA, Miselis RR (1989) Viscerotopic representation of the upper alimentary tract in the rat: sensory ganglia and nuclei of the solitary and spinal trigeminal tracts. J Comp Neurol 283: 248–262

Andrew BL (1956) The nervous control of the cervical esophagus of the rat during swallowing. J Physiol (Lond) 134: 729–740

Andrews PL, Lang KM (1982) Vagal afferent discharge from mechanoreceptors in the lower esophagus of the ferret. J Physiol (Lond) 332: 29P

Berthoud H-R (1995) Anatomical demonstration of vagal input to nicotinamide acetamide dinucleotide phosphate diaphorase-positive (nitrergic) neurons in rat fundic stomach. J Comp Neurol 358: 428–439

Berthoud H-R, Neuhuber WL (1994) Distribution and morphology of vagal afferents and efferents supplying the digestive system. In: Tache Y, Wingate DL (eds) Brain-gut interactions. CRC Press, Boca Raton, pp 43–65

Berthoud H-R, Powley TL (1992) Vagal afferent innervation of the rat fundic stomach: morphological characterization of the gastric tension receptor. J Comp Neurol 319: 261–276

Berthoud H-R, Kressel M, Raybould HE, Neuhuber WL (1995) Vagal sensors in the rat duodenal mucosa: distribution and structure as revealed by in vivo DiI-tracing. Anat Embryol 191: 203–212

Clerc N, Condamin, M (1987) Selective labeling of vagal sensory nerve fibers in the lower esophageal sphincter with anterogradely transported WGA-HRP. Brain Res 424: 216–224

Clerc N, Mei N (1983) Vagal mechanoreceptors located in the lower esophageal sphincter of the cat. J Physiol (Lond) 336: 487–498

Duc C, Barakat-Walter I, Droz B (1993) Peripheral projections of calretinin-immunoreactive primary sensory neurons in chick hindlimbs. Brain Res 622: 321–324

Holzer P (1994) Spinal afferent nerves: sensory, afferent, and effector functions. In: Tache Y, Wingate DL, Burks TF (eds) Innervation of the gut: pathophysiological implications. CRC Press, Boca Raton, pp 123–136

Kolossow NG, Milochin AA (1963) Morphologischer Nachweis der afferenten (sensiblen) Innervation der peripheren Neurone des vegetativen Nervensystems. Z Mikrosk Anat Forsch 70: 427–464

Kressel M, Berthoud H-R, Neuhuber WL (1994) Vagal innervation of the rat pylorus: an anterograde tracing study using carbocyanine dyes and laser scanning confocal microscopy. Cell Tissue Res 275: 109–123

Neuhuber WL (1987) Sensory vagal innervation of the rat esophagus and cardia: a light and electron microscopic anterograde tracing study. J Auton Nerv Syst 20: 243–255

Neuhuber WL, Clerc N (1990) Afferent innervation of rat esophagus in cat and rat. In: Zenker W, Neuhuber WL (eds) The primary afferent neuron: a survey of recent morpho-functional aspects. Plenum Press, New York, pp 93–107

Neuhuber, WL, Kressel M, Duetsch M, Woerl J, Berthoud H-R (1995) Relationships of IGLEs to enteric glia and neurons in the rat esophagus: further indications of a mechanosensor-local effector role. Soc Neurosci Abstr 21: 1633

Nonidez JF (1946) Afferent nerve endings in the ganglia of the intermuscular plexus of the dog's esophagus. J Comp Neurol 85: 177–185

Powley TL, Berthoud H-R (1991) A fluorescent labeling strategy for staining the enteric nervous system. J Neurosci Methods 36: 9–15

Rodrigo J, Hernandez CJ, Vidal MA, Pedrosa JA (1975) Vegetative innervation of the esophagus. II. Intraganglionic laminar endings. Acta Anat 92: 79–100

Rodrigo J, Felipe J de, Robles-Chillida EM, Perez-Anton JA, Mayo I, Gomez A (1982) Sensory nature and anatomical access paths to esophagus laminar nerve endings in myenteric ganglia. Determination by surgical degeneration methods. Acta Anat 112: 47–57

Sato M, Koyano H (1987) Autoradiographic study on the distribution of vagal afferent nerve fibers in the gastroduodenal wall of the rabbit. Brain Res 400:101–109

Sengupta JN, Kauvar D, Goyal RK (1989) Characteristics of vagal esophageal tension-sensitive afferent fibers in the opossum. J Neurophysiol 61: 1001–1010