cmgh
CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

REVIEW

Neuropods

Rodger A. Liddle

Duke University Medical Center, Durham Veterans Affairs Health Care System, Durham, North Carolina

SUMMARY

Enteroendocrine cells (EECs) are sensory cells of the gut that communicate by releasing hormones locally through paracrine action or into the blood stream. Recently it has been discovered that EECs possess cytoplasmic processes known as neuropods that extend to distant cells including nerves. Thus, EECs regulate ingestive behavior through local, hormonal and neural signaling.

Enteroendocrine cells (EECs) are sensory cells of the gastrointestinal tract. Most EECs reside in the mucosal lining of the stomach or intestine and sense food in the gut lumen. Food signals stimulate the release of hormones into the paracellular space where they either act locally or are taken up into the blood and circulate to distant organs. It recently was recognized that many EECs possess basal processes known as neuropods that not only contain hormones but also connect to nerves. This review describes how neuropods contribute to EEC function beyond typical hormonal actions. For example, gastrointestinal hormones not only act on distant organs, but, through neuropods, some act locally to stimulate other mucosal cells such as intestinal stem cells, enterocytes, or other EECs. With the recent discovery that EECs communicate directly with enteric nerves, EECs not only have the ability to sense food and bacteria in the gastrointestinal tract, but can communicate these signals directly to the nervous system. *(Cell Mol Gastroenterol Hepatol 2019;7:739–747; https://doi.org/10.1016/j.jcmgh.2019.01.006)*

Keywords: Enteroendocrine cell; Gut Hormone; Paracrine; Neuron; Neurotransmission.

Traditionally, enteroendocrine cells (EECs) have been viewed as spindle or flask-shaped cells that reside in the mucosa of the gastrointestinal tract.[1,2] Most EECs are open to the intestinal lumen where a small portion of their apical surface is exposed to intestinal contents. Similar to enterocytes, microvilli cover their luminal surface. In this manner, EECs can sample food or microbes in the intestine. A small number of EECs are of the closed type, and even though their cell bodies are contained in the mucosa, they do not come into contact with the lumen of the intestine.

Hormones are stored within vesicles that are concentrated in the basal region of the EEC and are released into the paracellular space when the cell is stimulated. Secreted hormones can act locally on adjacent cells or be taken up by the blood stream where they can bind to cell surface receptors on distant organs.[3] In this manner, EECs can sense gut contents and communicate these signals throughout the body.

Most EECs have been identified through their expression of a predominant gastrointestinal peptide and were assigned a single letter designation.[4] For example, in the stomach, gastrin-containing cells were called G cells, while somatostatin cells, whether they are found in the stomach or pancreatic islets, have been referred to as D cells. With the exception of L cells, which produce both peptide YY (PYY) and glucagon-like peptides (GLPs), it was believed that a single EEC produced only 1 peptide hormone. Recently, however, using mice with transgenic expression of fluorescent markers, it has been shown that EECs previously thought to express only a single hormone actually produce many gastrointestinal peptides.[5] In fact, it appears that secretin is expressed in most, if not all, EECs of the intestine.[5] Thus, not only is the terminology for describing specific EECs incomplete, the diversity of EECs is wider than ever expected. The variety of hormones expressed in EECs has broad implications for the function of these cells that is only beginning to be explored. For the purposes of this review, we refer to EECs by the hormone(s) they produce.

Anatomic Features

Within the gastrointestinal tract, the anatomy of EECs has been guided largely through microscopic analysis of tissues stained for chemical properties such as silver (eg, argentaffin), or immunohistochemical staining using general vesicle markers (eg, chromogranin) or specific hormone antibodies.[6] These methods have shown that EECs comprise approximately 1% of the intestinal mucosal cells, are dispersed among enterocytes, and contain secretory vesicles along their basal pole. Electron microscopy has shown that microvilli cover the luminal surface of EECs and that both electron-dense core vesicles typical of hormone-containing granules and clear vesicles resembling neurotransmitter-containing granules comprise the vesicular compartment of EECs.[2]

Abbreviations used in this paper: CCK, cholecystokinin; EEC, enteroendocrine cell; EGF, epidermal growth factor; GFP, green fluorescent protein; GLP, glucagon-like peptide; IGF-1, insulin-like growth factor-1; iSEMF, intestinal subepithelial myofibroblast; PYY, peptide YY.

Most current article

© 2019 The Author. Published by Elsevier Inc. on behalf of the AGA Institute. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
2352-345X
https://doi.org/10.1016/j.jcmgh.2019.01.006

Because traditional microscopy generally is performed in 2 dimensions, it has been difficult to appreciate all of the anatomic features of EECs that deviate from the traditional spindle or flask-shape view. The exception has been somatostatin-containing cells of the stomach.[7] Somatostatin, which has broad inhibitory effects, is found in gastric cells that have long cytoplasmic processes that extend to adjacent gastrin-producing EECs and hydrochloric acid–producing parietal cells. Thus, it appeared that somatostatin could be delivered to and released from cytoplasmic processes. This discovery provided compelling evidence for paracrine control of cell function in the gastrointestinal tract.[8]

It was assumed that paracrine effects made manifest through cytoplasmic extensions were unique to somatostatin cells and were most apparent in the stomach despite several curious observations. Serotonin-containing enterochromaffin cells, a subtype of EECs, also are believed to exert paracrine effects within the gastrointestinal tract. By using immunohistochemical staining and careful cell dissection techniques, a physical connection was observed between EECs and other cells through an axon-like process.[9] However, the presence of such processes was not limited to somatostatin cells.[10]

In the intestine, mucosal cells arise from crypt stem cells and migrate toward the lumen. Within 4–5 days most mucosal cells reach the villus tip, undergo apoptosis, and are sloughed into the intestinal lumen. Although it was observed that an occasional EEC of the distal small intestine possessed a lagging segment of cytoplasm along the basal surface, it was assumed this occurred as the EEC was being pushed upward by younger cells arising from the crypt.[11] Even though this explanation seems unlikely because lagging cytoplasm has not been observed for other migrating mucosal cells such as enterocytes or goblet cells, it largely has gone unchallenged.

With the development of transgenic mice expressing green fluorescent protein (GFP) downstream from the specific gastrointestinal hormone promoters such as cholecystokinin (CCK) and PYY, EECs could be analyzed using confocal fluorescence microscopy.[12,13] With the ability to visualize cells in 3 dimensions, it was possible to trace cellular processes that extended outside a simple plane. Remarkably, a large number of EECs from the proximal small intestine of CCK-GFP mice showed basal cytoplasmic processes. These processes generally extended from the basal portion of the cell but occasionally arose from the midportion of the cell body. They were usually less than 10 μm in length and radiated in all directions from the EEC. Their multidirectionality clearly differed from the unidirectional cytoplasmic processes reported previously and were not consistent with the notion that they were caused by EECs being pushed toward the villus tip.

However, where these cytoplasmic processes went and what they did was not known. In contrast to CCK cells in the proximal small intestine, PYY cells had only 1 process per cell, although they often were much longer, with some extending over 70 μm in length. Long cytoplasmic processes tunneled under enterocytes along the lamina propria (Figure 1). How to assess the ultrastructure of EECs that contained long

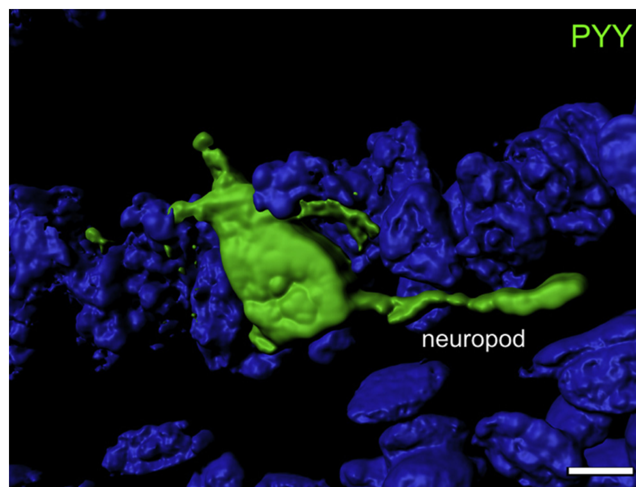

Figure 1. An enteroendocrine cell neuropod. A confocal microphotograph shows an enteroendocrine cell within the mouse small intestine. An enteroendocrine cell (green) stained with a fluorescently labeled PYY antibody possesses a neuropod that extends below the basal surface of surrounding mucosal cells (eg, enterocytes). Nuclei are stained blue with 4′,6-diamidino-2-phenylindole. *Scale bar*: 10 μm.

cytoplasmic extensions posed a formidable problem. First, it was difficult to locate a rare cell type (an EEC) among a field of enterocytes and other mucosal cells using traditional electron microscopy. Second, by its very nature, electron microscopy is ideal for analyzing a small region of a cell, but does not lend itself to tracing a long and possibly tortuous cellular process that may not reside in a single plane. To circumvent these problems, confocal microscopy was used to locate a fluorescent EEC combined with a newly developed technique of 3-dimensional scanning electron microscopy.[14] This approach enabled imaging an entire EEC with its cytoplasmic process from the distal small intestine. Several unique features of the cytoplasmic process were revealed. First, 70% of the EEC's secretory vesicles were contained within the process. Second, both small, clear and large, dense core vesicles were identified. Third, the process was packed with mitochondria. Fourth, running down the core of the process was a ribbon-like band absent of ribosomes that upon immunohistochemical staining was found to contain neurofilament proteins, the structural component of neuronal axons. Finally, this axon-like process came into contact with glia in the submucosa. These findings indicated that EECs possess many neuron-like properties and the cytoplasmic process became known as a *neuropod* (Figure 2).

Paracrine Actions of EECs

Somatostatin

The prototypical paracrine cell is the somatostatin-containing D cell found in the intestine and pancreatic islets. In the gut, somatostatin cells were notable for their dendritic-like processes that extended to adjacent cells, including other EECs.[7] The dictum that structure determines function was keenly illustrated by somatostatin cells when it was recognized that the broad inhibitory actions of

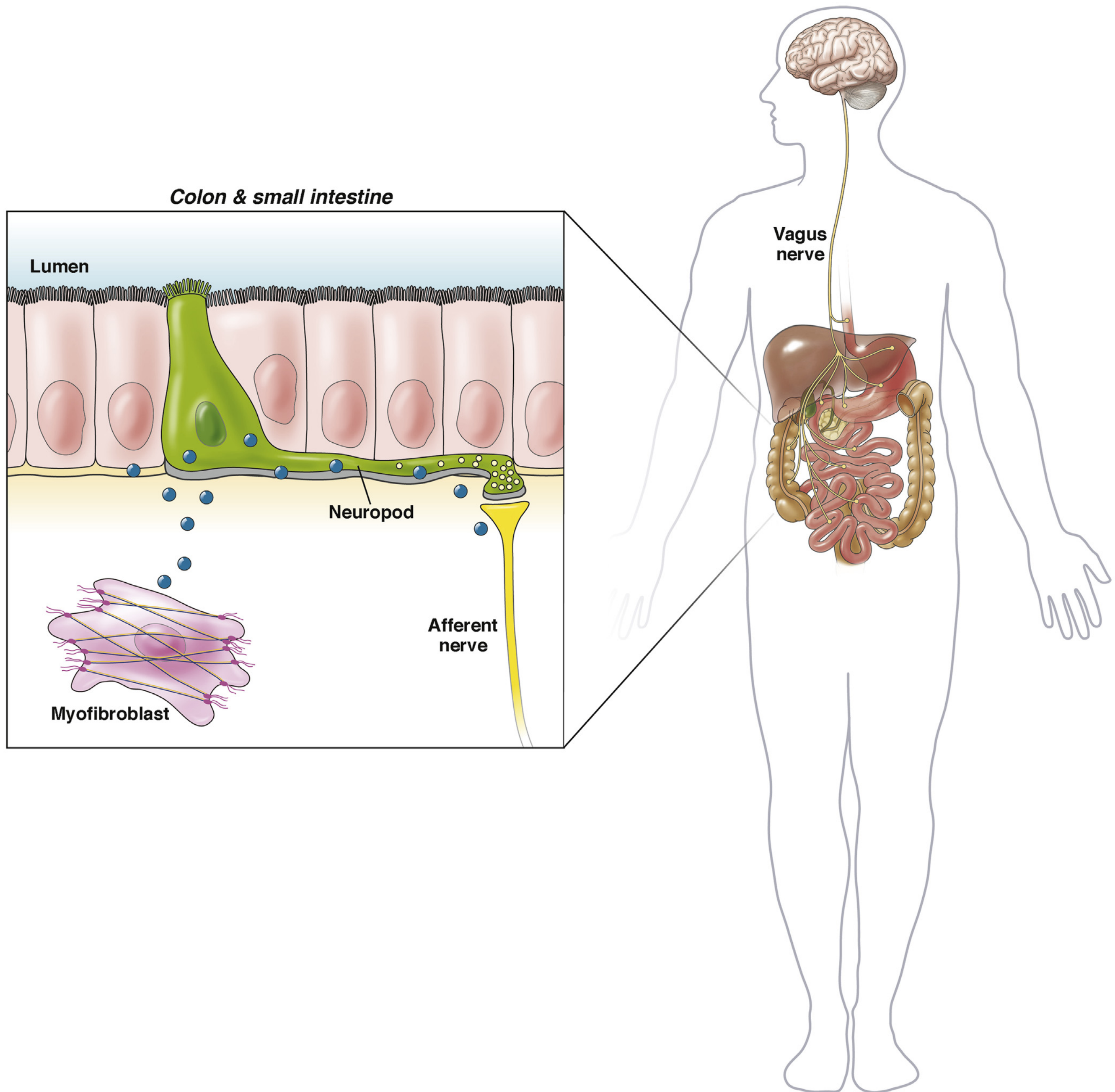

Figure 2. Enteroendocrine cell communication within the gut. A model of an enteroendocrine cell (green) residing in the epithelium of the gut shows that EECs contain both large, dense (blue) and small, clear (yellow) secretory granules that are believed to contain peptide hormones (eg, CCK, PYY, and GLPs) and neurotransmitters (eg, glutamate), respectively. Neurotransmitters are released at synaptic connections with sensory neurons. Peptides such as PYY or GLP-2 are released locally and bind to proliferative cells in the crypt or intestinal subepithelial myofibroblasts, respectively. Peptides (eg, CCK, PYY, and GLPs) also may bind locally to nerves.

somatostatin were not well suited for precise control of individual cells. In contrast, when cellular extensions were seen to innervate other cells it was easy to appreciate that somatostatin could be delivered at a specific site. Thus, the cellular processes on somatostatin cells are ideal for targeted delivery of a transmitter onto a single cell.

In the stomach, cellular processes extend from the cell body of D cells through which somatostatin exerts a tonic inhibitory action on gastrin-containing cells to block gastrin release in the antrum.[15] From somatostatin cells in the body and fundus of the stomach, cellular processes extend to parietal cells to directly inhibit gastric acid secretion.[16] Dual feedback loops regulate somatostatin cell function. First, somatostatin cells that are open to the gastric lumen are stimulated by gastric acid. Somatostatin released from these cells then inhibits both G cells and parietal cells to reduce gastrin and gastric acid secretion. Second, somatostatin cells possess gastrin (CCK2) receptors, activation of which also

stimulates somatostatin release, resulting in reduced gastrin and acid secretion.[17]

Glucagon-Like Peptides

GLP-1 and GLP-2 are synthesized in L cells, which are most abundant in the ileum and colon.[18] These are the same cells that produce PYY. It is believed that stimulation of L cells causes the release of all 3 peptides. GLP-1 and GLP-2 are products of post-translational processing of the pro-hormone proglucagon. GLP-1 and GLP-2 are secreted in response to ingested nutrients, including glucose and fat, and their main biological actions are to enhance insulin secretion and intestinal epithelial growth, respectively.[19] The growth-stimulating effects of GLP-2 accompany nutrient ingestion and result from both increased epithelial proliferation and decreased apoptosis.[20] GLP-2 also enhances nutrient digestion and absorption, increases intestinal transit time, increases intestinal blood flow, improves epithelial barrier function, and enhances immune protection by increasing Paneth cell function. GLP-2 also has protective effects in animal models of intestinal inflammation.

Despite the prominent effects of GLP-2 on the intestinal epithelium, these actions appear to be indirect because GLP-2 receptors are not expressed on intestinal stem cells (either Lgr5+ crypt base or in Bmi1+ cells that comprise the proliferative reserve stem cell population).[20] Although GLP-2 receptors are expressed almost exclusively in the gastrointestinal tract, they are found on 3 distinct cell types, but not intestinal epithelial cells. The most prominent cell type expressing GLP-2 receptors are intestinal subepithelial myofibroblasts (iSEMFs), which lie below the intestinal mucosa.[21–23] GLP-2 receptors also are expressed in enteric neurons,[24–26] including vagal afferent nerves[27] and a subpopulation of EECs.[28] It is quite remarkable that despite the lack of receptors on intestinal stem cells or cells in the transit-amplifying region of the crypt, GLP-2 increases mucosal and crypt cell proliferation, increases the length of microvilli on enterocytes, improves intestinal barrier function and decreases intestinal permeability, facilitates nutrient digestion and absorption, and enhances enterocyte survival.[29–41]

Similar to most gastrointestinal hormones, upon release from its EEC, GLP-2 enters the blood stream or is taken up in lymphatic vessels.[42,43] However, based on the lessons learned from somatostatin cells, together with the discovery of neuropods in L cells, it is just as likely that the local effects of GLP-2 are the result of paracrine actions, but the actions are likely on a cell type expressing GLP-2 receptors that reside in the intestinal submucosa. A strong case can be made for iSEMFs mediating the growth-promoting effects of GLP-2. Not only do iSEMFs express GLP-2 receptors, but they produce the growth factors insulin-like growth factor-1 (IGF-1) and epidermal growth factor (EGF).[23,44–46] Both IGF-1 and EGF receptors are expressed in intestinal stem cells where they could affect intestinal growth. Importantly, administration of IGF-1 and EGF each have been shown to exert mucosal growth-promoting effects.[23,47–49] Interestingly, EGF, but not IGF-1, restores intestinal growth in GLP-2–receptor knockout mice.[50]

These findings show that GLP-2 has prominent local, growth-promoting effects on the intestinal epithelium. GLP-2 receptors are expressed on cells that lie in close proximity to the GLP-producing EECs, and the target cells (such as iSEMFs) are capable of mediating the growth-promoting effects of GLP-2. Thus, it appears that L cells release GLP-2, which stimulates iSEMFs to produce IGF-1 and EGF, which exert their own paracrine effects on the intestinal mucosa.

Even though GLP-2 receptors have been identified on a subpopulation of EECs, there are insufficient data to invoke a role for another EEC type to mediate the growth-promoting effects of GLP-2 on the intestine. Nevertheless, it is interesting that in Drosophila, EECs provide local cues for maintaining intestinal stem cells.[51] Therefore, it is possible that EECs exert as yet unrecognized direct effects on intestinal stem cells.[52]

GLP-1 receptors are expressed on β cells of the pancreatic islet and GLP-1 increases insulin synthesis and secretion.[53–55] Even though the effects of GLP-1 on the pancreas are well established and therapeutic GLP-1 mimetics are used to treat patients with diabetes mellitus, there is evidence that endogenously released GLP-1 may influence insulin secretion through a paracrine action by acting locally on afferent nerves.[56,57] Local paracrine actions of GLP-1 also may influence immune function through effects on intraepithelial lymphocytes and afferent neurons.[58] Thus, despite our historical focus on the actions of circulating glucagon-like peptides and their exploitation for therapeutic purposes, locally released GLP-1 and GLP-2 may exert important and previously unappreciated physiological effects.

Peptide YY

A notable feature of some L cells is their long neuropods, which are packed with both large, dense and small, clear secretory vesicles that are believed to contain hormones and neurotransmitters, respectively. One function of neuropods is neurotransmission, with glutamate as a candidate neurotransmitter.[59] However, that does not explain the functional significance of the abundant peptide-containing vesicles that reside in neuropods.

Although these, too, could function as neuropeptides, it is striking that EECs are found as single cells in the mucosa, surrounded by enterocytes. In this orientation, only a few enterocytes actually come into contact with an EEC. Neuropods lie between the base of enterocytes and the lamina propria, but their long extension allows EECs to reach many more cells in the mucosa than those that lie next to the EEC cell body. Similar to the prototypical paracrine cell—the somatostatin cell with its cellular extensions—the neuropods of L cells provide a convenient mechanism for delivering peptide to enterocytes throughout the mucosa.

This possibility took on more meaning when it was discovered that PYY induces proliferation of intestinal cells in vivo and in vitro and activates mitogenic signaling pathways,[60] and gut epithelial cells express Y1-type PYY receptors.[61] Y1-receptor messenger RNA is expressed in basal crypt cells consistent with the transit-amplifying cell region

of the intestinal mucosa.[62] In this location, the receptor is in a position to mediate the growth-promoting effects of PYY. Expression of the Y1 receptor protein has been found throughout the colonic crypt epithelium, suggesting that the receptor protein is long lived even though the proliferative effects of PYY would be realized only in the intestinal stem cell or transit-amplifying cell region. Thus, it appears that PYY, unlike GLP-2, exerts direct effects on intestinal epithelial cells to stimulate growth. In addition, confocal microscopy of fluorescently labeled Y1 receptor localized the receptor to the basolateral surface of human colonocytes. In this location, cells are ideally positioned to respond to locally released PYY.[61]

Serotonin

One of the earliest recognized EEC signaling actions onto nerves in the gut was the regulation of peristalsis initiated by the local release of serotonin from enterochromaffin cells (a major EEC subtype).[63,64] Locally released serotonin also stimulates nerves in the gut to transmit signals to the brain and mediates chemotherapy-induced nausea.[65] This pathway is the basis for the use of 5-hydroxytryptamine-3 antagonists in the treatment of nausea.[66] Although much of the serotonin effect was believed to be paracrine, a direct functional connection between enterochromaffin cells and gut nerves recently was shown.[67] Upon activation, enterochromaffin cells were shown to transmit an electrical signal onto serotonin-sensitive primary sensory afferent nerve fibers via synaptic connections, enabling them to transduce information from the gut lumen directly to the nervous system. Thus, what once was believed to be exclusively a paracrine action between serotonin-producing cells and cells within the gut has taken on a new level of communication because the local actions of serotonin may be either paracrine or neural.

This pathway has implications for gut–brain communication and diseases in which the brain perceives sensory signals from the gut. Abnormal serotonin signaling has been implicated in intestinal hypersensitivity and irritable bowel syndrome.[68] Tissue concentrations of serotonin appear to be more abundant in patients with irritable bowel syndrome[69] and 5-hydroxytryptamine-3 antagonists have been used to ameliorate symptoms in patients with irritable bowel syndrome.[70]

Neuron-Like Features

EECs are terminally differentiated and, after in vitro isolation, single cells do not grow in culture. However, if maintained in intestinal organoids, EECs arise from crypt stem cells and in organoids prepared from the intestine of CCK-GFP or PYY-GFP mice, fluorescent EECs are not only present, but they flourish.[14] They even express neuropods. EECs were found to express neurotrophin receptors including TrkA and glial-derived neurotrophic factor family receptor α-3 and the neurotrophins, nerve growth factor-β, and artemin were discovered to induce neuropod elongation and branching, respectively.

The neural network within the intestinal villus is extensive and neuropods come into close contact with neurons lying beneath the intestinal mucosa. It seemed natural to ask if EECs connect with neurons. This possibility was investigated using several complementary approaches.[71] First, using a variety of neuronal cell markers, immunohistochemical staining showed that EECs contacted nerves expressing neurofilament proteins, the pan-neuronal marker protein gene product 9.5, calbindin, and calcitonin gene-related peptide, a marker of sensory neurons.[72] Thus, it appeared that EECs were capable of connecting to sensory neurons. EECs were not seen connecting to vasoactive intestinal peptide-containing nerves, which has been used as a marker of motor neurons.

Next, the connection of EECs to sensory neurons was recapitulated in vitro.[71] Despite EECs not growing in culture, a small number survive cell isolation and can be maintained in vitro, making it possible to co-culture EECs from CCK-GFP mice together with sensory neurons from trigeminal or dorsal root ganglia. By using time-lapse photomicroscopy it was observed that fluorescent EECs interacted with sensory neurons labeled with the lipophilic dye, DiI.[71] With an EEC and sensory nerve lying in close proximity, the nerve extended a small neurite toward the EEC. The EEC responded by elongating a process to contact the nerve's neurite. The EEC then withdrew from the nerve but the cells remained connected through an axon-like structure. These studies show that EECs and neurons have an affinity for one another that likely results from the release of chemical signals. We propose that chemical attractants are released from EECs and neurons and form chemical gradients that induce directional extensions of cellular processes (either neuropods or neurites) to produce axon-like connections.

EECs express a number of genes that encode neuronal proteins, including those for presynaptic and postsynaptic proteins.[71] For example, genes for the presynaptic proteins synapsin 1, piccolo, bassoon, MUNC13B, RIMS2, latrophilin 1, and transsynaptic neurexin 2 have been identified in EECs. In addition, these cells express postsynaptic genes for neuroligins 2 and 3, homer 3, and postsynaptic density 95. Thus, the connection between EECs and neurons appeared to be a synapse.

A synaptic connection between EECs and neurons was proven using monosynaptic rabies virus neurotracing. By using a modified rabies virus in which the envelope glycoprotein rabG was replaced with green fluorescent protein (rabG-GFP),[73] it was possible to show that rabies virus placed into the lumen of mouse colon infected PYY-containing EECs.[71] Because rabG is responsible for the spread of rabies from neuron to neuron through synapses, the modified rabies virus could not move beyond the EEC. In separate experiments, using a mouse with targeted expression of the envelope protein rabG exclusively in PYY cells, instillation of rabG-GFP into the colon infected EECs, and because the virus could be packaged with the viral coat expressed within the PYY cells, the virus jumped onto underlying nerves, indicating that EECs connected to nerves

through a synaptic connection. These studies established that a neural circuit exists connecting EECs to the nervous system.

This new neural circuit has been shown to connect EECs directly to the brainstem.[59] By using modified rabies tracing, some EECs from both the small intestine and colon connected to nerves in the vagal nodose ganglia and projected into the nucleus tractus solitarius of the brainstem, indicating that the EECs communicate with vagal neurons and connect the gut to the brain through a single synapse. Interestingly, labeled nerve fibers also were seen in the dorsal root ganglia, indicating that EECs connect with nerves innervating the spinal cord.

EECs have been characterized by the hormones they express and because individual EECs were thought to produce only a single hormone, the possibility of an EEC producing other transmitters, not to mention neurotransmitters, had been largely overlooked. However, when it was appreciated that EECs synapse with neurons, it was natural to ask why and what transmitters were involved. RNA analysis of GFP-expressing EECs provided a hint when it was discovered that EECs express the vesicular glutamate transporter 1 protein. Subsequently, glutamate was shown to be released from EECs in vitro and to transmit a synaptic signal in EECs co-cultured with sensory neurons.[59] Thus, it appears that the synaptic signal emanating from EECs involves the classic neurotransmitter glutamate.

Electrical recordings from synaptically connected EECs and vagal nodose neurons in a co-culture system was used to dissect the synaptic transmission properties of the EEC-neuron connection.[59] By using selective CCK and glutamate-receptor blockers, it was shown that CCK-GFP EECs release both CCK and glutamate, which stimulate vagal neurons in a time-dependent manner. Glutamate activation of nodose neurons occurred within milliseconds of EEC stimulation, indicating synaptic transmission. In contrast, the CCK-receptor blocker devazepide inhibited a slower phase of nerve activation consistent with CCK having a paracrine action. Thus, EECs use both CCK and glutamate as transmitters to activate vagal sensory nerves through both paracrine and synaptic pathways.

Gut signaling through the vagus nerve generally has been thought to mediate negative feedback mechanisms to control meal size.[74] Recently, however, using selective optogenetic techniques to stimulate select fibers in the vagus, it was shown that vagal sensory pathways from the gut project to reward regions in the brain including the substantia nigra.[75] Thus, not only do vagal signals from the gut control satiety, but select neurons can affect motivation and pleasure.

The function of the EEC-neural circuit is only beginning to be appreciated, however, it is evident that nutrient signals such as glucose can activate a signal within an EEC that is transmitted onto sensory neurons that extend to the brain. Therefore, it is highly likely that other signals from ingested nutrients or microbiota in the gut may be transmitted to the brain in the same way. This circuit establishes a direct sensory neural link between the gut and the brain.

EEC Life Span

In general, it had been believed that EECs, similar to other epithelial cells of the intestinal mucosa, turn over every 4–5 days.[76] If correct, the dynamics of EEC migration from the crypt to the villus tip and the rapid turnover of cells may restrict the ability of EECs to connect with nerves and limit the duration of the connection. However, some studies have suggested that EECs live longer than enterocytes.[77] By using bromodeoxyuridine labeling of cells, it was observed that some EECs reside in the intestinal mucosa for at least 60 days,[71] and likely longer. These findings indicate that EECs have a life span similar to that of other sensory cells such as taste cells and olfactory neurons.[78,79]

Final Note on EEC Sensing

Accumulating evidence has indicated that EECs respond not only to food in the lumen of the intestine, but also are stimulated by absorbed nutrients.[80] Because neuropods extend throughout the gut mucosa, they are ideally positioned to receive signals from enterocytes and other neighboring cells. These signals may be in the form of absorbed nutrients, such as lipids that stimulate CCK secretion.[81] Although an attractive hypothesis, it remains to be determined if neuropods receive and transmit signals from the paracellular space to the EEC.

It recently became apparent that EECs respond to a variety of chemical and mechanical stimuli and transmit sensory signals from the gut to the brain to inform us of what we eat and warn us of ingested dangers. We interpret those signals by modifying what, when, and how we eat.

In addition, EECs act locally in the gut to regulate intestinal secretion, motility, and growth to facilitate the ingestion, digestion, and absorption of nutrients. However, it is not yet understood how EECs know to do all of these things. Although we understand a little bit about how EECs send signals from the gut, we know EECs also express postsynaptic proteins, implying that they receive efferent signals from the nervous system.[71] On the frontier is understanding how the EEC learns from the brain.

References

1. Solcia E, Capella C, Buffa R, Usellini L, Frigerio B, Fontana P. Endocrine cells of the gastrointestinal tract and related tumors. Pathobiol Annu 1979;9:163–204.
2. Solcia E, Usellini L, Buffa R, Rindi G, Villani L, Zampatti C, Silini E. Endocrine cells producing regulatory peptides. Experientia 1987;43:839–850.
3. Wade PR, Westfall JA. Ultrastructure of enterochromaffin cells and associated neural and vascular elements in the mouse duodenum. Cell Tissue Res 1985;241:557–563.
4. Helander HF, Fandriks L. The enteroendocrine "letter cells" - time for a new nomenclature? Scand J Gastroenterol 2012;47:3–12.
5. Haber AL, Biton M, Rogel N, Herbst RH, Shekhar K, Smillie C, Burgin G, Delorey TM, Howitt MR, Katz Y, Tirosh I, Beyaz S, Dionne D, Zhang M, Raychowdhury R, Garrett WS, Rozenblatt-Rosen O, Shi HN, Yilmaz O,

Xavier RJ, Regev A. A single-cell survey of the small intestinal epithelium. Nature 2017;551:333–339.

6. Grimelius L. Methods in neuroendocrine histopathology, a methodological overview. Ups J Med Sci 2008;113:243–260.
7. Larsson LI, Goltermann N, de Magistris L, Rehfeld JF, Schwartz TW. Somatostatin cell processes as pathways for paracrine secretion. Science 1979;205:1393–1395.
8. Larsson LI. Peptide secretory pathways in GI tract: cytochemical contributions to regulatory physiology of the gut. Am J Physiol 1980;239:G237–G246.
9. Gustafsson BI, Bakke I, Tommeras K, Waldum HL. A new method for visualization of gut mucosal cells, describing the enterochromaffin cell in the rat gastrointestinal tract. Scand J Gastroenterol 2006;41:390–395.
10. Sjolund K, Sanden G, Hakanson R, Sundler F. Endocrine cells in human intestine: an immunocytochemical study. Gastroenterology 1983;85:1120–1130.
11. Cheng H, Leblond CP. Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. III. Entero-endocrine cells. Am J Anat 1974;141:503–519.
12. Chandra R, Samsa LA, Vigna SR, Liddle RA. Pseudopod-like basal cell processes in intestinal cholecystokinin cells. Cell Tissue Res 2010;341:289–297.
13. Bohórquez DV, Liddle RA. Axon-like basal processes in enteroendocrine cells: characteristics and potential targets. Clin Transl Sci 2011;4:387–391.
14. Bohórquez DV, Samsa LA, Roholt A, Medicetty S, Chandra R, Liddle RA. An enteroendocrine cell-enteric glia connection revealed by 3D electron microscopy. PLoS One 2014;9:e89881.
15. Makhlouf GM, Schubert ML. Gastric somatostatin: a paracrine regulator of acid secretion. Metabolism 1990;39:138–142.
16. Schubert ML, Edwards NF, Arimura A, Makhlouf GM. Paracrine regulation of gastric acid secretion by fundic somatostatin. Am J Physiol 1987;252:G485–G490.
17. DelValle J, Chiba T, Park J, Yamada T. Distinct receptors for cholecystokinin and gastrin on canine fundic D-cells. Am J Physiol 1993;264:G811–G815.
18. Drucker DJ, Habener JF, Holst JJ. Discovery, characterization, and clinical development of the glucagon-like peptides. J Clin Invest 2017;127:4217–4227.
19. Holst JJ. Enteroglucagon. Annu Rev Physiol 1997;59:257–271.
20. Brubaker PL. Glucagon-like peptide-2 and the regulation of intestinal growth and function. Compr Physiol 2018;8:1185–1210.
21. Orskov C, Hartmann B, Poulsen SS, Thulesen J, Hare KJ, Holst JJ. GLP-2 stimulates colonic growth via KGF, released by subepithelial myofibroblasts with GLP-2 receptors. Regul Pept 2005;124:105–112.
22. Ramsanahie A, Duxbury MS, Grikscheit TC, Perez A, Rhoads DB, Gardner-Thorpe J, Ogilvie J, Ashley SW, Vacanti JP, Whang EE. Effect of GLP-2 on mucosal morphology and SGLT1 expression in tissue-engineered neointestine. Am J Physiol Gastrointest Liver Physiol 2003;285:G1345–G1352.
23. Leen JL, Izzo A, Upadhyay C, Rowland KJ, Dube PE, Gu S, Heximer SP, Rhodes CJ, Storm DR, Lund PK, Brubaker PL. Mechanism of action of glucagon-like peptide-2 to increase IGF-I mRNA in intestinal subepithelial fibroblasts. Endocrinology 2011;152:436–446.
24. Bjerknes M, Cheng H. Modulation of specific intestinal epithelial progenitors by enteric neurons. Proc Natl Acad Sci U S A 2001;98:12497–12502.
25. de Heuvel E, Wallace L, Sharkey KA, Sigalet DL. Glucagon-like peptide 2 induces vasoactive intestinal polypeptide expression in enteric neurons via phophatidylinositol 3-kinase-gamma signaling. Am J Physiol Endocrinol Metab 2012;303:E994–E1005.
26. Guan X, Karpen HE, Stephens J, Bukowski JT, Niu S, Zhang G, Stoll B, Finegold MJ, Holst JJ, Hadsell D, Nichols BL, Burrin DG. GLP-2 receptor localizes to enteric neurons and endocrine cells expressing vasoactive peptides and mediates increased blood flow. Gastroenterology 2006;130:150–164.
27. Nelson DW, Sharp JW, Brownfield MS, Raybould HE, Ney DM. Localization and activation of glucagon-like peptide-2 receptors on vagal afferents in the rat. Endocrinology 2007;148:1954–1962.
28. Yusta B, Huang L, Munroe D, Wolff G, Fantaske R, Sharma S, Demchyshyn L, Asa SL, Drucker DJ. Enteroendocrine localization of GLP-2 receptor expression in humans and rodents. Gastroenterology 2000;119:744–755.
29. Drucker DJ, Erlich P, Asa SL, Brubaker PL. Induction of intestinal epithelial proliferation by glucagon-like peptide 2. Proc Natl Acad Sci U S A 1996;93:7911–7916.
30. Tsai CH, Hill M, Asa SL, Brubaker PL, Drucker DJ. Intestinal growth-promoting properties of glucagon-like peptide-2 in mice. Am J Physiol 1997;273:E77–E84.
31. Litvak DA, Hellmich MR, Evers BM, Banker NA, Townsend CM Jr. Glucagon-like peptide 2 is a potent growth factor for small intestine and colon. J Gastrointest Surg 1998;2:146–150.
32. Smither BR, Pang HY, Brubaker PL. Glucagon-like peptide-2 requires a full complement of Bmi-1 for its proliferative effects in the murine small intestine. Endocrinology 2016;157:2660–2670.
33. Sigalet DL, de Heuvel E, Wallace L, Bulloch E, Turner J, Wales PW, Nation P, Wizzard PR, Hartmann B, Assad M, Holst JJ. Effects of chronic glucagon-like peptide-2 therapy during weaning in neonatal pigs. Regul Pept 2014;188:70–80.
34. Burrin DG, Stoll B, Guan X, Cui L, Chang X, Holst JJ. Glucagon-like peptide 2 dose-dependently activates intestinal cell survival and proliferation in neonatal piglets. Endocrinology 2005;146:22–32.
35. Benjamin MA, McKay DM, Yang PC, Cameron H, Perdue MH. Glucagon-like peptide-2 enhances intestinal epithelial barrier function of both transcellular and paracellular pathways in the mouse. Gut 2000;47:112–119.
36. Cameron HL, Perdue MH. Stress impairs murine intestinal barrier function: improvement by glucagon-like peptide-2. J Pharmacol Exp Ther 2005;314:214–220.
37. Hadjiyanni I, Li KK, Drucker DJ. Glucagon-like peptide-2 reduces intestinal permeability but does not modify the

onset of type 1 diabetes in the nonobese diabetic mouse. Endocrinology 2009;150:592–599.

38. Dong CX, Zhao W, Solomon C, Rowland KJ, Ackerley C, Robine S, Holzenberger M, Gonska T, Brubaker PL. The intestinal epithelial insulin-like growth factor-1 receptor links glucagon-like peptide-2 action to gut barrier function. Endocrinology 2014;155:370–379.
39. Brubaker PL, Izzo A, Hill M, Drucker DJ. Intestinal function in mice with small bowel growth induced by glucagon-like peptide-2. Am J Physiol 1997; 272:E1050–E1058.
40. Cheeseman CI, Tsang R. The effect of GIP and glucagon-like peptides on intestinal basolateral membrane hexose transport. Am J Physiol 1996; 271:G477–G482.
41. Hsieh J, Longuet C, Maida A, Bahrami J, Xu E, Baker CL, Brubaker PL, Drucker DJ, Adeli K. Glucagon-like peptide-2 increases intestinal lipid absorption and chylomicron production via CD36. Gastroenterology 2009;137, 997–1005, 1005 e1-4.
42. Lu WJ, Yang Q, Yang L, Lee D, D'Alessio D, Tso P. Chylomicron formation and secretion is required for lipid-stimulated release of incretins GLP-1 and GIP. Lipids 2012;47:571–580.
43. Ohlsson L, Kohan AB, Tso P, Ahren B. GLP-1 released to the mesenteric lymph duct in mice: effects of glucose and fat. Regul Pept 2014;189:40–45.
44. Ohneda K, Ulshen MH, Fuller CR, D'Ercole AJ, Lund PK. Enhanced growth of small bowel in transgenic mice expressing human insulin-like growth factor I. Gastroenterology 1997;112:444–454.
45. Simmons JG, Pucilowska JB, Lund PK. Autocrine and paracrine actions of intestinal fibroblast-derived insulin-like growth factors. Am J Physiol 1999;276:G817–G827.
46. Murali SG, Brinkman AS, Solverson P, Pun W, Pintar JE, Ney DM. Exogenous GLP-2 and IGF-I induce a differential intestinal response in IGF binding protein-3 and -5 double knockout mice. Am J Physiol Gastrointest Liver Physiol 2012;302:G794–G804.
47. Ney DM, Huss DJ, Gillingham MB, Kritsch KR, Dahly EM, Talamantez JL, Adamo ML. Investigation of insulin-like growth factor (IGF)-I and insulin receptor binding and expression in jejunum of parenterally fed rats treated with IGF-I or growth hormone. Endocrinology 1999; 140:4850–4860.
48. Grun D, Lyubimova A, Kester L, Wiebrands K, Basak O, Sasaki N, Clevers H, van Oudenaarden A. Single-cell messenger RNA sequencing reveals rare intestinal cell types. Nature 2015;525:251–255.
49. Yan KS, Gevaert O, Zheng GXY, Anchang B, Probert CS, Larkin KA, Davies PS, Cheng ZF, Kaddis JS, Han A, Roelf K, Calderon RI, Cynn E, Hu X, Mandleywala K, Wilhelmy J, Grimes SM, Corney DC, Boutet SC, Terry JM, Belgrader P, Ziraldo SB, Mikkelsen TS, Wang F, von Furstenberg RJ, Smith NR, Chandrakesan P, May R, Chrissy MAS, Jain R, Cartwright CA, Niland JC, Hong YK, Carrington J, Breault DT, Epstein J, Houchen CW, Lynch JP, Martin MG, Plevritis SK, Curtis C, Ji HP, Li L, Henning SJ, Wong MH, Kuo CJ. Intestinal enteroendocrine lineage cells possess homeostatic and injury-inducible stem cell activity. Cell Stem Cell 2017;21:78–90 e6.
50. Bahrami J, Yusta B, Drucker DJ. ErbB activity links the glucagon-like peptide-2 receptor to refeeding-induced adaptation in the murine small bowel. Gastroenterology 2010;138:2447–2456.
51. Scopelliti A, Cordero JB, Diao F, Strathdee K, White BH, Sansom OJ, Vidal M. Local control of intestinal stem cell homeostasis by enteroendocrine cells in the adult Drosophila midgut. Curr Biol 2014;24:1199–1211.
52. Sei Y, Feng J, Samsel L, White AO, Zhao X, Yun S, Citrin D, McCoy JP, Sundaresan S, Hayes MM, Merchant JL, Leiter AB, Wank SA. Mature enteroendocrine cells contributes to basal and pathological stem cell dynamics in the small intestine. Am J Physiol Gastrointest Liver Physiol 2018;315:G495–G510.
53. Pyke C, Heller RS, Kirk RK, Orskov C, Reedtz-Runge S, Kaastrup P, Hvelplund A, Bardram L, Calatayud D, Knudsen LB. GLP-1 receptor localization in monkey and human tissue: novel distribution revealed with extensively validated monoclonal antibody. Endocrinology 2014;155:1280–1290.
54. Richards P, Parker HE, Adriaenssens AE, Hodgson JM, Cork SC, Trapp S, Gribble FM, Reimann F. Identification and characterization of GLP-1 receptor-expressing cells using a new transgenic mouse model. Diabetes 2014; 63:1224–1233.
55. Campbell JE, Drucker DJ. Pharmacology, physiology, and mechanisms of incretin hormone action. Cell Metab 2013;17:819–837.
56. D'Alessio D. Is GLP-1 a hormone: whether and when? J Diabetes Investig 2016;7(Suppl 1):50–55.
57. D'Alessio DA. What if gut hormones aren't really hormones: DPP-4 inhibition and local action of GLP-1 in the gastrointestinal tract. Endocrinology 2011; 152:2925–2926.
58. Yusta B, Baggio LL, Koehler J, Holland D, Cao X, Pinnell LJ, Johnson-Henry KC, Yeung W, Surette MG, Bang KW, Sherman PM, Drucker DJ. GLP-1R agonists modulate enteric immune responses through the intestinal intraepithelial lymphocyte GLP-1R. Diabetes 2015; 64:2537–2549.
59. Kaelberer MM, Buchanan KL, Klein ME, Barth BB, Montoya MM, Shen X, Bohórquez DV. A gut-brain neural circuit for nutrient sensory transduction. Science 2018; 361:6408.
60. Mannon PJ. Peptide YY as a growth factor for intestinal epithelium. Peptides 2002;23:383–388.
61. Mannon PJ, Kanungo A, Mannon RB, Ludwig KA. Peptide YY/neuropeptide Y Y1 receptor expression in the epithelium and mucosal nerves of the human colon. Regul Pept 1999;83:11–19.
62. Wharton J, Gordon L, Byrne J, Herzog H, Selbie LA, Moore K, Sullivan MH, Elder MG, Moscoso G, Taylor KM, et al. Expression of the human neuropeptide tyrosine Y1 receptor. Proc Natl Acad Sci U S A 1993; 90:687–691.
63. Bulbring E, Lin RC. The effect of intraluminal application of 5-hydroxytryptamine and 5-hydroxytryptophan on peristalsis; the local production of 5-HT and its release in

relation to intraluminal pressure and propulsive activity. J Physiol 1958;140:381–407.
64. Bulbring E, Crema A. The release of 5-hydroxytryptamine in relation to pressure exerted on the intestinal mucosa. J Physiol 1959;146:18–28.
65. Blackshaw LA, Grundy D. Effects of 5-hydroxytryptamine on discharge of vagal mucosal afferent fibres from the upper gastrointestinal tract of the ferret. J Auton Nerv Syst 1993;45:41–50.
66. Gershon MD, Tack J. The serotonin signaling system: from basic understanding to drug development for functional GI disorders. Gastroenterology 2007;132:397–414.
67. Bellono NW, Bayrer JR, Leitch DB, Castro J, Zhang C, O'Donnell TA, Brierley SM, Ingraham HA, Julius D. Enterochromaffin cells are gut chemosensors that couple to sensory neural pathways. Cell 2017;170:185–198 e16.
68. Enck P, Aziz Q, Barbara G, Farmer AD, Fukudo S, Mayer EA, Niesler B, Quigley EM, Rajilic-Stojanovic M, Schemann M, Schwille-Kiuntke J, Simren M, Zipfel S, Spiller RC. Irritable bowel syndrome. Nat Rev Dis Primers 2016;2:16014.
69. Faure C, Patey N, Gauthier C, Brooks EM, Mawe GM. Serotonin signaling is altered in irritable bowel syndrome with diarrhea but not in functional dyspepsia in pediatric age patients. Gastroenterology 2010;139:249–258.
70. Camilleri M, Boeckxstaens G. Dietary and pharmacological treatment of abdominal pain in IBS. Gut 2017;66:966–974.
71. Bohórquez DV, Shahid RA, Erdmann A, Kreger AM, Wang Y, Calakos N, Wang F, Liddle RA. Neuroepithelial circuit formed by innervation of sensory enteroendocrine cells. J Clin Invest 2015;125:782–786.
72. McCoy ES, Taylor-Blake B, Street SE, Pribisko AL, Zheng J, Zylka MJ. Peptidergic CGRPalpha primary sensory neurons encode heat and itch and tonically suppress sensitivity to cold. Neuron 2013;78:138–151.
73. Wall NR, Wickersham IR, Cetin A, De La Parra M, Callaway EM. Monosynaptic circuit tracing in vivo through Cre-dependent targeting and complementation of modified rabies virus. Proc Natl Acad Sci U S A 2010;107:21848–21853.
74. Schwartz GJ. The role of gastrointestinal vagal afferents in the control of food intake: current prospects. Nutrition 2000;16:866–873.
75. Han W, Tellez LA, Perkins MH, Perez IO, Qu T, Ferreira J, Ferreira TL, Quinn D, Liu ZW, Gao XB, Kaelberer MM, Bohórquez DV, Shammah-Lagnado SJ, de Lartigue G, de Araujo IE. A neural circuit for gut-induced reward. Cell 2018;175:887–888.
76. Bertrand PP. The cornucopia of intestinal chemosensory transduction. Front Neurosci 2009;3:48.
77. Tsubouchi S, Leblond CP. Migration and turnover of entero-endocrine and caveolated cells in the epithelium of the descending colon, as shown by radioautography after continuous infusion of 3H-thymidine into mice. Am J Anat 1979;156:431–451.
78. Hamamichi R, Asano-Miyoshi M, Emori Y. Taste bud contains both short-lived and long-lived cell populations. Neuroscience 2006;141:2129–2138.
79. Hinds JW, Hinds PL, McNelly NA. An autoradiographic study of the mouse olfactory epithelium: evidence for long-lived receptors. Anat Rec 1984;210:375–383.
80. Gribble FM, Reimann F. Enteroendocrine cells: chemosensors in the intestinal epithelium. Annu Rev Physiol 2016;78:277–299.
81. Chandra R, Wang Y, Shahid RA, Vigna SR, Freedman NJ, Liddle RA. Immunoglobulin-like domain containing receptor 1 mediates fat-stimulated cholecystokinin secretion. J Clin Invest 2013;123:3343–3352.

Received September 24, 2018. Accepted January 22, 2019.

Correspondence
Address correspondence to: Rodger A. Liddle, MD, Duke University Medical Center, 1033A Genome Science Research Building 1, 905 LaSalle Street, Durham, North Carolina 27710. e-mail: rodger.liddle@duke.edu; fax: (919) 684-4983.

Acknowledgments
The author thanks Drs Steven Vigna and Diego Bohórquez for thoughtful review of the manuscript, and Dr Rashmi Chandra for the enteroendocrine cell photomicrograph.

Conflicts of interest
The author discloses no conflicts.

Funding
This work was supported by National Institutes of Health grants DK098796 and 109368, and the Department of Veterans Affairs grant BX002230.