Crohn's & Colitis 360, 2021, **3**, 1–7
https://doi.org/10.1093/crocol/otab040
Advance access publication 7 July 2021
Review Article

Pain in Inflammatory Bowel Disease: Optogenetic Strategies for Study of Neural–Epithelial Signaling

Sarah A. Najjar, PhD* and Kathryn M. Albers, PhD

Department of Neurobiology and Pittsburgh Center for Pain Research, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA
*Present address: Department of Pediatrics, Columbia University Medical Center, New York, New York, USA

Address correspondence to: Kathryn M. Albers, PhD, Department of Neurobiology, University of Pittsburgh, 200 Lothrop Street, Pittsburgh, PA 15216, USA (kaa2@pitt.edu).

Abdominal pain is common in patients with active inflammation of the colon but can persist even in its absence, suggesting other mechanisms of pain signaling. Recent findings suggest colon epithelial cells are direct regulators of pain-sensing neurons. Optogenetic activation of epithelial cells evoked nerve firing and pain-like behaviors. Inhibition of epithelial cells caused the opposite effect, reducing responses to colon distension and inflammatory hypersensitivity. Thus, epithelial cells alone can regulate the activation of pain circuits. Future goals are to define the anatomical and cellular mechanisms that underlie epithelial–neural pain signaling and how it is altered in response to colon inflammation.

Lay Summary

New studies using optogenetic mouse models support a role for epithelial cells that line the colon as important contributors to pain signaling. Increasing epithelial activity was found to stimulate pain-sensing neurons, whereas inhibition of epithelial cells reduced pain.

Key Words: optogenetics, colon, sensory neuron, hypersensitivity, mouse, channelrhodopsin

Introduction

Chronic abdominal pain is a major burden for patients with inflammatory bowel disease (IBD) and other conditions of colitis.[1] Histological healing of the mucosal lining is associated with disease remission and reduced pain and is the desired treatment outcome. However, it is not uncommon for patients who exhibit healing and reduced inflammation to continue to experience pain,[2, 3] suggesting factors other than the inflammatory milieu drive pain signaling. Such factors may include changes in the central nervous system (CNS; ie, central facilitation) that affect the psychological state of patients, leading to increased anxiety, catastrophizing, and hypervigilance.[4] Persistent pain in remission may also reflect yet-to-be-defined changes that occur in the peripheral tissue that sensitizes nerve fibers within the colon. In this regard, here we review recent studies that support a role for the lining epithelial cells of the colon as contributors to pain signaling in the absence of elevated inflammation.[5, 6] Analysis of optogenetic mouse models that allow specific targeting of the epithelium has shown that increasing the activity of epithelial cells is sufficient to activate pain-sensing neurons and cause an increase in behavioral pain responses.[5] In other studies, it was found that decreasing activity of epithelial cells reduced pain associated with colon inflammation.[6] These findings contribute to a growing body of research that is defining how epithelial cells communicate with nerve fibers, glial cells, and immune cells within the gut and the functional significance of these interactions. They also support the prioritization of treatment strategies for IBD that promote repair of the epithelial lining as a means to reduce pain. Topics to be discussed are Pain in IBD, Epithelial Cell Types of the Colon, Nerve Innervation of the Colon, Optogenetic Models to Study Epithelial–Neural Communication in the Colon, Activation of Epithelial Cells and Nerve Activity, Inhibition of Epithelial Cells and Nerve Activity, and Future Directions for Research.

Pain in IBD

The complex pathophysiology of IBD, for example, changes in gut permeability, immune signaling, and stress level, has hindered detailed understanding of mechanisms underlying its etiology and the pain signaling pathways engaged. Preclinical and clinical studies have suggested a process in which damage to the epithelial lining leads to infiltration of bacteria that causes an inflammatory immune cell infiltration.[7–10] Release of cytokines and other neuroactivators (adenosine triphosphate [ATP], chemokines, interferon, dopamine, and proteases) from immune cells, which can bind receptor proteins on terminals of sensory neurons within the colon, is postulated to increase neuronal activity and stimulate pain signaling circuits.[11–13] Paradoxically, pain can be experienced in both active and quiescent IBD; patients with pain often exhibit inflammation,[11] but patients with no evidence of epithelial damage or elevated inflammation may report pain as well.[3] The disconnect between inflammation and pain, as well as a new understanding of the impact of the microbiome profile on pain,[14–16] suggests pain may involve changes unrelated to immune signaling. In this regard, recent studies of epithelial

Received for publications: March 26, 2021. **Editorial Decision:** May 17, 2021
© The Author(s) 2021. Published by Oxford University Press on behalf of Crohn's & Colitis Foundation.
This is an Open Access article distributed under the terms of the Creative Commons Attribution-Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

cells of the skin[17] and colon[5, 6] using optogenetic approaches suggest a direct role for the epithelium in driving neural activity and activation of pain pathways.

The bowel is richly innervated by neurons that have cell bodies located in both extrinsic (spinal dorsal root ganglia [DRG] and autonomic) and intrinsic (myenteric and submucosal) ganglionic structures. Pain is perceived in response to the activity of extrinsic sensory neurons within the DRG.[18] These sensory neurons project to all regions of the gut and function as both homeostatic sensors and as transducers to the CNS (spinal cord and brain) sensations of pain, bloating, fullness, and urgency.[19] In addition to afferent activity, sensory nerves also have efferent functions. Under inflammatory conditions, the neuronal release of neuropeptides (eg, calcitonin gene-related peptide [CGRP], substance P) can activate immune cells (eg, macrophages, mast cells), which lead to increased cytokine and protease release, driving a circular inflammatory cycle termed neurogenic inflammation.[12, 20] Such neural–immune signaling, in both peripheral tissues and spinal cord, is thought to be a significant factor in maintaining chronic pain conditions.

In addition to neural–immune signaling, recent studies in the skin have revealed a direct role for epithelial cells (keratinocytes) in neural activation and pain signaling.[17, 21] Keratinocyte activity was shown to cause action potential firing in diverse subtypes of cutaneous afferents, ranging from large myelinated fibers to unmyelinated nociceptor subtypes. These findings have implications for understanding the mechanisms that underlie the pain, itch, and changes in thermal sensation that can accompany chronic inflammatory conditions of the skin. They also lead to the question of whether epithelial cells of other barrier tissues, such as the colon, can similarly drive neural activity and influence pain signaling. Here we review findings that have shown, using optogenetic strategies in the mouse colon, that colonic epithelial lining cells do indeed have an active and independent role in visceral pain signaling.

Epithelial Cell Types of the Colon

Colon epithelial cells are diverse in chemical phenotype and function and have long been known to have a critical role in the pathophysiology of visceral inflammatory diseases. They form a simple columnar epithelial lining that absorbs water and nutrients that facilitate the formation and removal of fecal material. Epithelial cells undergo a constant, highly regulated renewal of constituent secretory and signaling cell types. Secretory cells include Goblet cells, which are enriched in the colon and produce a thick layer of protective mucus that is the first line of immune defense, being impenetrable to bacteria.[22, 23] Tuft cells and numerous subtypes of enteroendocrine cells (EECs; eg, L cells, enterochromaffin cells [ECs], D cells) are more specialized, secreting peptide/hormone transmitters such as cholecystokinin, peptide YY, and somatostatin.[24–28] Epithelial cells also synthesize and release classical neurotransmitters such as glutamate,[29] 5-hydroxytryptamine (5-HT, serotonin),[30] acetylcholine,[31] and ATP,[32] all of which can stimulate neuronal activity.[33] In this regard, EC cells are of particular interest as they function as electrically excitable chemosensors.[34] ECs express the transient receptor protein ankyrin repeat 1 ion channel,[35] agonists of which include chemical irritants such as mustard oil.[36, 37] ECs also release 5-HT that, through activation of 5-HT3 receptors on sensory afferents, can evoke changes in neurons that lead to sensitization, which may cause pain associated with irritable bowel syndrome (IBS) and IBD.[38]

Nerve Innervation of the Colon

Nerve innervation of the colon is comprised of intrinsic (enteric) and extrinsic neuron populations. Intrinsic neurons of the colon have cell bodies in ganglia located between submucosal and myenteric smooth muscle layers and are quite diverse in phenotype.[39, 40] Intrinsic neurons have complex anatomical and functional connections with surrounding glial cells, interstitial cells of Cajal, and sympathetic and parasympathetic neurons (autonomic input), which allow coordinated regulation of colon motility and movement of gut contents.[41]

Extrinsic sensory neurons (primary afferents) convey sensory information from the colon to the CNS and are also diverse in phenotype.[42] Cell bodies of extrinsic neurons are located in DRG that is positioned laterally along with thoracolumbar and lumbosacral spinal levels. In the colon, anterograde tracing studies show branching of sensory nerves around myenteric ganglia, circular smooth muscle layers (but rarely to longitudinal muscle), the submucosa, and mucosal layers.[40] Immunolabeling also shows nerve fibers that contain the transient receptor potential vanilloid type 1 (TRPV1) ion channel and the neuropeptide CGRP to be closely associated with overlying epithelial cells.[5, 40] Interestingly, some epithelial EECs exhibit specialized cytoplasmic processes known as neuropods.[43] Electron microscopic and viral tracing studies have shown neuropod containing EECs form synaptic-like connections with local neural fibers, providing a means for fast neurotransmission between the epithelial layer and the nervous system.[44]

Numerous studies have linked dysregulation of sensory neuron activity to IBD and functional gastrointestinal disorders (eg, IBS).[3, 16, 20] Electrophysiological and molecular properties of colon sensory neurons indicate a functional role as nociceptors, for example, they are activated in response to colon distension and express inflammatory neuropeptides (eg, CGRP) and the capsaicin receptor TRPV1.[12, 20, 45] Phenotypic changes in sensory afferents, for example, changes in ion channel gene expression and membrane properties, are also considered factors in the development and transition to chronic visceral pain.[46–49] Sensory neuron communication with sympathetic and parasympathetic systems through spinal pathways, which are regulators of bowel motility, is also a possible means by which disturbance in normal bowel function could lead to hypersensitivity.[50, 51]

Optogenetic Models to Study Epithelial–Neural Communication in the Colon

While there is evidence of functional communication between sensory neurons and epithelial cells, mechanisms underlying this signaling have been difficult to define because of the intimate association of nerves with epithelial, immune, and glial cell types. To address this complexity, optogenetic models to allow specific activation of epithelial cells alone were developed.[5] In these studies, Cre/Lox recombination technology was used to drive expression of the excitatory channelrhodopsin (ChR2) protein or the inhibitory archaerhodopsin (Arch) in colon epi-

thelial cells. ChR2 is a blue light-activated nonselective cation (Ca^{2+}, Na^{+}) channel that enables depolarization of the cell, whereas Arch is a yellow light-activated proton (H^{+}) pump that causes cell hyperpolarization. Hyperpolarization inhibits cell activity by blocking the influx of divalent cations, especially Ca^{2+}, which requires a negative membrane potential (which is normally maintained by K^{+} and Na^{+} channel activity). To target each opsin to colon epithelial cells, mice that express Cre recombinase under the control of the villin gene were crossed with mice with a floxed transcriptional stop element upstream of gene sequences encoding either ChR2-YFP or Arch-YFP fusion proteins. Because villin, an actin-binding protein, is expressed in all lining epithelial cells of the colon, all types (enterocytes, goblet cells, EECs, etc.) express the selected opsin.

Activation of Epithelial Cells and Nerve Activity

To determine if epithelial stimulation could engage neural signaling, sensory neuron activity was examined in an ex vivo open colon preparation from mice that express ChR2 in colon epithelial cells (Vil-ChR2 mice; Figure 1A). In response to blue light (473 nm) stimulation of epithelial cells, teased fiber analysis of the pelvic nerve showed that nearly half of all fibers fired high-frequency trains of action potentials, often in patterns similar to those evoked by mechanical stimulation of the colon surface.[5] In addition, the response of 70% of the epithelial-activated neurons showed a decrease in firing frequency when assayed in the presence of purinergic receptor (P2X and P2Y) antagonists, supporting the role of ATP and/or UTP as major regulators of epithelial–sensory neuron signaling in the colon.

To determine if epithelial stimulation could engage neural circuitry in vivo, the effect of ChR2-mediated epithelial activation on nerve activity was assessed by recording visceromotor behavioral responses (VMRs), which are a surrogate for visceral pain-like behavior, in Vil-ChR2 mice. Colon epithelium was stimulated by the insertion of a blue light fiber optic probe into the colorectum of a lightly anesthetized mouse. Blue light illumination of the colon epithelial cells evoked VMRs, as measured by electromyographic recordings of the abdominal musculature, to a similar extent as those recorded in response to balloon distension (stretching) of the colon (Figure 1B and C).[5] That ChR2-mediated activity of epithelial cells alone is sufficient to evoke pain-like behaviors shows the capability of the epithelium to directly engage neural circuitry and supports the hypothesis that visceral hypersensitivity results, at least in part, from functional changes in epithelial–neuronal communication.

Inhibition of Epithelial Cells and Nerve Activity

Stimulation of the colon epithelium engages neural circuits, suggesting that a reduction in epithelial activity might inhibit neuronal firing and in so doing, reduce visceral nociception (VMRs). Studies done using mice that express the inhibitory yellow light-activated Arch-EGFP protein in colon epithelial cells (Vil-Arch mice) showed this to be the case[6] (Figure 2A). The ability of Arch-mediated inhibition of epithelial cells to block pain was assessed by in vivo measure of VMRs in response to colorectal distension in the presence or absence of Arch stimulation. Epithelial inhibition was produced using a yellow laser (589 nm) fiber optic probe equipped with a balloon distension device inserted into the colorectum. In Vil-Arch mice, but not control mice, Arch-mediated inhibition of

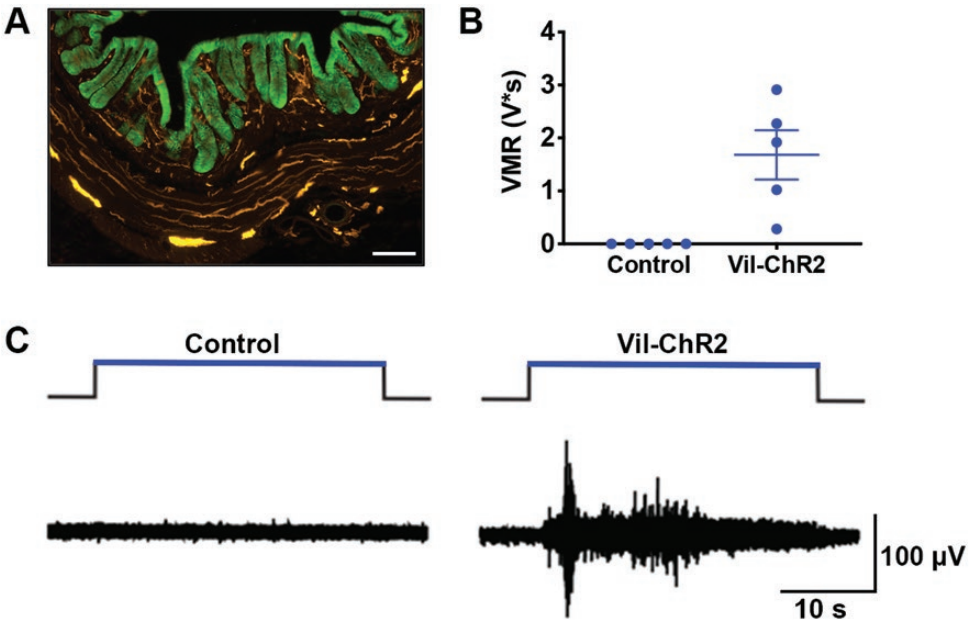

Figure 1. Optogenetic activation of the colon epithelium initiates nociceptive responses. A, The excitatory channelrhodopsin (ChR2) is conjugated to a yellow fluorescent protein (YFP) to enable visualization. ChR2-YFP (green) expression is restricted to the colon epithelium in Vil-ChR2 mice. Nerve fibers throughout colon layers are labeled with the pan-neuronal marker PGP9.5 (yellow). B, Visceromotor responses (VMRs) to blue light stimulation of the colon lumen were measured in control littermate mice (not expressing ChR2) and Vil-ChR2 mice. Control mice did not display VMRs to laser, whereas Vil-ChR2 mice displayed robust VMRs, indicating nociceptive responses. C, Example electromyographic recordings of VMR in control and Vil-ChR2 mice in response to blue light stimulation. Scale bar = 100 µM (A). Adapted with permission from Ref. [5].

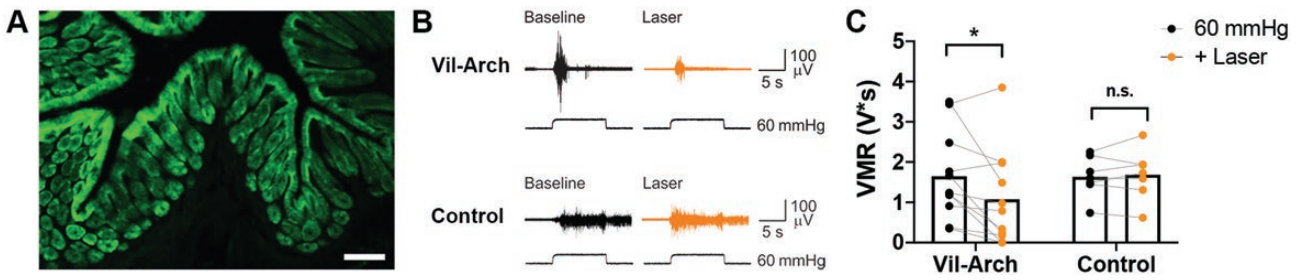

Figure 2. Optogenetic inhibition of the colon epithelium reduces visceromotor responses (VMRs) to colorectal distension (CRD). A, The inhibitory archaerhodopsin (Arch) is conjugated to an enhanced green fluorescent protein (EGFP) to enable visualization. Arch-EGFP (green) is specifically expressed in colon epithelial cells under the villin-Cre driver in Vil-Arch mice. B, VMRs to 60 mmHg CRD were recorded in Vil-Arch and control littermate mice before (Baseline) and during yellow light application to the colon lumen (+ Laser). C, Yellow light significantly reduced the VMR to CRD in Vil-Arch mice ($n = 11$; $^*P < .05$) but not in control littermate mice ($n = 7$; $P = .93$). From Ref. [6]; for permission to reuse, please contact Wolters Kluwer Health, Inc.

colon epithelial cells diminished VMRs in response to colon distension by 76% (Figure 2B and C). Importantly, in mice with colitis induced by dextran sodium sulfate (DSS; 3% in drinking water), Arch-mediated inhibition of the epithelium reduced VMRs by 55%.[6] Thus, inhibition of colon epithelial cells reduced pain-like responses to distension in both healthy mice and in mice with DSS-induced inflammation.

How, mechanistically, Arch-mediated inhibition and cell hyperpolarization cause a reduction in colon nociception is as yet unclear. For neurons, light activation of Arch and subsequent hyperpolarization are known to inhibit action potential firing by blocking Ca^{2+} influx.[52] For nonneuronal epithelial cells that do not have action potential events, a similar block in Ca^{2+} signaling is predicted to occur, which could functionally decrease a distension-mediated (Ca^{2+} dependent) release of ATP (or other neuromodulators). Reduced extracellular ATP would reduce the activity of purinergic receptors expressed on extrinsic sensory terminals and inhibit firing.[5, 32] Arch activity could also impact 5-HT signaling, by inhibition of the electrically excitable ECs, which release 5-HT in an activity-dependent manner.[34, 53]

Future Directions in Research

That experimentally induced changes in the activity of epithelial cells alone can either generate or block activity in sensory afferents strongly support a role for colon epithelial cells in pain signaling (Figure 3). Breakdown in mucosal permeability, changes in the mucus barrier, or alterations in products produced by commensal bacteria, all may impact epithelial properties and in so doing change neural activity. In the context of IBD, most studies of epithelial cells have focused on disruption in the epithelial barrier, enabling bacterial infiltration leading to immune infiltration and resultant neural sensitization.[9] However, the findings described here suggest the epithelium itself can be a regulator of neural sensitization in the absence of barrier disruption, suggesting a more direct role in pain signaling.

Further research to define the changes that occur in colon epithelial cells in IBD and to determine how these changes relate to neuronal sensitization and hypersensitivity is needed. As visceral pain is difficult to treat and can persist long after resolution of the precipitating injury or disease, changes in epithelial properties have been considered as a driver of sensitization.[9, 10, 54] Indeed, studies showed that bladder epithelial cells from patients with interstitial cystitis pain were more sensitive to pharmacological stimulation than epithelial cells derived from control patients.[55] Colon epithelial cells may also exhibit changes in response to inflammation that could alter communication with surrounding nerve fibers. For example, biopsies and feces from IBD patients show increased serine protease activity and a 100-fold elevation in active thrombin that is derived, at least in part, from the epithelium.[54, 56–60] Proteases can act on receptors expressed by sensory neurons that can sensitize ion channels expressed on afferent terminals (eg, TRPV1)[61] and in so doing, may serve as a link between IBD pathogenesis and pain.[62, 63]

Whether specialized subtypes of epithelial cells preferentially communicate with neurons is also unknown. EEC cells, which express neurotransmitters critical to gut function (5-HT) and activate neurons, appear to be central players.[34] Studies in animal models and of human colon tissue have shown that inflammation impacts EEC activity and phenotype,[64] supporting the idea that inflammation alters normal communication between EECs and sensory afferents. Future studies could employ additional Cre-lines to target specific EEC subtypes and examine how activation of each affects nociception under normal and inflammatory conditions. Detailed understanding of the neurotransmitters and receptors that underlie epithelial–neural signaling and how immune and glial cells factor into this signaling are also needed areas of investigation.[65]

Another area of intense interest is to define the types of sensory afferents that are engaged by colon epithelial activation. This can be accomplished using the expanding list of mouse strains that express genetically encoded calcium indicators (eg, GCaMPs) and voltage indicators that allow real-time imaging of nerve activity. Defining afferent subtypes would allow the development of more refined, pharmacologic or natural product (peppermint) approaches that target specific afferent types and receptors, for example, TRP channel receptors (TRPM8[66]), cannabinoid receptors.[67] Defining afferent pathways can also reveal their input and circuitry within the spinal cord and brain, broadening targets for treatment. In this regard, recent work has shown that sensory afferents activate parasympathetic pathways and that sympathetic input to the colon can evoke calcium transients in lining epithelial cells.[50, 51]

Finally, although changes in epithelial cell properties can activate or inhibit neuron activity, it is not clear if this occurs via direct or indirect communication. Direct fast communication would be predicted to occur via synaptic structures, which have been shown for EECs and nerves.[43] Whether these connections transmit pain signals and if similar relationships exist with other epithelial cell types are still undefined. The role of intermediate cell types such as immune cells and glial cells and how they impact epithelial–neural transmission are

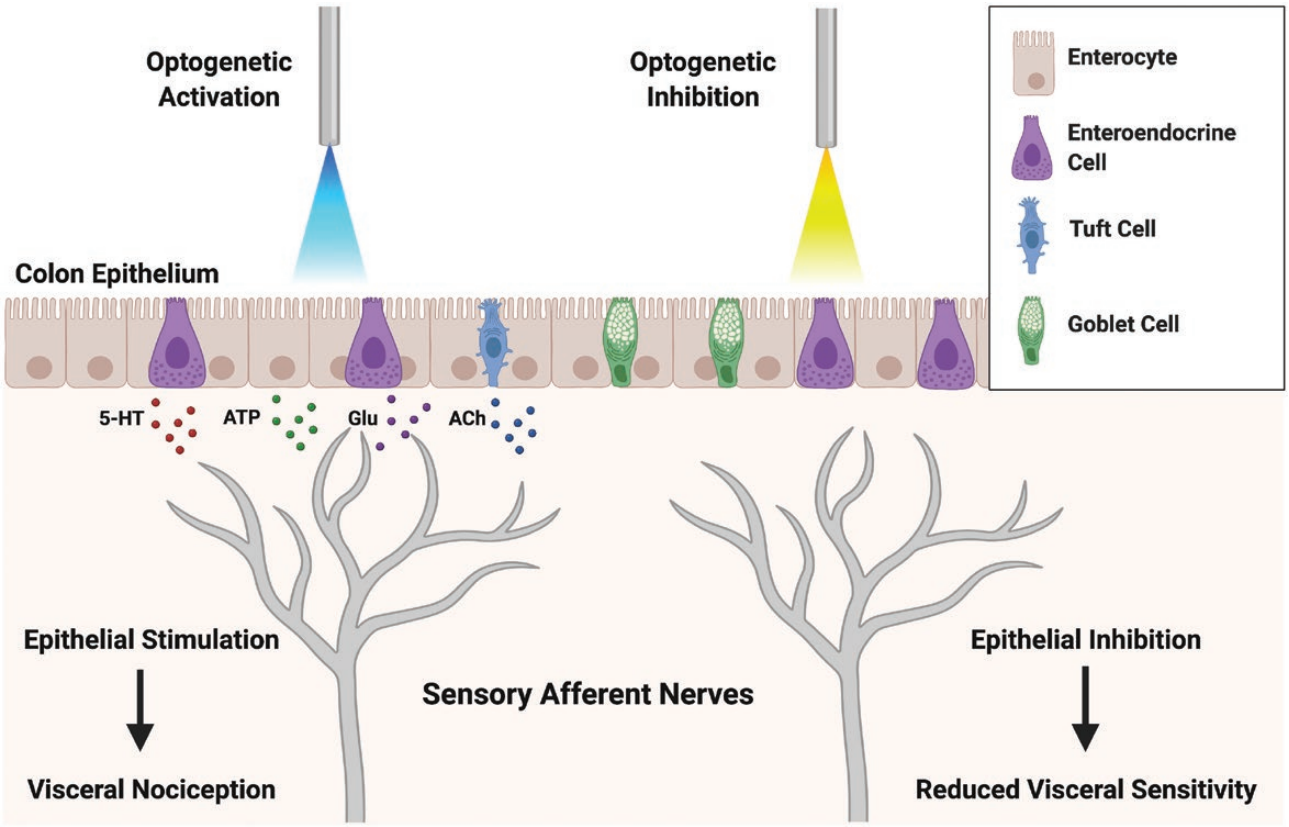

Figure 3. Epithelial cell regulation of neural signaling in the colon. The colon is comprised of a heterogeneous cell population including enterocytes, enteroendocrine cells (EECs), goblet cells, and tuft cells. These cells release neurotransmitters such as ATP (enterocytes and EECs), acetylcholine (ACh; tuft cells), serotonin (5-HT), and glutamate (Glu; EECs). These are hypothesized to act on local sensory afferent nerve fibers, which express receptors for all of the aforementioned transmitters. Sensory afferent nerve fibers convey pain stimuli from the colon and epithelial-released neurotransmitters can sensitize these fibers. Optogenetic activation of the colon epithelium leads to neurotransmitter release that activates sensory afferent fibers, leading to a nociceptive response. Optogenetic inhibition of the colon epithelium during distension of the colon reduces epithelial-released neurotransmitters, diminishing the sensory afferent responses and therefore reducing visceral sensitivity. Graphic created with BioRender (BioRender.com).

other areas of interest. Although many questions remain, defining how epithelial–neural communication occurs in the colon is essential to fully understand how pain develops and for the design of new strategies to relieve pain.

Funding

This work was supported by funding from the National Institutes of Health (NIAMS AR069951, NIDDK DK124955, OD DK122798).

Conflicts of Interest

None declared.

Data Availability

Data in this review are available in doi:10.1523/JNEUROSCI.0837-18.2018 and doi:10.1097/j.pain.0000000000002110.

References

1. Coates MD, Lahoti M, Binion DG, et al. Abdominal pain in ulcerative colitis. *Inflamm Bowel Dis.* 2013;19(10):2207–2214.
2. Coates MD, Johri A, Gorrepati VS, et al. Abdominal pain in quiescent inflammatory bowel disease. *Int J Colorectal Dis.* 2021;36(1):93–102.
3. Bielefeldt K, Davis B, Binion DG. Pain and inflammatory bowel disease. *Inflamm Bowel Dis.* 2009;15(5):778–788.
4. Szigethy EM, Allen JI, Reiss M, et al. White Paper AGA: the impact of mental and psychosocial factors on the care of patients with inflammatory bowel disease. *Clin Gastroenterol Hepatol.* 2017;15(7):986–997.
5. Makadia PA, Najjar SA, Saloman JL, et al. Optogenetic activation of colon epithelium of the mouse produces high-frequency bursting in extrinsic colon afferents and engages visceromotor responses. *J Neurosci.* 2018;38(25):5788–5798.
6. Najjar SA, Ejoh LL, Loeza-Alcocer E, et al. Optogenetic inhibition of the colon epithelium reduces hypersensitivity in a mouse model of inflammatory bowel disease. *Pain.* 2021;162(4):1126–1134.
7. Allaire JM, Crowley SM, Law HT, et al. The intestinal epithelium: central coordinator of mucosal immunity. *Trends Immunol.* 2018;39(9):677–696.
8. Grundy L, Erickson A, Brierley SM. Visceral pain. *Annu Rev Physiol.* 2019;81:261–284.
9. Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. *Nat Rev Immunol.* 2014;14(3):141–153.
10. Roda G, Sartini A, Zambon E, et al. Intestinal epithelial cells in inflammatory bowel diseases. *World J Gastroenterol.* 2010;16(34):4264–4271.

11. Akbar A, Walters JR, Ghosh S. Review article: visceral hypersensitivity in irritable bowel syndrome: molecular mechanisms and therapeutic agents. *Aliment Pharmacol Ther.* 2009;30(5):423–435.
12. Esquerre N, Basso L, Defaye M, et al. Colitis-induced microbial perturbation promotes postinflammatory visceral hypersensitivity. *Cell Mol Gastroenterol Hepatol.* 2020;10(2):225–244.
13. Sharkey KA, Kroese AB. Consequences of intestinal inflammation on the enteric nervous system: neuronal activation induced by inflammatory mediators. *Anat Rec.* 2001;262(1):79–90.
14. Labus JS, Osadchiy V, Hsiao EY, et al. Evidence for an association of gut microbial Clostridia with brain functional connectivity and gastrointestinal sensorimotor function in patients with irritable bowel syndrome, based on tripartite network analysis. *Microbiome.* 2019;7(1):45.
15. Lai NY, Musser MA, Pinho-Ribeiro FA, et al. Gut-innervating nociceptor neurons regulate Peyer's patch microfold cells and SFB levels to mediate salmonella host defense. *Cell.* 2020;180(1):33–49.e22.
16. Mayer EA, Labus JS, Tillisch K, et al. Towards a systems view of IBS. *Nat Rev Gastroenterol Hepatol.* 2015;12(10):592–605.
17. Baumbauer KM, DeBerry JJ, Adelman PC, et al. Keratinocytes can modulate and directly initiate nociceptive responses. *Elife.* 2015;4:1–14.
18. Wang G, Tang B, Traub RJ. Differential processing of noxious colonic input by thoracolumbar and lumbosacral dorsal horn neurons in the rat. *J Neurophysiol.* 2005;94(6):3788–3794.
19. Brierley SM, Hibberd TJ, Spencer NJ. Spinal afferent innervation of the colon and rectum. *Front Cell Neurosci.* 2018;12:467.
20. Gebhart GF, Bielefeldt K. Physiology of visceral pain. *Compr Physiol.* 2016;6(4):1609–1633.
21. Sadler KE, Moehring F, Stucky CL. Keratinocytes contribute to normal cold and heat sensation. *Elife.* 2020;9:1–14.
22. Johansson ME, Hansson GC. Immunological aspects of intestinal mucus and mucins. *Nat Rev Immunol.* 2016;16(10):639–649.
23. Nystrom EEL, Martinez-Abad B, Arike L, et al. An intercrypt subpopulation of goblet cells is essential for colonic mucus barrier function. *Science.* 2021;372(6539).
24. Rindi G, Leiter AB, Kopin AS, et al. The "normal" endocrine cell of the gut: changing concepts and new evidences. *Ann N Y Acad Sci.* 2004;1014:1–12.
25. Bertrand PP. The cornucopia of intestinal chemosensory transduction. *Front Neurosci.* 2009;3(3):48.
26. Gunawardene AR, Corfe BM, Staton CA. Classification and functions of enteroendocrine cells of the lower gastrointestinal tract. *Int J Exp Pathol.* 2011;92(4):219–231.
27. Furness JB. Integrated neural and endocrine control of gastrointestinal function. *Adv Exp Med Biol.* 2016;891:159–173.
28. Fakhry J, Wang J, Martins P, et al. Distribution and characterisation of CCK containing enteroendocrine cells of the mouse small and large intestine. *Cell Tissue Res.* 2017;369(2):245–253.
29. Uehara S, Jung SK, Morimoto R, et al. Vesicular storage and secretion of L-glutamate from glucagon-like peptide 1-secreting clonal intestinal L cells. *J Neurochem.* 2006;96(2):550–560.
30. Gershon MD. 5-Hydroxytryptamine (serotonin) in the gastrointestinal tract. *Curr Opin Endocrinol Diabetes Obes.* 2013;20(1):14–21.
31. Klapproth H, Reinheimer T, Metzen J, et al. Non-neuronal acetylcholine, a signalling molecule synthezised by surface cells of rat and man. *Naunyn Schmiedebergs Arch Pharmacol.* 1997;355(4):515–523.
32. Burnstock G. Purine-mediated signalling in pain and visceral perception. *Trends Pharmacol Sci.* 2001;22(4):182–188.
33. Najjar SA, Davis BM, Albers KM. Epithelial-neuronal communication in the colon: implications for visceral pain. *Trends Neurosci.* 2020;43(3):170–181.
34. Bellono NW, Bayrer JR, Leitch DB, et al. Enterochromaffin cells are gut chemosensors that couple to sensory neural pathways. *Cell.* 2017;170(1):185–198.e16.
35. Nozawa K, Kawabata-Shoda E, Doihara H, et al. TRPA1 regulates gastrointestinal motility through serotonin release from enterochromaffin cells. *Proc Natl Acad Sci U S A.* 2009;106(9):3408–3413.
36. Fothergill LJ, Callaghan B, Rivera LR, et al. Effects of food components that activate TRPA1 receptors on mucosal ion transport in the mouse intestine. *Nutrients.* 2016;8(10):1–11.
37. Gonzalez-Cano R, Montilla-García Á, Perazzoli G, et al. Intracolonic mustard oil induces visceral pain in mice by TRPA1-dependent and -independent mechanisms: role of tissue injury and P2X receptors. *Front Pharmacol.* 2020;11:613068.
38. Mawe GM, Hoffman JM. Serotonin signalling in the gut—functions, dysfunctions and therapeutic targets. *Nat Rev Gastroenterol Hepatol.* 2013;10(8):473–486.
39. Drokhlyansky E, Smillie CS, Van Wittenberghe N, et al. The human and mouse enteric nervous system at single-cell resolution. *Cell.* 2020;182(6):1606–1622.e23.
40. Spencer NJ, Hu H. Enteric nervous system: sensory transduction, neural circuits and gastrointestinal motility. *Nat Rev Gastroenterol Hepatol.* 2020;17(6):338–351.
41. Graham KD, López SH, Sengupta R, et al. Robust, 3-dimensional visualization of human colon enteric nervous system without tissue sectioning. *Gastroenterology.* 2020;158(8):2221–2235.e5.
42. Hockley JRF, Taylor TS, Callejo G, et al. Single-cell RNAseq reveals seven classes of colonic sensory neuron. *Gut.* 2019;68(4):633–644.
43. Bohórquez DV, Shahid RA, Erdmann A, et al. Neuroepithelial circuit formed by innervation of sensory enteroendocrine cells. *J Clin Invest.* 2015;125(2):782–786.
44. Kaelberer MM, Rupprecht LE, Liu WW, et al. Neuropod cells: the emerging biology of gut-brain sensory transduction. *Annu Rev Neurosci.* 2020;43:337–353.
45. Spencer NJ, Kyloh MA, Travis L, Dodds KN. Sensory nerve endings arising from single spinal afferent neurons that innervate both circular muscle and myenteric ganglia in mouse colon: colon-brain axis. *Cell Tissue Res.* 2020;381(1):25–34.
46. Feng B, La JH, Schwartz ES, Gebhart GF. Irritable bowel syndrome: methods, mechanisms, and pathophysiology. Neural and neuro-immune mechanisms of visceral hypersensitivity in irritable bowel syndrome. *Am J Physiol Gastrointest Liver Physiol.* 2012;302(10):G1085–G1098.
47. Feng B, La JH, Schwartz ES, et al. Long-term sensitization of mechanosensitive and -insensitive afferents in mice with persistent colorectal hypersensitivity. *Am J Physiol Gastrointest Liver Physiol.* 2012;302(7):G676–G683.
48. Gold MS, Gebhart GF. Nociceptor sensitization in pain pathogenesis. *Nat Med.* 2010;16(11):1248–1257.
49. Verne GN, Sen A, Price DD. Intrarectal lidocaine is an effective treatment for abdominal pain associated with diarrhea-predominant irritable bowel syndrome. *J Pain.* 2005;6(8):493–496.
50. Smith-Edwards KM, Edwards BS, Wright CM, et al. Sympathetic input to multiple cell types in mouse and human colon produces region-specific responses. *Gastroenterology.* 2021;160(4):1208–1223.e4.
51. Smith-Edwards KM, Najjar SA, Edwards BS, et al. Extrinsic primary afferent neurons link visceral pain to colon motility through a spinal reflex in mice. *Gastroenterology.* 2019;157(2):522–536.e2.
52. Chow BY, Han X, Dobry AS, et al. High-performance genetically targetable optical neural silencing by light-driven proton pumps. *Nature.* 2010;463(7277):98–102.
53. Alcaino C, Knutson KR, Treichel AJ, et al. A population of gut epithelial enterochromaffin cells is mechanosensitive and requires Piezo2 to convert force into serotonin release. *Proc Natl Acad Sci U S A.* 2018;115(32):E7632–E7641.
54. Motta JP, Palese S, Giorgio C, et al. Increased mucosal thrombin is associated with Crohn's disease and causes inflammatory damage through protease-activated receptors activation. *J Crohns Colitis.* 2021;15(5):787–799.
55. Gupta GN, Lu SG, Gold MS, Chai TC. Bladder urothelial cells from patients with interstitial cystitis have an increased sensitivity to carbachol. *Neurourol Urodyn.* 2009;28(8):1022–1027.
56. Cenac N, Andrews CN, Holzhausen M, et al. Role for protease activity in visceral pain in irritable bowel syndrome. *J Clin Invest.* 2007;117(3):636–647.

57. Denadai-Souza A, Bonnart C, Tapias NS, et al. Functional proteomic profiling of secreted serine proteases in health and inflammatory bowel disease. *Sci Rep*. 2018;8(1):7834.
58. Róka R, Rosztóczy A, Leveque M, et al. A pilot study of fecal serine-protease activity: a pathophysiologic factor in diarrhea-predominant irritable bowel syndrome. *Clin Gastroenterol Hepatol*. 2007;5(5):550–555.
59. Rolland-Fourcade C, Denadai-Souza A, Cirillo C, et al. Epithelial expression and function of trypsin-3 in irritable bowel syndrome. *Gut*. 2017;66(10):1767–1778.
60. Motta JP, Denadai-Souza A, Sagnat D, et al. Active thrombin produced by the intestinal epithelium controls mucosal biofilms. *Nat Commun*. 2019;10(1):3224.
61. Sadeghi M, Erickson A, Castro J, et al. Contribution of membrane receptor signalling to chronic visceral pain. *Int J Biochem Cell Biol*. 2018;98:10–23.
62. Ceuleers H, Van Spaendonk H, Hanning N, et al. Visceral hypersensitivity in inflammatory bowel diseases and irritable bowel syndrome: the role of proteases. *World J Gastroenterol*. 2016;22(47):10275–10286.
63. Desormeaux C, Bautzova T, Garcia-Caraballo S, et al. Protease-activated receptor 1 is implicated in irritable bowel syndrome mediators-induced signaling to thoracic human sensory neurons. *Pain*. 2018;159(7):1257–1267.
64. Lomax AE, Linden DR, Mawe GM, Sharkey KA. Effects of gastrointestinal inflammation on enteroendocrine cells and enteric neural reflex circuits. *Auton Neurosci*. 2006;126–127:250–257.
65. Grubišić V, McClain JL, Fried DE, et al. Enteric glia modulate macrophage phenotype and visceral sensitivity following inflammation. *Cell Rep*. 2020;32(10):108100.
66. Chumpitazi BP, Kearns GL, Shulman RJ. Review article: the physiological effects and safety of peppermint oil and its efficacy in irritable bowel syndrome and other functional disorders. *Aliment Pharmacol Ther*. 2018;47(6):738–752.
67. Castro J, Garcia-Caraballo S, Maddern J, et al. Olorinab (APD371), a peripherally acting, highly selective, full agonist of the cannabinoid receptor 2, reduces colitis-induced acute and chronic visceral hypersensitivity in rodents. *Pain*. 2021. PMID: 33863856.