OPEN ACCESS
Journal of Innovative Optical Health Sciences
Vol. 16, No. 3 (2023) 2230013 (7 pages)

DOI: 10.1142/S1793545822300130

World Scientific
www.worldscientific.com

Application of optogenetics in the study of gastrointestinal motility: A mini review

Song Zhao*, Ting Zhang† and Weidong Tong*,‡
*Department of General Surgery, Gastric and Colorectal Surgery Division, Army Medical Center (Daping Hospital) Army Medical University Chongqing, P. R. China
†Department of General Surgery The 983th Hospital of Joint Logistic Support Force of People's Liberation Army Tianjin, P. R. China
‡vdtong@163.com

Received 3 August 2022
Accepted 13 September 2022
Published 19 October 2022

Disorders of gastrointestinal (GI) motility are associated with various symptoms such as nausea, vomiting, and constipation. However, the underlying causes of impaired GI motility remain unclear, which has led to variation in the efficacy of therapies to treat GI dysfunction. Optogenetics is a novel approach through which target cells can be precisely controlled by light and has shown great potential in GI motility research. Here, we summarized recent studies of GI motility patterns utilizing optogenetic devices and focused on the ability of opsins, which are genetically expressed in different types of cells in the gut, to regulate the excitability of target cells. We hope that our review of recent findings regarding optogenetic control of GI cells broadens the scope of application for optogenetics in GI motility studies.

Keywords: Gastrointestinal; motility; optogenetics.

1. Introduction

Although gastrointestinal (GI) motility, which is related to many physiological functions (such as peristalsis, digestion, and defecation) and pathological conditions (such as gastroparesis, constipation, and irritable bowel syndrome), is essential, its detailed mechanisms have not been fully elucidated.

The enteric nervous system (ENS) is thought to regulate GI motility by interacting with the smooth muscle cells (SMCs), interstitial cells of Cajal (ICC), and platelet-derived growth factor receptor (PDGFR) α^+ cells, which form a multicellular syncytium known as the SIP syncytium.[1] In addition, other types of cells, such as enteric glial cells

‡Corresponding author.

and enterochromaffin (EC) cells, are involved in GI motility patterns, though some aspects of this involvement remain unclear.[2–6] Furthermore, the central nervous system (CNS) participates in the modulation of GI motility by cross-talk with the ENS through the sympathetic and/or parasympathetic pathways; this modulation is known as the gut-brain axis.[3–7]

Contractions of the GI tract can be evoked by electrical field stimulation (EFS) and pharmacological stimulations, which are common physical and chemical methods for studying GI motility patterns. However, these methods suffer from insufficient spatiotemporal resolution to varying degrees.[8,9]

Optogenetics uses various approaches to genetically encode light-sensitive channels in the cells of interest (target cells) and modulates the functions of these cells when they are illuminated with certain wavelengths of light. It was primarily applied in neuroscience research in the early 21st century, and later expanded to research on oncotherapy, diabetes, cardiovascular disease, etc.[8–10] In the last decade, optogenetics has become increasingly popular in studies of GI motility due to the advantages of precise control over the excitability of target cells and high spatiotemporal resolution. In addition, with the refinement of the light delivery system, it has become possible to evoke and record GI motility in freely moving animals, which is important to fully elucidate the motility patterns of the gut in awake organisms.[11–13]

In this mini review, we summarized recent studies regarding GI motility patterns that used optogenetics and focused on the ingenious application of optogenetics to solve problems in GI motility research.

2. Optogenetics and Gi Motility

Broadly speaking, there are two classes of optogenetic devices: Sensors (or indicators), which emit optical signals, and actuators, which are driven by optical signals.[10,14] Back in 2003, Nagel *et al.* reported that channelrhodopsin-2 (ChR2) was a light-gated cation-selective membrane channel.[15] Over the next three years, four separate groups (Yawo *et al.*) reported their more in-depth studies of ChR2.[16–19] It wasn't until 2006 that the term 'optogenetics' first appeared in an article reported by Karl Deisseroth and coworker.[20] Since ChR2 is the most typical optogenetic tool, some researchers also use the term 'actuator' to refer to optogenetics.

2.1. *Sensors*

Sensors are broadly used to trace specific cell clusters or visualize changes in intracellular function. Over the past century, it has been difficult to label ICC or PDGFRα^+ cells with corresponding antibodies in mixed cell suspensions resulting from enzymatic dispersion of the GI tract due to the limited number of target cells and damage to extracellular epitopes.[2,21] In 2010, Ro and coworkers.[22] reported that they inserted the complementary DNA of the green fluorescent protein derived from a copepod genome (copGFP) at the Kit locus of mice. They found that copGFP colocalized with KIT immunofluorescence in the GI tract of Kit$^{+/\text{copGFP}}$ mice, revealing a powerful approach to tracking the ICC (kit$^+$ cells). Along with the ability to translate the physiological signals of cells into an optical signal, the application of this reporter mouse strain and fluorescence-activated cell sorting (FACS) enabled high-quality purification of the primary SMC, ICC, and PDGFRα^+ cells, ultimately facilitated the construction of the *Smooth Muscle Transcriptome Browser* and the understanding of the role of SIP in GI motility.[23,24]

Another type of sensor is genetically encoded calcium indicators (GECIs), including the enhanced green fluorescent protein-calmodulin-M13 (GCaMP) family.[25] These endogenous calcium indicators were expressed within the target cells, application of light stimuli increased fluorescence when they bound with Ca^{2+}. GECIs overcome the poor cellular specificity resulting from the loading of exogenous Ca^{2+} indicators and can be applied in wide-field microscopy systems.[26] Baker and coworkers crossbred Kit-Cre mice with GCaMP3 mice and obtained offspring mice (Kit-Cre-GCaMP3). After these mice were injected with tamoxifen, GCaMP3$^+$ cells were predominantly colocalized with c-Kit$^+$ cells in the jejunum of mice.[27] By utilizing this method, the characteristics of ICC, the well-known pacemaker cells of GI motility, could be investigated in depth.[28–31] GECIs have also been applied in imaging the Ca^{2+} levels of enteric motor neurons and glia, which are related to gut motility patterns.[32]

2.2. *Actuators*

Genetically expressed actuators encompass a variety of proteins, including but not limited to ion channels or pumps, G protein-coupled receptors (GPCRs), protein-protein interaction domains, and caging groups, which have been reviewed in greater detail elsewhere.[8] The most representative actuator is the ion channel, which has been frequently used to control the excitability of neurons.[9] When stimulating cells that express the ion channels with a suitable wavelength and power density of light, depolarization or hyperpolarization is induced (depending on the characteristics of the different ion channels), followed by corresponding activation or inhibition of the cells.

3. Activation

ChR2, the *Chlamydomonas reinhardtii*-derived cation channel sensitive to blue-wavelength light, allows cations to pass through and enter cells under light stimulation.[9,15] To date, many ChR2-derived actuators have been developed that differ in the kinetics of the photocurrent and speed of response to light. Further details are provided in a study by Mattis.[33]

In 2016, Fattahi and coworkers reported that ENS progenitors derived from human pluripotent stem cells (hPSCs) could differentiate into functional enteric neurons. This finding is expected to facilitate the development of therapeutic strategies for Hirschsprung's disease, which is closely related to the loss of enteric neurons.[34] In their study, ChR2 was genetically expressed in hPSCs under the control of the human synapsin promoter (which encodes a neuron-specific phosphoprotein). Blue light stimulation induced contractions of cocultured SMCs) which are closely associated with ChR2$^+$ neurons. Using optogenetic methods, the authors validated the function of these hESC-derived enteric neurons in regulating SMC contraction as well as the specificity of this method to only activate a subgroup of enteric neurons. A similar optogenetic strategy was employed by Stamp *et al.*[35] They sought to verify the hypothesis that transplanted enteric neurons facilitated functional innervation of SMCs. In this study, they used the Cre-loxP system to ectopically express ChR2 in wnt1$^+$ enteric neurons and glia in Wnt1-Cre-ChR2-eYFP mice. ChR2$^+$ cells were harvested from the gut of embryonic or postnatal mice and then transplanted into the colon of C57BL/6 mice. They found that the graft-derived neurons matured and that blue light evoked both inhibitory junction potentials (IJPs) and excitatory junction potentials (EJPs) of the SMCs of colon strips *in vitro*.

Hibbard and coworkers further defined enteric neurons involved in colonic motility activated by light stimulation.[36] In this study, ChR2 with the H134R mutation (ChR2(H134R)), which results in greater photocurrent of target cells than wild-type ChR2,[37] was expressed in calretinin$^+$ (encoded by the gene calbindin 2) myenteric neurons, which are thought to be involved in GI motility.[38] Light stimulation evoked excitation of ChR2$^+$ myenteric neurons isolated acutely from the mouse colon. Additionally, a proximal contraction and/or distal relaxation, depending on the stimulation site, were evoked by light stimulation in the full length of the colon *in vitro*. Moreover, light stimulation of the proximal colon *in vivo* enhanced colon transit and increased pellet output.[36] Afterward, the same model was used by the same team to investigate the role of optogenetics in improving small intestinal transit. Surprisingly, blue light stimulation evoked only local contraction, not premature propagating contraction, in the isolated small intestine.[38] They ascribed these results to the inherent characteristics of the small intestine, as it is refractory to premature activation and the calretinin$^+$ myenteric neuron types differ between the small intestine and colon (e.g., the population of calretinin$^+$ neurons containing nitric oxide synthase is absent in the small intestine).[38]

These findings reflect the high cell-level specificity of optogenetics, which facilitates the identification of neurons or neurotransmitters with different roles in GI motility patterns. The same optogenetic strategies (and different animal models) have been used in several studies to investigate the distinct role of enteric neuron subpopulations, such as cholinergic neurons (ChAT-ChR2-YPF-BAC mice[39]), nitrergic neurons (nNOS-ChR2 mice[13,40,41]), and HCN4$^+$ neurons (gSAIGFF249A-UAS: ChRWR-YFP zebrafish[42]) (ChRWR is a type of channelrhodopsin).

In addition to the enteric neurons, the peripheral nervous system (PNS) is also involved in gut motility. Not surprisingly, studies have also investigated the role of these neurons in relation to GI motility patterns. In a study by Smith-Edwards *et al.*,

a mouse model that expressed ChR2 in neuropeptide Y-positive (NPY^+) neurons was used to investigate the extent of sympathetic innervations to the colon.[43] When the inferior mesenteric ganglion was exposed to the blue light, the local contractions and the length of migrating motor complexes of the colon preparation decreased.

Many studies have proposed that intestinal macrophages play a critical role in GI motility (reviewed elsewhere[44,45]). In 2018, Luo and his colleagues reported a direct interaction of macrophages and SMCs independent of the ENS in mouse colon contraction.[46] A subpopulation of $TRPV4^+$ muscularis macrophages (MMs) was identified; upon light stimulation, colon strips of CX3CR-1-creER-ChR2 mice produced contractile responses, even in the presence of tetrodotoxin (TTX).[46] Their study was the first to utilize optogenetic approaches to induce smooth muscle contraction by directly stimulating nonneuronal cells. In 2021, Vogt and coworkers examined gastroparesis by expressing ChR2 in SMCs.[47] Upon stimulation with 460 nm blue light, contractions in antral smooth muscle strips were evoked, and light-evoked food propulsion was observed. Additionally, in a recent study by Wagdi and coworkers, a transgenic strategy similar to that of Vogt *et al.* was adopted. A G_q signaling-related actuator, human neuropsin (hOPN5), was expressed in SMCs of the mouse small intestine.[48] With UV stimulation, the muscle strips from the small intestine exhibited enhanced contraction. This finding further demonstrates the feasibility of using optogenetic tools to control smooth muscle contractility as well as the promising potential of using light-responsive GPCRs to study GI motility. Taken together, these findings suggest that nonneuronal cells in the GI tract (present in vast numbers) play a role in GI motility and expand potential targets for the treatment of motility disorders.

4. Inhibition

Other types of light-sensitive ion channels or light-driven pumps include halorhodopsin (NpHR), a chloride pump sensitive to yellow light.[49] NpHR hyperpolarizes target cells under light stimulation and can be used together with ChR2 to exert opposing effects on target cells.[50] The details of these actuators are beyond the scope of this review and are reviewed elsewhere.[9,49] By utilizing these opsins in combination, the components involved in regulating GI motility patterns can be precisely controlled, manipulating both excitation and inhibition, which may help reveal their role under different conditions.

5. Challenges

However, use of this technology faces several important challenges. The first issue concerns the power of light. Extremely high-power light will cause photobleaching of the opsin, while extremely low-power light cannot activate the opsin. Thus, a gradient array of light power density and efficacy should be used to identify the optimal parameters in novel animal models.[36,39] The second challenge is the manner of light delivery. Light is attenuated as it travels through tissue. Thus, the efficiency of the light delivery device should be carefully considered, especially for *in vivo* applications. This challenge is less difficult for *in vitro* experiments, which usually use commercially available fibers optic with a diode-pumped solid-state laser system. In contrast, *in vivo* experiments with free-moving animal models are challenging. Theoretically, there are two routes for light delivery, the intraperitoneal and GI luminal pathways. Indeed, studies have reported several different devices,[11–13,36,38] but more convenient and cost-efficient devices with reliable light power supplies are needed. Third, optogenetics-based GI motility studies are limited to transgenic mice. As rodents differ in GI motility from other mammals and humans, it is an open question as to how optogenetics can be applied in other species. Finally, before clinical application, researchers must overcome the problem faced by all transgenic technologies, including optogenetics: The immune response of the body to the carrier or the light-sensitive protein itself.

6. Conclusions

In the past decade, optogenetics has achieved rapid development and great success in the field of neuroscience; this technique has gradually been applied to other research fields, including GI motility, in recent years. As previously described, use of genetically encoded light-sensitive opsins has revealed novel mechanisms by which certain subtypes of

ENS or PNS neurons as well as other nonneuronal cells regulate GI motility. Additionally, optogenetics provides a good tool for identifying the respective roles of various GI cells in propulsive motility patterns. We believe that further application of optogenetics in the field of GI motility will reveal further mechanisms of GI motility patterns that involve nonneuronal cells. For example, the well-known pacemaker cells, ICC, have network-like properties and excitability, making them ideal for optogenetic modulation. Furthermore, the combination of optogenetic techniques with other experimental approaches will allow us to fully elucidate the mechanisms of GI motility.

In this review, we have summarized the recent optogenetic applications in GI motility research and focused on studies of representative actuators that control nerve cells and nonneural cells. However, the promise of optogenetics is far greater than that described in this paper. In the future, the increase in the breadth and depth of optogenetics used to explore GI motility are expected to provide extensive contributions to our understanding and illuminate new strategies for the clinical treatment of GI motility diseases.

Conflicts of Interest

The authors declare no conflicts of interest relevant to this article.

Funding

This work was funded by the National Natural Science Foundation of China (No. 81770541) to WT.

Acknowledgment

Song Zhao and Ting Zhang contributed equally to this work.

References

1. K. M. Sanders, S. M. Ward, S. D. Koh, "Interstitial cells: Regulators of smooth muscle function," *Physiol. Rev.* **94**(3), 859–907 (2014), doi: 10.1152/physrev.00037.2013.
2. K. M. Sanders, "Spontaneous electrical activity and rhythmicity in gastrointestinal smooth muscles," *Adv. Exp. Med. Biol.* **1124**, 3–46 (2019), doi: 10.1007/978-981-13-5895-1_1.
3. S. Schneider, C. M. Wright, R. O. Heuckeroth, "Unexpected roles for the second brain: Enteric nervous system as master regulator of bowel function," *Annu. Rev. Physiol.* **81**(1), 235–259 (2019), doi: 10.1146/annurev-physiol-021317-121515.
4. B. D. Gulbransen, "Emerging tools to study enteric neuromuscular function," *Am. J. Physiol. Gastrointest. Liver Physiol.* **312**(5), G420–G426 (2017), doi: 10.1152/ajpgi.00049.2017.
5. G. Bassotti, V. Villanacci, D. Cretoiu, S. M. Cretoiu, G. Becheanu, "Cellular and molecular basis of chronic constipation: Taking the functional/idiopathic label out," *World J. Gastroenterol.* **19**(26), 4099–4105 (2013), doi: 10.3748/wjg.v19.i26.4099.
6. M. Camilleri, A. C. Ford, G. M. Mawe *et al.*, "Chronic constipation," *Nat. Rev. Dis. Primers.* **3**, 17095 (2017), doi: 10.1038/nrdp.2017.95.
7. J. B. Furness, "The enteric nervous system and neurogastroenterology," *Nat. Rev. Gastroenterol. Hepatol.* **9**(5), 286–294 (2012), doi: 10.1038/nrgastro.2012.32.
8. H. Ye, M. Fussenegger, "Optogenetic medicine: Synthetic therapeutic solutions precision-guided by light," *Cold Spring Harb Perspect Med.* **9**(9), a034371 (2019), doi: 10.1101/cshperspect.a034371.
9. W. Boesmans, M. M. Hao, P. Vanden Berghe, "Optogenetic and chemogenetic techniques for neurogastroenterology," *Nat. Rev. Gastroenterol. Hepatol.* **15**(1), 21–38 (2018), doi: 10.1038/nrgastro.2017.151.
10. G. Miesenbock, "The optogenetic catechism," *Science* **326**(5951), 395–399 (2009), doi: 10.1126/science.1174520.
11. K. L. Montgomery, A. J. Yeh, J. S. Ho *et al.*, "Wirelessly powered, fully internal optogenetics for brain, spinal and peripheral circuits in mice," *Nat. Meth.* **12**(10), 969–974 (2015), doi: 10.1038/nmeth.3536.
12. G. Shin, A. M. Gomez, R. Al-Hasani *et al.*, "Flexible near-field wireless optoelectronics as subdermal implants for broad applications in optogenetics," *Neuron* **93**(3), 509–521. e3 (2017), doi: 10.1016/j.neuron.2016.12.031.
13. N. Rakhilin, B. Barth, J. Choi *et al.*, "Simultaneous optical and electrical *in vivo* analysis of the enteric nervous system," *Nat. Commun.* **7**, 11800 (2016), doi: 10.1038/ncomms11800.
14. G. Miesenbock, I. G. Kevrekidis, "Optical imaging and control of genetically designated neurons in

functioning circuits," *Annu. Rev. Neurosci.* **28**, 533–563 (2005), doi: 10.1146/annurev.neuro.28.051804.101610.
15. G. Nagel, T. Szellas, W. Huhn *et al.*, "Channelrhodopsin-2, a directly light-gated cation-selective membrane channel," *Proc. Natl. Acad. Sci. USA* **100**(24), 13940–13945 (2003), doi: 10.1073/pnas.1936192100.
16. T. Ishizuka, M. Kakuda, R. Araki, H. Yawo, "Kinetic evaluation of photosensitivity in genetically engineered neurons expressing green algae light-gated channels," *Neurosci. Res.* **54**(2), 85–94 (2006), doi: 10.1016/j.neures.2005.10.009.
17. A. Bi, J. Cui, Y. P. Ma *et al.*, "Ectopic expression of a microbial-type rhodopsin restores visual responses in mice with photoreceptor degeneration," *Neuron* **50**(1), 23–33 (2006), doi: 10.1016/j.neuron.2006.02.026.
18. X. Li, D. V. Gutierrez, M. G. Hanson *et al.*, "Fast noninvasive activation and inhibition of neural and network activity by vertebrate rhodopsin and green algae channelrhodopsin," *Proc. Natl. Acad. Sci. USA* **102**(49), 17816–17821 (2005), doi: 10.1073/pnas.0509030102.
19. E. S. Boyden, F. Zhang, E. Bamberg, G. Nagel, K. Deisseroth, "Millisecond-timescale, genetically targeted optical control of neural activity," *Nat. Neurosci.* **8**(9), 1263–1268 (2005), doi: 10.1038/nn1525.
20. K. Deisseroth, G. Feng, A. K. Majewska, G. Miesenbock, A. Ting, M. J. Schnitzer, "Next-generation optical technologies for illuminating genetically targeted brain circuits," *J. Neurosci.* **26**(41), 10380–10386 (2006), doi: 10.1523/JNEUROSCI.3863-06.2006.
21. K. M. Sanders, Y. Kito, S. J. Hwang, S. M. Ward, "Regulation of Gastrointestinal smooth muscle function by interstitial cells," *Physiology (Bethesda)* **31**(5), 316–326 (2016), doi: 10.1152/physiol.00006.2016.
22. S. Ro, C. Park, J. Jin *et al.*, "A model to study the phenotypic changes of interstitial cells of Cajal in gastrointestinal diseases," *Gastroenterology* **138**(3), 1068–1078 e1-2 (2010), doi: 10.1053/j.gastro.2009.11.007.
23. A. Breland, S. E. Ha, B. G. Jorgensen *et al.*, "Smooth muscle transcriptome browser: Offering genome-wide references and expression profiles of transcripts expressed in intestinal SMC, ICC, and PDGFRalpha(+) cells," *Sci. Rep.* **9**(1), 387 (2019), doi: 10.1038/s41598-018-36607-6.
24. L. E. Peri, K. M. Sanders, V. N. Mutafova-Yambolieva, "Differential expression of genes related to purinergic signaling in smooth muscle cells, PDGFRalpha-positive cells, and interstitial cells of Cajal in the murine colon," *Neurogastroenterol. Motil.* **25**(9), e609-20 (2013), doi: 10.1111/nmo.12174.
25. T. A. Pologruto, R. Yasuda, K. Svoboda, "Monitoring neural activity and [Ca2+] with genetically encoded Ca2+ indicators," *J. Neurosci.* **24**(43), 9572–9579 (2004), doi: 10.1523/JNEUROSCI.2854-04.2004.
26. B. T. Drumm, G. W. Hennig, S. A. Baker, K. M. Sanders, "Applications of spatio-temporal mapping and particle analysis techniques to quantify intracellular Ca2+ signaling in situ," *J. Vis. Exp.* **7**(143), 10.3791/58989 (2019), doi: 10.3791/58989.
27. S. A. Baker, B. T. Drumm, D. Saur, G. W. Hennig, S. M. Ward, K. M. Sanders, "Spontaneous Ca(2+) transients in interstitial cells of Cajal located within the deep muscular plexus of the murine small intestine," *J. Physiol.* **594**(12), 3317–3338 (2016), doi: 10.1113/JP271699.
28. S. A. Baker, W. A. Leigh, G. Del Valle *et al.*, "Ca(2+) signaling driving pacemaker activity in submucosal interstitial cells of Cajal in the murine colon," *Elife* **10**, e64099 (2021), doi: 10.7554/eLife.64099.
29. T. S. Sung, S. J. Hwang, S. D. Koh *et al.*, "The cells and conductance mediating cholinergic neurotransmission in the murine proximal stomach," *J. Physiol.* **596**(9), 1549–1574 (2018), doi: 10.1113/JP275478.
30. B. T. Drumm, T. S. Sung, H. Zheng, S. A. Baker, S. D. Koh, K. M. Sanders, "The effects of mitochondrial inhibitors on Ca(2+) signalling and electrical conductances required for pacemaking in interstitial cells of Cajal in the mouse small intestine," *Cell Calcium* **72**, 1–17 (2018), doi: 10.1016/j.ceca.2018.01.003.
31. B. T. Drumm, G. W. Hennig, M. J. Battersby *et al.*, "Clustering of Ca(2+) transients in interstitial cells of Cajal defines slow wave duration," *J. Gen. Physiol.* **149**(7), 703–725 (2017), doi: 10.1085/jgp.201711771.
32. G. W. Hennig, T. W. Gould, S. D. Koh *et al.*, "Use of genetically encoded calcium indicators (GECIs) Combined with advanced motion tracking techniques to examine the behavior of neurons and Glia in the enteric nervous system of the intact murine colon," *Front Cell Neurosci.* **9**, 436 (2015), doi: 10.3389/fncel.2015.00436.
33. J. Mattis, K. M. Tye, E. A. Ferenczi *et al.*, "Principles for applying optogenetic tools derived from direct comparative analysis of microbial opsins," *Nat. Meth.* **9**(2), 159–172 (2011), doi: 10.1038/nmeth.1808.
34. F. Fattahi, J. A. Steinbeck, S. Kriks *et al.*, "Deriving human ENS lineages for cell therapy and drug discovery in Hirschsprung disease," *Nature*

531(7592), 105–109 (2016), doi: 10.1038/nature 16951.

35. L. A. Stamp, R. M. Gwynne, J. P. P. Foong *et al.*, "Optogenetic demonstration of functional innervation of mouse colon by neurons derived from transplanted neural cells," *Gastroenterology* **152**(6), 1407–1418 (2017), doi: 10.1053/j.gastro. 2017. 01.005.
36. T. J. Hibberd, J. Feng, J. Luo *et al.*, "Optogenetic induction of colonic motility in mice," *Gastroenterology* **155**(2), 514–528 e6 (2018), doi: 10.1053/j. gastro.2018.05.029.
37. G. Nagel, M. Brauner, J. F. Liewald, N. Adeishvili, E. Bamberg, A. Gottschalk, "Light activation of channelrhodopsin-2 in excitable cells of Caenorhabditis elegans triggers rapid behavioral responses," *Curr. Biol.* **15**(24), 2279–2284 (2005), doi: 10.1016/j.cub.2005.11.032.
38. N. J. Spencer, L. Travis, T. Hibberd, N. Kelly, J. Feng, H. Hu, "Effects of optogenetic activation of the enteric nervous system on gastrointestinal motility in mouse small intestine," *Auton. Neurosci.* **229**, 102733 (2020), doi: 10.1016/j. autneu.2020.102733.
39. A. L. Perez-Medina, J. J. Galligan, "Optogenetic analysis of neuromuscular transmission in the colon of ChAT-ChR2-YFP BAC transgenic mice," *Am. J. Physiol. Gastrointest. Liver Physiol.* **317**(5), G569–G579 (2019), doi: 10.1152/ajpgi.00089.2019.
40. T. W. Gould, W. A. Swope, D. J. Heredia, R. D. Corrigan, T. K. Smith, "Activity within specific enteric neurochemical subtypes is correlated with distinct patterns of gastrointestinal motility in the murine colon," *Am. J. Physiol. Gastrointest. Liver Physiol.* **317**(2), G210–G221 (2019), doi: 10.1152/ ajpgi.00252.2018.
41. S. D. Koh, B. T. Drumm, H. Lu *et al.*, "Propulsive colonic contractions are mediated by inhibition-driven poststimulus responses that originate in interstitial cells of Cajal," *Proc. Natl. Acad. Sci. USA* **119**(18), e2123020119 (2022), doi: 10.1073/ pnas.2123020119.
42. K. Fujii, K. Nakajo, Y. Egashira *et al.*, "Gastrointestinal neurons expressing HCN4 regulate retrograde peristalsis," *Cell Rep.* **30**(9), 2879–2888. e3 (2020), doi: 10.1016/j.celrep.2020.02.024.
43. K. M. Smith-Edwards, B. S. Edwards, C. M. Wright *et al.*, "Sympathetic input to multiple cell types in mouse and human colon produces region-specific responses," *Gastroenterology* **160**(4), 1208–1223.e4 (2021), doi: 10.1053/j.gastro.2020.09.030.
44. K. M. Sanders, T. Ordog, S. M. Ward, "Physiology and pathophysiology of the interstitial cells of Cajal: From bench to bedside. IV. Genetic and animal models of GI motility disorders caused by loss of interstitial cells of Cajal," *Am. J. Physiol. Gastrointest. Liver Physiol.* **282**(5), G747–G756 (2022), doi: 10.1152/ajpgi.00362.2001.
45. M. F. Viola, G. Boeckxstaens, "Intestinal resident macrophages: Multitaskers of the gut," *Neurogastroenterol. Motil* **32**(8), e13843 (2020), doi: 10.1111/ nmo.13843.
46. J. Luo, A. Qian, L. K. Oetjen *et al.*, "TRPV4 channel signaling in macrophages promotes gastrointestinal motility via direct effects on smooth muscle cells," *Immunity* **49**(1), 107–119 e4 (2018), doi: 10.1016/j.immuni.2018.04.021.
47. M. Vogt, B. Schulz, A. Wagdi *et al.*, "Direct optogenetic stimulation of smooth muscle cells to control gastric contractility," *Theranostics* **11**(11), 5569–5584 (2021), doi: 10.7150/thno.53883.
48. A. Wagdi, D. Malan, U. Sathyanarayanan *et al.*, "Selective optogenetic control of Gq signaling using human neuropsin," *Nat. Commun.* **13**(1), 1765 (2022), doi: 10.1038/s41467-022-29265-w.
49. W. Wang, "Optogenetic manipulation of ENS - The brain in the gut," *Life Sci.* **192**, 18–25 (2018), doi: 10.1016/j.lfs.2017.11.010.
50. F. Zhang, L. P. Wang, M. Brauner *et al.*, "Multimodal fast optical interrogation of neural circuitry," *Nature* **446**(7136), 633–639 (2007), doi: 10.1038/nature05744.