Published in final edited form as:

Expert Rev Gastroenterol Hepatol. 2019 April ; 13(4): 281–284. doi:10.1080/17474124.2019.1581061.

Optogenetic control of the enteric nervous system and gastrointestinal transit

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Abstract

Introduction: There are limited effective therapies available for improving gastrointestinal (GI) transit in mammals with intractable or chronic constipation. Current therapeutics to improve GI-transit usually require oral ingestion of therapeutic drugs, such as the serotonin receptor agonist prucalopride. However, most receptors are distributed all over the body and unsurprisingly drugs like prucalopride stimulate multiple organs, often leading to unwanted side effects. There is a desperate need in the community to improve GI-transit selectively without effects on other organs.

Areas covered: We performed a systematic review of the literature on Pubmed and report significant technical advances in optogenetic control of the GI-tract. We discuss recent demonstrations that optogenetics can be used to potently control the activity of subsets of enteric neurons. Special focus is made of the first recent demonstration that wireless optogenetics can be used to stimulate the colon in conscious, freely-moving, untethered mice causing a significant increase in fecal pellet output. This is a significant technical breakthrough with a major therapeutic potential application to improve GI-transit.

Expert opinion: The ability to selectively stimulate the ENS to modulate GI-transit in live mammals using light, avoids the need for oral consumption of any drugs and side effects; by stimulating only the GI-tract.

Keywords

Enteric nervous system; colon; peristalsis; gastrointestinal transit; optogenetics; wireless optogenetics

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Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

1. Introduction

The basic principle of optogenetics is to use light to manipulate activity in cells of interest, often neurons. The GI-tract contains an independent nervous system, known as the enteric nervous system (ENS), which comprises many hundreds of thousands of neurons, forming ganglionated neural networks around the gut circumference [1,2]. A large amount of work has been put into the classification of enteric neurons according to their axonal projections, morphological and electrophysiological characteristics, enabling inferences about their functional roles (sensory, inter and motor). Within a particular gut region and species, classes of enteric neurons express fixed combinations of proteins, neuro-transmitters, and other neurochemicals, comprising their 'neurochemical code' [3]. Long used as a classification and identification tool, neurochemical coding of enteric neurons may now also be used for neurogenetic targeting; to selectively express light-sensitive ion channels in specific classes of enteric neuron. Therefore, the presence of an ENS in the gut wall provides a unique potential to selectively control the activity of specific classes of neurons using optogenetics, based on their neurochemical coding, for precise temporal activation or inhibition.

2. How does optogenetics work?

In order for optogenetics to work, it requires the genetic implantation of light-sensitive ion channels into living cells of interest, providing for the first time, the unique ability to probe the function of specific neuron types in complex neural circuits [4]. Light-sensitive optogenetic actuators may be used to induce depolarization, hyperpolarization, intracellular signaling cascades, or changes in gene expression [5]. Over the past 10 years, there has been a progressive increase in the number of laboratories effectively using optogenetics to control neural circuits in the brain and spinal cord [4]. It is therefore surprising that optogenetics has only been successfully demonstrated recently in the ENS [2,6,7].

Channelrhodopsins are naturally occurring light-gated cation channels, originally extracted from algae [8,9]. Channelrhodopsin-2 (ChR2; and its variants [10]) has proved to be extremely useful in neuroscience due to their large photo-evoked currents with fast on/off kinetics, enabling temporally precise control of cell depolarization [4]. To date, there have been five independent reports utilizing an optogenetic approach to directly control enteric neurons [11–15]. In all cases, a variant of ChR2 has been the optogenetic actuator of choice.

3. Different studies using optogenetics to control ENS activity

[12] demonstrated ChR2-mediated excitation of human embryonic stem cell-derived enteric neurons in a co-culture system with smooth muscle cells, *in vitro*. Here, ChR2 expression was driven by the human synapsin gene promoter. Studies by [11] and [13] demonstrated local optogenetic control of inhibitory motor function. They induced Cre-driven ChR2 expression in nitric oxide synthase (NOS)-expressing enteric neurons, via crossbreeding transgenic mice and viral transduction approaches, respectively. Most inhibitory motor neurons contain NOS in their neurochemical code, and nitric oxide is a major transmitter used by enteric inhibitory motor neurons. Consistent with this, photostimulation of colonic

NOS neurons evoked IJPs mediated by purines and nitric oxide, *in vitro* [13]. Photostimulation of NOS neurons in small intestine *in vivo* led to quiescence of smooth muscle action potential discharge, consistent with smooth muscle hyperpolarization [11].

In a study by Stamp et al. (2017), channelrhodopsin-expressing neural crest-derived enteric neurons were generated using Cre-driven ChR2 expression under the control of the Wnt1 gene promoter. Neurons from postnatal or fetal Wnt1::Cre;ChR2-EYFP mice were isolated and transplanted into the distal colon of 3–4 week-old wild-type recipient mice [14]. Intracellular recordings of smooth muscle cells in recipient mice demonstrated blue light stimulation of graft-derived cells readily evoked inhibitory and excitatory junction potentials in local smooth muscle cells that could lead to muscle contraction. Evidence of graft-derived inter-neuronal functions was also demonstrated, providing proof-of-principle that functional integration of transplanted neurons in gut can occur [14].

Another study recently generated mice with Cre-dependent expression of channelrhodopsin-H134R (ChR2-H134R [16]) under the promoter of calcium binding protein, calretinin (CAL) [2,15]. Calretinin-immunoreactive neurons comprise a major population of predominantly cholinergic excitatory neurons. These include virtually all putative intrinsic sensory neurons [17], as well as excitatory motor neurons and a subset of interneurons [18]. Focal photo-stimulations (5 Hz, 10–20 sec, 10 ms pulse width, 473 nm λ , ~0.1 mm²) in whole colon *ex vivo* preparations consistently evoked polarized motor reflexes, followed by premature propagating neurogenic contractions (also known as colonic migrating motor complexes, CMMCs [15];. Photo-stimulation of calretinin neurons also increased propulsion of fecal content *ex vivo* (Figure 1) [15]. Interestingly, single light pulses did not evoke detectable contractions (unpublished observations). We recently demonstrated CMMC generation involves tens of thousands of enteric neurons across large numbers of neighboring myenteric ganglia in a temporally synchronized discharge [19]. Single pulse optical stimuli, similar to electrical stimuli [20], may be insufficient to coordinate the firing of such large populations of ascending and descending interneurons to generate a CMMC contraction [2,15]. Given the large numbers of neurons involved in the coordinated discharge underlying CMMCs, it is perhaps not surprising that using the same approach described in Hibberd et al. (2018), focal photostimulation of calretinin neurons expressing the inhibitory light-sensitive proton pump, archaerhodopsin-3 [21], had no detectable suppressing effect on CMMCs or GI-transit (unpublished observations). It is possible that larger areas may be required to suppress motility via excitatory neurons than that required to enhance motility. Alternatively, activation of inhibitory enteric neurons [11,13] may enable suppression of GI motility *in vivo*. This possibility remains to be tested.

Details on the electrical and chemical effects of ChR2 in enteric neurons are currently sparse. Serendipitous impalements of single, graft-derived myenteric neurons by [14] revealed light evoked both depolarization, with or without an action potential, as well as fast EPSPs for the duration of a 100 ms stimulus. Isolated enteric calretinin neurons were also depolarized and fired single action potentials at the onset of long duration light pulses (500 ms; [15]). However, a subset of these neurons would be expected to fire repetitively during depolarization [22]. These phasic discharge responses to prolonged photostimulation may reflect an inability to repolarize under strong ongoing ChR2 currents [10]. Nevertheless, the

observation that smooth muscle IJPs were graded in amplitude and duration in proportion to the number of repetitive photostimuli suggests that enteric neurons can follow repetitive Chr2 stimulation [13]. How Chr2 (and other opsins) interacts with different types of enteric neurons and whether its expression or activation affects enteric neural circuits and glial cells will be important to clarify, particularly in longer term studies.

4. Development of wireless optogenetics

A major technical advance in the past few years is wireless optogenetics [23]. This exciting technique enables implantation of wireless miniature light emitting diodes into animals for targeted photostimulation, *in vivo*. Here, animals are not tethered to implanted fiber optics and are thus fully free to move. Although wireless optogenetics has been used for about 3–4 years to control CNS neural pathways, it was only shown for the first time recently that wireless micro-light-emitting diodes (micro-LEDs; e.g. in Figures 2 and 3) with appropriate dimensions can be implanted onto the gut wall of living mice [15]. Following *ex vivo* demonstration that Chr2 photostimulation of calretinin neurons could increase colonic transit, it was found micro-LED activation also increased fecal pellet output *in vivo* [15]. The latter study was performed in conscious, freely-moving mice, offering great potential for other mammals that have impaired colonic transit. Technology now exists to induce Chr2 expression in nontransgenic animals, affording the potential for new therapeutic strategies. This can be performed by delivering genetic material to the gut by viral vectors [24]. Widespread induction of novel gene expression in enteric neurons has been achieved by intravenous or intraperitoneal administration of adeno-associated viruses (AAV) in mice [25–27], guinea pigs [28] and monkeys [28]. Local expression in the colonic ENS was achieved by focal intramural injections in rats and mice [29]. Notably, single intramural AAV injections in rat colon transduced neurons over an area of ~47 mm² without spreading to other tissues. This is compatible with the ~0.1 mm² used for optogenetic induction of CMMCs in mice [15]. Low transduction efficiency can limit the effectiveness of viral delivery technique; reported efficiencies in ENS currently range from ~1% to 57%, depending on the choice of viral vector dosage, serotype and promotor, the gut region and enteric plexus, and the timing and routes of administration [24,30]. Promisingly, viral transduction of Chr2 in the ENS of NOS-Cre mice was sufficient to evoke IJPs *in vitro* and in other areas, these viruses generate sufficient levels of light-sensitive ion channels in peripheral neurons to potentially control the behavior of non-transgenic mammals, *in vivo* [31].

5. Expert opinion

The ability to now improve bowel transit with targeted light delivery to the gut offers major hope for millions of patients with chronic constipation or other disorders associated with impaired intestinal transit. Wireless optogenetic control of the ENS and GI-motility offers significant advantages over current pharmacological agents used to stimulate GI-motility. Commonly, current therapeutic agents used to stimulate the ENS are orally ingested agonists (e.g. 5-HT agonist, prucalopride). The unfortunate problem with drugs like 5-HT agonists is that while they stimulate the ENS, the same receptors expressed on enteric neurons are also expressed on many other organs, generating unwanted side effects. Wireless optogenetic stimulation not only targets specific and spatially restricted neuronal populations but it is

also temporally precise – delivering stimuli on demand, without off-target stimulation of other central or peripheral neural circuits. This provides the community with a new and unique opportunity to stimulate specific gut regions in live animals to modify muscle contractility, leading to a realistic potential to increase expulsion of ingested content, particularly during conditions of impaired transit or chronic constipation.

Optogenetics offers great potential for high fidelity temporal and spatial control of the excitability of the ENS *in vitro* and *in vivo*. The ability to selectively improve transit along the gut in live mammals using light could avoid the need to continue prescription drugs, which take time to be absorbed and unavoidably act in multiple organ systems, typically inducing numerous undesirable side effects. A major advantage of wireless optogenetic control of the ENS is that the light can be activated instantaneously to stimulate only the localized region of gut of interest, without requiring a transcutaneous power supply and without directly activating receptors in other neural pathways of peripheral or central ganglia. The recent demonstration that wireless optogenetics can potently modulate activity in peripheral nerve pathways in conscious freely-moving **non-transgenic** mice is particularly exciting, because this means that all the technology now exists to effectively apply wireless optogenetics to the GI-tract of larger non-transgenic mammals (including humans) to improve GI-transit. To do this, adeno-associated viruses can be injected in the gut wall that would be transported retrograde along enteric nerve axons, allowing expression of ChR2 without the need to use transgenic mice.

Funding

This work was supported by grants from the NIH, R01GM101218, R01DK103901 (to HH), Washington University School of Medicine Digestive Disease Research Core Center (NIDDK P30 DK052574), The Center for the Study of Itch of Department of Anesthesiology at Washington University School of Medicine. N J Spencer is supported by NH&MRC of Australia, grants 1156427 & 1156416.

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Wood JD. Enteric nervous system: brain in the Gut In: Said HM, editor. Handbook of Physiology. Academic Press; London, UK 2018p. 361–372.
2. Hu H, Spencer NJ. Enteric nervous system structure and neuro-chemistry related to function and neuropathology In: Said HM, editor. Physiology of the gastrointestinal tract. 6th ed. Cambridge: Elsevier/Academic Press; 2018 p. 629–669.
3. Costa M, Brookes SJ, Steele PA, et al. Neurochemical classification of myenteric neurons in the guinea-pig ileum. Neuroscience. 1996;75(3):949–967. [PubMed: 8951887]
4. Kim CK, Adhikari A, Deisseroth K. Integration of optogenetics with complementary methodologies in systems neuroscience. Nat Rev Neurosci. 2017;18(4):222–235. [PubMed: 28303019]
5. Guru A, Post RJ, Ho YY, et al. Making sense of optogenetics. Int J Neuropsychopharmacol. 2015;18(11):pyv079. [PubMed: 26209858]
6. Wang W Optogenetic manipulation of ENS - the brain in the gut. Life Sci. 2018;192:18–25. [PubMed: 29155296]
7. Boesmans W, Hao MM, Vanden Berghe P. Optogenetic and chemo-genetic techniques for neurogastroenterology. Nat Rev Gastroenterol Hepatol. 2018;15(1):21–38. [PubMed: 29184183]
8. Nagel G, Ollig D, Fuhrmann M, et al. Channelrhodopsin-1: a light-gated proton channel in green algae. Science. 2002;296 (5577):2395–2398. [PubMed: 12089443]

9. Nagel G, Szellas T, Huhn W, et al. Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. *Proc Natl Acad Sci U S A*. 2003;100(24):13940–13945. [PubMed: 14615590]
10. Lin JY. A user's guide to channelrhodopsin variants: features, limitations and future developments. *Exp Physiol*. 2011;96(1):19–25. [PubMed: 20621963]
11. Rakhilin N, Barth B, Choi J, et al. Simultaneous optical and electrical *in vivo* analysis of the enteric nervous system. *Nat Commun*. 2016;7:11800. [PubMed: 27270085]
12. Fattahi F, Steinbeck JA, Kriks S, et al. Deriving human ENS lineages for cell therapy and drug discovery in hirschsprung disease. *Nature*. 2016;531(7592):105–109. [PubMed: 26863197]
13. Perez-Medina A, Manfredson F, Galligan J. Selective stimulation of inhibitory motoneurons in the mouse colon following light activation of channel rhodopsin-2 (ChR2) *Ex Vivo*. *FASEB J*. 2017;31 (Supplement 1). Experimental Biology, Conference Abstract LB572.
14. Stamp LA, Gwynne RM, Foong JPP, et al. Optogenetic demonstration of functional innervation of mouse colon by neurons derived from transplanted neural cells. *Gastroenterology*. 2017;152(6): 1407–1418. [PubMed: 28115057]
15. Hibberd TJ, Feng J, Luo J, et al. Optogenetic induction of colonic motility in Mice. *Gastroenterology*. 2018;155:514–528. [PubMed: 29782847] •• This study is the first to demonstrate that optogenetics can be used successfully in vitro and in vivo to significantly increase colonic transit.
16. Nagel G, Brauner M, Liewald JF, et al. Light activation of channelrhodopsin-2 in excitable cells of *Caenorhabditis elegans* triggers rapid behavioral responses. *Curr Biol*. 2005;15(24):2279–2284. [PubMed: 16360690]
17. Furness JB, Robbins HL, Xiao J, et al. Projections and chemistry of dogiel type II neurons in the mouse colon. *Cell Tissue Res*. 2004;317(1):1–12. [PubMed: 15170562]
18. Sang Q, Young HM. The identification and chemical coding of cholinergic neurons in the small and large intestine of the mouse. *Anat Rec*. 1998;251(2):185–199. [PubMed: 9624448]
19. Spencer NJ, Hibberd TJ, Travis L, et al. Identification of a rhythmic firing pattern in the enteric nervous system that generates rhythmic electrical activity in smooth muscle. *J Neurosci*. 2018;38(24):5507–5522. [PubMed: 29807910]
20. Spencer NJ, Bywater RA. Enteric nerve stimulation evokes a premature colonic migrating motor complex in mouse. *Neurogastroenterol Motil*. 2002;14(6):657–665. [PubMed: 12464088]
21. 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. [PubMed: 20054397]
22. Nurgali K, Stebbing MJ, Furness JB. Correlation of electrophysiological and morphological characteristics of enteric neurons in the mouse colon. *J Comp Neurol*. 2004;468(1):112–124. [PubMed: 14648694]
23. Montgomery KL, Yeh AJ, Ho JS, et al. Wirelessly powered, fully internal optogenetics for brain, spinal and peripheral circuits in mice. *Nat Methods*. 2015;12(10):969–974. [PubMed: 26280330]
24. Buckinx R, Timmermans JP. Targeting the gastrointestinal tract with viral vectors: state of the art and possible applications in research and therapy. *Histochem Cell Biol*. 2016;146(6):709–720. [PubMed: 27665281]
25. Buckinx R, Van Remoortel S, Gijsbers R, et al. Proof-of-concept: neonatal intravenous injection of adeno-associated virus vectors results in successful transduction of myenteric and submucosal neurons in the mouse small and large intestine. *Neurogastroenterol Motil*. 2016;28(2):299–305. [PubMed: 26564813]
26. Gombash SE, Cowley CJ, Fitzgerald JA, et al. Intravenous AAV9 efficiently transduces myenteric neurons in neonate and juvenile mice. *Front Mol Neurosci*. 2014;7:81. [PubMed: 25360081]
27. Rahim AA, Wong AM, Hoefer K, et al. Intravenous administration of AAV2/9 to the fetal and neonatal mouse leads to differential targeting of CNS cell types and extensive transduction of the nervous system. *Faseb J*. 2011;25(10):3505–3518. [PubMed: 21746868]
28. Gombash SE, Cowley CJ, Fitzgerald JA, et al. Systemic gene delivery transduces the enteric nervous system of guinea pigs and cynomolgusAll macaques. *Gene Ther*. 2017;24(10):640–648. [PubMed: 28771235]

29. Benskey MJ, Kuhn NC, Galligan JJ, et al. Targeted gene delivery to the enteric nervous system using AAV: a comparison across sero-types and capsid mutants. *Mol Ther*. 2015;23(3):488–500. [PubMed: 25592336]
30. Gombash SE. Adeno-associated viral vector delivery to the enteric nervous system: a review. *Postdoc Journal: a journal of Postdoctoral Research and Postdoctoral Affairs*. 2015;3(8):1–12.
31. Iyer SM, Montgomery KL, Towne C, et al. Virally mediated optogenetic excitation and inhibition of pain in freely moving nontransgenic mice. *Nat Biotechnol*. 2014;32(3):274–278. [PubMed: 24531797]

Article highlights

- Optogenetics can be used to successfully target specific neurons of interest in the ENS leading to direct stimulation of enteric neurons.
- Wireless optogenetics can now be used to increase gastrointestinal transit *in vivo* in conscious freely moving animals. This offers many advantages over other conventional therapeutic agents to stimulate the ENS, like tegaserod, that involve oral consumption and can act in many different organs with unwanted side effects.
- There are major advantages of using optogenetics to stimulate the ENS; that includes being able to selectively stimulate only the region of gut that is focally illuminated and being able to instantaneously stimulate the ENS to induce GI motility.
- Disadvantages of using optogenetics to stimulate the ENS include the fact that specific cells of interest must be genetically modified and incorporate foreign DNA. Also, light illumination can inadvertently heat the tissue of interest.
- Recent studies have now shown that harmless adeno-associated viruses can be used to express functional light-sensitive ion channels in non-transgenic animals and respond to photo-illumination.

This box summarizes key points contained in the article.

Figure 1. shows a pulse of blue light and the spatial field of colon influenced by the fiber optic light source. The train of blue light pulses evoked muscle contraction of the colon that led to the expulsion of fecal content along the bowel. Modified from Hibberd T et al. (2018) *Gastroenterology* 155, 514–528.

Figure 2.

A single micro-light-emitting diode (micro-LED). The entire micro-LED can be implanted onto the colon to emit pulses of blue light to stimulate the ENS instantaneously leading to an increase colonic motility and transit *in vivo*.

Figure 3. Wireless activation of a blue micro-LED above a resonant cavity custom made at Flinders University by Nigel Kelly in Biomedical Engineering.