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Optogenetic Induction of Colonic Motility in Mice

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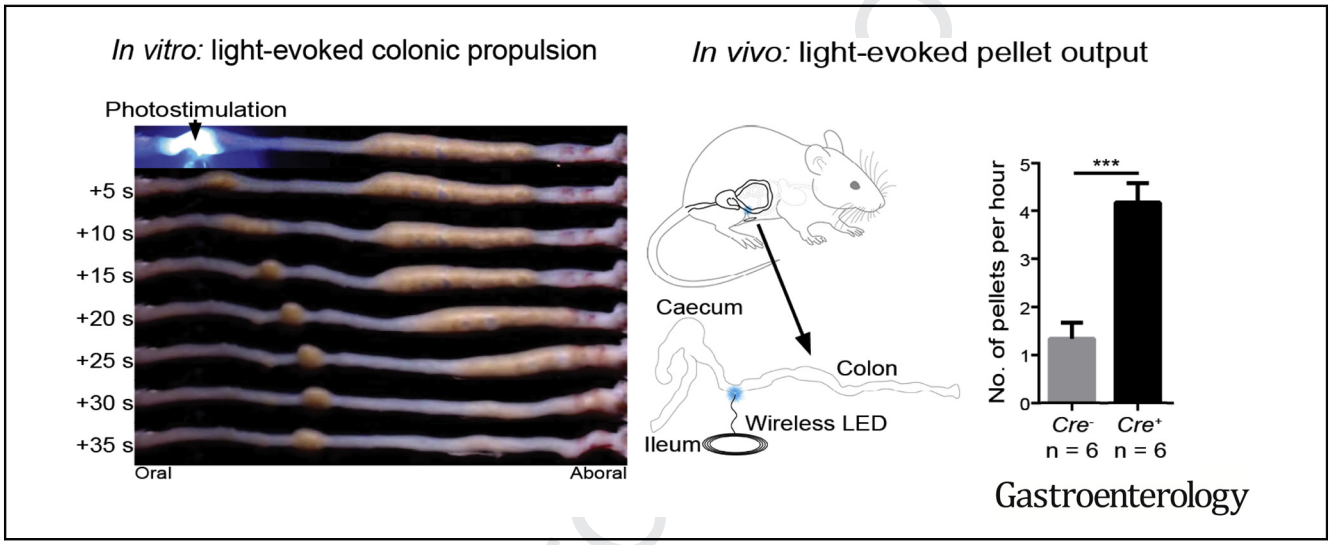

BACKGROUND & AIMS: Strategies are needed to increase gastrointestinal transit without systemic pharmacologic agents. We investigated whether optogenetics, focal application of light to control enteric nervous system excitability, could be used to evoke propagating contractions and increase colonic transit in mice. **METHODS:** We generated transgenic mice with Cre-mediated expression of light-sensitive channelrhodopsin-2 (ChR2) in calretinin neurons (CAL-ChR2 *Cre*+ mice); *Cre*– littermates served as controls. Colonic myenteric neurons were analyzed by immunohistochemistry, patch-clamp, and calcium imaging studies. Motility was assessed by mechanical, electrophysiological, and video recording in vitro and by fecal output in vivo. **RESULTS:** In isolated colons, focal light stimulation of calretinin enteric neurons evoked classic polarized motor reflexes (50/58 stimulations), followed by premature anterograde propagating contractions (39/58 stimulations). Light stimulation could evoke motility from sites along the entire colon. These effects were prevented by neural blockade with tetrodotoxin (n = 2), and did not occur in control mice (n = 5). Light stimulation of proximal colon increased the proportion of natural fecal pellets expelled over 15 minutes in vitro (75% ± 17% vs 32% ± 8% for controls) ($P < .05$). In vivo, activation of wireless light-emitting diodes implanted onto the colon wall significantly increased hourly fecal pellet output in conscious, freely moving mice (4.17 ± 0.4 vs 1.3 ± 0.3 in controls) ($P < .001$). **CONCLUSIONS:** In studies of mice, we found that focal activation of a subset of enteric neurons can increase motility of the entire colon in vitro, and fecal output in vivo. Optogenetic control of enteric neurons might therefore be used to modify gut motility.

Keywords: Peristalsis; Gut Motility; Colonic Migrating Motor Complex; Myoelectric Complex.

Compared with all other internal organs, the gastrointestinal tract is unique in that it possesses an independent nervous system, known as the enteric nervous system (ENS).[1,2] Comprising similar numbers of neurons as the spinal cord, the ENS has intrinsic sensory neurons within the gut wall, in addition to populations of interneurons and excitatory and inhibitory motor neurons.

*Authors share co-first authorship; §Authors share co-senior authorship.

Abbreviations used in this paper: **CAL, calretinin; ChAT, choline acetyltransferase; CNS, central nervous system; ENS, enteric nervous system; NOS, nitric oxide synthase; ChR2, channelrhodopsin-2; Cre-recombinase, causes recombination; eYFP, enhanced yellow fluorescent protein; PBS, phosphate-buffered saline; TTX, tetrodotoxin.**

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WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

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NEW FINDINGS

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LIMITATIONS

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Enteric neurons coordinate complex behaviors fully autonomous of the central nervous system (CNS). Perhaps the best demonstration of this, is that isolated segments of bowel are capable of generating propulsive gut motility without CNS input.[3] ENS activity is essential for the coordinated release of excitatory neurotransmitters onto smooth muscle, facilitating contraction of smooth muscle and propulsion of ingested content. Optogenetics has been well established in the CNS, but comparatively less so in the peripheral nervous system. The possibility of using focal application of light to control ENS excitability and improve transit of contents without using agonists that act in multiple organ systems with off-target effects, is particularly attractive.

Calretinin is a calcium-binding protein encoded by the gene calbindin 2. Calretinin is expressed by a subset of myenteric neurons involved in gut motility. These include cholinergic motor neurons, interneurons, and putative sensory neurons.[4,5] Propagating contractions of the murine large intestine has a neural origin (neurogenic).[6] Intrinsic primary sensory neurons, which express calretinin, may initiate propagating neurogenic contractions.[7] We hypothesized that expression of the light-sensitive cation channel, channelrhodopsin2 in calretinin neurons would enable control of colonic neurogenic contractions.

In this study, we generated mice expressing channelrhodopsin2 (ChR2) and enhanced yellow fluorescent reporter protein (eYFP), in cells expressing calretinin (*CAL-ChR2*$^{Cre+}$; see Methods section). Focal light-activation of calretinin enteric neurons evoked polarized motor reflexes and propulsive neurogenic contractions in vitro, leading to significantly increased expulsion of natural fecal pellets both in vitro and in vivo. Parts of this study have been published in abstract form.[8]

Methods

Animals

Calb2-IRES-Cre (B6(Cg)-*Calb2*$^{tm1(cre)Zjh}$/J) and Rosa-CAG-LSL-ChR2(H134R)-eYFP-WPRE (Ai32,[9] B6.Cg-*Gt(ROSA)26Sor*$^{tm32(CAG-COP4*H134R/EYFP)Hze}$/J) mice were obtained from Jackson Laboratories (Bar Harbor, ME). Mice expressing Cre-recombinase (causes recombination) were crossbred with Ai32 mice (Figure 1*C*). Resulting *CAL-ChR2*$^{Cre+}$ progeny expressed the ChR2(H134R)-eYFP fusion gene in cells expressing the CALB2 gene product, CAL. All mice were housed under a 12-hour light/dark cycle with food and water provided ad libitum. Experiments were done by observers blind to the treatments or genotypes of animals. All mice used for behavior tests were genotyped and allocated to experimental groups or control groups. *Cre*$^{-}$ littermates were used as controls in all experiments. Strictly matched mice (gender, age) were used at 8 to 12 weeks old for all the experiments. Animal studies are reported in compliance with the ARRIVE guidelines. All experiments were performed in accordance with the guidelines of the National Institutes of Health and the International Association for the Study of Pain, and were approved by the Animal Studies Committee at Washington University School of Medicine.

Tissue Preparation

Mice were killed by cervical dislocation. The abdomen was immediately opened and the entire colon from caecum to terminal rectum was removed. In all experiments except the imaging of natural pellet expulsion, the full length of colon was placed in a Petri dish filled with carbogen-gassed (95% O_2 / 5% CO_2) Krebs solution (25–30°C; in 10^{-3} M concentrations: NaCl 118; KCl 4.7; NaH_2PO_4 1; $NaHCO_3$ 25; $MgCl_2$ 1.2; D-Glucose 11; $CaCl_2$ 2.5). Residual pellets were gently flushed from the colon using Krebs solution and mesentery was removed. For recording natural pellet expulsion in vitro, the terminal rectum was occluded by tying off with fine suture thread before the colon was removed from the animal.

Immunohistochemistry

For immunohistochemical analysis, the colon was pinned into Krebs-filled, Slygard-lined Petri dish. Preparations were made into a flat sheet by cutting longitudinally along the mesenteric attachment. Preparations were fixed under maximal stretch in modified Zamboni fixative (2% formaldehyde, 0.2% saturated picric acid in 0.1M phosphate buffer, pH 7.0) for approximately 24 hours at 4°C. Tissue was cleared with 3 washes of 100% dimethyl sulfoxide, and stored in phosphate-buffered saline (PBS) at 4°C. To improve visualization of myenteric ganglia, the mucosa, submucosa, and longitudinal muscle layers were removed by sharp dissection.

Preparations of circular muscle and myenteric plexus were incubated with antisera to calretinin (1:500, AB5054; Millipore, Bedford, MA), choline acetyl-transferase (1:50, AB144P; Millipore) or nitric oxide synthase (NOS) (1:300, AB1529; Millipore) at room temperature for 2 days. Preparations were then rinsed 3 times using PBS and incubated with appropriate secondary antisera (1:300, donkey anti-rabbit, catalog no. 711165152; donkey anti-goat, catalog no. A11055, Invitrogen, Carlsbad, CA, or donkey anti-sheep, catalog no. 713095147,