Title: Supplementary Data 1

Description: 3D printing files for gut organ cultures device.

Title: Supplementary Data 2.

Description: Arduino Code for controlling LED light patterns.

Title: Supplementary Data 3

Description: Quantification of gut motility dynamics and pixel-based transitions across frames in Cre+ and control (Cre-) groups.

Title: Supplementary Data 4

Description: Differential gene expression analysis for colon tissues dissected from ChAT-ChR2 mice and illuminated by 2Hz blue light vs. yellow light (CTRL), using the optogenetics-integrated gut culture system. Tissues were collected following 2h in culture.

Title: Supplementary Data 5

Description: Differential gene expression analysis for colon tissues dissected from ChAT-ChR2 mice and illuminated by 10Hz blue light vs. yellow light (CTRL), using the optogenetics-integrated gut culture system. Tissues were collected following 2h in culture.

Title: Supplementary Data 6

Description: Genes within enteric neuron classes (per Morarach et al., Nature Neuroscience, 2021) that exhibited significant up- or down-regulation in response to optogenetic stimulation 2Hz/10Hz blue light vs. yellow light (CTRL).

Title: Supplementary Data 7

Description: Differential gene expression analysis for colon tissues dissected from ChAT-ChR2 mice and illuminated by 10Hz yellow light vs. no light (darkness), using the optogenetics-integrated gut culture system. Tissues were collected following 2h in culture.

Title: Supplementary Data 8

Description: Differential taxa abundance at the species level in colon tissues dissected from ChAT-ChR2 mice and illuminated with 10 Hz blue light vs. yellow light (CTRL), using the optogenetics-integrated gut culture system. The microbiome was collected from tissues following 2h in culture.

Title: Supplementary Data 9

Description: Differential gene expression analysis for colon tissues dissected from Tac1-Cre mice and illuminated by 10Hz blue light comparing Tac1-Cre-Positive-ChR2 vs. Tac1-Cre-Negative-ChR2, using the optogenetics-integrated gut culture system. Tissues were collected following 2h in culture.

Title: Supplementary Data 10

Description: Differential gene expression analysis for colon tissues dissected from ChAT-ChR2 mice and illuminated by 10Hz blue light and infused with sterile culture medium (without *C. ramosum*) vs. yellow light (CTRL), using the optogenetics-integrated gut culture system. Tissues were collected following 2h in culture.

Title: Supplementary Data 11

Description: Differential gene expression analysis for colon tissues dissected from ChAT-ChR2 mice and illuminated by 10Hz blue light and infused with *C. ramosum* vs. yellow light (CTRL), using the optogenetics-integrated gut culture system. Tissues were collected following 2h in culture.

Title: Supplementary Data 12

Description: Gene set enrichment analysis (GSEA) with M5 ontology gene sets identified pathways significantly (FDR q<=0.05) up- or downregulated following the optogenetics stimulation as described.

Title: Supplementary Movie 1

Description: Live imaging of light-stimulated ChAT-ChR2 colonic tissues show intracellular calcium influx in myenteric neurons in response to blue light illumination.

Title: Supplementary Movie 2

Description: Optogenetic stimulation of ChAT-ChR2 small intestinal tissues significantly enhanced gut motility.