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Enteric neuron activity in the mouse colon and responses to lumbosacral stimulation V.2

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We use this protocol and it's working

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Abstract

This protocol describes the steps for calcium imaging activity in the enteric nervous system (ENS) and interstitial cells of Cajal (ICC) in isolated colons and ex vivo lumbosacral spinal cord-colon preparations from GCaMP mice.

Troubleshooting

Isolated Colon Preparation

1 Euthanize Ella-GCaMP mice (offspring from the pairing of B6.FVB-Tg(Ella-cre)C5379Lmgd/J [RRID:IMSR_JAX:003724; cat. 003724; Jax Labs] and B6J.Cg-Gt(ROSA)26Sortm96(CAG-GCaMP6s)Hze/MwarJ [RRID:IMSR_JAX:028866; cat. 028865; Jax Labs]), by inhalation of isoflurane and thoracotomy. Remove the entire colon from mouse and place into a Sylgard-lined Petri dish (Dow Corning). Circulate oxygenated artificial cerebral spinal fluid at room-temp (ACSF, containing in mM: 117.9 NaCl [cat. S9888, Sigma], 4.7 KCL [cat. P3911; Sigma], 25 $NaHCO_3$[catS6014; Sigma], 1.3 NaH_2PO_4, [cat. S8282; Sigma] 1.2 $MgSO_47H_2O$ [cat. 230391; Sigma], 2.5 $CaCl_2$ [cat. C1016; Sigma], 11.1 D-glucose [cat. G5767; Sigma], 2 sodium butyrate [cat. B5887; Sigma], and 20 sodium acetate [cat. S2889; Sigma]) with 4µM nifedipine (cat. N7634; Sigma) and 3µM indomethacin (cat. I7378; Sigma) added. Cut the colon open longitudinally and pin flat using minutien pins (Fine Science Tools; cat. 26002-20) with mucosal side facing down. A small amount of stretch should be applied when pinning the colon tissue for optimal imaging conditions. Transfer to the stage of an upright fluorescence microscope (DM6 FS, Leica) equipped with camera (Prime 95B, Photometrics, Roper Scientific) and software (Metamorph, Molecular Devices) for calcium imaging and slowly heat up circulating fluid to 35-37 C (or desired temperature) using a heated water bath.

2 Image calcium signals from myenteric neurons, submucosal neurons, and interstitial cells of Cajal (ICC) in the submucosa. Set acquisition parameters to: 25ms exposure time (for 40Hz sampling rate), 800 frames, binning 2×2. Using a 20X or 40X objective lens, image spontaneous activity as well as responses to colon stimulation using a stimulus isolator (A365, World Precision Instruments) and concentric bipolar electrodes (cat. CBJRJ75; FHC) placed 5 mm oral and 5 mm anal to the field of view. Using one electrode at a time, record the response to electrical stimulation by delivering 20 pulses (100µs duration) at 20Hz, set to occur 10s into the recording (CED 1401 micro4 and Spike 2 software). Then repeat using the other electrode in the same field of view, allowing 2 minutes between each stimulus.

Lumbosacral Spinal Cord Colon Preparation (with spinal cord circuits intact)

3 Euthanize mice by inhalation of isoflurane and thoracotomy. Perform cardiac perfusion using oxygenated ice-cold sucrose ACSF containing in mM: 234 sucrose [cat. S0389, Sigma], 2.5 KCL [cat. P3911; Sigma], 26 $NaHCO_3$[catS6014; Sigma], 1.3 NaH_2PO_4, [cat. S8282; Sigma] 10 $MgSO_47H_2O$ [cat. 230391; Sigma], 0.5 $CaCl_2$ [cat. C1016; Sigma], 11 D-glucose [cat. G5767; Sigma], 2 sodium butyrate [cat. B5887; Sigma], and 20 sodium acetate [cat. S2889; Sigma] and do a laminectomy to expose spinal cord. Dissect spinal cord (T12-S3), pelvic nerve and 4 cm of distal colon and place into Sylgard-lined Petri

dish with circulating oxygenated ice-cold sucrose ACSF, making sure that the spinal cord and colon are still connected via the pelvic nerve. Cut the colon open longitudinally adjacent to the mesenteric border and pin flat (applying some stretch), mucosal side down, and pin spinal cord in place. Remove dura from spinal cord and isolate the dorsal and ventral roots of L6 spinal cord. Replace fluid with room-temp oxygenated ACSF with 4µM nifedipine and 3µM indomethacin added (NOT sucrose ACSF) and move preparation to the stage of an upright fluorescent microscope for calcium imaging and slowly heat up circulating fluid to 31 C (this lower temperature increases the viability of spinal cord tissue).

4 Place two stimulating electrodes on the L6 dorsal and ventral roots to stimulate either the dorsal or ventral root using the same parameters as above except 100 pulses for a total of 5 sec. To verify electrode placement, image over the L6 dorsal root ganglion to confirm activation of neurons (the majority will be activated with dorsal root stimulation, whereas approx 5-10% will be activated with ventral root stimulation). Using the same imaging parameters as above, image myenteric neuron responses to dorsal or ventral root stimulation. Ventral root stimulation should be used as a positive control to make sure the preparation is still viable and intact. To determine the role of spinal cord circuits, cut the roots away from the cord and stimulate the ends of the roots that are still connected to the nerve that innervates the colon. Stimulating ventral roots should still evoke responses, whereas dorsal root should not.

Lumbosacral Colon Preparation with Dorsal and Ventral Roots (spinal cord not intact)

5 Euthanize mice by inhalation of isoflurane and thoracotomy. Perform cardiac perfusion using oxygenated ACSF and do a laminectomy to expose spinal cord. Dissect lumbosacral spinal cord, pelvic nerve and 4 cm of distal colon and place into Sylgard-lined Petri dish with circulating oxygenated ACSF, making sure that the spinal cord and colon are still connected via the pelvic nerve. Remove dura from spinal cord and isolate the L6 dorsal and ventral roots of spinal cord. Cut both roots at the entry point into the spinal cord and remove the spinal cord from the spinal column while leaving behind the L6 dorsal root ganglion with dorsal and ventral roots still attached. Cut the colon open longitudinally, adjacent to the mesenteric border and pin flat (applying some stretch), mucosal side down, and pin spinal column with roots in place. Move preparation to fluorescent microscope for calcium imaging and slowly heat up circulating, oxygenated ACSF (with 4µM nifedipine and 3µM indomethacin added) to 35-37 C.

6 Place two stimulating electrodes on the L6 dorsal and ventral roots to stimulate either the dorsal or ventral root using the same parameters as above except 100 pulses for a total of 5 sec. To verify electrode placement, image over the L6 dorsal root ganglion to confirm activation of neurons (the majority will be activated with dorsal root stimulation, whereas approx 5-10% will be activated with ventral root stimulation). Using the same

imaging parameters as above, image myenteric neuron responses to dorsal or ventral root stimulation. Ventral root stimulation should be used as a positive control to make sure the preparation is still viable and intact.