Enteric neuron activity during spontaneous motor complexes in mouse colon V.1

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ABSTRACT

This protocol describes the steps to prepare mouse colon and image GCaMP calcium activity in myenteric neurons during spontaneous colonic motor complexes.

1 Euthanize EIIa-GCaMP mice (offspring from the pairing of B6.FVB-Tg(EIIa-cre)C5379Lmgd/J

[RRID:IMSR_JAX:003724;cat. 003724; Jax Labs] and B6J.Cg-Gt(ROSA)26Sortm95.1(CAG-GCaMP6f)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 NaHCO3[catS6014; Sigma], 1.3 NaH2PO4, [cat. S8282; Sigma] 1.2 MgSO47H2O [cat. 230391; Sigma], 2.5 CaCl2 [cat. C1016; Sigma], 11.1 D-glucose [cat. G5767; Sigma], 2 sodium butyrate [cat. B5887; Sigma], and 20 sodium acetate [cat. S2889; Sigma]). 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 for optimal imaging conditions. The full-length, full-thickness colon should remain intact for imaging activity during spontaneous motor complexes. 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 using a heated water bath.

2 After the colon has warmed up and equilibrated (10-15 minutes), use a 10X objective, focus on the myenteric neurons and image calcium signals during spontaneous motility behavior. Myenteric neurons can be distinguished from other cell types based on 1) their location in ganglia within the myenteric plexus between the longitudinal and circular muscle layers, 2) cell body shape and size, and 3) the kinetics of their calcium transients. Set imaging parameters to 150ms exposure time, 1200 frames, 2x2 binning to obtain 3-min duration imaging files. Collect several consecutive 3-min imaging files for a total of 9-15 min to capture multiple colon motor complexes (CMCs), which typically occur every 2-5 min depending on the amount of stretch applied to the colon tissue.

3 Export imaging files to ImageJ and open as a multi-TIFF stack. Use the ImageJ plugin "Template Matching, Align Slices in Stack" to quantify and correct for tissue displacement in the x and y axis of the field of view. Save the x and y displacement values for all 1200 frames, convert frames to time by multiplying the frame by 0.150, and plot as a motility trace (displacement over time) in Excel.

4 After imaging files have been corrected for movement in ImageJ and displacement data saved, myenteric neuron activity can be analyzed by drawing regions of interests over cell bodies and plotting the mean intensity changes over time.