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Mechanical colorectal stimuli for GCaMP6f characterization

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We use this protocol and it's working

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Abstract

By adopting our developed high-throughput optical recording system, a large scale of dorsal root ganglia (DRG) neurons were recorded and identified from a whole DRG. Based on this custom-built platform, we functionally characterize numerous mechanosensitive colorectal DRG neurons and plot their topological distribution on the DRG. This protocol includes the steps for tissue preparation (colorectum, pelvic nerve, lumbar splanchnic nerve, thoracolumbar, and lumbosacral DRG), optical signal acquisition (GCaMP signals), and processing. Following this protocol, responses of colorectal DRG neurons to mechanical stimuli were compared between zymosan-treated and control groups.

Materials

Ai95 mice (The Jackson Laboratory, CT, strain# 028865, RRID:IMSR_JAX:028865)
VGLUT2-Cre mice (Jackson Laboratory, CT, strain# 028863, RRID:IMSR_JAX:028863)
Microscope platform (BX51WI, Olympus, Waltham, MA)
10x objective (UMPLFLN 10XW, 0.3 NA)
sCMOS camera (Xyla-4.2P, 82% quantum efficiency, Andor Technology, South Windsor, CT)
SigmaPlot v11.0 (Systat software, Inc., San Jose, CA)
ImageJ software (v. 1.8.0; National Institutes of Health, Bethesda, MD)
MATLAB v2022 (Mathworks Inc., Natick, MA)

Tissue preparation for GCaMP recording

1 Mice (VGU/GCaMP) 8-14 weeks of age were deeply anesthetized by intraperitoneal and intramuscular injection of a 0.4 mL cocktail of ketamine (120 mg/kg) and xylazine (10 mg/kg).

2 Mice were then euthanized by perfusion from the left ventricle with modified ice-cold Krebs solution (in mM: 117.9 NaCl, 4.7 KCl, 25 $NaHCO_3$, 1.3 NaH_2PO_4, 1.2 $MgSO_4$, 2.5 $CaCl_2$, 11.1 D-glucose).

3 A dorsal laminectomy was performed to expose the thoracic and lumbar spinal cord.

4 The colorectum with attached pelvic and lumbar splanchnic nerves and DRGs were carefully dissected via blunt dissection.

5 The tissue was then transferred to a recording chamber perfused with 32-34 degree Krebs solution (in mM: 117.9 NaCl, 4.7 KCl, 25 $NaHCO_3$, 1.3 NaH_2PO_4, 1.2 $MgSO_4$, 2.5 $CaCl_2$, 11.1 D-glucose) bubbled with carbogen (95% O_2, 5% CO_2).

GCaMP signals recording

6 Turn on the light source and set up the microscope settings. An upright microscope platform was used to capture one whole DRG with a water immersed 10x objective using a halogen epi-illumination light source.

7 Turn on the camera. The video was captured using a high-speed sCMOS camera. The resolution of video is 1920×1920. The sampling speed is 65 frames per second. Spatial resolution is 1.6 micrometer.

8 Open the softwares that are used for the experiment. The software ImageJ was used to capture the video. A Matlab GUI software was used to control the custom-built system to distend the colorectum for mechanical stimulation.

9 Distend the colorectum. The cannulated colorectum was distended by four ascending hydrostatic pressures (15, 30, 45, 60 mmHg). Each pressure lasts for 5 sec.

10 Stimulate colorectum by shear flow. The colorectum was then flushed by a shear flow (20-30 mL/min) lasting for 23 sec.

Image analysis and functional characterization of mechanosensitive neurons

11 Align the image stack using ImageJ to remove the spatial movement of the DRG.

12 Extract the potential active neurons based on intensity fluctuation across different frames.

13 Locate the neurons using marker-based watershed segmentation algorithm.

14 Active neurons are classified into four classes based on their response profiles. Low-threshold (LT) muscular neurons are activated by all pressures. High-threshold (HT) muscular neurons are evoked by pressures no lower than 30 mmHg. Mucosal neurons are exclusively activated by shear flow, and muscular-mucosal type is responsive to both distension and shear flow.

15 The topological distribution of responsive neurons is plot using Matlab and statistical analysis is performed using SigmaPlot.