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Imaging and stimulating enteric neurons in the murine large intestine

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Protocol for imaging neurons within the murine myenteric plexus.

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This protocol applies to transgenic animals expressing fluorescent calcium indicators in neuronal cells.

1 A ventral midline incision is made and the whole colon is carefully excised into a Sylguard lined dissection dish containing oxygenated Krebs-ringer solution.

2 Using scissors, cut along the mesenteric border until the colonic tube is now a flat sheet.

3 Carefully pin region of interest (proximal, middle or distal) mucosal side down. Allow for end sections of the proximal and distal regions to be pinned mucosal side up to allow for mucosal brush stimulation. Place two platinum electrode wires across serosal layer in perpendicular direction.

4 Perfuse with oxygenated Krebs-ringers solution at 36-37 degrees Celcius

5 Functional imaging was performed on a Nikon Eclipse FN1 upright fluorescence microscope using Nikon Plan Fluor 20x lens. Image sequences were captured using an Photometrics Prime 95B sCMOS camera and captured on a Windows-based PC using Nikon NIS Elements 4.1. Image sequences were recorded at 25 frames per second.

6 A camel hair paintbrush was used for mucosal stimulation, where each stimulation consisted of a sequence of five brush strokes in approximately 5 seconds. Electronic field stimulation was applied at 20 hertz for 10 seconds; 6 volts .01 millisecond pulse duration.

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