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Measuring tension, pellet transit, and calcium imaging within cell subtypes in response to pelvic nerve stimulation

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A protocol to measure the effects of pelvic nerve stimulation on colonic motility

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Nothing beyond steps

1 After sacrifice, the caudal half of the animal is pinned out. A ventral midline incision is made and the colon is cut at the cecum and separated from the small intestine, which is removed. Then the pelvic nerve is dissected by slowly removing all of the muscle bone and cartilage surrounding the distal colon. Leave the tuft of tiisue surrounding the pelvic nerve intact. For a good reference for the dissection, see: Feng and Gebhart, 2015, J Vis Exp,(95), 52310. The

colon plus pelvic nerves are then placed into a sylguard-lined dissection dish filled with oxygenated Krebs-Ringers solution. The colon is then gently flushed of its contents. For all experiments, the colon is perfused with 35-degree, oxygenated Krebs-Ringers for the duration of the experiment. A suction electrode connected to a 10ml syringe and tubing is then placed over tuft of tissue with pelvic nerve, and upward pressure applied to suck in nerve.

2 For tension, the colon is then drawn over a 1.5-mm diameter fire-polished capillary tube, whose length exceeds that of the colon. An artificial pellet is mounted to the capillary glass and the colon is positioned with the pellet in the middle. The capillary glass is then fixed to the bottom of the organ bath by the ends protruding from each colonic opening. Suture silk is used to connect three force transducers (model TST125C; Biopac Systems, Santa Barbara, CA) to the proximal, transverse and distal segments of the colon. Resting tension is initially set at 8 mN and monitored using an MP100 interface and recorded on a PC running Acqknowledge software 3.2.6 (Biopac Systems). Spontaneous and stimulation-induced responses (20 HZ for 10s) are recorded.

3 For pellet transit, the colon is pinned loosely into a dish and placed under a camera. A mouse fecal pellet that has been dried, then coated with epoxy, and dried again, is placed with forceps into bottom of proximal colon (end of chevrons).The time it takes to begin movement, as well as the speed of movement, through the colon is recorded, both spontaneously and in response to pelvic nerve stimulation at 20 HZ for 10s.

4 For calcium imaging, either Kit-GCaMP6f, ChAT-GCaMP6f, or nNOS-GCaM6f mice are used. In this dissection, the middle colon only is slit from the ventral midline, and pinned with gentle tension. This is the area of the colon that is imaged. Then calcium responses are measured in response to pelvic nerve stimulation at 20 HZ for 10s.

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