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Optogenetically inhibiting enteric neurons in the murine large intestine

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Protocol for optogenetically inhibiting neuronal subtypes in murine colonic myenteric plexus.

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This protocol applies to transgenic animals expressing the optogenetic protein halorhodopsin in colonic myenteric neurons.

Although this technique apparently inhibits some populations of cells, we were never able to see any effects of any amount of light stimulation of cholinergic or nitrergic myenteric neurons on colonic smooth muscle tension.

ChAT-Cre transgenic mice
nNOS-CreERT2 transgenic mice
HaloRhodopsin knockin conditionally-expressing mice

1 Dissection and experiments are done in the dark under infrared illumination. 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 The colon is then drawn over a 1.5-mm diameter fire-polished capillary tube, whose length exceeds that of the colon.

3 An artificial pellet is mounted to the capillary glass and the colon is positioned with the pellet in the middle.

4 The capillary glass is then fixed to the bottom of the organ bath by the ends protruding from each colonic opening.

5 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.

6 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).

7 After spontaneous colonic migrating motor complexes (CMMCs) are detected, yellow-green light from a 532 nm, 300 mW laser (MGL-III-532, OptoEngine) is shined at 5Hz with a pulsewidth of 30ms for 10-60 seconds in duration, either during spontaneous CMMCs or in between them.

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