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Optogenetically stimulating enteric neurons in the murine large intestine.

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Protocol status: Working
We use this protocol in our group and it is working. We are moving to a TTL-coupled laser-emitting diode system to control timing of laser pulses for nNOS-ChR2 mice, similar to the timed LED pulses that are sufficient to drive contractions in the colon of ChAT-ChR2 mice.

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Abstract

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

Guidelines

This protocol applies to transgenic animals expressing the optogenetic protein channelrhodopsin-2 in colonic myenteric neurons.

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, blue light is shined continuously for 20s by a hand-held laser (450 nm Sapphire Galaxy 3; ZBolt; Happy Valley, OR) mounted on a clamp stand, positioned 30 cm above the preparation, or by a combination of 4 pairs of LEDs placed on either side of the colon, connected to a wire driven by an SD9 stimulator at 5Hz (20ms pulsewidth) for 5, 20 or 60 seconds.

8 After several control light stimulations, drugs are perfused and light stimulations are obtained again.