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Measuring tension, pellet transit, and calcium imaging within cell subtypes in response to direct electrical field stimulation of colon

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Measurement of the effects of direct electrical stimulation of the mouse colon

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Nothing more than in steps.

Same as in dx.doi.org/10/17504/protocols.io.82fhybn

not applicable

Make sure there is enough gas.

1 A ventral midline incision is made and the entire colon is excised and 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 expt.

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). Two platinum wire electodes are placed on top of proximal or distal colon. Square wave electrical stimulation, pulsewidth 0.3 ms, is applied at various frequencies and durations.

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 electrical stimulation at various locations and frequencies/durations.

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 electrical stimulation at various locations and frequencies/durations.

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