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Measuring tension and calcium imaging within cell subtypes of the colon in response to vagus nerve stimulation

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Dante Heredia¹, Thomas Gould¹

¹University of Nevada, Reno

SPARC

Tech. support email: info@neuinfo.org



Thomas Gould

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Protocol status: Working

We use this protocol and it's working

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Abstract

Protocol for measuring tension and activity in distinct cell types of the colon in response to stimulation of the vagus nerve in an ex vivo preparation lacking the afferent brainstem targets of the vagus nerve.

Guidelines

Nothing more than in steps.

Materials

Same as in dx.doi.org/10.17504/protocols.io.82fhybn

Safety warnings

 Not applicable

- 1 The entire gut, including esophagus with vagus nerve trunks attached, and stomach and intestines and mesentery, is dissected and placed in a dish with oxygenated Krebs-Ringers solution. The small intestine, as well as pancreas and spleen, are removed carefully. The colon is pinned and gently flushed of its contents. The right vagus nerve trunk is gently dissected away from the esophagus. The tissue is perfused with 35-degree, oxygenated Krebs-Ringers for the duration of the experiment.
- 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). A suction electrode connected to a 10ml syringe and tubing is then placed over the right vagus nerve trunk and upward pressure is applied to suck in the nerve. Stimulation is then performed with a variety of pulse duration durations and frequencies.
- 3 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. A suction electrode connected to a 10ml syringe and tubing is then placed over the right vagus nerve trunk and upward pressure is applied to suck in the nerve. Stimulation is then performed with a variety of pulse duration durations and frequencies, and calcium responses are imaged.