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Contractile response to chemogenetic activation or inhibition of cholinergic or nitrergic myenteric neurons of the mouse colon

Thomas Gould[1], Dante Heredia[1]

[1][University of Nevada Reno School of Medicine]

1 *Works for me* dx.doi.org/10.17504/protocols.io.btuznnx6

Thomas Gould

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ABSTRACT

Protocol for measuring colonic migrating motor complexes in response to chemogenetic activation or inhibition of cholinergic or nitrergic myenteric neurons of the mouse colon

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GUIDELINES

This protocol applies to transgenic animals expressing the chemogenetic proteins hM3Dq or hM4Di in cholinergic or nitrergic colonic myenteric neurons.

We were able to see distinct effects of activating or silencing cholinergic or nitrergic neurons by this technique.

MATERIALS TEXT

ChAT-Cre transgenic mice
nNOS-CreERT2 transgenic mice
hM3Dq or hM4Di conditionally-expressing mice
Clozapine N-Oxide

1 A ventral midline incision is made and the whole colon is carefully excised into a Sylgard-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, 10 mM clozapine-n-oxide is perfused into the bath.

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