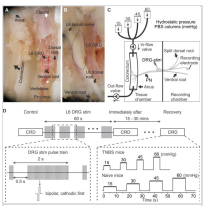

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Dorsal Root Ganglia stimulation-block colorectal afferents

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To test the feasibility of transmission block in visceral afferents by DRG stimulation, we developed an ex vivo
preparation in which mouse colorectum, pelvic nerve (PN), L6 DRG, and dorsal root (DR) were harvested in continuity. We conducted experiments with tissues harvested from mice receiving intracolonic treatment of 2,4,6-trinitrobenzenesulfonic acid (TNBS), a model of postinfectious Irritable bowel syndrome (IBS). We then systematically investigated the blocking effect of DRG stimulation by conducting single-fiber recordings from split L6 DRs of action potentials evoked from peripheral endings in the colorectum through colorectal distension (CRD).

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Animals:
C57BL/6 mice (C57BL/6NTac, RRID:IMSR_TAC:b6, Taconic, Germantown, NJ)

TNBS (Sigma-Aldrich, St. Louis, MO)
Dietary gel (NGB-1; Bio-Serv, Flemington, NJ)
Isoflurane (Hospira Inc., Lake Forest, IL)

22-gauge feeding needle(#18061-22; Fine Science Tools, Foster City, CA)
TDT system (PZ5-32, RZ5D, IZ2H, Tucker-Davis Technologies [TDT], Alachua, FL)
Custom-built colorectal distension device
Needle electrode (FHC, platinum-iridium)
Custom-built recording electrodes
Custom-built perfusion chamber

Software:
SigmaPlot v11.0 (Systat Software, Inc., San Jose, CA)
MATLAB v2022 (Mathworks Inc., Natick, MA)

Intracolonic TNBS treatment

1 C57BL/6 mice (8-16 weeks, 25-35 g, and either sex) were anesthetized by isoflurane inhalation.

2 Then, mice were transanally administered with 2,4,6-trinitrobenzene sulfonic acid (TNBS) (0.2 mL at 10 mg/mL in 50% ethanol; Sigma-Aldrich, St. Louis, MO) using a 22-gauge feeding needle (#18061-22; Fine Science Tools, Foster City, CA), and held in a head-down position (~30°) for 5 minutes to preserve TNBS in the colorectum.

3 Dietary gel (NGB-1; Bio-Serv, Flemington, NJ) was provided to mice showing severe weight loss (0.5% original body weight).

Ex vivo colorectum-Pelvic Nerve-Dorsal Root Ganglia-Dorsal Root (PN-DRG-DR) preparation

4 Mice at 7 to 14 days after TNBS treatment were used, a time span of colorectal hypersensitivity. C57BL/6 mice without TNBS treatment were used as control.

5 Mice were anesthetized by 2% isoflurane inhalation followed by intraperitoneal and intramuscular injection of a ketamine/xylazine cocktail (100/10 mg per kg weight).

6 Mice were then euthanized by exsanguination from the right atrium and transcardiac perfusion from the left ventricle with oxygenated (95% O2, 5% CO2) ice-cold Krebs solution containing (mM) 117.9 NaCl, 4.7 KCl, 25 NaHCO3, 1.3 NaH2PO4, 1.2 MgSO4, 2.5 CaCl2, and 11.1 D-glucose.

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7 Dorsal pediculectomy was performed to expose the spinal cord and DRG from T12 to S1 segments.

8 Exsanguinated mouse carcass was then placed in a dissection chamber circulated with oxygenated ice-cold Krebs solution.

9 The colorectum-PN-DRG-DR were carefully dissected and transferred to a custom-built chamber consisting of a tissue chamber and an adjacent recording chamber.

10 The colorectum, PN, and L6 DRG were placed in the tissue chamber perfused with oxygenated Krebs solution at 30°C, and the DR was gently pulled into the recording chamber filled with mineral oil (Fisher Scientific, East Greenwich, RI).

11 The L6 DR was split into fine filaments (~10 um) for single-fiber recordings from individual afferent axons using a custom-built microwire electrode array.

12 The colorectum was cannulated and connected to custom-built colorectal distension (CRD) device consisting of 4 hydrostatic columns of phosphate-buffered saline (PBS) set at 15, 30, 45, and 60 mmHg pressures, respectively.

Protocol for DRG stimulation

13 A blunt-tipped needle electrode (FHC, platinum-iridium, tip size Φ ~25μm) was used to deliver constant current stimulation to the caudal region of the L6 DRG, where the somata of high-threshold colorectal afferents are clustered.

14 Biphasic constant current stimuli (charge-balanced bipolar) generated by an IZ2H stimulator (Tucker-Davis Technologies Inc, Alachua, FL) were delivered to the L6 DRG at a wide frequency range from 10 to 1000 Hz.

15 The stimulus pulse width was set to be either 0.1 or 0.2 ms based on the chronaxie measurement.

16 The amplitude of DRG stimulation was set to be suprathreshold so as to evoke action potentials in single-fiber recordings. In some experiments, subthreshold DRG stimulation was also tested during which no action potentials were evoked.

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17 A typical DRG stimulation protocol consisted of 30 pulse trains at 0.5 Hz train frequency and 0.5-second intertrain intervals (60 seconds in total).

Protocol for colorectal distension

18 The CRD protocol was performed before (control), immediately after, and 15 to 30 minutes after DRG stimulation (recovery).

19 The CRD protocol consisted of 4 ascending pressure steps of 5 and 10 seconds duration for TNBS-treated and naive mice, respectively, and 8 seconds interstep intervals (15, 30, 45, and 60 mmHg).

Data processing

20 Recorded data were processed offline to identify individual action potentials.

21 The number of action potentials evoked by each CRD protocol was normalized to the number of action potentials evoked by 60 mmHg distension (=100%) in the control trial.

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