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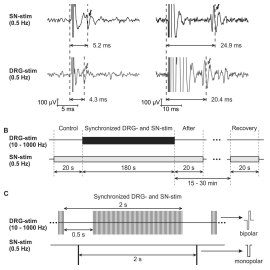
Synchronized spinal nerve and dorsal root ganglia stimulation



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We use this protocol and it's working

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Abstract

Assessing the instantaneous effect of dorsal root ganglia (DRG) stimulation on the transmission of electrically evoked action potentials from the spinal nerve via the ex vivo preparation with mouse L6 spinal nerve (SN), DRG, and dorsal root (DR) in continuity, i.e., the SN-DRG-DR preparation. Synchronized spinal nerve and DRG stimulation reveal a progressive increase in conduction delay by DRG stimulation, suggesting an activity-dependent slowing in blocked fibers. Midrange frequencies (50-500 Hz) are more efficient at blocking transmission than lower or higher frequencies.

Materials

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

Isoflurane (Hospira Inc., Lake Forest, IL)

TDT system (PZ5-32, RZ5D, IZ2H, Tucker-Davis Technologies [TDT], Alachua, FL)
Needle electrode (FHC, platinum-iridium)
Suction electrode (quartz glass capillary tip dia~300 um)
Custom-built recording electrodes
Custom-built perfusion chamber

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

Troubleshooting

Ex vivo spinal nerve-dorsal root ganglia-dorsal root (SN-DRG-DR) preparation

1 C57BL/6 mice (8-16 weeks, 25-35 g, and either sex) were anesthetized by isoflurane inhalation followed by intraperitoneal and intramuscular injection of a ketamine/xylazine cocktail (100/10 mg per kg weight).

2 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.

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

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

5 The spinal nerve (SN), dorsal root ganglia (DRG), and the attached dorsal root (DR), i.e. SN-DRG-DR preparation were carefully dissected and transferred to a custom-built chamber consisting of a tissue chamber and an adjacent recording chamber.

6 The SN 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).

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

Determine DRG stimulation location and amplitude

8 Neural transmission from the SN to the DR was evoked by electrical stimulation of the SN through a suction electrode pulled from a quartz glass capillary (tip Φ ~300 µm), which delivered cathodic constant current stimulation (0.2 ms pulse width and 0.2-2 mA pulse amplitude) through a stimulus isolator (A365; World Precision Instruments, New Haven, CT).

9 A blunt-tipped needle electrode (FHC, platinum-iridium, tip size Φ ~25µm) was used to deliver biphasic constant-current stimulation to the L6 DRG, which was generated by an IZ2H stimulator (Tucker-Davis Technologies Inc, Alachua, FL). The stimulus pulse width was set to be either 0.1 or 0.2 ms based on the chronaxie measurement.

10 To determine the threshold amplitude of DRG stimulation, we first evoked action potentials in the DR by stimulation of the SN or DRG at 0.5 Hz, which can evoke activity

in the same afferent as evidenced by identical waveforms in the single-fiber recording but different conduction delays.

11 Then, the threshold amplitude of DRG stimulation was determined by stimulating 3 locations along the axial length of the DRG to determine the minimal current amplitude to evoke 3 to 5 action potentials with every 10 stimulus pulses (0.5 Hz). We set that DRG stimulation location for that afferent throughout an experiment.

12 The DRG stimulus intensities were set to be either subthreshold or suprathreshold, corresponding to 70% to 80% and 120% to 150% of the threshold current amplitude, respectively.

Synchronized SN and DRG stimulation

13 Run spinal nerve stimulation for 20 seconds (10 stimulations at 0.5 Hz) as a control group and simultaneously record action potentials from the split dorsal root.

14 Run the temporally synchronized SN and DRG stimulation protocol. The train frequencies of DRG stimulation and pulse frequency of SN stimulation were both set to be 0.5 Hz. The intertrain interval of DRG stimulation was set to be 0.5 seconds and in the middle of which SN stimulation was delivered. The synchronized SN and DRG stimulation protocol consisted of 180 seconds of combined SN and DRG stimulation. In each DRG stimulation protocol, pulse frequency was set to be one of the following: 10, 50, 100, 500, or 1000 Hz.

15 Run spinal nerve stimulation immediately after the termination of DRG stimulation for 20 seconds (10 stimulations at 0.5 Hz) and simultaneously record action potentials from the split dorsal root.

16 After 15-30 minutes, run another spinal nerve stimulation for 20 seconds (10 stimulations at 0.5 Hz) as a recovery group.