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🌐 Current clamp recordings

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We use this protocol and it's working

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Abstract

To better understand LRRK2 effects on intrinsic iSPNs adaptations, *Drd2-eGFP* BAC reporter mice were injected with haloperidol, haloperidol + MLi-2, or vehicle controls for 14 days. Then, we performed whole-cell current-clamp recordings on identified iSPNs in ex vivo striatal slices.

Troubleshooting

Acute Slice Preparation

1   *Drd2-eGFP* mice were treated with either haloperidol or haloperidol plus LRRK2 inhibitor(MLi-2) for 14 days.

2   On day 15 or 16, mice were deeply anesthetized on isoflurane (3%) prior to perfusion with ice-cold aCSF containing in (mM): 127 NaCl, 2.5 KCl, 1.25 NaH2PO4, 25 NaHCO3, 20 glucose, 2 CaCl2, and 1 MgCl2.

3   Brains were dissected and sectioned on a Leica vibratome (VT1000S) to 300 um thickness.

4   Slices were transferred to a holding chamber and incubated at 34°C for 20 minutes prior to recordings.

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5   Slices were placed in the recording chamber at RT and continually perfused with oxygenated aCSF with 1mM scopolamine to block cholinergic interneuron firing.

6   The dorsal striatum was located under a 10x air objective, then switched to a 60x water immersion objective was used to target neurons for recording (Olympus, Tokyo, Japan). Slices were visualized using DIC and a QIClick microscope camera (QImaging, Surrey, Canada). GFP-positive cells were selected using a CoolLED pe4000 system (CoolLED Ltd., Andover,UK).

7   Patch electrodes were pulled from borosilicate capillary glass to a resistance of 2.5–6 MΩ. Electrodes were filled with an intracellular solution comprised of (in mM) 135 K-gluconate, 4 KCl, 10 HEPES, 10 Na-phosphocreatine, 4 MgATP, 0.4 Na2GTP, 1 EGTA, 20 μM Alexa 594 (pH 7.28, 298-305 mOsm/L).

8   After break-in, cells were held for ~5-10 minutes prior to current clamp recordings.

9   Recordings were acquired using a Multiclamp 700B amplifier (Axon Instruments, Union City, CA), digitized at a rate of 20 kHz, and filtered at a rate of 3-4 kHz.

10 Five second-long sweeps were acquired using a version of the MATLAB-based (MathWorks, Natick, MA) acquisition suite, ScanImage.

11 A series of 500 ms-long current steps from -50 to 300 pA were pseudorandomized. Current steps occurred one second after the start of the sweep, with a 20 ms-long, -20 pA current step added at the end of the sweep to test input resistance throughout the recording.

12 Cells with input resistance that changed >10% over the recording were discarded. Sweeps occurred with an inter-sweep interval of 10-20 seconds. A minimum of five sweeps for each current step magnitude was obtained.

Current clamp recording analysis

13 Average firing rate at each current step was determined using custom built MatLab code.

14 All values are reported as Hz. Firing rate values were then exported to GraphPad Prism (GraphPad, LaJolla, CA) for data visualization and statistics.