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In Vivo Opto-Tagging and Extracellular Recording (Acute and Chronic)

In 1 collection

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We use this protocol and it's working

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Abstract

This protocol describes in vivo extracellular recordings with opto-tagging of VTA units in acute and chronic configurations. Extracellular data are acquired using the Open Ephys acquisition system with Intan headstages at 30 kHz sampling. Opto-tagging is performed using brief light pulses to identify ChR2- and Chrimson-expressing units, followed by additional stimulation protocols as required.

Materials

- Stereotaxic frame and isoflurane anesthesia system for acute experiments.
- Acute optrodes: Cambridge Neurotech H3 and H10 (64-channel; 1200 µm recording span).
- Chronic optrodes: Cambridge Neurotech H8b and H10b (32-channel) with integrated 200 µm fiber (NA 0.39) mounted on a nano-drive.
- Laser sources for 470 nm (ChR2) and 635 nm (Chrimson) stimulation.
- Patch cords and commutator as needed.
- Synchronization: TTL lines recorded alongside neural data (stimulation TTLs).

A. Acute Opto-Tagging Recordings

1 After 4-5 weeks of viral expression, anesthetize the mouse with isoflurane (3-4% induction; 1.0-1.5% maintenance) and secure in a stereotaxic frame.

2 Open a small craniotomy over VTA and lower a 64-channel acute optrode (H3 or H10) to VTA (first penetration: AP -3.25 mm, ML +0.50 mm, DV -4.90 mm; tip from bregma).

3 Perform 3-4 penetrations per experiment, shifting the entry site by approximately 200 µm (anterior, posterior, medial) while targeting the same depth.

4 Connect the probe to an Intan RHD2164 headstage and start acquisition in the Open Ephys GUI at 30 kHz.

5 Record a baseline epoch.

6 Perform opto-tagging with 1 ms light pulses at 5 Hz delivered as 3 s ON, 10 s OFF (210 pulses total): 470 nm at 15 mW for ChR2-DAT units and 635 nm at 5 mW for Chrimson-tagged VTAPnO units.

7 After tagging, deliver continuous 635 nm light (1 s) every 10 s for 3 min to probe pathway recruitment (if included in the experimental plan).

8 At the end of the session, transcardially perfuse for histology as required.

B. Chronic Recordings and Opto-Tagging

9 Connect the chronically implanted mouse (optrode with integrated fiber) to the headstage and patch cord.

10 For chronic recordings, use an Intan RHD2132 headstage and acquire data in the Open Ephys GUI at 30 kHz.

11 Record baseline activity and perform opto-tagging using 1 ms pulses: 470 nm for ChR2-DAT units and 635 nm for Chrimson-tagged VTAPnO units.

12 Proceed with the behavioral paradigm as required (See protocol for rotarod forced forward or reverse locomotion).

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Acknowledgements

Outputs
- Raw extracellular recordings (.continuous or equivalent Open Ephys formats) at 30 kHz.
- Stimulation TTL timestamps recorded synchronously with neural data.
- Behavioral synchronization signals for peri-event analyses (when applicable).

Critical Steps
- Confirm stable headstage connection and low-noise baseline before stimulation.
- Record all TTLs through the acquisition system to ensure precise alignment.
- Maintain consistent light power settings at the fiber tip as specified in the relevant stimulation protocols.