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In Vivo Fiber Photometry Recording With Concurrent Optogenetic Stimulation

In 1 collection

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol describes in vivo fiber photometry recordings from freely moving DAT-Cre mice expressing jGCaMP8f in VTA neurons. Dual-wavelength excitation (470 nm signal channel and 405 nm isosbestic channel) was sinusoidally modulated and demodulated using a Doric fluorescence minicube system. In some experiments, concurrent optogenetic stimulation of Chrimson-expressing neurons was delivered through the same ferrule.

Guidelines

- Avoid spectral overlap in filter configuration.
- Use non-opsin controls to confirm absence of optical artifacts.

Materials

Viral Strategy and Implant
- DAT-Cre mice.
- VTA injection: AAV5-Syn-DIO-jGCaMP8f and AAV8-CAG-FLPX-rc[ChrimsonR-tdTomato].
- PnO injection: retroAAV-Ef1a-FlpO.
- Optical fiber: 400 µm core diameter, NA 0.66, implanted above VTA.

Recording Setup
- Doric fluorescence minicube system.
- Silicon photodiode detector.
- Lock-in demodulation via Doric acquisition system.
- Behavioral arena: 20 × 30 cm.
- Freely moving configuration.

Excitation Parameters

GCaMP Signal Channel
- 470 nm excitation.
- Bandpass: 460-490 nm.
- Emission: 500-540 nm.
- Power at ferrule tip: approximately 70 µW.
- Sinusoidal modulation frequency: 208.616 Hz.

Isosbestic Control Channel
- 405 nm excitation.
- Power at ferrule tip: approximately 20 µW.
- Sinusoidal modulation frequency: 333.786 Hz.

Signal Detection
- Fluorescence collected through implanted 400 µm fiber.
- Signal detected via silicon photodiode.
- Channels demodulated by Doric system.
- Demodulated signals exported for downstream processing.

Concurrent Optogenetic Stimulation (When Performed)
- Chrimson stimulation delivered through the same ferrule.
- Wavelength: 568 nm.
- Power at ferrule tip: approximately 150 µW.
- Delivered via external TTL trigger.

Optical Separation Safeguards
- Separate excitation and emission bands for GCaMP.

- Dedicated return paths for green emission.
- 568 nm channel not modulated at 405 or 470 carrier frequencies.
- Non-opsin controls confirmed negligible 568 nm influence on GCaMP detection channel.

Procedure

1 Confirm excitation powers at the ferrule tip.

2 Connect mouse to patch cord.

3 Verify stable fluorescence baseline.

4 Start sinusoidal LED modulation.

5 Begin demodulated signal acquisition.

6 Deliver optogenetic stimulation if applicable.

7 Record synchronized stimulation TTLs.

Outputs

8 Demodulated 470 nm signal trace.

9 Demodulated 405 nm isosbestic trace.

10 Stimulation TTL timestamps.

11 Continuous behavioral session recording.

Critical Steps

12 Maintain stable patch cord coupling.

13 Confirm modulation frequencies remain distinct and stable.

14 Verify optical power before each session.

15 Avoid spectral overlap in filter configuration.

16 Use non-opsin controls to confirm absence of optical artifacts.