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Open-Field Locomotion Assay with Optogenetic Stimulation

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

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Abstract

This protocol describes the open-field assay used to quantify locomotor responses during optogenetic manipulation of the VTA–PnO pathway. Experiments were conducted under controlled illumination conditions during the light phase and synchronized with optical stimulation delivered either to VTA somata (DAT-Cre experiments) or to presynaptic terminals (GAD2 experiments). The VTAPnO projection-defined subpopulation was selectively labeled using a retrograde Flp-dependent targeting strategy, enabling specific manipulation of VTA neurons projecting to the PnO. Locomotor behavior was recorded using overhead video acquisition and analyzed offline for directional movement parameters.

Guidelines

Critical Steps
- Maintain consistent illumination (50 lux) across sessions.
- Measure optical output at the fiber tip immediately before each session and adjust to 5 mW.
- Ensure stable patch cord positioning to avoid torque-induced artifacts.
- Maintain consistent stimulation timing parameters across animals.

Materials

Arena
- Square open-field arena: 50 cm × 50 cm
- Opaque walls
- Uniform illumination: 50 lux
- Experiments performed during the light phase

Video Recording
- Overhead camera
- Frame rate: 30 frames per second
- Continuous recording throughout session
- Stimulation synchronized via TTL or equivalent digital trigger

Before start

Immediately before each behavioral session, optical output was measured at the tip of the implanted fiber using a calibrated power meter. Output power was adjusted to 5 mW at the fiber tip before starting the experiment.

Procedure

1 Connect the optic patch cord to the implanted ferrule. Verify proper connection and ensure free cable movement. Place the mouse in the center of the open-field arena.

2 Begin video acquisition.
Deliver repeated stimulation cycles consisting of:
3 seconds stimulation
10 seconds pause
Continue cycles for a total duration of 5 minutes. Ensure synchronization between stimulation onset and video timestamps.

3 After 5 minutes, stop stimulation and recording. Disconnect the patch cord and return the animal to its home cage.

Data Acquisition and Synchronization

4 Stimulation onset timestamps were recorded.
Video frames were aligned to stimulation epochs for peri-stimulus analysis.
Directional locomotion metrics were extracted offline.