Feb 03, 2025

In vivo voltammetry and fiber photometry in the mouse striatum

In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.dm6gp32kpvzp/v1

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DOI: dx.doi.org/10.17504/protocols.io.dm6gp32kpvzp/v1

Protocol Citation: Yan-Feng Zhang, Stephanie J Cragg 2025. In vivo voltammetry and fiber photometry in the mouse striatum. **protocols.io https://dx.doi.org/10.17504/protocols.io.dm6gp32kpvzp/v1**

Manuscript citation:
Zhang et al. (2024) **An axonal brake on striatal dopamine output by cholinergic interneurons**, bioRxiv

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

Created: November 28, 2023

Last Modified: February 03, 2025

Protocol Integer ID: 91485

Keywords: in vivo, voltammetry, fiber photometry

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: ASAP-020370

Abstract

This protocol describes how to measure extracellular dopamine concentrations and calcium dynamics in striatal dopaminergic axons in response to electrical stimulations using *in vivo* voltammetry and fiber photometry.

Before start

The *in vivo* voltammetry was performed in wild-type mice (C57BL/6J, 21-40 days).

The *in vivo* fiber photometry was performed in two different animal backgrounds:

(A) For measuring calcium dynamics, we use heterozygous DAT-Cre:Ai95D (4-7 weeks) mice that were bred from homozygous DAT-Cre mice (B6.SJL-Slc6a3tm1.1(cre)Bkmn/J, JAX stock number 006660) crossed with homozygous Ai95D mice (B6;129S-Gt(ROSA)26Sortm95.1(CAG-GCaMP6f)Hze/J, JAX stock number 028865). These mice express the genetically encoded calcium indicator GCaMP6f in striatal dopaminergic axons.

(B) For measuring evoked extracellular dopamine concentrations, we followed the steps described in the **Protocol: Intracranial injections of viral vectors in mouse midbrain and striatum** to inject the AAV serotype 5 vector containing GRAB-DA_{2m} (AAV5-hSyn-GRABDA2m) in the dorsal striatum of wild-type mice. The titer dilution was 1E+13 vg/mL.

Preparing the mouse for surgery

1 Anesthetize the mouse in an induction chamber with urethane (1.4–1.9 g/kg, i.p.; Biolab), supplemented with additional urethane (0.2 g/kg) every 1-2 hr as required.

2 Infiltrate all wounds and pressure points with bupivacaine (0.5%).

3 Upon reaching surgical anaesthesia, fix the head in a stereotaxic frame.

4 Monitor core temperature using a rectal probe and maintain it at 35-36°C using a homeothermic blanket.

5 Administer mecamylamine (2 mg/kg) by intraperitoneal injection to block nAChRs in the striatum.

Measuring evoked extracellular dopamine (DA) concentration using fast-scan cyclic voltammetry (FCV)

6 **Surgical Procedure:**

6.1 Remove a round piece of skull overlying the left hemisphere to target the dorsolateral striatum (DLS) (AP +1.0 mm, ML 1.6 mm, DV 2.2 mm to bregma).

6.2 Position a stimulating and recording array consisting of a 7 µm-diameter carbon fiber microelectrodes (CFMs; tip length 50-100 µm) and a bipolar stimulating electrode (MS303/3-A/SPC, P1 Technology) in the DLS.

6.3 Implant the Ag/AgCl reference electrode in another part of the forebrain.

7 **Determining evoked extracellular dopamine (DA) concentration using electrical stimulations:**

8 Use fast-scan cycling voltammetry (FCV) with carbon fiber microelectrodes and a Tarheel system (University of Washington, Seattle, US) to measure evoked extracellular dopamine concentration.

9 Apply voltage as a triangular waveform (-0.4 to +1.3 V range versus Ag/AgCl) at a scan rate of 400 V/s.

10 Sample data at 10 Hz.

11 Apply electrical stimulus (0.65 mA current, 200 µs) using a bipolar stimulating electrode (0.005 inch, MS303/3-A/SPC, P1 Technologies).

Note

Separate the stimulating electrode tips by ~500 µm and glued them to the FCV recording electrode to fix the tip of the FCV electrode between the two stimulating poles.

Measuring axonal calcium dynamics and evoked extracellular dopamine (DA) concentration using fiber photometry

12 **Surgical Procedure:**
These steps were performed in heterozygous DAT-Cre:Ai95D (4-7 weeks) mice expressing the genetically encoded calcium indicator GCaMP6f in striatal dopaminergic axons, or in wild-type mice expressing $GRAB_{DA2m}$ in the dorsal striatum.

12.1 Remove a round piece of skull overlying the left hemisphere to target the dorsolateral striatum (DLS) (AP +1.0 mm, ML 1.6 mm, DV -2.2 mm to bregma) and susbtantia nigra (SNc) (AP -3.1mm, ML 0.8mm, DV -4.3mm to bregma).

12.2 Position the injection and recording array, consisting of a glass pipette and a 200 µm diameter fibre, in the DLS.

13 **Determining axonal calcium dynamics in striatal dopaminergic (DA) axons using electrical stimulations:**

13.1 Activate GCaMP6f expression in DA axons with 480 nm light (76 µW).

13.2 Sample the intensity of GCaMP6f at 40 Hz with Neurophotometrics (FP3001).

13.3 Apply electrical stimulus (0.5 mA current, 500 µs) using a bipolar stimulating electrode (0.005 inch, MS303/3-A/SPC, P1 Technologies) at 0.1 Hz.

Note

Separate the electrode tips by ~500 µm.

14 **Determining evoked extracellular dopamine (DA) concentration using electrical stimulations:**
These steps were performed in wild-type mice expressing $GRAB_{DA2m}$ in the dorsal striatum.

14.1 Activate GRAB-DA_{2m} with 480 nm light (76 µW).

14.2 Sample the intensity of GRAB-DA_{2m} at 40 Hz with Neurophotometrics (FP3001).

14.3 Apply electrical stimulus (0.5 mA current, 500 µs) using a bipolar stimulating electrode (0.005 inch, MS303/3-A/SPC, P1 Technologies) at 0.05 Hz.

Verification of carbon-fibre locations in dorsal striatum

15 Sacrifice anesthetised mice and quickly remove brains.

16 Fix whole brains overnight in 4% PFA.

17 Section fixed brains into 50 µm slices using a vibratome.

18 Mount the individual slices on glass slides and image under microscope to identify the location of recording sites.