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Fast scan cyclic voltammetry

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes the ex vivo fast-scan cyclic voltammetry (FSCV) technique for detecting dopamine within the dorsal striatum in mice.

Carbon fiber electrode preparation

- 1 Suction a single carbon fiber (7 μ m diameter, Goodfellow) into a capillary tube (Sutter).
- 2 Load the carbon fiber/capillary tube into a puller (Narishinge), and under the microscope, hand-cut (scalpel blade) fiber tips 30–100 μ m past the capillary tip.

Brain sections preparation

- 3 Anesthetize mice at postnatal day 90–110 with a ketamine-xylazine mixture.
- 4 Perfuse the mice transcardially with ice-cold artificial cerebrospinal fluid (aCSF) containing the following (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 2.0 CaCl₂, 1.0 MgCl₂, 25 NaHCO₃, and 12.5 glucose, bubbled continuously with carbogen (95% O₂ and 5% CO₂).
- 5 Expose the skull by a caudal to rostral midline incision and gently remove the brain with a spatula
- 6 Glue the brain to the stage of a vibrating microtome (Leica Instrument) and immerse it in ice cold aCSF.
- 7 Cut parasagittal slices containing the dorsal striatum at a thickness of 240 μ m
- 8 Transfer the slices to a holding chamber where they are submerged in aCSF at 37 °C

Fast scan cyclic voltammetry set up

- 9 Recording dish with cover stimulating electrode and carbon fiber electrode, Amplifier (Molecular Devices), a digitizer (Molecular Devices), and pClamp (Molecular Devices)

Voltammetric measurements

- 10 Before recording, condition the electrodes by running the ramp at 60 Hz for 15 min and 10 Hz for another 15 min.
Deliver a voltage ramp to and from 1.2 V (400 V/s) every 100 ms (10 Hz) to detect the oxidation and redox peak for DA.
- 11 Before recording, calibrate the carbon fiber electrode by applying a flow of dopamine diluted in aSCF in known concentrations until dopamine signals are stable
- 12 Dopamine transients are evoked by electrical stimulation delivered through a concentric, bipolar electrode (FHC) placed in the rostradorsal striatum.
- 13 Use a single electrical pulse (**300 MA, 0.2 ms**).
- 14 Perform voltammetric measurements by sampling at dorsostriatal sites ~200–400 μm ventral and posterior from the forceps minor corpus callosum.
- 15 Take four recordings each site with two-minute intervals between recordings and then averaged as a reported measure.
- 16 The voltammogram and peak oxidative current amplitudes of the dopamine transient are measured.
Note: Experiments were rejected when the evoked current did not have the characteristic electrochemical signature of dopamine.

General experimental considerations

- 17
 - Four recordings taken at each site with two-minute intervals between recordings and then averaged as a reported measure.
 - Considering the slice where the GPe was first evidenced as the most lateral slice.*
 - *consecutive slices 480 μm apart and medial of this lateral slice should be considered intermediate and medial ones.*
 - The recording order of the slices is randomized to reduce potential recording biases and mitigate possible electrode sensitivity issues.
 - Electrode sensitivity is to be retested at the end of recordings with a freshly made dopamine stock used for calibration.
 - Recordings from electrodes with a larger than 10% change from calibrated dopamine solution are subtracted.