Oct 08, 2022

Fast scan cyclic voltammetry

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dx.doi.org/10.17504/protocols.io.kxygx9py4g8j/v1

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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.

DOI

dx.doi.org/10.17504/protocols.io.kxygx9py4g8j/v1

PROTOCOL CITATION

Harry Xenias, Savio Chan, Loukia Parisiadou 2022. Fast scan cyclic voltammetry. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kxygx9py4g8j/v1

FUNDERS ACKNOWLEDGEMENT

Aligning Science Across Parkinson's through the Michael J. Fox Foundation for Parkinson's Research (MJFF)
Grant ID: [ASAP-020600]

CREATED

Oct 08, 2022

LAST MODIFIED

Oct 08, 2022

PROTOCOL INTEGER ID

71032

Carbon fiber electrode preparation

1 Suction a single carbon fiber (7µm 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 30-100µm 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 NaH_2PO_4, 2.0 $CaCl_2$, 1.0 $MgCl_2$, 25 $NaHCO_3$, and 12.5 glucose, bubbled continuously with carbogen (95% O_2 and 5% CO_2).

5 Expose the skull by a caudal to rostral midline incision and gentle 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 µm

8 Transfer the slices to a holding chamber where there are submerged in aCSF at 37 ºC

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

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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 rostrodorsal striatum.

13 Use a single electrical pulse (**300 MA**, **0.2 ms**).

14 Perforvoltammetricic 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.

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