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Fast-scan cyclic voltammetry to assess dopamine release in *ex vivo* mouse brain slices

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We use this protocol and it's working

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Abstract

This protocol is to assess changes in extracellular dopamine concentration following electrical or optogenetic stimulations in *ex vivo* mouse brain slices.

Materials

Equipment:

- Ag/AgCl Reference pellet (WPI, SKU# EP08)
- Concentric bipolar stimulating electrode (FHC, SKU# 30200)
- Gilson Peristaltic Mini-Pump (Gilson)
- In-line heater (made in-house)
- Isolated current stimulator (Digitimer Ltd)
- Master8 (AMPI)
- Millar Voltammeter (Julian Millar, Barts and the London School of Medicine and Dentistry)
- PVC connecting tubing bore 1.01, wall 0.838 mm (Altec, SKU# 116-0536-09)
- PVC inlet. 2 stop tubing bore 1.02mm (white/white) (Elkay, SKU# 116-0549-C59)
- PVC outlet. 2 stop tubing bore 1.42mm (yellow/yellow) (Elkay, SKU# 116-0549-120)
- Surgical microscope Leica MZ6 (Leica)
- Leica VT1200 S Fully Automated Vibrating Blade Microtome (Leica)
- 473 nm diode laser (DL-473, Rapp Optoelectronic) coupled to the microscope with a fiber optic cable (200 µm multimode, NA 0.22)

Software:

- Digidata 1440A (Molecular Devices)

Virus:

- Channelrhodopsin-2 (ChR2) fused in-frame with a gene encoding enhanced yellow fluorescent protein (pAAV5-hEF1α-DIO-hChR2(H134R)-EYFP-WPRE-pA) from University of North Carolina Vector Core
- Cre-inducible recombinant AAV serotype 5 vector containing an inverted gene for Chrimson (ssAAV-5/2-hEF1α/hTLV1-dlox-ChrimsonR-tdTomato(rev)-dlox-WPRE-bGHp(A), v288-5) from ETH Zurich

CUTTING

Preparing high Mg2+ solution:

- 2.46 mM $MgCl_2$
- 1.25 mM NaH_2PO_4
- 25 mM KCl
- 25 mM $NaHCO_3$
- 85 mM NaCl
- 0.5 mM $CaCl_2$
- 10 mM glucose
- 65 mM sucrose

RECORDING

Preparing bicarbonate-buffered artificial cerebrospinal fluid (aCSF) solution:

- 130 mM NaCl
- 25 mM $NaHCO_3$

- 2.5 mM KCl
- 1.25 mM NaH_2PO_4
- 2.5 mM $CaCl_2$
- 2 mM $MgCl_2$
- 10 mM glucose

Troubleshooting

Before start

For experiments using electrical stimulation, we followed the steps described in **Protocol: Intracranial injections of viral vectors in mouse midbrain and striatum** to inject the desired virus in the midbrain (1 µL per site) of heterozygous $DAT^{IRES\text{-}Cre}$ mice. We injected a Cre-inducible recombinant AAV serotype 5 vector containing an inverted gene for channelrhodopsin-2 (ChR2) fused in-frame with a gene encoding enhanced yellow fluorescent protein (pAAV5-hEF1α-DIO-hChR2(H134R)-EYFP-WPRE-pA). The titer dilution was 1E+12 vg/ml.

The same surgical procedure was performed for experiments using optogenetic stimulation of cholinergic interneurons (ChI) or dopaminergic axons (DA). We injected a Cre-inducible recombinant AAV serotype 5 vector containing an inverted gene for Chrimson (ssAAV-5/2-hEF1α/hTLV1-dlox-ChrimsonR-tdTomato(rev)-dlox-WPRE-bGHp(A), v288-5) in the midbrain, or a Cre-inducible recombinant AAV serotype 2 vector containing an inverted gene for channelrhodopsin-2 fused in-frame with a gene encoding enhanced yellow fluorescent protein (pAAV-double floxed-hChR2(H134R)-EYFP-WPRE-pA) in the striatum of heterozygote DAT-Cre: ChAT-Cre mice.

The coordinates used by us for targeting the midbrain were as follows:

Ventral tegmental area (VTA) (AP = -3.1 mm, ML = ± 0.5 mm, DV = -4.4 mm)
Substantia nigra pars compacta (SNc) (AP = -3.5 mm, ML = ± 1.2 mm , DV = -4.0 mm)
Striatum (AP = +1.0 mm, ML = ± 2.0 mm , DV = -1.8 mm, or AP +1.0 mm, ML ± 1.1 mm, DV -3.8 mm)

Animals were maintained for at least three weeks following surgery to allow virus expression in the midbrain and striatum.

Preparation of *ex vivo* mouse brain slices

1 Prepare cutting solution (see **Materials**), chill and oxygenate.

2 Prepare vibratome settings: 300 µm slices, 0.44 mm/s speed, Δ1.45 mm vibration. Chill plate and buffer tray in freezer, rinse razor blade in acetone.

3 Kill mouse by cervical dislocation, confirm death by exsanguination.

4 Decapitate mouse and take ear-clip for post-hoc genotyping where required (put in 2.5 ml Eppendorf).

5 Remove brain.

6 Block brain with razor blade to remove olfactory bulb.

7 Section brain to 300 µm-thick coronal slices between +1.5 to +0.5 mm from bregma containing caudate-putamen and nucleus accumbens.

8 Leave slices to recover at 32° for 30 to 40 minutes.

9 Move slices to slice saver chamber at room temperature for 1 hour.

Note

The slice saver is made by removing base of plastic beaker and gluing stretched nylon tights over base. Place this into a larger glass beaker filled with bicarbonate-buffered artificial cerebrospinal fluid (aCSF) solution (see **Materials**) and oxygenate.

Setting-up rig

10 Prepare bicarbonate-buffered artificial cerebrospinal fluid (aCSF) solution (see **Materials**).

11 Circulate dH_2O through inflow pipes and recording chamber.

12 Transfer silver weight pins from isopropanol to recording chamber to rinse.

13 Circulate aCSF, ensuring no wicking up temp probe or reference $Ag/AgCl_2$ pellet.

Note

The vast majority of issues affecting experiment quality are due to issues with aCSF flow.

Before adding slice, ensure flow is stable and there is a good flow in the chamber from the inflow to the outflow to ensure slice is being constantly superfused with fresh aCSF.

You can inspect the flow by introducing fluorescent beads (e.g., fluospheres TehrmosFisher F8803) near the inflow and watch them move to the outflow.

Flow can be optimised by ensuring inflow pipe comes into the bottom of the chamber and the outflow and inflow are opposite one another directly across the slice.

14 Heater on, light on, stim box on, computer on. Master8 and VOLTAMMETER* must stay off until in circuit.

15 Place coronal section in recording chamber and place silver pins on cortex to keep slice in place.

Note

Do not stretch the brain tissue.

If recording from NAcc core or shell, be careful with pin placement on the ventral region of the slice to avoid damage or obscuring areas.

16 Insert the recording electrode ~100 µm into the tissue at 45^o angle, connect it to voltammeter headstage, and switch on voltammeter.

17 Check waveform of electrode, and perform a quality control of the electrode.

18 Allow electrode to charge for >30 mins to gain capacitance to ensure stable baseline of DA detection before start of stimulation.

Determining evoked extracellular dopamine (DA) concentration using electrical stimulations

19 The below parameters were used to assess the effect of application of a drug (DHBE or mecamylamine) on dopamine release evoked by single electrical pulses.

Stimulation: 200 µs, 0.65 mA
Temperature: 32-33ºC
Voltammeter sweep: -0.7 to 1.3 V versus Ag/AgCl, 800 V/s. Repeated at 8 Hz. Switches out of circuit between scans.

20 Check settings on axoscope and set file directory and filenames.

21 Make up drugs and ensure fully thawed.

22 Place stimulation electrode (bipolar concentric electrode) on surface of tissue. Surface location minimises damge.

23 Place recording electrode ~100 µm from recording electrode, 100 µm into the tissue.

24 Record dopamine (DA) release.

24.1 Before recording, “refresh” voltammeter. (Specific for acquisition and use of Millar Voltammeter)

24.2 Record >1s of baseline prior to then delivering 1p electrical stimulation.

24.3 Record for sufficient time to allow DA to return to baseline (in drug free condition ~3 s, in the presence of DAT inhibitors will need to record for longer).

24.4 Note approximate peak voltage at DA oxidation potential in a lab book to alllow investigator to follow the approximate outcome of experiments during progress.

Note

Be sure to note any information about signal quality or artefacts in a lab book (e.g., stimulation artefacts, pH shifts, changes in bath level or deviations of temperature).

Ensure stimulation occurs when voltammeter is out-of-circuit to avoid stimulation artefacts.

25 Wait 2.5 mins.

26 Repeat **steps 26** and **27** until >6 stable recordings in control condition.

Note

A stable recording will depend slightly on the expected drug effect. For instance, a small effect will require a more stable baseline.

27 Move inflow pipe to drug cylinder try to minimise bubbles.

28 As drug washes into the recording chamber, watch the oscilloscope in real time as drug washes on for indication of electroactive drugs.

29 Continue **steps 26 and 27** until 12 recordings in drug condition.

Note

Some drugs may take longer to wash on than 2.5 mins.

Stimulations every 2.5 mins should be maintained whilst washing on the drugs. **Do not stop stimulating and recording whilst washing on drugs.**

30 If wash-off is required, move inflow pipe to control solutions and repeat data acquisition for approximately 20 stims.

Note

Wash-offs tend to take longer than wash-ons. Some drugs wash off faster than others. If complete wash-off is required may need to wash on drug for shorter time.

31 After experiment has finished, remove electrodes and ensure voltammeter is switched off when out-of-circuit.

32 Remove slice.

Note

Fix if necessary, take tissue punch for content if necessary.

33 Allow aCSF to run through rig before placing next slice for more experiments or before electrode calibration.

34 At end of the day, wash through with dH20 and then air to ensure clean and dry. Use cotton bud with HCl to clean bath and check no salt build up around temperature probe or ref.

35 Rinse bubblers and bubble dry to ensure they do not backfill with liquid and become contaminated or grow mould.

36 Release pressure points from the peristaltic pump on inflow and outflow tubes to prolong the life of the tubing. **Do not stretch tubing beyond where is necessary to hook on as they may break.**

Note

Replace tubing ~every month depending on use frequency of equipment.

Nomifensine, GBR and citalopram are very sticky drugs. If you use these drugs, make sure you replace inflow tubing between every experiment.

Determining evoked extracellular dopamine (DA) concentration using optogenetic stimulations

37 **Activating ChR2-expressing cholinergic interneurons or dopaminergic axons:**

37.1 Use a 473 nm diode laser coupled to the microscope with a fiber optic cable.

37.2 Spot illumination had a 30 µm diameter under x40 immersion objective.

37.3 Laser pulses were 2 ms duration, 5-23 mW/mm^2 at specimen.

38 **Activating Chrimson-expressing dopaminergic axons:**

38.1 Use a LED with a 585 ± 22 nm filter.

38.2 LED pulses were 2 ms duration, 2.5-3.4 mW/mm^2.

38.3 Lstim$_0$ is achieved by lowering the laser intensity to the point at which there was no detectable evoked FCV signal above noise, in on-line or off-line analyses.