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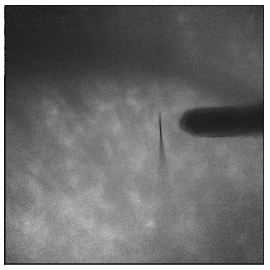
Fast-scan cyclic voltammetry to assess dopamine release in ex vivo mouse brain slices while optogenetically activating astrocytes



Forked from 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 whether optogenetically activating astrocytes affects the dopamine concentration released following electrical stimulation.

Materials

Drugs:

- Dihydro-β-erythroidine hydrobromide (DHβE, 1 μM, Tocris, Cat# 2349)
- (+)-bicuculline (10 μM, Abcam, Cat# ab120107)
- CGP 55845 hydrochloride (CGP, 4 μM, Tocris, Cat# 1248)
- 8-Cyclopentyl-1,3-dimethylxanthine (CPT, 10 μM, Abcam, Cat# ab144745)
- Oxotremorine (10 μM, Tocris, Cat# 1067)

Equipment:

- Millar Voltammeter (Julian Millar, Barts and the London School of Medicine and Dentistry)
- Digidata 1440A (Molecular Devices)
- **Multichannel Systems stimulus generator** (Multichannel Systems, STG3000)
- Microscope BX50WI Olympus
- Microscope C-mount Camera Adapter U-CMAD-2
- Cairn Research OptoLED Light Source
- **Isolated current stimulator** (Digitimer Ltd)
- In-line heater (made in-house)
- **Gilson Peristaltic Mini-Pump** (Gilson)
- **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)
- **Concentric bipolar stimulating electrode** (FHC, SKU# 30200)
- **Ag/AgCl Reference pellet** (WPI, SKU# EP08)

SLICING SOLUTION

Preparing Cutting Solution:

1. Mix in dH_20 for final volume of 1 L:

- 194 mM sucrose
- 30 mM NaCl
- 4.5 mM KCl
- 1 mM $MgCl_2$
- 26 mM $NaHCO_3$
- 1.2 mM NaH_2PO_4
- 10 mM D-glucose

HOLDING AND RECORDING SOLUTIONS

Preparing artificial CerebroSpinal Fluid (aCSF) (10x):

1. Mix in dH_20 for final volume of 1 L:

- 130 mM NaCl
- 2.5 mM KCl
- 26 mM $NaHCO_3$

- 1.25 mM $NaHCl_2$
- 2 mM $MgCl_2$,
- 1.25 mM NaH_2PO_4
- 10 mM glucose

Preparing working solution of aCSF:

1. Mix and dilute in dH_20 for final volume of 1 L

- 100 mL of 10x aCSF
- 2.5 mM $CaCl_2$

CALIBRATION

Preparing Dopamine (DA) solution (2 µM):

1. Add to 25 mL aCSF:

- 20 µL stock DA solution (0.1 M HClO4).

Stock DA solution is stored in fridge wrapped in foil (use by ~1-2 months). Before use, hold up to the light and check it is not pink/brown (oxidation). If solution is oxidised, make up new stock.

Software:

- **Axoscope** (version 11.3)
- **MC Stimulus II** (version 3.5.11)
- **FIJI** (version 1.54f)

Before start

Stereotaxic intracranial injections were performed to deliver AAV5/hgfaABC1D-ChR2(H134R)_EYFP in the mouse striatum. After injection, mice were kept for 3 weeks to ensure appropriate construct expression. These animals were then used in the subsequent steps.

Prior to starting the experiment, you first need to determine a stable baseline. The variance permitted in the baseline will depend upon the effect size of your manipulation. We recommend you first perform a pilot experiment to determine the approximate effect size and then determine a cut-off for any change in dopamine release over time, allowing you to pre-determine an exclusion criteria based on your control data.

If your cut-off is too stringent, you might use more animal tissue than is necessary (not in accordance with the **3Rs**), but also you may be sub-selecting a populations of release-sites that may not reflect the properties of the striatum you hope to generalise to. Setting a cut-off too permissive may result in a larger decay component due to time and may either over-estimate or underestimate your effect.

Consider designing your experiment with a time-matched control. Having a pre-determined exclusion criteria will prevent you from erroneously excluding data that does not match your hypothesis and ensures your findings are more reproducible.

Preparation of *ex vivo* mouse brain slices

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

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 (Schedule 1 method), confirm death by exsanguination.

4 Decapitate mouse and remove brain on to iced plate.

5 Block brain with razor blade to remove olfactory bulb and posterior part of brain.

6 Add small amount of cyanoacrylate glue to magnetic chuck, smooth with pipette tip.

7 Transfer brain containing striatum to glue, mounted on the posterior surface, using filter paper to pick up block, and dampening filter paper to remove paper.

8 **This step is optional.**

Save posterior brain block by transferring to Eppendorf containing 4 % PFA to allow fixation of midbrain (SNc and VTA) containing dopaminergic neurons. Tissue block can be sectioned once fixed to 40 µm sections and used for IHC.

9 Section mounted striatum to 300 µm-thick coronal slices.

10 Move slices to slice saver chamber at room temperature for >1 hr.

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.

11 **Optional step**: Take ear-clip for post-hoc genotyping where required (put in 2.5 ml Eppendorf).

Setting-up rig

12 Prepare artificial cerebrospinal fluid (aCSF) solution and oxygenate (see **Materials**).

13 Pump in dH_2O through inflow pipes and recording chamber to ensure flushed clean before placing slice.

14 Transfer silver weight pins from isopropanol storage vial to recording chamber to rinse.

15 Pump aCSF in to recording chamber, ensuring no wicking up the temperature probe or reference electrode ($Ag/AgCl_2$ 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 ThermoFisher 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 that outflow is at opposite side of chamber ensuring flow of solution directly across the slice.

16 Camera on, heater on, light on, stimulating box on, computer on. Voltammeter* stays off until electrode is in circuit.

17 Place striatal 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 NAc core or shell, be careful with pin placement on the ventral region of the slice to avoid damage or obscuring areas.

18 Visualised using microscope, insert the recording electrode ~100 µm into the tissue at ~45^{o} angle, connect it to voltammeter headstage, and switch on voltammeter.

19 Check waveform applied to electrode, and read detected current to ensure electrode viability and low noise.

20 Allow electrode to charge and stabilise for >30 mins in tissue.

Determining evoked extracellular dopamine (DA) concentration

21 The below parameters were used to assess the effect of optogenetically activating astrocytes on dopamine release evoked by single electrical pulses.

Stimulation: 200 µs, 0.6 mA
Temperature: 31-33°C
Perfusion speed: >1.8 ml/min
Inter-stimulation interval: 2.5 min
Voltammeter sweep: -700V to +1300V to -700V, 800 V/s. Repeated at 8 Hz. Switches out of circuit between scans.
Default gain settings: Full signal gain: ~3-10 mV/nA, to avoid saturation of amplifier. Additional faradaic current gain: x20.
Light stimulation: 470 nm wavelength light, 4-5 mW/mm^2, 500 ms.
Drugs: Control experiments conducted in drug-free aCSF, dihydro-β-erythroidine hydrobromide (DHβE, 1 µM), (+)-bicuculline (10 µM), CGP 55845 hydrochloride (CGP, 4 µM), 8-cyclopentyl-1,3-dimethylxanthine (CPT, 10 µM), oxotremorine (10 µM).

Note

Tips to keep a good record of the parameters used in each set of experiments.

Note on a lab book the gain settings, stimulation settings, aCSF composition, mouse details, inter-stimulation time (typically 2.5 min), recording sites (draw diagram of slice, noting the shape of corpus callosum, distance of anterior commissure, to check AP position), drugs (e.g. DHBE, CPT, etc.), and vehicles used (DMSO, ethanol etc).

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

23 Make up drugs, if using.

24 Visualised through a microscope (and/or camera), place stimulation electrode (bipolar concentric electrode) on surface of tissue. Surface location minimises damage.

25 Place recording electrode ~100 µm from stimulating electrode, 100 µm vertical depth into the tissue.

26 Activate data capture on computer recording system, stimulate and record evoked signals until return to baseline.

27 Wait 2.5 mins.

28 Repeat **steps 26** and **27** until around 6 approximately 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.

28.1 If applying a drug, continue stimulations at same interval during drug equilibration period. This period will vary significantly depending on drug characteristics (e.g. molecular weight, diffusion profile in brain tissue, absorption, uptake/breakdown, hydrophobicity/hydrophilicity).

29 When stable recordings are obtained with 1p stimulation, follow the stimulation protocol outlined for a baseline set of recordings.

1p

Wait 2.5 min

5p at 50Hz

Wait 2.5 min

1p

29.1 Repeat **step 29** only for the baseline recordings.

30 To explore impact of prior light activation of ChR2-expressing astrocytes, repeat **step 29** pairing each electrical stimulation with a 470 nm light stimulation starting at 400 ms preceding the electrical stimulation.

Note

The duration of the light stimulation would be 500 ms with an overlap with the electrical stimulation.

31 Repeat alternatively **step 29 and step 30** for example x3 to gain dataset in triplicate.

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

33 Remove slice.

Note

If post-recording molecular labelling or tissue content analysis is desired, prepare section accordingly, e.g. add to fixative or take a tissue punch as appropriate.

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

35 **Calibration**
Immediately before calibration make up 2 µM DA (see **Materials**).

35.1 Allow aCSF to flow at high speed (~4 ml/min).

35.2 Place electrode directly in front of inflow tube.

35.3 Connect electrode to voltammeter and turn on voltammeter to allow the electrode to settle for >10 mins.

35.4 Switch trigger settings on axoscope to “immediate” to record without triggering stim electrode.

35.5 Rapidly switch inflow tube from aCSF to 2 µM DA solution. Minimise bubbles which interfere with the electrode.

35.6 Watch bubbles to know when DA is washing on.

35.7 Start recording axoscope.

35.8 Refresh voltammeter immediately before DA washes on.

35.9 Watch oxidation peak rise with DA wash-on, for ~30 s.

Note

DA will adsorb to electrode and so will continue to rise.

35.10 Switch tube to aCSF, and repeat as for wash-on to record negative oxidation potential for wash-off.

35.11 Repeat as desired to gain a reproducible estimate of electrode sensitivity.

35.12 Plot peak oxidation potential over time for an example DA wash-on.

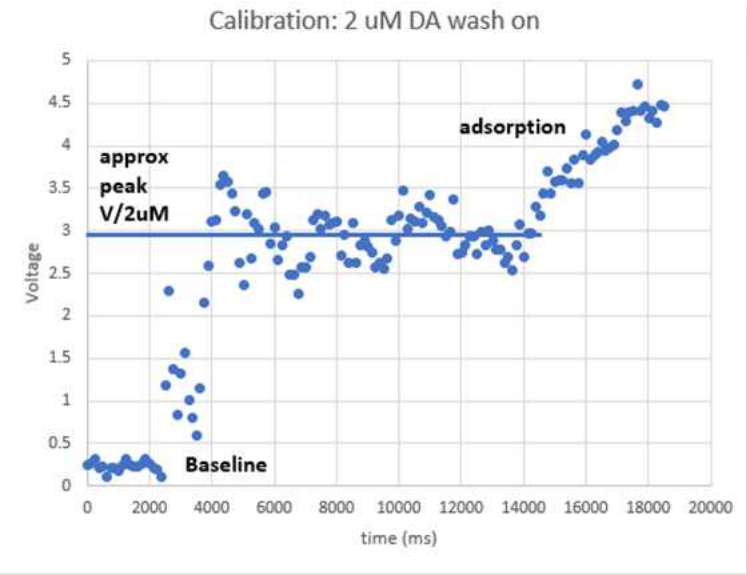

Figure 1: Calibration of 2 µM DA wash-on.

Note

Do not overestimate accuracy of this step (i.e. only accurate to ~5-10%).

35.13 Repeat calibration for each recording solution used (i.e. each drug you use) to check drugs are not affecting electrode sensitivity. This is especially important if changing divalent ion concentration.

Note

If the electrodes are being re-used, electrode sensitivity tends to decrease and on/off kinetics will slow.

35.14 Keep electrode until data has been analysed.

Note

Calibration factors differ for electrodes before being in tissue, immediately after being in tissue or days/weeks after being used. Therefore, to best compare DA concentrations between experiments always calibrate immediately after finished recording in tissue.

36 At end of the day, wash recording chamber through with dH20 and then empty. Use cotton bud with HCl to clean bath and dissolve any salt build-up around temperature probe or ref electrode.

37 Rinse aCSF bubblers and aerate them until dry to ensure they do not remain full with liquid and become contaminated or grow mould.

38 Release pressure points from the peristaltic pump on inflow and outflow tubes to prolong the life of the tubing.

Note

Replace tubing ~every month if in regular use.

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