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Fast-scan cyclic voltammetry to assess dopamine release in ex vivo mouse brain slices V.2

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ABSTRACT

This protocol is to assess whether a drug changes the dopamine concentration released following a single pulse (1p) electrical stimulation.

MATERIALS

Equipment:

- Millar Voltammeter (Julian Millar, Barts and the London School of Medicine and Dentistry)
- Digidata 1440A (Molecular Devices)
- Master8 (AMPI)
- Isolated current stimulator (Digitimer Ltd)
- In-line heater (made in-house)
- Surgical microscope Leica MZ6 (Leica)
- 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 & HOLDING

Preparing stock solution 5x HEPES:

1. Dilute in 1L dH_2O:

- 4.29 g HEPES sodium salt (final concentration 3.3 mM)
- 7.98 g HEPES acid (final concentration 6.7 mM)
- 2.46 g $MgSO_4(7H_2O)$ (final concentration 2mM)

MANUSCRIPT CITATION:
Brimblecombe KR, Connor-Robson N, Bataille CJR, Roberts BM, Gracie C, O'Connor B, Te Water Naude R, Karthik G, Russell AJ, Wade-Martins R, Cragg SJ. Inhibition of striatal dopamine release by the L-type calcium channel inhibitor isradipine co-varies with risk factors for Parkinson's. Eur J Neurosci. 2023 Nov 8. doi: 10.1111/ejn.16180. Epub ahead of print. PMID: 37941514.

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We use this protocol and it's working

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- 0.85 g KH_2PO_4 (final concentration 1.25 mM)
- 1.86 g KCl (final concentration 5 mM)
- 8.40 g $NaHCO_3$ (final concentration 20 mM)
- 35.07 g NaCl (final concentration 120 mM)

Preparing HEPES working solution:

- 0.90 g Glucose (final concentration 10 mM)
- 1 mL CaCl2 solution [1M] (final concentration 2 mM)
- 100 mL 5x HEPES
- 400 mL dH2O

RECORDING

Preparing stock solution $NaHCO_3$:

1. Add 50.4 g $NaHCO_3$ to 2 L dH_2O

2. Presaturate with CO2 bubble ~1 hour

Preparing stock solution ~6.8x Krebs:

1. Dilute in 2L dH_2O:

- 4.40 g $MgSO_4(7H_2O)$ (final concentration in aCSF 1.3 mM)
- 99.5 g NaCl (final concentration in aCSF 125 mM)
- 3.85 g KCl (final concentration in aCSF 3.8 mM)
- 2.30 g KH2PO4 (final concentration in aCSF 1.2 mM)

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

- 4.10 g Glucose (final concentration 10 mM)
- 0.797 g CaCl22H2O (final concentration 2.4 mM)
- 1730 mL dH2O
- 330 mL stock solution Krebs (final concentration 5 mM)
- **200 mL stock solution $NaHCO_3$** (final concentration 26 mM)

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.

BEFORE START INSTRUCTIONS

Prior to drug application, you first need to determine a stable baseline. The variance permitted in the baseline will depend upon the effect size of your drug of interest. 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 (pre-drug) 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 drug 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 HEPES buffer 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 and brain posterior to ~-1.055 mm bregma.

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

8 Transfer brain containing striatum to glue using filter paper to pick up block, and dampening fliter paper to reomve paper.

9 **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.

10 Section striatum to 300 µm-thick coronal slices.

11 Move slices to slice saver chamber at room temperature >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.

Optional: If comparing drug vs vehicle control from tissue tak...

12 Set up needle bubbler (carbogen inlet connected to needle (26G) that rests above glass vial

containing 10 mls aCSF).

Note

Ensure constant trickle of bubbles that doesn't cause the aCSF to vortex, which would disrupt the slice.

13 Add required drug or vehicle to 10 mL of aCSF in the vial and clearly label vial.

14 Bisect slice and put each hemisphere into appropriate vial.

Note

Note if slice comes from left (L) or right (R) hemisphere. This will ensure that when you transfer the slice to the recording chamber, you record from the surface that was not in contact with bottom of vial. It is possible to have both hemispheres labelled as L or R.

15 Allow slices to be incubated in drug or vehicle for typically 30 mins.

Setting-up rig

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

17 Circulate dH_2O through inflow pipes and recording chamber.

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

19 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.

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

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

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

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

24 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

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

Stimulation: 200 µs, 0.6 mA
Temperature: 31-33ºC
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: faradaic gain: x20, full signal gain: ~3-10 mV/nA, to avoid saturation of amplifier.
Drugs: record in DHBE 1 µM throughout to exclude effect of co-activation of ACh release and nAChR activation. Wash on drug of interest added to this solution.

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, isradipine, cocaine, etc.), and vehicles used (DMSO, ethanol etc).

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

27 Make up drugs and ensure fully thawed.

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

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

30 Record dopamine (DA) release.

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

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

30.3 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).

30.4 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.

31 Wait 2.5 mins.

32 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.

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

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

35 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.**

36 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.

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

38 Remove slice.

Note

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

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

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

40.1 Allow aCSF to circulate at high speed (~4 ml/min).

40.2 Place electrode directly in front of inflow tube.

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

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

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

40.6 Watch bubbles to know when DA is washing on.

40.7 Start recording axoscope.

40.8 Refresh voltammeter immediately before DA washes on.

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

> **Note**
>
> DA will adsorb to electrode and so will continue to rise.

40.10 Switch tube to aCSF, and repeat as for wash-on to record negative ox potential for wash off

40.11 Repeat 3x and average wash-on and wash-off for approx. electrode sensitivity.

40.12 Plot peak oxidation potential over time for each DA wash on.

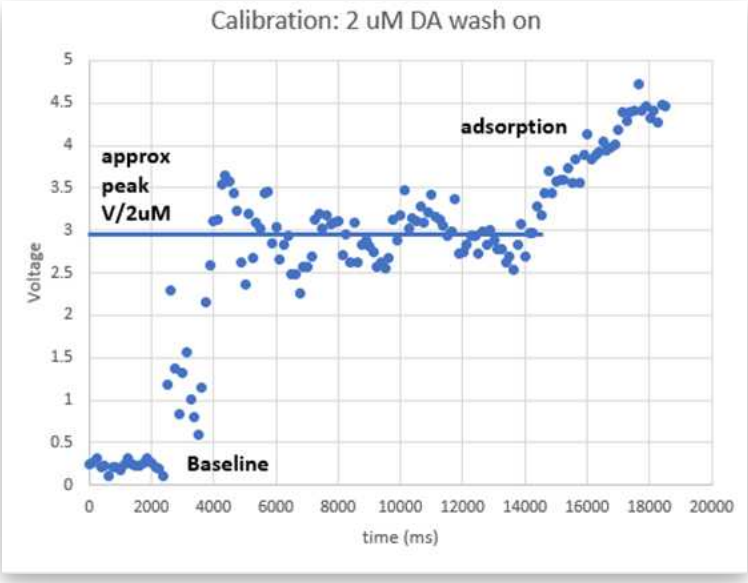

Figure 1: Calibration of 2 µM DA wash-on.

Note

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

40.13 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, the sensitivity tends to decrease and kinetics will slow.

Always use a new electrode for kinetic analysis.

40.14 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.

41 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.

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

43 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.

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