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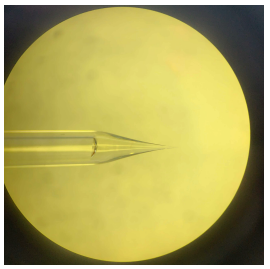
Making Ca^{2+} - selective microelectrodes



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We use this protocol and it's working

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Disclaimer

Earlier versions of this protocol were developed in collaboration with Dorly Verdier and Arlette Kolta.

Abstract

The following protocol describes how to make Ca^{2+} sensitive electrodes to measure extracellular Ca^{2+} concentrations at high spatiotemporal resolution in acute *ex vivo* brain slices. Glass micropipettes can be precisely (µm precision) located at/near a particular brain region/cell type of interest to measure rapid changes in Ca^{2+} concentrations.

Image Attribution

J. Stedehouder. Glass micropipette visualized under light microscope, backfilled with calcium ionophore I cocktail A.

Guidelines

Follow all the steps carefully and pay particular attention to the warning signs.

In our experience, manifacturing electrodes fresh on the day gave the optimal results, and disposing of the electrodes after one day. Re-usage of the same electrode within a single day for several recordings provided reliable results.

Materials

Equipment:

- P-1000 Flaming/Brown Horizontal Micropipette Puller (Sutter Instruments) or equivalent
- P100 Pipettes (e.g. Gilson)
- Long 20 µl pipette tips (e.g. X192 Tip Eppendorf Microloader 0.5ul to 20ul, Sigma-Aldrich)
- Fume hood with small-size oven (up to ~120 C)
- Borosilicate glass capillaries (30-0057; GC150F-10; 1.5 OD x 0.86 IK x 100 L mm; Harvard Apparatus)
- Heat-resistant glass jar (~7-10 ml) with screw-on lid
- Electrophysiological set-up with amplifier to perform recordings

Reagents:

- Calcium ionophore I cocktail A (Sigma-Aldrich, St Louis, Missouri, USA)
- Dimethyldichlorosilane (40136 selectophore, FLUKA)
- aCSF with increasing Ca^{2+} concentrations (0.1 mM - 2 mM) to validate electrodes
- $CaCl_2$ 2M for backfilling

Safety warnings

!!!

Dimethyldichlorosilane is a dangerous chemical that must be handled with extreme care
Use a fume hood, wear gloves, goggles, a mask and a lab coat
Close the fume hood window as far as possible
Dispose of all materials as per local guidelines

!!!

Ethics statement

All procedures on animals were conducted in accordance with the Animals (Scientific Procedures) Act 1986 (United Kingdom), approved by an Animal Welfare and Ethical Review Board at the University of Oxford, under the authority of Licenses from the Home Office (UK).

Cleaning and Pulling

1 Pull glass capillaries on the horizontal puller to get a sharp tip, with opening ~1-2 µm and roughly a ~3-5 MOhm resistance.

2 **Optional:** Break electrode tips under a microscope by gently pushing them against a clean glass rod to reach the intended diameter.

3 Store the pipettes upright in the top cap of one of the pre-drilled glass containers until the next step.

Silanization

4

Safety information

Dimethyldichlorosilane is a dangerous chemical that must be handled with extreme care. Wear gloves, goggles, a mask and a lab coat. The next steps should be performed in a fume hood which must be in proper working order with the sash closed as far as possible. Remnant dimethyldichlorosilane must be carefully disposed of as per local guidelines.

5 Preheat the oven in the fume hood to 120°C. Make sure everything is clean and ready to go.

6 Pour approx. ~5-10 ml undiluted dimethyldichlorosilane into the bottom glass vial to fill it approximately half-way.

7 Carefully place the cap with the inserted pipettes onto the bottom glass vial and close the vial.

8 Expose the pipettes to the dimethyldichlorosilane vapor for ~50 seconds.

9 Place the cap with the pipettes (without the glass bottle) in the preheated oven and bake for 2 hours at 120°C.

10 After 2 hours, take the cap out of the oven and place the cap in the hood to cool down. The oven can be turned off.

Backfilling of Pipettes

11

Safety information

Use gloves when handling the silanized pipettes.

12 Place a long 20 µl pipette tip on a p20 pipette.

13 Fill the pipette tip with ~1-2 µl of calcium ionophore cocktail A. Calcium ionophore cocktail A should be prevented from extensive light exposure.

14 Carefully backfill the cocktail to the outmost tip of the silanized glass pipettes (~1 µl per pipette). See main image for example.

15 Carefully check the tip and filling under the coarse light microscope. Discard badly filled pipettes or carefully attempt to remove the bubbles. The ionophore must be clean and homogenous.

16 If correct, carefully fill the rest of the pipette with $CaCl_2$ (2 M).

17 Leave the electrodes to rest for at least ~30 mins before any next steps.

Calibration

18 Electrodes should be calibrated at experimental temperatures (~33°C) with standard aCSF solutions containing increasing concentrations of Ca^{2+} (0, 0.1, 0.2, 0.4, 0.8, 1.2, 1.6 and 2 mM), the potentials for each concentration recorded, and a calibration curve obtained fitted with a logarithmic function.

19 **Optional:** Comparison experiments to examine sensitivity for other ionic solutions (Zn^{2+}, Mg^{2+}, K+) can be performed in aCSF with or without 1.6 mM Ca^{2+} present.

20 Only electrodes exhibiting a potential jump exceeding 20 mV between concentrations 0.2 and 2 mM ('sensitivity') for Ca^{2+} should be used for experiments.

21 **Optional:** Further sensitivity can be tested in situ by applying either a calcium chelator (eg. EGTA, BAPTA) or high applying Ca2+ concentrations (eg. 20 mM).