# Making Carbon-Fibre Microelectrode (CFM) for electrochemical recordings of monoamines in ex vivo mouse brain slices V.2

In 1 collection

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## ABSTRACT

This protocol is to make carbon-fibre microelectrode (CFM) for electrochemical recordings of monoamines in *ex-vivo* mouse brain slices. This protocol is optimised for the detection of dopamine from 300 µm coronal striatal sections, using a Miller voltammeter.

## GUIDELINES

We make a new electrode per experimental day. If you reuse electrodes the kinetics slow over time, but they become increasingly stable, therefore if you are less interested in kinetics and would rather a less sensitive, more stable electrode, you may rather use a single electrode repeatedly.

We connect CFM to our head-stage via a crocodile clip. If you have a different headstage, you will need to adapt how to wire the CFM. When you start making CFMs, it can be a highly frustrating exercise; remember to take lots of breaks, it gets easier with experience!

**MANUSCRIPT CITATION:**
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**Protocol status:** Working
We use this protocol and it's working

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MATERIALS

**Reagents:**

- Acetone
- Conductive silver paint (RS 186-3600)
- Cyanoacrylate glue (Loctite superglue)

**Equipment:**

- Light box
- Test tube
- Forceps with rubber ends (push small piece of rig tubing over each prong of forceps to stop snapping carbon fibre)
- Small scissors
- Scalpel
- Microscope with 10X objective and graticule eye piece
- Puller
- Capillary tubes 2.0 OD 1.16 X 100 mm (Harvard Apparatus, CAT# 30-0074)
- Wire (RS PRO Data Cable, 9 cores (7/0.1 mm), 30 AWG, PVC coated)
- Glass Slides
- Blu Tack

**For each electrode:**

- Capillary tubes
- Wire (use 1 core per electrode)
- Conductive silver paint
- Cyanoacrylate glue

**Making a cutting block:**

- Stick two capillary tubes to a glass slide, slightly further apart than a capillary tube.
- Cut glass slide in half and on one half score a single groove the full width of the slide with a diamond knife and a metal ruler, sufficiently deep to hold a scalpel in place when drawn down it.
- Stick this half slide to the first glass slide (see **Figure A** for clarification).

## Threading Carbon Fibre

1 Fill test tube with acetone (flammables cupboard) and add capillary tubes.

2 Stick test tube to light box with Blu Tack.

Note

Careful not to spill acetone on light box.

3 Select a single carbon fibre using rubber tipped forceps.

4 Thread carbon fibre down capillary tube.

Note

Ensure capillary tube is completely filled with acetone.

Ensure ~ 0.5 cm carbon fibre is exposed at the end of the capillary tube to stop it from falling out of the end of the tube.

5 Lift the tube out of the acetone and allow acetone to evaporate.

## Pulling Carbon Fibre

6 Place capillary tube into puller.

7 Tighten holder “finger-tight” and raise tube for 3 clicks and secure in place “finger tight”.

8 **Settings:**

- Magnet: off

- Heat: ~6.7

Note

**The higher the heat, the longer/more gradual the seal will be.**

**Golden Rule:**

- Too cold or short seal: easier to see the capillary tube being cut, but it can make it leaky.
- Too hot or long seal: harder to see the capillary tube being cut, but also fine class seal can break during an experiment suddendly exposing extra carbon fibre, leading to a noisy and unreliable electrode.

9 Check which end of the tube the carbon is in and carefully remove from puller.

10 Trim excess exposed fibre with fine scissors to stop waving extended fibre from stressing the glass seal.

## Cutting Carbon Fibre

11 Carefully place electrode between capillary tubes on cutting block.

12 Approximately line up seal with cut ridge.

Note

If you have problems seeing the seal, breathe on the fibre and its flex point will indicate the approximate point it joins the glass.

Roll the capillary tube, often one side is more clear.

When looking, start at the fully exposed carbon fibre end and travel towards the capillary tube, your eye finds spotting the increase from a constant easier than a change to a constant (IMO).

13 Make a cut at ~ 300 µm from seal, by running scalpel down cut ridge, mark this as 0 on the graticule.

14 Move electrode so there is 50-100 µm exposed carbon fibre and run scalpel down cut ridge.

Note

**This step takes practice. Don't be discouraged and take your time!**

The length of exposed carbon fibre is another golden rule.

- The longer the exposed carbon fibre, the more sensitive your electrode will be (bigger surface to detect dopamine), however, there is more area to detect noise too.
- A shorter electrode will be less sensitive, but also often quieter. We can amplify the signal from a shorter electrode, however, remember it will also amplify the noise.

Given we record dopamine from ~100 µm sphere, it makes sense to try to sample the most amount of dopamine available, but not over-sample. An electrode length of 50-100 µm will allow an amplification of 3-10 nA/mV, which we have determined to be optimal for our set up.

15 Carefully remove electrode from cutting block.

## Wiring Electrode

16 Cut wire to slightly longer than your capillary tube.

17 Strip off ~1cm of the plastic coating from each end.

18 On one end trim exposed wires so only 1 is sticking out.

19 Dip this end in the conductive silver paint and gently thread down the capillary so the paint makes contact with the carbon fibre.

20 Glue exposed end of wire in place by dripping glue down capillary tube, exposed wire can be connected to voltammetry head-stage using croc-clip.

21 Allow to set overnight before use.