1. Calibration of carbon fiber electrodes (CFE) in a beaker using three concentrations of analyte.

This is the simplest approach to calibration that we incorporated in Invilog In Vivo Voltammetry Setup. The following tools are used:

Figure 1. Plastic beaker for CFE calibration in stereotaxic frame

- 1.5 - 2 ml beaker made from cut to size plastic test tube (D=15mm) attached with a hot glue to a 20 ml plastic bottle (see Figure 1, small holes for ear bars are shown by arrows).
- stereotaxic frame with arm and CFE holder used as a chemical stand and second arm or Invilog manipulator as a holder for reference electrode.
- syringe 1 ml with 0.6-0.8 mm needle for filling the beaker
- plastic pipette, 1-2 ml for mixing dopamine in the beaker
- Hamilton syringe, 10-50 µl

We use the following chemicals:

- 100 µM dopamine hydrochloride dissolved in 1 ml phosphate buffered saline (pH=7.4) in Eppendorf tubes. We prepare many tubes at once and keep this stock at -20-25^0
- phosphate buffered saline 1-2 l. Note that ACSF can be also used, but results of calibration will be slightly different.

After experiment we remove animal from the frame and fix the beaker between ear bars (see Figure 2, Invilog micro stirrer is fixed between the bars; plastic beaker is fixed in the same way). Wash CFE in saline.

Figure 2. Beaker (Invilog micro-stirrer) fixed between ear bars

Add 1 ml of medium to the beaker and immerse CFE and reference electrodes in it. You may use compact Invilog manipulator or additional stereotaxic arm for fixation of the reference electrode.

Ensure that expected shape of voltammogram is present and wait for stabilization of the background current.

Add unfrozen stock solution (keep stock solution on ice or at 4^{0}C in refrigerator if calibrating several electrodes). We calibrate CFE with three concentrations of dopamine: 200, 400 and 600 nM (to obtain 200 nM final concentrations add 2 µl of stock solution to 1 ml of medium). Calibrate CFE with 1, 2 and 3 µM dopamine concentrations when measuring stimulated dopamine release in a micromolar range.

Add bolus of stock solution in the beaker with Hamilton syringe or micropipette and mix medium with help of plastic pipette:

- gently press the pipette and insert the nose of the pipette in the medium
- suck a small amount of medium in the nose of the pipette and release it back to the medium
- repeat manipulation 10-12 times trying not to produce bubbling or expose CFE tip to air.

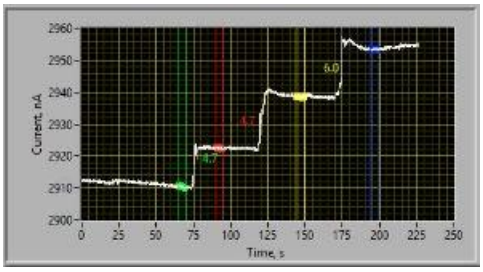

Figure 3. Example of CFE calibration

Do not wait too long between testing different concentrations since drift in the background current will affect results of calibration calculated automatically by the program. A typical calibration of the CFE takes only 3-4 minutes (Figure 3).

Note 1. Invilog Setup software automatically approximates data obtained with three concentrations by linear regression. Indeed, any other approaches to calibration can be used after collecting raw data.

Note 2. Invilog reference electrode should be immersed in buffer several hours before measurements. Its ceramic end soaks solvent and will be active after the first immersion. Wash it with saline after measurements and keep in saline in small Eppendorf tube at 4^0C (make hole in the cap of the tube so that the reference electrode tightly fits in it).

Note 3. CFE can be re-calibrated or used for analytical measurements in the beaker as many times as needed. However, **we recommend to use a new CFE for each measurement in the brain**. On rear occasions repeated use of the CFEs after brain implantation is also possible:

- we use twice CFE when operating two animals one after another in one day; the CFE is kept in saline between implantations
- 30 microns CFE can be clean mechanically with a sharp blade under binocular microscope; if the tip is cleaned without damage it should be re-calibrated in the flow cell to ensure that temporal characteristics of the CFE are preserved.

2. Calibration of carbon fiber electrodes in a flow cell.

For the flow cell calibration we place tip of the CF electrode in the plastic tubing via a small opening in the wall or in the cut end. Medium (ACSF or a phosphate buffer) steadily flows via the tubing. Bolus of the analyte solution is briefly injected in the stream (for estimation of the temporal response of the CFE) or the flow cell is switched to a medium with a new specific concentration of analyte (for obtaining stable concentration levels).

Useful side of the flow cell calibration is its ability to obtain temporal characteristics of the CFE. This is important when one initiates experimenting with a new electrode's material or a new electrochemical pre-treatment. Over-treated CFE while demonstrates high sensitivity, loses time resolution. As a result, it starts to work as a low pass filter decreasing response to fast changes in concentrations of analyte (e.g. dopamine transients).

Note that calibration in the flow cell overestimates CFE sensitivity as a results of faster renewing medium on the CFE surface (in comparison with calibration in the beaker).

We do not use calibration of the CFE in the flow cell for routine pharmacological experiments with a standard Invilog CFE and for calibration of the CFE after standard manufacturing process. Instead we employ calibration in the beaker.