Microwave-assisted Chemical Fixation of Acute Hippocampal Slices

0. Safety precautions
- This procedure involves the use of hazardous chemicals (including embryotoxins). Review MSDS for information on exposure limit, health risks, first aid, handling, etc.
- You must be trained to break open glass ampules.
- Perform this procedure only in the designated area.
- Wear appropriate Personal Protective Equipment for this procedure:
  - Lab coat
  - Nitrile gloves (double-layer; regularly check for holes)
  - Eye goggles
  - Mask (optional)
  - Plastic apron (optional)
  - Shoulder-length gloves (optional)
  - Place a piece of absorbent sheet on the work surface before starting the procedure.
- Have all waste containers ready (see Clean-up).
- Read the following papers:

1. Reagents and supplies:
- For fixative solution:
  - A 500-ml graduated cylinder (This cylinder should be dedicated for use with aldehydes.)
  - Pasteur pipette and bulb
  - 1-ml micropipet and tips
  - Erlenmeyer flasks, beakers, stir bars
  - 5-ml cryo-tubes labeled with date and contents (ask Masa for the labels)
  - purified water (Rica Chemical 915025 or Fisher 9150-25)
  - sodium cacodylate trihydrate (solid; Ladd Research 20305)
  - glutaraldehyde (70% aqueous solution in 10-ml ampules stored at 4°C; Ladd Research 20108)
  - formaldehyde (20% aqueous solution in 10-ml ampules stored at 4°C; Ladd Research 20300)
  - calcium chloride dihydrate (CaCl₂·2H₂O)
    - Make a 0.4 M stock solution by adding 1.176 g into 20 ml of purified water. This can be stored in a glass vial at 4°C.
    - magnesium sulfate heptahydrate (MgSO₄·7H₂O)
      - Make a 0.8 M stock solution by adding 3.944 g into 20 ml of purified water. This can be stored in a glass vial at 4°C.
      - pH meter (with calibration standards)
      - 1N NaOH (aq) for adjusting pH
      - 1N HCl (aq) for adjusting pH
    - For microwave-assisted fixation:
      - 2-6 tubes of 6% glutaraldehyde/2% paraformaldehyde (stored in the freezer in NHB 3.360E)
      - tri-pour beaker
      - 6-well plate or 35 mm plastic Petri dish (12-well plate can be used for small slices)
      - 4 rings (used to make nets for interface chamber)
      - microwave oven (exhaust must be properly vented; we currently use a consumer model with 1500 W output)
      - an array of neon bulbs (e.g., EMS 97036-01)

2. Fixative Solution
1. Remove 70% glutaraldehyde from refrigerator (and leave at RT) 3 days prior to fixative preparation.
2. In an Erlenmeyer flask, dissolve sodium cacodylate, calcium chloride, and magnesium sulfate into purified water. See the table below for the amounts.
3. Adjust pH to 7.4 with 1N NaOH or 1N HCl.
1. Approximately 15-20 minutes before the end of your experiment, begin prepping for fixation.
2. Take 2 tubes of 6% glutaraldehyde/2% paraformaldehyde out of the freezer in the processing room.
3. Place tubes in a tri-pour beaker, place beaker in sink, and allow warm water to run over the tubes to thaw the fixative (should be warmed to 31°C).
4. Grab a 6-well plate on the shelf above the vibrotome and 4 rings (ring only, no net) from the net-making bucket.
5. Place each of the 4 rings in a separate well of the 6-well plate.
6. Place neon bulb array in microwave, and run microwave for ~ 30 seconds to warm up the magnetron. Make sure none of the bulbs light up (which indicates a hot spot). Take array out of microwave and leave door ajar. Set microwave to 20 seconds (or less depending on the method of subsequent tissue processing).
7. Once fixative is thawed, add ~5 ml of fixative with a transfer pipette to each of the 4 wells (basically one tube's worth).
8. After last data point is collected and the Synchrobrain program ends, push the cameras out of the way, withdraw all of the stimulating and recording electrodes out of the chambers and fully retract manipulators.
9. Bring 6-well plate with fixative over to rig and starting with the first chamber, take off the cover, pick up net by edge using tweezers (marked with red tape), flip net over onto ring in 12 well plate so that slice is completely submerged, but not touching the bottom of the well.
10. Repeat step 9 with remaining 3 chambers. Note to be very CAREFUL of recording electrodes.
11. After microwave is done, take 6-well plate out and add remaining tube of fixative to all 4 wells. Place lid back on plate and leave in the fume hood overnight.
   a. The fixed tissue should be vibrasliced on the following day.
12. Place empty tubes and any other waste in solid waste bag on top of microwave.

3. Microwave-assisted chemical fixation

<table>
<thead>
<tr>
<th>[stock] or FW</th>
<th>(final)</th>
<th>Final volume = 350 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>purified water to start with</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Na cacodylate·3H₂O</td>
<td>214.03 g/mol</td>
<td>0.1 M</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>0.4 M</td>
<td>2 mM</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>0.8 M</td>
<td>4 mM</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>20%</td>
<td>2%</td>
</tr>
<tr>
<td>Glutaraldehyde</td>
<td>70%</td>
<td>6%</td>
</tr>
</tbody>
</table>

4. Clean-up

**Waste containers:**

- Hazardous Liquid Waste: Pour all waste into the proper waste collection bottles available in the fume hood in NHB 3.360E.
- Aldehyde-Cacodylate (fixative solution, cacodylate buffer)
- Hazardous Solid Waste: Place all contaminated solid waste (e.g., gloves, tubes, processing dishes, etc.) into hazardous waste bags in the fume hood. All tubes must be uncapped.
- Sharps: Pasteur pipettes must be discarded into a sharps container labeled "Pb-aldehydes"