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 (Ricca Chemical 915025 or Fisher 9150-25)
  - sodium cacodylate trihydrate (solid; Ladd Research 20305)
  - formaldehyde (20% aqueous solution in 1-ml ampules stored at 4°C; Ladd Research 20300)
  - magnesium sulfate heptahydrate (MgSO₄·7H₂O)
- 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.
- For microwave-assisted fixation:
  - 2-6 tubes of 6% glutaraldehyde/2% paraformaldehyde (stored in the freezer in NHB 3.360E)
  - tri-pour beaker
  - 12-well plate
  - 4 rings (used to make nets for interface chamber)
  - microwave oven (exhaust must be properly vented)
  - an array of neon bulbs
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.
4. Move the solution to a fume hood before adding the aldehydes. Keep stirring the solution.
5. Use a Pasteur pipet to dispense 70% glutaraldehyde. Rinse the ampules with small amounts of the solution to remove as much glutaraldehyde as possible.
6. Add formaldehyde to the flask.
7. Once everything is completely dissolved, usea graduated cylinder to bring to the final volume to 350 ml with purified water.
8. Dispense the fixative into 15-ml conical tubes (labeled with date and contents), and store at -20°C.

<table>
<thead>
<tr>
<th>purified water to start with</th>
<th>[stock] or FW</th>
<th>[final]</th>
<th>Final volume = 350 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na cacodylate·3H₂O</td>
<td>214.03 g/mol</td>
<td>0.1 M</td>
<td>0.749 g</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>0.4 M</td>
<td>2 mM</td>
<td>1.75 ml</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>0.8 M</td>
<td>4 mM</td>
<td>1.75 ml</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>20%</td>
<td>2%</td>
<td>35.0 ml</td>
</tr>
<tr>
<td>Glutaraldehyde</td>
<td>70%</td>
<td>6%</td>
<td>30 ml</td>
</tr>
</tbody>
</table>

3. Microwave-assisted chemical fixation

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 12-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 12-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 ~2 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 12-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. Place 12-well plate with all 4 slices in microwave, close door and press start.
12. After microwave is done, take 12-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.

1. The fixed tissue should be vibrasliced on the following day.
13. Place empty tubes and any other waste in solid waste bag on top of microwave.

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”