Preparation of Rodent Brain Slices Protocol V.1

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ABSTRACT

This is a protocol used to prepare and slice the brain for electrophysiological recordings or patching.

ATTACHMENTS

[Electrophysiology_PreparationOfRodentBrain_2009-.pdf](#)

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Materials

- 1
 - Extra-cellular solution (1x), fresh for every experiment, prepared from the 10x Stock solution – see protocol name *Protocol_Solutions_Extracellular_Solution_year.docx*
 - Guillotine
 - Scissors (to cut the scalp)
 - Forceps (to open up the skull)
 - Scalpel (to detach the olfactory bulbs and the spinal cord from the cranium)
 - Blade (to separate the hemispheres)
 - Knife (to break the Extracellular solution prior to slicing)
 - Spoon (to collect brain out of cranium)
 - Whatman filter paper

Protocol

NAME

Extracellular solution

CREATED BY

Anna-Kristin Kaufmann

PREVIEW

Preparations

- 2 Solution
 1. Bubble Extracellular Solution (1x) for 15-20 minutes with 95% O₂ - 5% CO₂.
 2. Into 2 plastic beakers add ~300 ml each of extracellular solution (1x). Place a petri dish on top of beaker to cover it. Keep at -80°C for 45min-1hr (solution should not be liquid!)
- 3 During the freezing, prepare the slicer, i.e. fix a blade to the slicer (HR-2 in Room AI 2210)
 1. Material needed: chamber, blade, glue (Loctite 401)
 2. "Physical" properties:
 3. Frequency: 50 Hertz
 - 4.
 5. Amplitude: 1 mm
 - 6.
 7. Forward: 0.04 mm/s
 - 8.
- 4 Water bath: Turn it on just before starting to cut; it should not warm the slices too much, maximum 37°C

Brain Extraction and Slicing

- 5 Notes:

Do not let the brain dry up in the air. Always keep the brain wet with extracellular solution! Carbogen (also called Meduna's Mixture) original formula was 30% CO₂ and 70% O₂, but the term Carbogen can refer to any mixture of these two gases, from 1.5% to 50% CO₂.

First check the position on the right, at the front of the slicer (the red point should be down). Wear lab coat and gloves

- 6 Using the knife, break the frozen extracellular solution.
- 7 Into 2 separate shallow and wide beakers, place some frozen extracellular solution and some liquid extracellular solution. These are for a. the detached skull and b. for the brain.
- 8 Cut the head with the guillotine, put it into a beaker with frozen and some liquid extracellular solution, wait that blood ends to flow (a few seconds)
- 9 Hold the head gently between your thumb and index finger (gloves) with the nose facing your left side. Using scissors cut a line along the medial line of the skull to remove the skin.
- 10 Place the scissors in the hole where the spinal cord connects to the brain, cut a little part of the skull on the right. Then continue on the left side towards the olfactory bulbs. With the forceps, lift off the dura mater entirely. Detach the olfactory bulbs and the spinal cord from the cranium using a scalpel. Finally, with a spoon collect the brain and place it in another beaker with frozen extracellular solution.
- 11 Put the brain on top of a Petri dish (the cover of the beaker that was at -80°C with the extracellular solution inside), separate the hemispheres.
- 12 At this moment: turn on the Carbogen!
- 13 Place the right hemisphere (medial side) on a blade and remove some of the excess liquid around the brain with a Whatman filter paper. Put a line of glue on the chamber's sample holder, then quickly place the hemisphere on the glue (medial side). Be careful, once the brain touches the glue it is fixed and cannot be moved!
- 14 Fix the chamber on the slicer, put ice in the chamber all around the brain and above. Add a little bit of cold extracellular solution so that the brain is submerged.

- 15 On the right part of the chamber, move ice on the right side so that you see the height of the blade. Place the blade just above the brain, but not cutting it yet. This is to indicate the top of the brain. Now place the blade behind the brain so it is not touching the brain.
- 16 Turn the wheel 20x (then you will have slices almost parallel!)
- 17 Start to cut at a frequency of 50 Hertz, 1mm Amplitude and 0.04 mm/s
- 18 While the slicer is cutting your slice (300 μ m), remove some of the “old” ice and put fresh one!
- 19 Each new slice needs 3 turns of the wheel to go down 300 μ m (the thickness of one slice) Put your slices in the bubbling chamber inside a beaker containing extracellular solution, leave them in there for 30 minutes @ max 37°C in water bath before patching.

Cleaning

- 20 Clean first with hot tap water, then Ethanol (EtOH 70%) and finally double distilled water. Let instruments dry on the desk.