Preparation of ex-vivo brain slices for physiology experiments V.2

Version 1 is forked from Preparation of tissue for Ribo-Tag/RNAseq analysis

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VERSION 2

NOV 16, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.dm6gp328jvzp/v2

Protocol Citation: enrico.zampese 2023. Preparation of ex-vivo brain slices for physiology experiments. **protocols.io** https://dx.doi.org/10.17504/protocols.io.dm6gp328jvzp/v2 Version created by enrico.zampese

MANUSCRIPT CITATION:
https://www.science.org/doi/10.1126/sciadv.abp8701

ABSTRACT

This protocols describes the procedure to obtain ex-vivo mouse brain slices with a vibratome.
This protocol is originally meant to collect mibrain coronal slices or coronal/parasagittal striatal slices, but can be adapted to other brain regions/configurations with the appropriate adjustments.

GUIDELINES

Follow institutional guidelines and protocols.
Wear appropriate PPE.

MATERIALS

Materials:

- Anesthetic (ketamine 50 mg/Kg and xylazine 4.5 mg/Kg – varies according to institutional protocols)

- Vibratome (VTS1200S Leica microsystems) with removable ice tray and cutting chamber and vibro-check tool

- 2 large ice trays

- Large beaker

- 2 glass petri dishes (one of the two can be substituted with a medium weighing

Created: Nov 16, 2023

Last Modified: Nov 16, 2023

PROTOCOL integer ID: 91038

Keywords: vibratome, slices, brain, slicing, ex vivo

Funders Acknowledgement:
JPB Foundation
Grant ID: n/a
MJF Foundation
Grant ID: n/a
NIH
Grant ID: NS121174
NIH
Grant ID: NS34696
ASAP
Grant ID: 020551
CHDI Foundation
Grant ID: n/a

boat)

- 1 circular filter paper
- Peristaltic pump (Gilson) with tubing and connectors
- Blood-gas mixture (95% O2, 5% CO2) tank connected to bubblers.
- Dissection tools (scissors, fine scissors, spring scissors, tweezers, spatula, according to preferences)
- Double-edged razor blades
- Single-edge razor blades
- artificial cerebro-spinal fluid (aCSF)
- Freshly made “slicing solution”: modified aCSF.
- Pre-frozen slicing solution
- Immersion blender
- Perfusion needle (preferred: 27 gauge ½ inch)
- Holding chamber for slices, filled with aCSF.
- Heated water bath
- Pre-solidified 2% agarose

- Superglue
- Precision wipes
- Water wash bottle
- Extra needles
- Carcass bag
- Plastic or glass transfer pipette
- 1.5ml Eppendorf tubes to collect samples

Recommended PPE:

- Lab gown/disposable gown
- Face mask
- Face shield/goggles
- Examination gloves (cut-resistant gloves are recommended)

Solutions:

Different types of aCSF are adopted by different groups and are optimized for different for different preparations.

For physiology experiements on **SNc DAergic neurons in midbrain coronal slices** we normally adopt:

aCSF (used in the holding chamber and for experiments): 135.75 mM NaCl, 2.5mM KCl, 1.25mM NaH2PO4, 25 mM NaHCO3, 2 mM CaCl2, 1 mM MgCl2, 3.5 mM glucose.

Slicing solution (modified aCSF): 49.14 mM NaCl, 2.5 mM KCl, 1.43 mM NaH2PO4, 25 mM NaHCO3, 25 mM glucose, 99.32 mM sucrose, 10 mM MgCl2 and 0.5 mM CaCl2.

All solutions have ~300 mOsm and pH ~7.4.

For physiology experiements on **MSNs neurons in striatal coronal or parasagittal slices** we normally adopt:

aCSF (in holding chamber and for experiments) in mM: in mM: 125 NaCl, 3 KCl, 1 MgCl2, 2 CaCl2, 25 NaHCO3, 1.25 NaH2PO4 and 10 glucose.

Slicing solution (modified aCSF) in mM: 125 NaCl, 3 KCl, 2.5 MgCl2, 0.5 CaCl2, 25 NaHCO3, 1.25 NaH2PO4 and 10 glucose.

All the solutions are continuously bubbled with blood-gas mixture before and during the procedure. This will maintain the proper oxygenation and pH.

We recommend preparing a small batch of slicing solutions a day or a few hours before the procedure and freeze it.

SAFETY WARNINGS

Please refer to the SDS of each reagent/product.
Please refer to the operating instruction manual of each equipment piece.

Before the procedure:

1 A few hours/day before the procedure it is recommended to prepare some slicing solution and freeze it (~200ml).

2 On the day of the extraction, prepare fresh aCSF and slicing solution and start bubbling them with the O2/CO2 gas mixture.

3 Break the pre-frozen slicing solution into a large beaker and add freshly-made slicing solution. With the help of the immersion blender transform the frozen/fresh mix of slicing solution into a 0-4 °C slushie.

4 Keep bubbling the slushie and start running it through the tubing connected to the peristaltic pump, with a 27G needle attached to the connector on the other end of the tubing. Place the needle back into the slushie so that the ice-cold solution will keep recirculating until the moment of the procedure.

5 Pour aCSF into the holding chamber where the slices will be collected and place it in a heated water bath at 34 °C , under continuous bubbling.

6 Fill the two large trays with ice.

7 In one of the trays, model the ice to create an inclined area where the mouse will be placed during the perfusion. Pre-chill the dissection tools in the remaining ice.

8 In the other tray, place a petri-dish upside-down, in contact with the ice, and cover it with the filter paper. Dig the other dish into the ice, leaving it empty. Later slicing slushie will be poured in this dish.

9 Lay a precision wipe over the ice and place the razor blades, a 0.5x0.5in block of agar and the holding stage of the vibratome chamber over it, so that they stay cold.

10 Turn on the vibratome, pre-adjust the settings: for midbrain coronal slices a thickness of 220um, a speed

of 0.06 mm/s, and an oscillation amplitude of of 1mm are recommended. For coronal or parasagittal striatal slices we recommend a thickness of 275um.

11 Attach the vibro-check tool, carefully insert the double-edge blade into the holder and perform the vibration check. Follow instruction to minimize the vibration.

12 Lift the blade holder, remove the vibro-check apparstus and leave blade in (vibration check should be performed each time a blade is inserted in the holder).

13 Place the vibratome slicing chamber in the vibratome ice tray, and fill the the area of the ice tray outside of the chamber with ice.

Procedure:

14 Terminally anesthetize the mouse according to institutional protocols.

15 Bring the anesthetized mouse to the dissection area and verify that the mouse is fully anesthetized. This can be performed by pinching one of the posterior paws and observing the presence (or lack of) pain reflex. The mouse must be fully anesthetized before starting the transcardiac perfusion.

16 Once full anesthesia is achieved, the mouse can be positioned and secured on the dissection area.

17 The mouse should be positioned in a supine position, with the head oriented away from the operator. If the operation area is inclined, the mouse should be oriented so that the head is facing down-ward.

18 Holding the skin just below the sternum with a tweezer, cut the skin just below the tweezer tips, exposing

the peritoneal cavity and the rib cage. The diaphragm is intact.

19 Expand the cut and with the scissors cut the fascia connecting the skin to the rib cage.

20 Once the rib cage is clearly visible, carefully cut the diaphragm without damaging the beating heart. Cut the chest cage and lift it toward the head. A needle can be used to hold it in position while operating. The liver should be visible in the abdominal cavity.

21 Carefully insert the needle connected to the peristaltic pump (where the slicing solution slushie is still circulating) in the left ventricle of the heart, and rapidly pinch the right atrium with the spring scissor. Dark-red blood should start flowing out of it immediately. Hold the needle in position, while the solution washes out the blood from the mouse. The heart should still be beating. A wash water bottle can be used to remove excess blood and see more clearly.

22 Within 10-20s the liver should start looking paler, and less blood should be flowing from the right atrium.

23 When the liver looks clear of blood, remove the needle, and quickly decapitate the mouse with the scissor.

24 Transfer the head into the empty petri dish on ice and fill it with some of the slicing slushie remaining from the perfusion. Don’t discard the rest. The head should be submerged in the solution.

25 With a fine scissor, cut the skin and expose the median line of the skull. Cut off the posterior part of the skull. Then, carefully cut along the median line, towards the rostral part of the head. Past bregma, apply two diagonal cuts toward the eyes, and two other later cuts along the lambdoidal sutures. During this stage, keep the head in the slicing slushie or remove it only briefly and put it back in to maintain the brain cold.

26 Carefully open the skull with the help of the tweezers and expose the brain. With the spatula, gently

remove the brain and slide it into the slicing slushie in the petri.

27 Discard the skull into the carcass bag. Remember to remove any ear-tag.

28 Wet the filter paper on top of the other petri dish with slicing slushie.

29 Take the vibratome holding stage from the ice, wipe it dry with a precision wipe and apply super glue to the center of it.

30 Gently move the brain from the slushie to the filter paper. With the single edge blade, carefully prepare the brain to optimize the slicing process in the desired orientation.

31 With a spatula, move the brain from the filter paper to the vibratome holding stage, on the glue in the desired orientation. Make sure that the brain and the agarose block are properly set by gently touching them with the spatula.

32 Move the holding stage into the slicing chamber.

33 Quickly pour slicing slushie into the chamber, covering the brain. Set the chamber on the vibratome. Add a small bubbler to the chamber.

34 With the spatula, gently move the residual icy part of the slushie toward the operator, leaving the brain visible.

35 Lower the blade into the solution, adjust its position/inclination, set the start/end point of the slicing cycle and manually lower the blade closer to the region where you expect to start collecting slices.

36 Start the vibratome.

37 Discard fragments of brain and slices from undesired area, until the region of interest is reached.

38 Immediately transfer each slice containing the region of interest from the slicing chamber into the holding chamber at 34 °C .

After slicing:

45m

39 Once all the desired slices are obtained, they should recover at 34 °C for 30 minutes, under cons 30m bubbling.

40 Once all the slices are collected, discard remaining tissue, wash the apparatus, the tools, and clean the working station.

41 A fragment of the tail might be collected in an eppendorf tube in case genotyping is needed.

42 Discard the mouse carcass and extra tissue in the designated bag.

43 Carefully dispose of all the sharps according to institutional guidelines.

44 After 30 minutes, remove the holding chamber from the heated water bath and leave it at room temperature. Slices should equilibrate at Room temperature for at least 15 minutes before starting experiments. 15m