Ex vivo Brain Slice Preparation for electrophysiology and 2PLSM recordings

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We use this protocol and it's working

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ABSTRACT

In this protocol we detail the steps to obtain brain slices containing the pedunculopontine nucleus (PPN) for ex vivo electrophysiology and 2PLSM recordings. We perform parasagital slices with a 20º angle to maximize the PPN area in each slice. We make both electrophysiological and imaging recording of PPN cholinergic neurons to understand the mechanisms that rely in their vulnerability in Parkinson's disease.

MATERIALS

Solutions to prepare (see preparation below):

- 1 x Slicing solution
- 1 x aCSF
- 2x Ice bucket with ice

Dissection, perfusion and slicing tools:

- Tubing to extend syringe needle
- 10mL syringe
- Barbed forceps
- Large surgical scissors
- Small surgical scissors
- 3x 95mm diameter glass petri dish bottom
- 27G needles for IP injection
- Scalpel
- Borosilicate glass tube
- Super glue
- 20° agarose wedge
- Glass wide bore transfer pipette

Stock Solutions:

All solutions are prepared as 10´ stock solutions and diluted for use on the day of the experiment. Stock solution can be stores at 4°C for a week.

Stock slicing

Solution 10x **1L**

	Compound	MW	g	conc (mM)
	NaCl	58.44	28.72	491.4
	KCl	74.55	1.86	25
	sodium bicarbonate	84.01	21	250
	sodium phosphate	120	1.72	14.3

Stock aCSF

Solution 10x **1L**

	Compound	MW	g	conc (mM)
	NaCl	58.44	79.33	1357.5
	KCl	74.55	1.86	25
	sodium bicarbonate	84.01	21	250
	sodium phosphate	120	1.5	12.5

Slicing solution

Solution 1x **1L**

	A	B	C	D
	Compound	**MW**	**g**	**conc (mM)**
	NaCl	**diluted from 10x**		**49.14**
	KCl	**diluted from 10x**		**2.5**
	sodium bicarbonate	**diluted from 10x**		**25**

	A	B	C	D
	sodium phosphate	**diluted from 10x**		**1.43**
	CaCl2	**1M solution**	0.5ml	**0.5**
	MgCl2	**1M solution**	10ml	**10**
	Glucose*		4.5	**25**
	Sucrose*		34	**99.3**

*add glucose and sucrose to working solution (do not include in 10x stock)

Physiological glucose

aCSF

Solution 1x **1L**

	A	B	C	D
	Compound	**MW**	**g**	**conc (mM)**
	NaCl	**diluted from 10x**		**125**
	KCl	**diluted from 10x**		**2.5**
	sodium bicarbonate	**diluted from 10x**		**25**
	sodium phosphate	**diluted from 10x**		**1.25**
	CaCl2	**1M solution**	2ml	**2**
	MgCl2	**1M solution**	1ml	**1**
	Glucose*		0.63	**3.5**

* add glucose to working solution (do not include in 10x stock)

High glucose aCSF

Solution 1x **1L**

	A	B	C	D
	Compound	**MW**	**g**	**conc (mM)**
	NaCl	**diluted from 10x**		**135.75**

	A	B	C	D
	KCl	**diluted from 10x**		**2.5**
	sodium bicarbonate	**diluted from 10x**		**25**
	sodium phosphate	**diluted from 10x**		**1.25**
	CaCl2	**1M solution**	2ml	**2**
	MgCl2	**1M solution**	1ml	**1**
	Glucose*		4.5	**25**

*add glucose to working solution (do not include in 10x stock)

Prepare Bubbling Solution

1 On the experiment day prepare the working aCSF and slicing solution in a 1:10 dilution from stock solutions

2 Place slicing solution in -20 °C freezer for 30-40 minutes.

2.1 Then place the slicing solution in a large Styrofoam container of ice to remain cold.

3 Place two 95mm diameter glass petri dishes on top of ice bucket and fill with cold slicing solution. Bubble the slicing solution in the bottle and the two petri dishes.

4 Turn on water bath and set to 34 °C °.

5 Fill a 250 mL beaker with 150 mL of slicing solution.

6 Place a second container that has a sieve bottom into the beaker containing the slicing solution.

7 Place the beaker with the slicing solution and second container in water bath and begin bubbling it.

8 Fill a 95mm diameter glass petri dish with slicing solution and set aside.

Prepare Vibratome

9 Cut a 1 cm piece of the 20° agarose wedge and super glue it to the specimen plate.

10 Insert razor blade into blade holder.

11 Set parameters (speed, amplitude, and section thickness) on the Vibratome control panel.

12 Place ice in the chamber below the specimen plate and prepare a glass with water next to the vibrotome.

Preparing Apparatus and Anesthesia:

13 Fill one 10 mL syringe with cold slicing solution, connect with tubing, and place 27G needle at end of tubing.

14 Fill a styrofoam tray with ice.

15 Anesthetize the mice with a mixture of ketamine (50 mg/kg) and xylazine (4.5mg/kg).

16 Use toe pinch-response method to determine depth of anesthesia.

17 Place the animals on ice in tray lying on the back with face upward.

Perfusion Surgery

18 Make an incision through the abdominal skin.

19 Make two additional skin incisions from the xiphoid process along the base of the ventral ribcage laterally.

20 Gently reflect the two flaps of skin to expose thoracic field completely.

21 Grasp the cartilage of the xiphoid process with blunt forceps and raise it slightly to insert pointed scissors. Cut through the thoracic musculature and ribcage between the breastbone and medial rib insertion points and extend the incision rostrally to the level of the clavicles.

22 Separate the diaphragm from the chest wall on both sides with scissor cuts.

23 Clamp the reflected ribcage laterally with a hemostat to expose the heart.

24 Secure the beating heart with fingers or blunt forceps, and immediately insert a blunt 27G syringe needle.

25 Cut the right atrium with scissors, and at the first sign of blood flow, begin the infusion of 1x slicing solution at 2-4 ml/min.

26 Continue perfusion with slicing solution until the 10 mL syringe is empty.

Dissection

27 Decapitate the mouse with large surgical scissors.

28 Place the decapitated head in one of the petri dishes containing cold, bubbling slicing solution. Removal of brain must be done in cold, bubbling slicing solution.

29 Cut down the midline to expose the skull.

30 Make two lateral and one dorsal cut using sharp surgical scissors on the base of the skull.

31 Cut the olfactory bulbs/optic nerve at rostral end of skull.

32 Gently peel off the skull using blunt forceps.

33 Once brain is fully exposed, remove it from the skull and place it in a petri dish with clean, cold and bubbled slicing solution.

34 Cut the brain in two halves along the midline.

35 Super glue the flat section (midline) onto the agarose wedge glued to the specimen plate so that the vibratome cuts along parasagittal plane with a 20° angle. Place the posterior part of the brain in the top of the agarose wedge. If bilateral sections are needed, super glue both hemisferes to the agarose wedge.

Slicing

1h

36 Place specimen plate in buffer tray and fill buffer tray with slicing solution.

37 Place O2/CO2 tubing into buffer tray to bubble slicing solution.

38 Play start to the vibrotome and cut the desired slices.

39 With the glass transfer pipette, transfer the slices to the beaker containing the sieve bottom container at 34 °C.

40 When finishing slicing, turn of the water bath and let it cool to room temperature.

41 Leave brain slices in beaker for 01:00:00 to recover. 1h