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# Brain Slice Preparation for electrophysiology patch-clamp recording

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**Protocol status:** Working
**We use this protocol and it's working**

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## Abstract

This protocol describes brain slice preparation for patch-clamp electrophysiology recording as described in Serra et al., 2023
doi: 10.1016/j.celrep.2023.113328.

## Guidelines

Please note that this protocol requires prior approval by the users' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee. Anyone carrying out this protocol must have valid authorisation for animal experimentation.

## Materials

**Solutions to prepare:**
- 1 x Sucrose aCSF (slicing solution)
- 1 x aCSF (holding solution)
- 1x Ice bucket with ice

**Dissection/perfusion and patching tools:**
- Tubing to extend syringe needle
- 10mL syringe
- Barbed forceps
- Large surgical scissors
- Small scissors
- Small surgical scissors
- 2× 95mm diameter glass petri dish bottom
- 27G needles for IP injection
- Scalpel
- Super glue
- Glass pipette
- Glass wide bore transfer pipette

## Before start

1. Wear PPEs before entering slicing room when making brain slice, i.e. gloves and coat.
2. Fill the register book with mouse genotype, date of birth and ID number.

## Prepare Solutions

1 On the prior day, prepare sucrose aCSF solution in a 1:10 dilution from stock solutions (1 part stock 10x aCSF, 1 part 10x bicarbonate, 8 part Milli-Q water). Note: sucrose must be added to sucrose aCSF while making working solution – not the stock solution.

2 On the prior day, place sucrose aCSF solution in -20° freezer overnight.

3 On the day of cutting, prepare 1x working aCSF solution.

4 On the day of cutting, remove sucrose aCSF from freezer and place in a bucket of hot water until sucrose aCSF become icy. Place in a container of ice to remain cold.

5 Place a 95mm diameter glass petri dish on top of ice bucket and fill with cold sucrose aCSF. Bubble the sucrose aCSF in the bottle and the petri dish.

6 Turn on water bath and set to 35°.

7 Fill a 250 mL beaker with 150-200 mL of working aCSF.

8 Place a plastic container that has sifted bottom into the beaker containing the working aCSF.

9 Place the beaker with the aCSF and container in water bath and begin bubbling working aCSF.

10 Fill a 95mm diameter glass petri dish with working aCSF and set aside.

## Prepare Vibratome

11 On the cutting head of the vibratome, rotate blade holder 90° using a Allen key.

12 Open the blade holder using a Allen key and insert razor blade into blade holder.

13 Clamp down the blade holder using the Allen key until hand-tight.

14 Set parameters (speed, amplitude, and section thickness) on the Vibratome control panel (ex: Speed: 0.10mm/s; amplitude: 2.00; thickness: 250µm).

15 Put ice around the buffer tray.

## Preparing Apparatus and Anesthesia

16 Fill one 10 mL syringe with cold sucrose aCSF, connect with tubing, and place 27G needle at end of tubing.

17 Fill metal tray with ice.

18 Weight the mouse to the nearest 0.1 gram. Anesthetize with Ketamine/Xylazine (7 5/10 mg/kg, i.p.). Place the mouse back to the home cage.

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

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

## Perfusion Surgery

21 Make an incision through the abdominal skin.

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

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

24 Grasp the cartilage of the xiphoid process with 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.

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

26 Clamp the reflected ribcage laterally to expose the heart.

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

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

29 Continue perfusion with sucrose aCSF until the 10 mL syringe is empty.

## Dissection

30 Decapitate the mouse with large surgical scissors.

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

32 Cut down the midline to expose the skull.

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

34 Gently peel off the skull using blunt forceps.

35 Once brain is fully exposed, make an incision along the coronal plane to remove the cerebellum and make a sagittal incision to separate the hemispheres.

36 Glue the flat section (medial portion) of each hemisphere onto the specimen plate so that the vibratome cuts along sagittal plane.

37 Give a small incision with the scalpel on the cortex of one hemisphere to identify the origin of the slices.

## Slicing

38 Place specimen plate in buffer tray and fill buffer tray with sucrose aCSF.

39 Place O2/CO2tubing into buffer tray to bubble sucrose aCSF.

40 Using the manual mode of the vibratome VT1200 from Leica:

41 Select the automatic mode (Auto/Man button) and the single mode (Single/Cont. button) on the control panel.

42 Set a cutting window above the brain samples (using forward/backward and up/down buttons on control panel) and press the window buttons (on left side of the control panel).

43 Start collecting slices using the “RUN/STOP” on the control panel after blade has successfully cut through the entire brain.

44 Press “RUN/STOP” again after blade has successfully cut through the entire brain until cutting the region of interest.

45 After each slice, remove brain slice from buffer tray using a glass wide bore transfer pipette and place into the second petri dish on ice containing bubbling sucrose aCSF.

46 Once all brain slices are collected, move brain slices from the petri dish containing sucrose aCSF to a room-temperature petri dish containing working aCSF.

47 Move brain slices from the petri dish containing working aCSF to beaker in the water bath with bubbling working aCSF.

48 After 60 minutes, turn off water bath and leave brain slices in beaker for 1 hour to recover.

## Protocol references

Serra GP, Guillaumin A, VIcek B, Delgado-Zabalza L, Ricci A, Rubino E, Dumas S, Baufreton J, Georges F, Wallén-Mackenzie A. A role for the subthalamic nucleus in aversive learning *Cell Reports*. (2023) 42(11):113328. doi: 10.1016/j.celrep.2023.113328.