



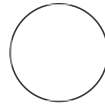
JUN 05, 2023

Brain Slice Preparation for electrophysiology recording

Hong-Yuan
Chu^{1,2},

Hong-yuan
Chu^{1,2}

¹Van Andel Institute; ²ASAP



johnson.agniswamy

ABSTRACT

This protocol describes brain slice preparation for electrophysiology recording.

ATTACHMENTS

[732-1843.docx](#)

MATERIALS

- Electrophysiology solutions are prepared as 10× stock solutions and diluted for use on the day of the experiment.
- Bicarbonate is added to all working solutions.
- For example, working sucrose aCSF

A	B
stock sucrose aCSF	50 mL
stock bicarbonate	50 mL
sucrose	39.36 g

made up to 500 mL with deionized water.

- At 4 °C the shelf life of stock bicarbonate and sucrose aCSF is ~1 week; stock aCSF and SIF have a shelf life of ~2 weeks.

A	B	C	D
Bicarbonate			
	mM	MW	10× g/L
NaHCO₃	26	84.01	21.8426

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.36wgqj2eovk5/v1

Protocol Citation: Hong-Yuan Chu, Hong-yuan Chu 2023. Brain Slice Preparation for electrophysiology recording. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.36wgqj2eovk5/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: May 31, 2023

Last Modified: Jun 05, 2023

PROTOCOL integer ID:
82690

Keywords: Brain Slice Preparation, electrophysiology recording, Perfusion Surgery

A	B	C	D	E
Sucrose aCSF (slicing solution)				
	mM	MW	10×, g/L	10×, g/500 mL
KCl	2.5	74.55	1.8638	0.9319
NaH₂PO₄·H₂O	1.25	137.99	1.7249	0.8624
CaCl₂·2H₂O	0.5	147.02	0.7351	0.3676
MgSO₄·7H₂O	10	246.48	24.6480	12.3240
D-Glucose	10	180.16	18.0160	9.0080
			g/500 mL	
Sucrose *	230	342.3	39.3645	

* add sucrose to working solution (do not include in 10× stock).

A	B	C	D	E
aCSF (holding solution)				
	mM	MW	10×, g/L	10×, g/500 mL
NaCl	126	58.44	73.6344	36.8172
KCl	2.5	74.55	1.8638	0.9319
NaH₂PO₄·H₂O	1.25	137.99	1.7249	0.8624
CaCl₂·2H₂O	2	147.02	2.9404	1.4702
MgSO₄·7H₂O	2	246.48	4.9296	2.4648
D-Glucose	10	180.16	18.0160	9.0080

Solutions to prepare:

- 1 x Bicarbonate
- 1 x Sucrose aCSF (slicing solution)
- 1 x aCSF (holding solution)
- 2x Ice bucket with ice
- Ethanol

Dissection/perfusion and patching tools:

- Tubing to extend syringe needle
- 10 mL syringe
- Barbed forceps
- Hemostat
- Large surgical scissors
- Small scissors
- Small surgical scissors
- 3x 95 mm diameter glass petri dish bottom
- 27G needles for IP injection
- Scalpel
- Borosilicate glass tube
- Super glue
- Harp (slice anchor)
- Glass pipette
- Glass wide bore transfer pipette

BEFORE START INSTRUCTIONS

Before beginning


1. Wear PPEs before entering patch clamp room when making brain slice. i.e. gloves, coat, and face mask.
2. Make sure the sharps container is not full. Replace if full.




Prepare Bubbling Solutions

- 1 On the prior day, prepare the working aCSF and sucrose aCSF solution in a 1:10 dilution from stock solutions (1 part stock, 1 part bicarbonate, 8 part Milli-Q water).

Note

Remember: sucrose must be added to sucrose aCSF while making working solution – not the stock solution.

- 2 On day of cutting, place sucrose aCSF solution in  -20 °C freezer for 30-40 minutes.
- 3 Remove sucrose aCSF and place in large Styrofoam container of ice to remain cold.



- 4 Place two 95mm diameter glass petri dishes on top of ice bucket and fill with cold sucrose aCSF. Bubble the sucrose aCSF in the bottle and the two petri dishes.
- 5 Turn on water bath and set to  35 °C .

- 6 Fill a 250 mL beaker with  150 mL of working aCSF.
- 7 Place a second container that has a sieve bottom into the beaker containing the working aCSF.
- 8 Place the beaker with the aCSF and second container in water bath and begin bubbling working aCSF.
- 9 Fill a 95 mm diameter glass petri dish with working aCSF and set aside.

Prepare Vibratome

- 10 On the cutting head of the vibratome, rotate blade holder 45°-90° using a size 3 Allen key.
- 11 Open the blade holder using a size 3 Allen key and insert razor blade into blade holder.

- 12 Clamp down the blade holder using the size 3 Allen key until hand-tight.
- 13 Set parameters (speed, amplitude, and section thickness) on the Vibratome control panel.
- 14 Turn on Julabo FL300 (recirculating chiller/cooler) and set to desired temperature to cool buffer tray.
- 15 Cut a small piece of agarose (approximately 2x2cm square) and super glue to specimen plate so that it is standing on its side.

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 Avertin (dosage:  0.3 mL /  10 g body weight, 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 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.
- 25 Separate the diaphragm from the chest wall on both sides with scissor cuts.
- 26 Clamp the reflected ribcage laterally with a hemostat to expose the heart.
- 27 Secure the beating heart with fingers or blunt forceps, and immediately insert a blunt 27G syringe

needle.

Note

Optional: clamp the needle to the left ventricle using a hemostat.

- 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 decapitated head in one of the petri dishes containing cold, bubbling sucrose aCSF.

Note

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

- 35 Gently peel off the skull using blunt forceps.
- 36 Once brain is fully exposed, make an incision along the coronal plane of the cerebellum to create a flat surface.
- 37 Super glue the flat section onto the specimen plate so that the vibratome cuts along coronal plane.

Slicing

1h

- 38 Place specimen plate in buffer tray and fill buffer tray with sucrose aCSF.
- 39 Place O₂/CO₂ tubing into buffer tray to bubble sucrose aCSF.
- 40 If using **manual mode**:
 - 40.1 Press the “AUTO/MAN” button so that the bottom LED light is on, indicating semiautomatic/manual mode. In semiautomatic mode, only continuous slicing (SINGLE) is possible.

40.2 Position razor blade (using the blade forward/backward and "UP" / "DOWN" buttons on control panel) at desired location and perform first cut by pressing the "RUN/STOP" button.

Note

Typically, at bottom of olfactory bulb.

40.3 Press "RUN/STOP" on the control panel after blade has successfully cut through the entire brain.

40.4 Return the blade to the initial position using the blade backwards button on control panel.



40.5 Turn Dial 2 (location next to "Section Thickness" button on control panel) to desired slice thickness.

40.6 Press "RUN/STOP" to perform second cut.


40.7 Press "RUN/STOP" again after blade has successfully cut through the entire brain.

40.8 Repeat steps 40.3-40.6 until desired amount of tissue or brain region(s) is cut.

41 If using **continuous mode**:

- 41.1** Press the “AUTO/MAN” button so that the top LED light is on, indicating automatic mode.
- 41.2** Press the “SINGLE/CONT” button to toggle between single stroke and continuous stroke.
- 41.3** Position razor blade (using the blade forward/backward and “UP”/“DOWN” buttons on the control panel) above the brain at the initial window edge.
- 41.4** Press the first cutting window button to set the first window edge.
- 41.5** Move the blade forward to the second window edge and press the second window cutting edge button.
- 42** Steps about continuous slicing.
- 43** 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.
- 44** 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.
- 45** Move brain slices from the petri dish containing working aCSF to beaker in the water bath with

bubbling working aCSF.

- 46** After 30 minutes, turn off water bath and leave brain slices in beaker for  01:00:00 to recover.

1h