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Hippocampal slice preparation for electrophysiology

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We use this protocol and it's working

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Abstract

This protocol describes the preparation and maintenance of acute hippocampal slices from mice for electrophysiology. We describe the dissection, slicing and incubation methods to produce healthy slices. Slice preparation can be followed by same-day electrophysiology experiments (e.g. to assess synaptic plasticity). Acute slices can be further processed for protein detection (e.g. immunofluorescence, immunoblotting) as shown by our lab.

Materials

Solution Recipes:

	A	B	C	D	E
	Reagent	Mass (g) for 1 L	Mass (g) for 500 mL	MW	mM
	NaCl	7.2466	3.6233	58.44	124
	Glucose	1.8016	0.9008	180.156	10
	NaHCO3	2.1843	1.0922	84.007	26
	KCl	0.2236	0.1118	74.55	3
	NaH2PO4•H2O	0.1932	0.0966	137.99	1.4
	MgSO4	1 mL	0.5 mL	1M Solution	1
	CaCl2	2 mL	1 mL	1M Solution	2
	Osmolarity 300-310 mOsm				

ACSF Recipe

	A	B	C	D	E
	Reagent	Mass (g) for 1 L	Mass (g) for 500 mL	MW	mM
	Sucrose	17.1145	8.5573	342.3	50
	NaCl	5.3765	2.6883	58.44	92
	Glucose	2.7024	1.3512	180.156	15
	KCl	0.3728	0.1864	74.55	5
	NaH2PO4•H2O	0.1987	0.0994	137.99	1.4
	NaHCO3	2.1843	1.0922	84.007	26

	A	B	C	D	E
	CaCl2	0.5 mL	0.25 mL	1M Solution	0.5
	MgCl2•6H2O	1.4231	0.7116	203.30	7
	Kynurenic Acid	0.1892	0.0946	189.17	1
	Osmolarity 322-330 mOsm				

Sucrose Solution Recipe

Dissection tools:
- Fine scissors
- Angled fine scissors
- Surgical scissors
- Dumont Forceps
- Hemostat
- Dressing forceps
- Scalpel handle and blade (#21)
- Spatula
- Razor blades
- Glass petri dish

Other supplies:
- 25 or 50 mL syringe
- Tygon tubing
- 27g needle (for i.p. injection)
- 16g needle (for perfusion)
- Anesthetic (e.g. Avertin)
- Filter paper
- Tissue adhesive (e.g. 3M Vetbond)
- Ice
- 95% oxygen/5% carbon dioxide (carbogen tank and lines)
- Cell strainers, falcon tubes, super glue and a 250 mL beaker (for homemade brain slice keeper)
- Beakers (250-500 mL)

Equipment:
- Vibratome
- Brain slice keeper

Brain slice keeper

Troubleshooting

Safety warnings

If you choose to use this protocol, you do so at your own risk and must ensure that any local guidance is adhered to.

Ethics statement

Experiments involving animals must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s). Animal care and experimental procedures were reviewed and approved by the Animal Ethics and Compliance Program at the University of Toronto and conducted in accordance with the Canadian Council on Animal Care (CACC) guidelines.

Set up

55m

1 Prepare solutions (see Materials) 1 week to 1 day in advance (store at 4 °C)

2 Bubble 100 mL sucrose solution and 200 mL ACSF for 20 minutes in 95% oxygen/5% carbon dioxide for 00:20:00 — 20m

3 Freeze sucrose solution at -20 °C until a slurry begins to form (~ 00:15:00) — 15m

4 Prepare fresh ACSF (day of) that will be used for recording — 20m

5 Once prepared, continue bubbling all solutions with 95% oxygen/5% carbon dioxide throughout the duration of slice preparation

6 Add ACSF to a brain slice keeper (e.g. 250 mL beaker with cell strainers affixed to cut falcon tubes to ensure continuous solution flow; see Materials) and heat in a water bath to 28 °C . Ensure that the cell holders are submerged and the solution is bubbled with 95% oxygen/5% carbon dioxide for the duration of the procedure.

7 Prepare dissection area with dissection tools, an absorbent pad, and bench protectors. Add ice to vibratome well (around buffer tray)

Perfusion and brain dissection

12m

8 Weigh and anesthetize mouse with the appropriate dosage (i.p.) and place back into home cage. — 5m

9 Assess depth of anesthesia using toe-pinch response (ensure response is gone before beginning procedure)

10 Add ice cold sucrose solution to 50mL syringe attached to tubing and needle for perfusion

11 Make a lateral incision through the abdomen at the xiphoid process using fine scissors

12 Pulling up on the xiphoid process, carefully cut the diaphragm and then cut along the base of the ribcage on both sides to expose the heart

13 Clamp the xiphoid process using a hemostat to expose the chest cavity

14 Securing the heart with dressing forceps, insert 18g needle attached to a 50mL syringe with tubing into left ventricle

15 Make a small incision in the right ventricle using fine scissors

16 Compress the syringe slowly to perfuse the mouse until the liver is visibly clear or approximately 30-40 mL of solution has been used 2m

17 Decapitate the mouse with large surgical scissors

18 Pull the skin back and make two lateral incisions at the base of the skull using fine scissors

19 Make a cut down the midline of the skull using angled fine scissors, taking care not to damage the brain

20 Gently peel away the two sections of the skull with Dumont forceps

21 Scoop the brain out of the skull using a spatula and immediately submerge in cold, bubbling ACSF for 00:05:00 5m

Slicing 1h 45m

22 Wet filter paper in ice cold ACSF and place in a glass petri dish

23 Remove the brain from solution and place on the wet filter paper. Use a scalpel to remove the cerebellum and olfactory bulb, then make two thin cuts along the sagittal plane of the brain bilaterally to provide a flat surface for slicing

24 Make a cut down the midline of the brain and glue the two hemispheres of the brain to the vibratome platform, with the hippocampus facing up and cortex towards the blade

25 Fill the buffer chamber with ice cold ACSF and continuously bubble the solution. Attach the razor blade to the vibratome and position it right above the tissue as the starting position

15m

26 Slice 400 µm slices at 0.08 mm/s (on continuous slicing mode). Carefully remove the CA3 region of each slice using a scalpel. Remove any excess tissue around the dorsal hippocampal slices. Pause the vibratome when performing microdissections of slices.

27 Transfer each slice to 28 °C bubbling ACSF in the brain slice keeper to rest for a minimum of 01:30:00 before recording in a chamber maintained at 28 °C

1h 30m

28 If recording from multiple slices, turn off the water bath after 03:00:00 of incubation and allow slices to gradually come to room temperature.