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Protocol for ex vivo patch clamping

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Protocol status: Working

We use this protocol and it's working

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Abstract

Protocol for ex vivo patch clamping

Materials

Reagents, instruments and materials

- Ketamine / xylazine cocktail
- Fine science surgical tools, including scalpel, scissors, forceps
- Petri dishes
- Vibratome
- Blade

Solutions:

- Sucrose-based artificial cerebrospinal fluid (sucrose aCSF in mM): 230 sucrose, 26 NaHCO₃, 2.5 KCl, 1.25 NaH₂PO₄, 0.5 CaCl₂, 10 MgSO₄, 10 glucose, 1 Na-pyruvate, and 0.005 L-glutathione.
- Standard aCSF (in mM): 26 NaHCO₃, 126 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 2 CaCl₂, 2 MgSO₄, 10 glucose, 1 Na-pyruvate, and 0.005 L-glutathione.
- Synthetic interstitial fluid (SIF) warmed to 35C, equilibrated with 95% O₂ and 5% CO₂ and containing (in mM): 26 NaHCO₃, 126 NaCl, 3 KCl, 1.25 NaH₂PO₄, 1.6 CaCl₂, 1.5 MgSO₄, and 10 glucose.

Equipment Setup

- Vibratome

Preparation of acute brain slices

- 1 Chill sucrose aCSF up to 4 degrees and then keep it on the ice and bubble continuously by carbogen.
- 2 Place 150 ml standard aCSF in holding chamber. Warm it up to 34°C in water bath and bubble continuously by carbogen.
- 3 After 3-5 weeks of the viral injection, anesthetize the animal briefly with Isoflurane and then make IP injection of ketamine/xylazine cocktail (87/13 mg/kg i.p.).
- 4 Perfuse the animal transcardially with 4-6 ml ice-cold sucrose aCSF.
- 5 Open the scalp, block the brain between the hemispheres and transfer it to the petri dish filled with ice-cold oxygenated sucrose solution.
- 6 Glue the brain on the specimen plate and cut 250 µm thickness slices on the vibratome.
- 7 Wash the slices with standard aCSF and transfer them to the holding chamber. Keep the slices 30 min at 34°C and then at room temperature.