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

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Perforated patch electrophysiology recordings

Forked from Ex vivo mouse brain patch clamp recordings and Fura-2 imaging

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ABSTRACT

In this protocol we detail the steps to perform ex-vivo brain slices perforated patch electrophysiology recordings.
The protocol is originally meant to record from striatal MSNs, but can be adapted to other cell types.

GUIDELINES

Please adhere to institutional guidelines.

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91042

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MATERIALS

Please refer to existing protocols to obtain brain slices for electrophysiology experiments, including preparation of experimental solution (aCSF) and brain slices.

These experiments require a fully set-up electrophysiology rig, including a floating table, a microscope equipped with bright-field illumination and a fluorescent light source and appropriate filter-sets, a camera to visualize slices, micromanipulators, and a computer with the appropriate electrophysiology and imaging software.

Internal solution:
in mM: 120 potassium-D-gluconate, 13 KCl, 10 HEPES, 0.5 EGTA, 4 ATP-Mg, 0.5 GTP-Na, 10 phosphocreatine-di (tris); adjusted to pH 7.25 with KOH; 275-280 mOsm.

Miscellaneous:
- Blood-gas mixture (95% O2, 5% CO2) tank connected to bubblers.
- Slice holder
- Peristaltic pump or gravity flow perfusion with tubing and connectors, including inlet and outlet to microscope's imaging chamber
- Stage heating system with probe
- Waste solution collector
- 10% Ethanol in water (wash solution)
- syringe
- microfilter
- flexible microfil
- Alexa Fluor hydrazide Na2+ salt (if desired)

SAFETY WARNINGS

Wear recommended PPE.
Check SDS for each reagent.
Follow operating instruction manuals/obtain recommended training for experimental equipment.

BEFORE START INSTRUCTIONS

Please refer to existing protocols to obtain brain slices for electrophysiology experiments.

Prepare patch pipettes

1 Turn on the Sutter P-1000 puller and enter the desired pull protocol.

2 Insert a thick-walled borosilicate glass capillary and press pull.

3 Pipette resistance must be of 3 to 6 megaohms.

Setting up patch rig and environment

4 Turn on the MultiClamp 700B Amplifier, Axon Digidata 1550B digitizer, micromanipulator, computer tower and the associated software. Note: amplifier and digitizer must be turned on prior to opening software.

5 Turn on O2/CO2 tank and bubble aCSF solution.

6 Filter the internal solution containing Alexa dye in a clean eppendorf tube. Save a small amount of internal solution with Alexa dye in a separate tube or in the syringe.

7 Add appropriate amount of gramycidin solution to the desired final concentration (e.g. 10 microM, but the protocol might be optimized based on specific needs).

8 Collect the internal solution with gramicidin in a clean syringe and attach a microfil flexible needle (or alternative electrode filling method).

9 Start circuling experiemental aCSF through chamber, either using a gravity flow system or a peristaltic pump (a gravity flow system is recommended because of the reduced electrical noise).

10 Turn on water heater and set to desired temperature (~ 34 °C or Room temperature)

Examine slices and patching cells

11 Transfer brain slice from holding chamber to the recording chamber.

12 Secure down slice with a harp (slice anchor).

13 Locate and focus the desired brain region under the 4x objective.

14 Change the microscope lens to the 60x objective.

15 Focus on healthy neurons in slices for patching.

16 Front-fill the patch electrode with a small volume of gramicidin-free internal solution (possibly filling only the tip of the pipette - smaller volumes will result in faster achievement of perforated patch configuration, larger volumes will allow more time to patch cell, since once gramicidin at the tip of the

electrode will compromise the ability to obtain a seal).

16.1 Gently back fill the electrode with internal solution containing gramicidin. Avoid formation of bubbles, but move carefully to avoid mixing the two solutions too quickly.

17 Gently place the glass micropipette onto the wire electrode and tighten.

18 Position the electrode using a micromanipulator.

19 Under the 60x objective, bring the tip of the glass pipette above the slice.

20 Apply a positive pressure and maintain it.

21 Approach the cell diagonally. The positive pressure should create a small dimple on the cell.

22 Once a dimple is formed, release the positive pressure, and apply a small amount of negative pressure. The resistance should begin to increase rapidly.

23 As the resistance increases, clamp the cell at your resting potential of interest (typically - 60 mV).

24 After a giga-ohm seal is formed, monitor series resistance until <100MOhm and stabilized - usually 20-30 minutes).

25 Start recording.

After the recording

26 If Alexa dye was added to the internal solution, a fluorescent light source can be used to verify that the Alexa signal is not detected inside the patch cell.

27 Gently remove electrode.

28 Remove and discard slice (unless further processing/fixation is needed)

29 Rinse tubing and microscope chamber.

30 Save/export files

31 Shut down the system, dispose of waste according to guidelines, clean work station.