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🌐 Ex vivo mouse brain patch clamp recordings combined with optogenetic stimulation

Forked from <u>Ex vivo mouse brain patch clamp recordings combined with uncaging and optogenetic stimulation</u>

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Abstract

In this protocol we detail the steps to perform ex-vivo brain slice electrophysiology and optogenetic stimulation.

Guidelines

Please adhere to institutional guidelines and regulations.

Materials

Solutions:

Internal solution:
These are prepared prior to the experiment day and aliquoted in 1.5 ml tubes, and stored at -20°C until the day of experiment.

Internal solution composition:
(in mM) 120 potassium-D-gluconate, 13 KCl, 10 HEPES, 0.05 EGTA, 4 ATP-Mg2, 0.5 GTP-Na, 10 phosphocreatine-di (tris); pH was adjusted to 7.25 with KOH and osmolarity to 275-280 mOsm.

Recording solution
(in mM) 125 NaCl, 3 KCl, 1 MgCl2, 2 CaCl2, 25 NaHCO3, 1.25 NaH2PO4 and 10 glucose (saturated with 95% O2-5% CO2; pH 7.4; 300 mOsm/l

Low Ca Optogenetics Recording solution
(in mM) 124 NaCl, 4.5 KCl, 1.8 MgCl2, 1.2 CaCl2, 25 NaHCO3, 1.25 NaH2PO4 and 10 glucose (saturated with 95% O2-5% CO2; pH 7.4; 300 mOsm/l

Hardware and miscellaneous:

-    Targeted focal spot blue LED (473 nm pE-100, CoolLED)
-    Blood-gas mixture (95% O2, 5% CO2) tank connected to bubblers.
-    Slice holder
-    Brain slices expressing genetically encoded opsinsin holding chamber with aCSF
-    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)
-    Image analysis software

Troubleshooting

Safety warnings

> ❗ Please follow institutional safety guidelines and chemical safety datasheets.

Before start

Please note that optogenetic experiments require the expression of a genetically encoded opsin in neurons of interest. Mice genetically engineered to express opsin in specific neuronal populations are available. Alternatively, opsin expression can be obtained via injections of appropriate viral vectors.
Please refer to existing protocols for brain slices preparation and/or viral injections.

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 5 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   Take an aliquot of internal solution from the -20° fridge.

7   Fill syringe with internal solution, place a filter on the end of the syringe, and place a MicroFil Pipette Filler on the end of the filter.

8   Start perfusing microscope chamber with aCSF (either with a gravity system or a peristaltic pump; a gravity system is normally preferred because of the lower electrical noise).

9   Adjust the flow rate to the desired value (recommended at least 2 mL/min).

10   Depending on the experimental protocol, turn on stage heater and set to temperature.

Examine slices and patching cells

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

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

13 Change the microscope lens to the 60x objective.

14 Focus on healthy neurons in slices for patching.

15 For whole cell configuration, fill a glass micropipette one-third full of internal solution. For cell attached configuration fill it with internal solution or aCSF. Ensure there is no residual internal solution on exterior of glass micropipette, as this may introduce salts into the micromanipulator and add additional noise to recordings. Remove any air bubbles by gently flicking the glass micropipette.

16 Gently place the glass micropipette onto the electrode holder.

17 Position the electrode using a micromanipulator.

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

19 Apply a positive pressure and maintain it. Zero the voltage offset.

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

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

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

23 After a giga-ohm seal is formed, apply a few quick pulses of negative pressure to break into the cell to record in whole cell configuration.

24 Start recording.

Recordings and optogenetic stimulation `15m`

25 If in whole cell configuration, allow time for the internal solution to fill the cell. `15m`

26 Electrophysiological recordings and stimulation are acquired and synchronized via the pClamp software.

27 Single-photon optogenetic stimulation is obtained with a 473 nm LED, whose pulse number, intensity, and duration are controlled via pClamp.

After the experiments

28 Gently remove and discard the recording pipette

29 Remove slice from the recording chamber. If desired, the slice can be gently transferred into a plate and bathed with a fixative solution for further processing.

29.1 Note: use appropriate PPE when working with fixatives, including operating under a chemical hood.

30 Discard waste according to institutional protocols.

31 Rinse perfusion tubing microscope chamber with water and/or a 10% ethanol in water.

32 Save and export files.