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Ex Vivo Mouse Brain Slice Whole-Cell Patch-Clamp Electrophysiology

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We use this protocol and it's working

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Abstract

This protocol outlines steps for performing ex vivo slice whole-cell patch-clamp electrophysiology experiments, including voltage clamp and current clamp recordings as well as optogenetic stimulation. For optogenetic stimulation, this procedure is typically performed on mice expressing genetically encoded opsins, such as Channelrhodopsin2, which can be introduced through viral injection or transgenic expression.

Attachments



Ding Lab, Ex Vivo Mou...

19KB

Materials

- ACSF components (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 25 NaHCO₃, 15 glucose, 2 CaCl₂, and 1 MgCl₂.
- Cs-based internal solution components (in mM): 115 CsMeSO₃, 10 TEA, 10 HEPES, 5 QX-314 chloride, 4 Mg-ATP, 0.4 Na₃-GTP, 10 Na₂-phosphocreatine, 1 EGTA, 0.1 CaCl₂.
- K-based internal solution components (in mM): 135 KMeSO₃, 5 KCl, 10 HEPES, 4 Mg-ATP, 0.3 Na₃-GTP, 8 Na₂-phosphocreatine, 1 EGTA, 0.1 CaCl₂.
- 0.22 µm membrane filter (e.g. MilliporeSigmaTM).
- Vibratome (e.g., Leica VT1200s)
- Patch-clamp recording set up (specific components listing in protocol for Ding lab)
- 450 nm light laser (e.g. Opto Engine LLC) and a 594 nm light (e.g. Opto Engine LLC)



Safety warnings

! Wear appropriate PPE as required by your institution.

Ethics statement

Prior ethics approval (e.g. IACUC) should be obtained before performing these experiments. Approval was obtained by the Stanford University IACUC before any procedures were performed.



Prepare artificial cerebrospinal fluid (ACSF)

- 1 Prepare 1x ACSF fresh daily before slicing.
- 1.1 ACSF components (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 25 NaHCO₃, 15 glucose, 2 CaCl₂, and 1 MgCl₂.
- 1.2 Continuously bubble the ACSF solution with 95% O₂ and 5% CO₂ to maintain pH at 7.4 and osmolarity at 300-305 mOsm.

Brain extraction and slicing

- 2 Anesthetize mice following protocols approved by the Animal Care and Use Committee (e.g. 4% isoflurane).
- 3 Decapitate the animal, immediately extract the brain, and submerge it in ice-cold ACSF or other optimized cutting solutions (e.g. high sucrose, low-sodium solution).
- 4 Use a vibratome (e.g., Leica VT1200s) to prepare 300 µm-thick coronal slices containing the region of interest.
- 5 Prepare either a CS-based internal solution for whole-cell voltage clamp recordings or K-Based internal solution for whole-cell current clamp recordings. **This can be done ahead of time.**



STEP CASE

Cs-based internal solution 13 steps

Prepare either a CS-based internal solution for whole-cell voltage clamp recordings or K-Based internal solution for whole-cell current clamp recordings.

- 6 Prepare internal solution containing (in mM): 115 CsMeSO₃, 10 TEA, 10 HEPES, 5 QX-314 chloride, 4 Mg-ATP, 0.4 Na₃-GTP, 10 Na₂-phosphocreatine, 1 EGTA, 0.1 CaCl₂.
- 7 Adjust the solution to pH 7.3-7.4 with CsOH and osmolarity at 280-290 mOsm.

- 8 After fully dissolving, filter the solution twice using a 0.22 μm membrane filter (e.g., MilliporeSigmaTM).
- 9 Store in small aliquots (e.g. 100 μL per tube) at -20°C .

Patch-clamp recording setup

- 10 Transfer a slice to a recording chamber, secure with an anchor (e.g., Warner Instrument), and begin ACSF perfusion at 2-3 mL/min at 30°C .
- 11 Mount the chamber on a microscope (e.g. Olympus BX51), equipped with a Multiclamp 700B amplifier (e.g., Molecular Devices) and monitored by custom MATLAB software (Mathworks) or other recording software (e.g., WinWCP V5.7).
- 12 Use glass pipettes (2.5-4.5 $\text{M}\Omega$) prepared with a micropipette puller (e.g., Sutter P-97), filled with the appropriate internal solution (e.g., Cs-based or K-based).
- 13 Apply positive pressure ($\sim 5 \text{ Kp}$) to the glass pipette while approaching the targeted cell. Upon contact, remove pressure to form a "Giga Ohm seal."
- 14 Once the seal is formed, hold voltage at -70 mV and apply brief negative pressure to achieve whole-cell patch mode ("Break-in").
- 15 Low-pass filter the signal at 2.2 kHz and digitize at 10 kHz (e.g., National Instruments NI PCIe-6259 card or another commercial digitizer).
- 16 Exclude cells with series resistance $> 25 \text{ M}\Omega$ from analysis for quality control.

Recording optogenetically evoked EPSCs and IPSCs

- 17 Once a whole-cell patch is established, hold the membrane potential at $\sim -70 \text{ mV}$ for excitatory postsynaptic currents (EPSCs) and at $\sim 0 \text{ mV}$ for inhibitory postsynaptic currents (IPSCs).
- 18 Once a whole-cell patch is established, hold the membrane potential at $\sim -70 \text{ mV}$ for excitatory postsynaptic currents (EPSCs) and at $\sim 0 \text{ mV}$ for inhibitory postsynaptic currents (IPSCs).