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Whole-cell patch-clamp recordings

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We use this protocol and it's working

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Abstract

This protocol describes the preparation of hippocampal slices from mice for whole-cell patch-clamp recordings. Whole-cell recordings are made from CA1 pyramidal neurons, with AMPAR-mediated EPSCs evoked by stimulation in the stratum radiatum and monitored under voltage-clamp conditions.

Materials

BuffersCutting aCSF

- 210 mM sucrose
- 2.5 mM KCl
- 0.5 mM $CaCl_2$
- 7 mM $MgSO_4$
- 1.25 mM NaH_2PO_4
- 25 mM $NaHCO_3$
- 7 mM D-glucose
- 1.3 mM ascorbic acid
- Saturate with 95% O_2/5% CO_2
- pH 7.4, 305–310 mOsm

Recording aCSF

- 119 mM NaCl
- 2.5 mM KCl
- 2.5 mM $CaCl_2$
- 1.3 mM $MgSO_4$
- 1 mM NaH_2PO_4
- 26.2 mM $NaHCO_3$
- 11 mM d-glucose
- Saturate with 95% O_2/5% CO_2
- pH 7.4, 305–320 mOsm

Pipette solution

- 135 mM $CsMeSO_3$
- 8 mM NaCl
- 0.3 mM EGTA
- 4 mM Mg-ATP
- 0.3 mM Na-GTP
- 5 mM QX-314
- 0.1 mM spermine
- 10 mM HEPES
- pH 7.4, 290–295 mOsm

Troubleshooting

Patch pipette preparation

1 Pull pipettes from borosilicate glass using a Flaming/Brown puller (P-87).
Fill pipettes with pipette solution; typical resistance 3–5 MΩ.

Animal preparation and brain slicing

2 Anesthetize P21–P35 mice with ketamine/xylazine (i.p.).

3 Perfuse with ice-cold cutting aCSF saturated with 95% O_2/5% CO_2.

4 Dissect brains in ice-cold cutting aCSF. Slice into 300 µm near-horizontal sections using a vibratome in oxygenated cutting aCSF. Transfer slices to a holding chamber containing recording aCSF.

5 Incubate slices at 32°C for 30 min, then maintain at room temperature until recording. Perform a micro-cut between CA1 and CA3 to prevent network propagation.

Whole-cell recordings

6 Perform recordings in recording aCSF saturated with 95% O_2/5% CO_2 at 34 ± 0.5°C, perfusion rate 3 mL/min.

7 Evoke AMPAR-mediated responses at -70 mV holding potential in the presence of 10 µM AP-V and 100 µM picrotoxin.

7.1 Fill the recording pipette with intracellular solution and check the recording pipette under the microscope to remove any residual air bubbles.

7.2 Apply positive pressure using 1ml surge to clear the path, and once the recording electrode approaches the cell, the positive pressure will create a dimple as it touches.

7.3 Remove the positive pressure immediately and watch for a slight resistance increase as positive pressure relieve.

7.4 Apply gentle suction and monitor pipette resistance; once it exceeds 1 GΩ, hold the cells at -68 mV immediately and release the suction.

7.5 Apply stronger, brief negative suction to rupture the membrane patch.
Note: The pipette now has access to the cell's interior, forming the whole-cell configuration.

7.6 Allow intracellular solution to exchange (2 mins) before recording.

8 Stimulate the stratum radiatum using a tungsten bipolar electrode.

9 Monitor membrane current, input resistance, and series resistance. Discard cells if series resistance exceeds 30 MΩ. Accept recordings only if seal resistance >1 GΩ.

Data acquisition

10 Acquire data with MultiClamp 700B amplifier.

11 Filter at 2 kHz and digitize at 10 kHz. Store and analyze data using IgorPro v6.3.4.1. Peak EPSC amplitudes are determined from recorded currents