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ACUTE HIPPOCAMPAL SLICE EPHYS PROTOCOL

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol details the Electrophysiological recording of mouse CA1 hippocampal neurons from ex vivo thick slice preparations to analyze whole-cell spontaneous miniature excitatory postsynaptic current and long-term potentiation.

Materials

- Vibratome (Leica VT 1200S)

Equipment

Leica VT1200 S Fully automated vibrating blade microtome

NAME

Vibratome

TYPE

Leica

BRAND

VT1200S

SKU

<https://www.leicabiosystems.com/us/research/vibratomes/leica-vt1200-s/>^{LINK}

Sucrose 189, glucose 10, NaHCO3 26, KCl 3, MgSO4 5, CaCl2 0.1, NaH2PO4 1.25

	A	B
	Sucrose solution (in mM)	
	Sucrose	189
	Glucose	10
	NaHCO3	26
	KCl	3
	MgSO4	5
	CaCl2	0.1
	NaH2PO4	1.25

NaCl 124, KCl 2.69, KH2PO4 1.25, MgSO4 2, NaHCO3 26, CaCl2 2, ascorbic acid 0.4, and glucose 10

	A	B
	Artificial cerebrospinal fluid (ACSF) (in mM)	
	NaCl	124
	KCl	2.69

	A	B
	KH ₂ PO ₄	1.25
	MgSO ₄	2
	NaHCO ₃	26
	CaCl ₂	2
	Ascorbic acid	0.4
	Glucose	10

- Olympus BX50WI microscope

Cesium gluconate 117, HEPES 20, EGTA 0.4, NaCl 2.8, TEA-Cl 5, ATP 2, GTP 0.3 (pH 7.3)

	A	B
	Internal solution (in mM)	
	Cesium gluconate	117
	HEPES	20
	EGTA	0.4
	NaCl	2.8
	TEA-Cl	5
	ATP	2
	GTP	0.3 (pH 7.3)

- PC-ONE amplifier (Dagan Instruments; Minneapolis, MN, USA)
- Digidata 1440A digitizer (Molecular Devices; San Jose, CA, USA)
- pCLAMP 10.4 (Axon Instruments, Molecular Devices; San Jose, CA, USA)
- Temperature control system (Warner Instruments electric TC-324C)

- 1 Obtain acute dorsolateral hippocampal coronal slices ($\pm$ 350 μ m thick) from 2-3 months old mice using a vibratome (Leica VT 1200S).
- 2 Briefly, remove the brain quickly after decapitation and placed in ice-cold high sucrose solution of the following composition (in mM): sucrose 189, glucose 10, NaHCO₃ 26, KCl 3, MgSO₄ 5, CaCl₂ 0.1, NaH₂PO₄ 1.25.
- 3 Afterward, incubate slices for at least 01:00:00 at Room temperature (21 °C – 24 °C) in artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 124, KCl 2.69, KH₂PO₄ 1.25, MgSO₄ 2, NaHCO₃ 26, CaCl₂ 2, ascorbic acid 0.4, and glucose 10, and continuously bubbled with carbogen (95% O₂ and 5% CO₂) (pH 7.4).
- 4 Transfer slices to an immersion recording chamber and superfused at 2 μ L with gassed ACSF at 30 °C – 32 °C and visualized under an Olympus BX50WI microscope (Olympus Optical; Japan).
- 5 Identify CA1 hippocampal neurons visually based on morphology and location in the pyramidal layer.
- 6 To study excitatory postsynaptic currents (EPSCs) and add picrotoxin (50 micromolar (μ M)) and CGP54626 (1 micromolar (μ M)) are to the solution to block the GABAA and GABAB receptors, respectively.
- 7 Obtain Whole-cell electrophysiological recordings from CA1 pyramidal neurons using patch electrodes (3–10 M Ω) filled with an internal solution containing in mM: cesium gluconate 117, HEPES 20, EGTA 0.4, NaCl 2.8, TEA-Cl 5, ATP 2, GTP 0.3 (pH 7.3).
- 8 Obtain recordings with a PC-ONE amplifier (Dagan Instruments; Minneapolis, MN, USA).
- 9 Membrane potentials are held at –70 mV unless otherwise stated.
- 10 Filter signals at 1 kHz, acquired at a 10 kHz sampling rate, and fed to a Digidata 1440A digitizer (Molecular Devices; San Jose, CA, USA).
- 11 Monitor series and input resistances throughout the experiment using a –5 mV pulse.
- 12 Discard Cells when series and input resistances changed >20%.

1h





- 13 pCLAMP 10.4 (Axon Instruments, Molecular Devices; San Jose, CA, USA) is used for stimulus generation, data display, data acquisition, and data storage.
- 14 To record evoked EPSCs, theta capillaries filled with ACSF are used for bipolar stimulation and place in the stratum radiatum to stimulate the Schaffer collaterals.
- 15 Obtain Input–output curves of EPSCs by increasing stimulus intensities from 0 to 100 μ A.
- 16 Acquire Paired-pulse facilitation by applying paired pulses (2 ms duration) with 25, 50, 75, 100, 200, 300, and 500 ms inter-pulse intervals.
- 17 Calculate the paired-pulse ratio by dividing the amplitude of the second EPSC by the first (PPR=EPSC-2/EPSC-1).
- 18 Obtain AMPA currents at a holding potential of -70 mV.
- 19 Obtain NMDA currents at a holding potential of +40 mV.
- 20 To ascertain the AMPA to NMDA receptor current ratio, the NMDA component measure 50 msec after the stimulus, when the AMPA component had decayed.
- 21 Recordings of miniature EPSCs (mEPSCs) and slow inward currents (SICs) are made in the presence of tetrodotoxin (TTX; [1M] 1 micromolar (μ M)).
- 22 Define SICs as currents with a rise time >5 ms, Ton >5 and decay time >10 ms.
- 23 For long-term potentiation (LTP), CA1 pyramidal neurons first underwent a baseline recording of  00:10:00 followed by theta-burst tetanic stimulation in the Schaffer collaterals (4 trains at 100 Hz for  00:00:01 ;  00:00:30 intervals).
- 24 After LTP induction, record neurons for  01:00:00 .

10m 31s

1h



- 25 Determine differences in LTP by comparing the average EPSC amplitudes from the last of post-stimulus recordings with the average EPSC amplitudes from the baseline recordings.
- 26 Perform all experiments at - using a temperature control system (Warner Instruments electric TC-324C).

20m