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ACUTE HIPPOCAMPAL SLICE EPHYS PROTOCOL

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We use this protocol and it's working

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Disclaimer

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
	KH2PO4	1.25
	MgSO4	2
	NaHCO3	26
	CaCl2	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 (350 µm 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, $NaHCO_3$ 26, KCl 3, $MgSO_4$ 5, $CaCl_2$ 0.1, $NaH2PO_4$ 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, $KH2PO_4$ 1.25, $MgSO_4$ 2, $NaHCO_3$ 26, $CaCl_2$ 2, ascorbic acid 0.4, and glucose 10, and continuously bubbled with carbogen (95% O_2 and 5% CO_2) (pH 7.4). 1h

4 Transfer slices to an immersion recording chamber and superfused at 2 µL 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 ([M] 50 micromolar (µM)) and CGP54626 ([M] 1 micromolar (µM)) 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 MΩ) 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%.

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 µA.

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; [M] 1 micromolar (µM)).

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). 10m 31s

24 After LTP induction, record neurons for 01:00:00 . 1h

25 Determine differences in LTP by comparing the average EPSC amplitudes from the last 00:10:00 of post-stimulus recordings with the average EPSC amplitudes from the 00:10:00 baseline recordings. 20m

26 Perform all experiments at 30 °C - 32 °C using a temperature control system (Warner Instruments electric TC-324C).