Jul 11, 2024

Mouse brain slice electrophysiology

DOI
dx.doi.org/10.17504/protocols.io.rm7vzj7y8lx1/v1

Shiyi Wang[1]

[1]Duke University

ASAP Collaborative Rese…

Shiyi Wang
Duke University

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DOI: dx.doi.org/10.17504/protocols.io.rm7vzj7y8lx1/v1

Protocol Citation: Shiyi Wang 2024. Mouse brain slice electrophysiology. **protocols.io** https://dx.doi.org/10.17504/protocols.io.rm7vzj7y8lx1/v1

Protocol status: Working
We use this protocol and it's working

Created: July 11, 2024

Last Modified: July 11, 2024

Protocol Integer ID: 103202

Keywords: ASAPCRN

Funders Acknowledgement:
Aligning Science Across Parkinson's (ASAP) initiative
Grant ID: ASAP-020607

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Abstract

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1 **Animal Handling**

1.1 - Anesthetize 3-4 mice of each genotype (WT and LRRK2 G2019Ski/ki) and condition using 200 mg/kg tribromoethanol (Avertin).

1.2 - Ensure proper anesthesia depth and confirm absence of reflexes before proceeding.

2 **Brain Extraction and Slicing**

2.1 - Decapitate the mice and immediately immerse the brains in ice-cold artificial cerebrospinal fluid (aCSF) containing (in mM): 125 NaCl, 2.5 KCl, 3 mM MgCl2, 0.1 mM CaCl2, 10 glucose, 25 NaHCO3, 1.25 NaHPO4, 0.4 L-ascorbic acid, and 2 Na-pyruvate, pH 7.3-7.4 (310 mOsmol).

2.2 - Use a vibrating tissue slicer (Leica VT1200) to obtain 350 µm thick coronal brain slices containing the anterior cingulate cortex (ACC).

3 **Slice Incubation**

3.1 - Transfer slices immediately to standard aCSF maintained at 33°C and continuously bubbled with 95% O2 – 5% CO2.

3.2 - Incubate slices for 30 minutes at 33°C to recover.

4 **Transfer to Holding Chamber**

4.1 - After incubation, transfer slices to a holding chamber at room temperature (approximately 25°C) with the same extracellular buffer.

5 **Microscopy Setup**

5.1 - Visualize brain slices using an upright microscope (BX61WI, Olympus) equipped with a 40x water-immersion objective and infrared-differential interference contrast optics.

5.2 - Use a digital camera (ODA-IR2000WCTRL) for image acquisition.

6 **Patch-Clamp Setup**

6.1 - Perform patch-clamp recordings using an EPC 10 patch-clamp amplifier controlled by Patchmaster Software (HEKA).

6.2 - Set data acquisition at a sampling rate of 50 kHz and low-pass filter at 6 kHz.

7 **Recording Configuration**

7.1 - Use aCSF bath solution containing: For mEPSCs: 1 µM tetrodotoxin and 50 µM Picrotoxin, held at -70 mV in voltage-clamp mode; For mIPSCs: 1 µM tetrodotoxin, 10 µM CNQX, and 50 µM D-AP5, held at -70 mV in voltage-clamp mode.

8 **Internal Solution Preparation**

8.1 - Prepare internal solutions for patch pipettes: For mEPSCs: 125 K-gluconate, 10 NaCl, 10 HEPES, 0.2 EGTA, 4.5 MgATP, 0.3 NaGTP, and 10 Na-phosphocreatine, pH adjusted to 7.2 – 7.4 with KOH, osmolality ~300 mOsmol. For mIPSCs: 77 K-gluconate, 77 KCl, 10 HEPES, 0.2 EGTA, 4.5 MgATP, 0.3 NaGTP, and 10 Na-phosphocreatine, pH adjusted to 7.2 – 7.4 with KOH, osmolality ~300 mOsmol.

9 **Data Acquisition and Analysis**

9.1 - Record mEPSCs and mIPSCs using Minhee Analysis software.

9.2 - Analyze frequency by counting events over 5 minutes of recording.

9.3 - Calculate average events per cell based on at least 100 non-overlapping events.

9.4 - Measure peak amplitude of average mEPSCs relative to baseline current.