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Mouse brain slice electrophysiology

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We use this protocol and it's working

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Abstract

Mouse brain slice electrophysiology

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 μ M tetrodotoxin and 50 μ M Picrotoxin, held at -70 mV in voltage-clamp mode; For mIPSCs: 1 μ M tetrodotoxin, 10 μ M CNQX, and 50 μ M 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.

