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Mouse brain slice electrophysiology

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Abstract

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1. For whole-cell patch-clamp recordings, 3-4 mice of each genotype and condition were used for miniature excitatory postsynaptic current (mEPSC) and miniature inhibitory postsynaptic current (mIPSC) measurements.

2. WT and *LRRK2* G2019S$^{ki/ki}$ mice of both sexes were anesthetized with 200 mg/kg tribromoethanol (avertin) and decapitated.

3. After decapitation, the brains were immersed in ice-cold artificial cerebrospinal fluid (aCSF, in mM): 125 NaCl, 2.5 KCl, 3 mM $MgCl_2$, 0.1 mM $CaCl_2$, 10 glucose, 25 $NaHCO_3$, 1.25 $NaHPO_4$, 0.4 L-ascorbic acid, and 2 Na-pyruvate, pH 7.3-7.4 (310 mOsmol).

4. 350 µm thick coronal slices containing the ACC were obtained using a vibrating tissue slicer (Leica VT1200; Leica Biosystems).

5. Slices were immediately transferred to standard aCSF (33°C, continuously bubbled with 95% O_2– 5% CO_2) containing the same as the low-calcium aCSF but with 1 mM $MgCl_2$and 1-2 mM $CaCl_2$.

6. After 30-minute incubation at 33°C, slices were transferred to a holding chamber with the same extracellular buffer at room temperature (RT: ~25°C).

7. Brain slices were visualized by an upright microscope (BX61WI, Olympus) through a 40× water-immersion objective equipped with infrared-differential interference contrast optics in combination with a digital camera (ODA-IR2000WCTRL).

8. Patch-clamp recordings were performed by using an EPC 10 patch-clamp amplifier, controlled by Patchmaster Software (HEKA). Data were acquired at a sampling rate of 50 kHz and low-pass filtered at 6 kHz.

9. To measure mEPSCs, the internal solution contained the following (in mM): 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 and osmolality set to ~ 300 mOsmol.

10. mEPSCs were measured in the aCSF bath solution containing 1 µM tetrodotoxin and 50 µM Picrotoxin at -70 mV in voltage-clamp mode.

11. To measure mIPSCs, the internal solution contained the following (in mM): 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 and osmolality set to ~ 300 mOsmol.

12. mIPSCs were measured in the aCSF bath solution containing 1 µM tetrodotoxin and 10 µM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and 50 µM D-2-amino-5-phosphonopentanoate (D-AP5) at -70 mV in voltage-clamp mode.

13. mEPSCs and mIPSCs recorded at -70 mV were detected using Minhee Analysis software (https://github.com/parkgilbong/Minhee_Analysis_Pack).

14. To analyze the frequency, events were counted over 5 minutes of recording.

15. To obtain the average events for each cell, at least 100 non-overlapping events were detected and averaged. The peak amplitude of the average mEPSCs was measured relative to the baseline current.