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We use this protocol and it's working

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Ex vivo electrophysiology

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ABSTRACT

This protocol describes steps for ex vivo electrophysiology in mouse brain slices. This protocol also includes instructions for clozapine N-oxide (CNO) testing in DREADD-expressing neurons.

MATERIALS

- Isoflurane
- Rodent guillotine
- Vibratome (Campden Instruments, 7000smz-2)
- aCSF solution containing (in mM): 119 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 1.0 NaH_2PO_4, 2.5 $CaCl_2$, 26.2 $NaHCO_3$, and 11 glucose saturated with 95% O_2—5% CO_2
- 3 – 5 MOhm pipettes containing (in mM): 123 K-gluconate, 10 HEPES, 0.2 EGTA, 8 NaCl, 2 MgATP, and 0.3 Na3GTP, pH 7.2, osmolarity adjusted to 275. Biocytin (0.1%, Sigma) is included in the internal solution to identify neurons after recordings where desired.
- Axio Examiner A1 equipped with Dodt and IR optics
- Zeiss Axiocam 506 mono
- Neurolucida 2023 software
- Sutter IPA and SutterPatch v2.3.1 software (Sutter Instruments)
- Clozapine N-oxide (for DREADD-expressing neurons; Tocris)
- 4% formaldehyde in PBS

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Tissue Preparation

1 Deeply anesthetize the mouse with isofluorane, decapitate, and remove the brain.

2 Using a vibratome, cut 150mm horizontal slices containing the region of interest in ice-cold aCSF solution and allow to recover at 33˚ C in aCSF for at least one hour.

Recording

3 For fluorescent imaging, visualize slices under an Axio Examiner A1 equipped with Dodt and IR optics using a Zeiss Axiocam 506 mono and Neruolucida 2023 software.

4 Whole-cell patch-clamp recordings are made at 33˚ C using 3 – 5 MOhm pipettes. Recordings are made using Sutter IPA and SutterPatch v2.3.1 software (Sutter Instruments), filtered at 5 kHz and collected at 10 kHz.

4.1 For I_h: voltage clamp cells at -60mV and step to -40, -50, -70, -80, -90, -100, -110, and -120 mV. I_h magnitude is quantified as the difference between the initial steady-state response to the -120mV step and the asymptote of the slow current sag.

4.2 For spontaneous firing rates: record in current-clamp mode (*I*= 0 pA). Spontaneous firing rate is measured as the mean firing rate during the first 2 min of whole-cell recording.

4.3 Action potential (AP) waveform measurements are made from averages across at least 8 APs from the first 2 min of recording.

4.4 For input resistance: Apply a brief hyperpolarizing pulse once every 10 sec and average across the measurements made during the first 2 min of recording.

4.5 For CNO testing in DREADD-expressing neurons: spontaneous firing rate or resting membrane potential are monitored until a stable baseline is observed for at least 5 min. Then switch the perfusion solution to 1mM CNO for 5 min.

5 When recordings are complete, drop fix slices in 4% formaldehyde in PBS for at least 2 hr.

6 Complete statistical analyses in R, first testing whether data meet the criteria for parametric statistical evaluation.