Ex vivo mouse brain patch clamp recordings combined with uncaging and optogenetic stimulation

Forked from Ex vivo mouse brain patch clamp recordings and Fura-2 imaging

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We use this protocol and it's working

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ABSTRACT

In this protocol we detail the steps to perform ex-vivo brain slices electrophysiology and optogenetic stimulation and/or 2-photon uncaging recordings.

GUIDELINES

Please adhere to institutional guidelines and regulations.

MATERIALS

Solutions:

Internal solution:
These are prepared prior to the experiment day and aliquoted in 1.5 ml tubes, and stored at -20°C until the day of experiment.

Internal solution composition:

120 potassium-D-gluconate, 13 KCl, 10 HEPES, 0.05 EGTA, 4 ATP-Mg2, 0.5 GTP-Na, 10 phosphocreatine-di (tris); pH was adjusted to 7.25 with KOH and osmolarity to 275-280 mOsm
25 microM AlexaFluro 568 is added to the internal solution immediately before the experiment.

Recording solution

ACSF containing, in mM: 125 NaCl, 3 KCl, 1 MgCl2, 2 CaCl2, 25 NaHCO3, 1.25 NaH2PO4 and 10 glucose (saturated with 95% O2-5% CO2; pH 7.4; 300 mOsm/l. AP5 (50 µM), NBQX (5 µM), CGP-55845 (1 µM), MPEP (1 µM), and CPCCOEt (50 µM) are added to the recording solution for optogenetic/uncaging experiments.

Hardware and miscellaneous:

- 2PLSM optical workstation and computer with imaging softwares (see below)
- Targeted focal spot blue laser (473 nm Aurora laser launch, Prairie

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Technologies)

- Blood-gas mixture (95% O2, 5% CO2) tank connected to bubblers.
- Slice holder
- Brain slices expressing genetically encoded opsinsin holding chamber with aCSF
- Peristaltic pump or gravity flow perfusion with tubing and connectors, including inlet and outlet to microscope's imaging chamber
- Stage heating system with probe
- Waste solution collector
- 10% Ethanol in water (wash solution)
- Image analysis software

2PLSM optical workstation:

The laser scanning optical workstation embodies an Ultima dual-excitation-channel scan head (Bruker Nano Fluorescence
Microscopy Unit). The foundation of the system is the Olympus BX-51WIF upright microscope with a LUMPFL 60X/1.0NA
water-dipping objective lens. The automation of the XY stage motion, lens focus, and manipulator XYZ movement was
provided by FM-380 shifting stage, axial focus module for Olympus scopes, and manipulators (Luigs & Neumann). Cell
visualization and patching were made possible by a variable magnification changer, calibrated to 2x (100 µm FOV) as defined by the LSM bright-field transmission image, supporting a 1 Mpixel USB3.0 CMOS camera (DCC3240M; Thor Labs) with ~30%
quantum efficiency around 770 nm. Olympus NIR-1 bandpass filter, 770 nm/100 nm, and microManager software were used with the patch camera. The electrical signals were sent and collected with a 700B patch clamp amplifier and MultiClamp Commander software with computer input and output signals were controlled by Prairie View 5.3-5.5 using a National Instruments PCI6713 output card and PCI6052e input card.

The 2P excitation (2PE) imaging source was a Chameleon Ultra1 series tunable wavelength (690-1040 mm, 80 MHz, ~250 fs at sample) Ti: sapphire laser system (Coherent Laser Group); the excitation wavelength was selected based on the probe being imaged (see below). Each imaging laser output is shared (equal power to both sides) between two optical workstations on a single anti-vibration table (TMC). Workstation laser power attenuation was achieved with two Pockels' cell electro-optic modulators (models M350-80-02-BK and M350-50-02-BK, Con Optics) controlled by Prairie View 5.3–5.5 software. The two modulators were aligned in series to provide enhanced modulation range for fine control of the excitation dose (0.1% steps over five decades), to limit the sample maximum power, and to serve as a rapid shutter during line scan or time series acquisitions.

The 2PE generated fluorescence emission was collected by non–de-scanned photomultiplier tubes (PMTs). Green channel (490–560 nm) signals were detected by a Hamamatsu H7422P-40 select GaAsP PMT. Red channel (580–630 nm) signals were detected by a Hamamatsu R3982 side on PMT. Dodt-tube-based transmission detector with Hamamatsu R3982 side on PMT (Bruker Nano Fluorescence) allowed cell visualization during laser scanning. Scanning signals were sent and received by the NI PCI-6110 analog-to-digital converter card in the system computer (Bruker Nano Fluorescence).

SAFETY WARNINGS

Please follow institutional safety guidelines and chemical safety datasheets.

BEFORE START INSTRUCTIONS

Please note that optogenetic experiments require the expression of a genetically encoded opsin in neurons of interest. Mice genetically engineered to express opsin in specific neuronal populations are available. Alternatively, opsin expression can be obtained via injections of appropriate viral vectors.
Please refer to existing protocols for brain slices preparation and/or viral injections.

Prepare patch pipettes

1 Turn on the Sutter P-1000 puller and enter the desired pull protocol.

2 Insert a thick-walled borosilicate glass capillary and press pull.

3 Pipette resistance must be of 3 to 5 megaohms.

Setting up patch rig and environment

4 Turn on the MultiClamp 700B Amplifier, Axon Digidata 1550B digitizer, micromanipulator, computer tower and the associated software. Note: amplifier and digitizer must be turned on prior to opening software.

5 Turn on O2/CO2 tank and bubble aCSF solution.

6 Take an aliquot of internal solution from the -20° fridge.

7 Fill syringe with internal solution, place a filter on the end of the syringe, and place a MicroFil Pipette Filler on the end of the filter.

8 Start perfusing microscope chamber with aCSF (either with a gravity system or a peristaltic pump; a gravity system is normally preferred because of the lower electrical noise).

9 Adjust the flow rate to the desired value (recommended at least 2 mL/min).

10 Depending on the experimental protocol, turn on stage heater and set to temperature.

Examine slices and patching cells

11 Secure down slice with a harp (slice anchor).

12 Locate and focus the desired brain region under the 4x objective.

13 Change the microscope lens to the 60x objective.

14 Focus on healthy neurons in slices for patching.

15 For whole cell configuration, fill a glass micropipette one-third full of internal solution. Ensure there is no residual internal solution on exterior of glass micropipette, as this may introduce salts into the micromanipulator and add additional noise to recordings. Remove any air bubbles by gently flicking the glass micropipette.

16 Gently place the glass micropipette onto the electrode holder.

17 Position the electrode using a micromanipulator.

18 Under the 60x objective, bring the tip of the glass pipette above the slice.

19 Apply a positive pressure and maintain it. Zero the voltage offset.

20 Approach the cell diagonally. The positive pressure should create a small dimple on the cell.

21 Once a dimple is formed, release the positive pressure, and apply a small amount of negative pressure. The resistance should begin to increase rapidly.

22 As the resistance increases, clamp the cell at your resting potential of interest (typically - 60 mV).

23 After a giga-ohm seal is formed, apply a few quick pulses of negative pressure to break into the cell to record in whole cell configuration.

24 Start recording.

Recordings and uncaging/optogenetic stimulation

15m

25 Once in whole cell configuration, allow time (15-20 mins) for the internal solution containing the Alex 15m Fluor 568 to fill the cell so that the dendritic tree and individual spines will become visible under 2P imaging.

26 Change the setup configuration to 2-photon imaging, using PrairieView. Alexa Fluor 568 is excited with a 810 nm wavelength.

27 Experiments are performed on distal (>100 microns from soma) dendritic spines within planar sections (20 microns) of the same dendrite with zoom 4 and a field of view (POV) of 50 microns.

28 Electrophysiological recordings, 2-photon imaging, and stimulation are acquired and synchronized via the PrairieView software.

29 Single-photon optogenetic stimulation is obtained with a 473 nm laser, whose pulse number, intensity, and duration are controlled via PrairieView. We recommend starting with a single spot of 8 microns

diameter, 3ms duration.

30 For glutamate uncaging, DNI-glutamate (5 mM) is perfused via a microinjectior on the recorded area for x minutes before initiating the stimulation protocol. 1 ms pulses are applied in proximity of single spines located in the same focal plane. The laser power and the spot location should be calibrated to evoke a somatic excitatory postsynaptic potential of ~1-2mV.

31 For combined blue laser stimulation and glutamate uncaging, the 3ms optogenetic stimulation precedes the uncaging protocol (1ms delay), which normally includes the stimulation of 15 spines with 1ms pulses 1ms apart.

After the experiments

32 Gently remove and discard the recording pipette

33 Stop imaging protocols and close shutters to protect the imaging system.

34 Remove slice from the recording chamber. If desired, the slice can be gently transferred into a plate and bathed with a fixative solution for further processing.

34.1 Note: use appropriate PPE when working with fixatives, including operating under a chemical hood.

35 Discard waste according to institutional protocols.

36 Rinse perfusion tubing microscope chamber with water and/or a 10% ethanol in water.

37 Save and export files.