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

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Protocol status: Working
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 Fura-2 recordings. For both types of recordings, the brain slices with the desired nucleus must be placed into the recording chamber and patch clamp high resistance seal must be performed. For cell attached configuration recording, the sual must be kept unbroken. For whole cell configuration, quick pulses of negative pressure to break into the cell. For Tura-2 recordings, a whole cell patch clamp must be performed to allow the Fura-2 to enter the cell.

MATERIALS

Solutions to prepare:

Internal solutions:
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.

K-based internal solution for whole cell

Compound	M.W.	Conc (mM)
KMeSO4	150.20	126.00
EGTA	380.40	1.00
KCl	74.55	14.00
HEPES	238.30	10.00
Mg-ATP	551.10	2.00
Na3-GTP	523.20	0.50
CaCl2	110.98	0.50
MgCl2	203.32	3

Adjust pH to 7.3 with KOH

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Internal solution for Fura 2

Compound	M.W.	Conc (mM)
KMeSO4	150.20	135.00
EGTA	380.40	5.00
KCl	74.55	5.00
Na-PCr	255.10	10.00
HEPES	238.30	5.00
Mg-ATP	551.10	2.00
Na3-GTP	523.20	0.50
CaCl2	110.98	0.50

Adjust pH to 7.3 with KOH

Electrophysiology solutions:

These are prepared as 10´ stock solutions (below) and diluted for use on the day of the experiment.

Stock aCSF

Solution 10x **1L**

Compound	**MW**	**g**	**conc (mM)**
NaCl	58.44	79.33	1357.5
KCl	74.55	1.86	25
sodium bicarbonate	84.01	21	250
sodium phosphate	120	1.5	12.5

Physiological glucose

aCSF 1x **1L**

	A	B	C	D
	Compound	**MW**	**g**	**conc (mM)**
	NaCl	**diluted from 10x**		**125**
	KCl	**diluted from 10x**		**2.5**
	sodium bicarbonate	**diluted from 10x**		**25**
	sodium phosphate	**diluted from 10x**		**1.25**
	CaCl2	**1M solution**	2ml	**2**
	MgCl2	**1M solution**	1ml	**1**
	Glucose*		0.63	**3.5**

* add glucose to working solution (do not include in 10x stock)

High glucose aCSF
Solution 1x **1L**

	A	B	C	D
	Compound	**MW**	**g**	**conc (mM)**
	NaCl	**diluted from 10x**		**135.75**
	KCl	**diluted from 10x**		**2.5**
	sodium bicarbonate	**diluted from 10x**		**25**
	sodium phosphate	**diluted from 10x**		**1.25**
	CaCl2	**1M solution**	2ml	**2**
	MgCl2	**1M solution**	1ml	**1**
	Glucose*		4.5	**25**

* add glucose to working solution (do not include in 10x stock)

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 2.5 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 (or aCSF for cell attached recordings), place a filter on the end of the syringe, and place a MicroFil Pipette Filler on the end of the filter.

8 Turn on the pump and circulate recording aCSF solution through chamber.

9 Adjust and set the rate of the pump to 3-4 mL/min.

10 Turn on water heater and set to desired temperature (~ 34 °C)

Examine slices and patching cells

11 Transfer brain slice from incubation beaker to the recording chamber.

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

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

14 Change the microscope lens to the 60x objective.

15 Slowly focus on healthy neurons in slices for patching.

16 For whole cell configuration, fill a glass micropipette one-third full of internal solution. For cell attached configuration fill it with aCSF. 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.

17 Gently place the glass micropipette onto the wire electrode and tighten.

18 Position the electrode using a micromanipulator.

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

20 Apply a positive pressure and maintain it.

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

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

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

24 After a giga-ohm seal is formed, leave it this way to record in cell attached configuration or apply a few quick pulses of negative pressure to break into the cell to record in whole cell configuration.

25 Start recording.

Fura 2 imaging

26 Transfer slices to a 2PLSM and turn on the 780-nm wavelength laser.

27 Add Fura-2 (100 uM) and Alexa Fluor 594 hydrazide (25 uM) to the internal solution.

28 Fill the patch pipettes with the internal solution.

29 Perform a whole cell patch clamp recording as previously explained.

30 Wait 10 minutes until the cell is completely filled with the internal solution containing Fura-2.

31 Choose a dendrite from the patched neuron 20-100 um far from soma.

32 Draw a strait line ROI over the chosen dendrite.

33 Start line scan acquisitions with 0.195-m pixels and 12-s dwell time.