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Ex vivo electrophysiology

In 1 collection

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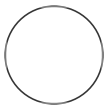

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ABSTRACT

This protocol describes the steps to perform whole-cell electrophysiology recordings in acute brain slices.

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86549

Keywords: ASAPCRN

Set up

1 Rig set-up.

1.1 Turn on required devices (computer, amplifier, manipulators, light sources, video camara controls, etc.)

1.2 Open the N_2 tank connected to the rig anti-vibration table.

1.3 Set 1X ACSF (add drugs needed for particular experiments) in a jug or bottle and bubble it with O_2/CO_2.

1.4 Place the intake line into the ACSF container and allow circulation. Wait until the fluid has entered the recording chamber, then turn on the in-line heater (Warner Instruments) and set it to desired temperature 32-34 °C.

2 Recording electrodes or patch pipettes:

2.1 Pull patch pipettes (1.5 – 2 MΩ) (World Precision Instruments) using an electrode puller (Narishige, PC-10).

2.2 Thaw an aliquot of the appropriate internal solution (stored at -80 °C (see 'Solutions' section below) (add ATP, GTP and Phosphocreatine if solution does not already have it). Fill a syringe with filter with the internal solution and keep on ice by the rig.

3 Electrical stimulation electrodes:

3.1 Pull electrodes (World Precision Instruments) using a puller (Narishige, PC-10).

3.2 Fill electrodes with 1X ACSF using a syringe with filter.

Whole-Cell recordings

5m

4 Transfer brain slice from incubation vial to the recording chamber and secure down the slice using a harp.

(The protocol for obtaining acute brain slices is linked below).

Protocol

NAME

Acute Brain Slices

CREATED BY

kelsey.barcomb

PREVIEW

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

6 Change the microscope lens to the 40x or 60x objective focus on healthy neurons selected for patching.

7 Fill a pipette tip with internal solution. Remove any air bubbles by gently flicking the glass pipette.

8 Place the pipette onto the wire electrode on the holder and tighten.

9 Apply a positive pressure and maintain it by quickly closing the stopcock.

10 Position the electrode using a micromanipulator.

11 Under the 40x-60x immersion objective, bring the tip of the pipette above the slice.

12 Approach the cell until the positive pressure create a small dimple.

13 Zero the pipette offset, release the positive pressure, and apply a small amount of negative pressure. The resistance should start to increase rapidly until a giga-Ohm seal is formed.

14 Clamp the cell at your resting potential of interest (typically -60 mV).

15 Apply a few quick pulses of negative pressure to break into the cell and reach the whole-cell configuration.

16 Wait 00:05:00 to allow the internal solution to dialyze the cell before start your recordings. 5m
Cells were discarded if series resistance was ≥15 MΩ.
Reordings can be made in voltage-clamp or current-clamp modes and the acquisition protocol will differ depending on the experiment.

16.1 Electrical stimulation: position the monopolar glass stimulating electrode filled with ACSF consistently 200 µm away from the recorded cell. Select the appropriate number of pulses, intensity and duration of electrical stimulation for the experiment.

16.2 Optogenetic stimulation: deliver light pulses using using a 478 nm LED. Select the appropriate number of pulses, intensity and duration of light stimulation for the experiment.

17 Once finished the recording of a particular cell, set the holding potential to 0 mV and remove the electrode. Discard the electrode in sharps container.

Solutions

18 Internal solutions:

- K-Gluconate + 10 mM BAPTA

	A	B	C
	Drug	[mM]	g/100 mL
	D-Gluconic Acid (K)	135	3.16
	HEPES (K)	10	0.28
	CaCl2	0.1	10 µL (1M stock)

	A	B	C
	MgCl2	2	200 µL (1M stock)
	BAPTA-tetra potassium	10	0.628

+ [M] 1 mg/mL ATP , [M] 0.1 mg/mL GTP and [M] 1.5 mg/mL phosphocreatine
pH=7.35, 275 mOsm

- K-Gluconate + 0.1 mM EGTA

	A	B	C
	Drug	[mM]	g/100 mL
	D-Gluconic Acid (K)	135	3.16
	HEPES (K)	10	0.28
	CaCl2	0.1	10 µL (1M stock)
	MgCl2	2	200 µL (1M stock)
	EGTA	0.1	0.0038

+ [M] 1 mg/mL ATP , [M] 0.1 mg/mL GTP and [M] 1.5 mg/mL phosphocreatine
pH=7.35, 275 mOsm

19 External solutions:
1X ACSF

For 1L:

- 900 mL MilliQH2O + 100 mL stock 10X ACSF + 1.8 g NaHCO3 + 2 g D-Glucose

10X ACSF stock

	A	B	C
	Drug	[mM]	10X Stock (g/4L)
	NaCl	126	294.52
	KCl	2.5	7.44
	MgCl2*6H2O	1.2	9.75
	NaH2PO4*H2O	1.2	6.64
	CaCl2*2H2O	2.5	14.7

	A	B	C
	NaHCO3	21.4	
	D-Glucose	11.1	