JUN 06, 2023

DOI:
dx.doi.org/10.17504/protocols.io.eq2ly7m2rlx9/v1

Protocol Citation: Hong-Yuan Chu 2023. Brain slice physiology and optogenetics. **protocols.io** https://dx.doi.org/10.17504/protocols.io.eq2ly7m2rlx9/v1

Protocol status: Working
We use this protocol and it's working

Created: Jun 01, 2023

Last Modified: Jun 06, 2023

PROTOCOL integer ID: 82723

Keywords: Brain slice physiology, Examine slices and patching cells , Optogenetics

Brain slice physiology and optogenetics

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ABSTRACT

This protocol describes Chu Lab standard external solutions for electrophysiology.

ATTACHMENTS

726-1837.docx

MATERIALS

Solutions to prepare:

Electrophysiology solutions are prepared as 10× stock solutions (below) and diluted for use on the day of the experiment. Bicarbonate is added to all working solutions.

A	B	C	D
Bicarbonate			
	mM	**MW**	**10×, g/L**
$NaHCO_3$	26	84.01	**21.8426**

A	B	C	D
Synthetic Interstitial Fluid (SIF) (recording solution)			
	mM	**MW**	**10×, g/L**
NaCl	126	58.44	**73.6344**
KCl	3	74.55	**2.2365**
$NaH_2PO_4.H_2O$	1.25	137.99	**1.7249**
$CaCl_2.2H_2O$	1.6	147.02	**2.3523**
$MgSO_4.7H_2O$	1.5	246.48	**3.6972**
D-Glucose	10	180.16	**18.0160**

Brain slice physiology: Setting up patch rig and environment

1 Turn on the MultiClamp 700B Amplifier, Axon Digidata 1550B digitizer, PatchStar Micromanipulator, PatchStar Slicescope, computer tower and the associated software.

Note

***Note**: amplifier and digitizer must be turned on prior to opening software.*

2 Turn on O_2/CO_2 tank and bubble SIF solution.

3 Take an aliquot of internal solution, ATP, and GTP from -20 °C freezer and thaw On ice.

4 Once thawed, add ATP and GTP (20 µL) to aliquot of internal solution and mix well with a pipette.

5 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.

6 Turn on Peri-Star Pro pump and circulate recording solution through chamber.

7 Adjust and set the rate of Peri-star pump to 3-4 mL /min.

8 Turn on water heater and set to desired temperature (~ 33-34 °C).

Brain slice physiology: Examine slices and patching cells

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

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

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

12 Change the microscope lens to the 60x objective.

Note

- Change slowly to ensure there is enough clearance and the lens does not get damaged.
- If there is not enough clearance, move back to the 4x and raise the objective up, and then try again.

13 Slowly focus on healthy neurons in slices for patching.

14 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.

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

16 Apply a positive pressure and maintain it.

17 Position the electrode using a micromanipulator.

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

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

20 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.

21 As the resistance increases, clamp the cell at your resting potential of interest (typically -70 mV).

22 Once gigaseal formed, you can perform fast/slow capacitance compensation.

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

24 Once whole cell configuration is formed, wait for 00:05:00 before the start of recording. 5m

Brain slice physiology: Optogenetics

25 Light pulses (1 ms duration) for optogenetic stimulation were delivered using a 478 nm LED through a 60x water immersion objective lens.

Note

Note: depending on the experimental design, synaptic blockers may be needed to isolate glutamatergic or GABAergic currents. Similarly, TTX and 4-AP can be applied to isolate monosynaptic responses from microcircuits with intensive local excitatory connections.

26 Under voltage clamp mode, adjust the intensity of LED to evoke synaptic currents of different amplitude. Repeat 3-5 sweeps per light intensity.

27 Similar procedures can be performed to evoke optogenetics-induced action potentials under current clamp mode.