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Electrophysiological recording from Brain Slices Protocol

In 1 collection

HaiDun[1]

[1]Duke University

ASAP Collaborative Research Network

Sasha Burwell
Duke University

ABSTRACT

This protocol details the steps for performing slice electrophysiological recordings with DART.

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MATERIALS

Glass recording pipette

Borosilicate glass with filament **Sutter Instruments Catalog #**BF150-110-10

Sutter Borosilicate Glass 1.5mm OD x .86mm x 10 cm with Filament **Sutter Instruments Catalog #**Su-BF150-86-10

Microfil (World Precision Instrument, Inc. 28GAUGE/97 MM, MF28G-5) **World Precision Instruments**

Solutions:

1. High sucrose cutting solution (1L)

	A	B
	Sucrose	220 mM
	KCl	3 mM
	NaH2PO4	1.25 mM
	NaHCO3	25 mM
	MgSO4.7H2O	12 mM
	D-glucose	10 mM
	CaCl2	0.2 mM
	L-Ascorbic acid (For VTA slice)	0.5 mM

Keep at 4 °C

2. aCSF (artificial cerebrospinal fluid) solution

	NaCl	120 mM
	KCl	3.3 mM
	NaH2PO4	1.23 mM
	MgSO4	1 mM
	CaCl2	2 mM
	NaHCO3	25 mM
	D-glucose	10 mM

a. First make 2 L of 10X aCSF solution, store at 4 °C to use.

	A	B	C	D	E
	aCSF				
		mM	FW	g/1 L(10X)	g / 2L (10X)
	NaCl	120	58.44	70.12	140.25
	KCl	3.3	74.55	2.46	4.92
	NaH2PO4.H2O	1.23	138	1.69	3.395
	(or NaH2PO4)		120	1.476	2.952
	NaHCO3	25	84.01	21	42

	A	B	C	D
	Stock solution			g/100 ml
	CaCl2.2H2O,	1M	147.12	14.71
	MgSO4.7H2O,	1M	246.48	24.65

	A	B	C	D
	Glucose	10 mM	180.1	1.8g/1L, 3.6g/2L

b. Experiment day, make 2 L of 1X aCSF solution

	A	B
	10X aCSF solution	200 mL
	1M MgSO4.7H2O stock solution	2 ml
	1M CaCl2.2H2O stock solution	4 ml
	D-glucose	3.6 g
	Add Milli-Q H2O up to 2 L, then saturated with 95% O2 and 5% CO2 at least for 15 min.	

3. Intracellular recording solution

For EPSC recording

	A	B	C	D
	Internal solution	mM	FW	g/50 ml

	A	B	C	D
	Cesium Mathanesulfonic acid	130 mM	228	1.48
	MgCl2	1		1M, 0.05 ml
	EGTA	0.5	380	0.0095
	HEPES	10	238.31	0.119
	Mg-ATP	4	507.2	0.101
	GTP-Na solte	0.5	523.2	0.01308
	Na2-phosphocreatine	10	255.1	0.1275
	QX314(Lidocaine N-ethyl Bromide)	4	343.3	0.0687
		pH 7.3 with CsOH		Osm 290

For IPSC recording

	A	B	C	D
	Internal solution	mM	FW	g/50 ml
	CsCl	135 mM	168.4	1.137
	MgCl2	2	203.31	1M, 0.1 ml
	EGTA	0.5	380	0.0095
	HEPES	10	238.31	0.119
	Mg-ATP	4	507.2	0.101
	GTP-Na solte	0.5	523.2	0.01308
	Na2-phosphocreatine	10	255.1	0.1275
	QX314(Lidocaine N-ethyl Bromide)	4	343.3	0.0687
			pH 7.3 with CsOH	Osm 290

Current clamp recording

	A	B	C	D
		mM	FW	g/50 ml
	K-gluconate (D-gluconic acid, Potassium salt)	130	234.2	1.5223
	NaCl	5	58.44	0.0146
	MgCl2	2	203.31	(1M, 0.1 ml)

	A	B	C	D
	EGTA	0.2	380.3	0.0038
	Hepes	10	238.3	0.11915
	Mg-ATP	4	507.2	0.10144
	NaGTP	0.5	523.18	0.01308
	Phosphocreatine (Creatin phosphate) Na	10		0.12755
		pH 7.3 with KOH		Osm 290

For all intracellular solutions, filter with syringe filter (0.2 µm diameter). Prepare intracellular recording solution aliquots of 1 ml (each tube) into 1.5 ml Eppendorf tubes to make 49-50 tubes. Store at -20 ^{o}C until needed.

Brain slice preparation

1h 35m

1 Take 150 mL - 200 mL high sucrose solution cutting solution, store at -80 °C for 00:15:00 - 00:20:00 to make chilled half ice solution. 35m

2 Saturate with carbogen (95% O_2/5% CO_2) for > 00:15:00 . 15m

3 Set up Vibratome (Leica VT 1200s) chamber and adjust the cutting speed 0.3, amplitude 0.7.

4 Deeply anesthetize the mouse with isoflurane, and then decapitate.

5 Take brain and place the brain into pre-chilled high sucrose cutting half ice solution. For VTA sections trim the brain by cutting off the cerebellum, which provides a flat surface to mount the brain with, and a small

part of the prefrontal cortex.

6 Mount the brain onto the Vibratome specimen disc using superglue, orienting the sample such that the cortex faces the razor blade. Add a supporting piece of agar (3-4%) behind the brain, away from the side of the Vibratome, to provide structural support during the slicing.

7 Use the Up-rocker button to move the buffer tray and brain to a position where the exposed surface is just below the razor blade edge and press start to begin the brain slicing (300 undetermined VTA, coronal section). Using a transfer pipette, transfer each individual brain slice containing the region of interest to a holding chamber pre-filled and with aCSF (saturated with carbogen).

8 Incubate the slices for 01:00:00 at 33 °C for 00:50:00 - 01:00:00 in the carbogen 2h 50m bubbled aCSF solution and then allowed to cool to Room temperature (22 °C - 24 °C) until the recordings initiate.

Electrophysiology recording

9 Fill up a bottle with aCSF solution bubble with carbogen and adjust the flow rate of aCSF solution to approximately 2 mL /min.

10 Place brain slice into recording chamber using small brush or transfer pipette.

11 Fill the glass recording pipette with intracellular solution using Microfil and filter again with syringe filter (0.2 µm), making sure the solution is all the way down at the tip of the pipette (no air).

12 Attach the pipettes to the electrode holders of the patch-clamp amplifier headstages and turn into position.

13 Using fine control micromanipulators, descend the recording pipettes to the region of interest within the brain slice.

14 If required, use a coarse-manipulator to position an appropriate stimulating electrode again to the appropriate region of the brain slice to stimulate inputs to recorded neurons (from soma 60 undetermined - 100 undetermined distance).

15 Once pipette is in contact with a neuron within the brain slice, apply negative pressure to pipette via 1 ml or 2 ml syringe. Monitor resistance of seal formation on oscilloscope or computer.

16 Once seal resistance has exceeded 1 GΩ, using amplifier and computer software to compensate transients and apply further negative pressure to rupture cell membrane gaining whole-cell access to neuron.

17 Perform current-voltage relationship using computer-controlled software to access neuronal health and to assess for presence of active membrane conductance.

18 Once happy with quality of recording, perform set experiment applying test compounds (DART, DNQX 20 µM, AP-V 50 µM, etc) via connected in-tube line with the aCSF flow.