Organotypic Hippocampal Slice Cultures

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Keywords: (maximum 10)

Hippocampus, Hippocampal formation, Brain Slices, Organotypic Cultures, Synaptic Transmission, Synaptic Physiology

Short Abstract: (50 words maximum)

We describe a method to prepare organotypic hippocampal slices that can be easily adapted to other brain regions. Brain slices are laid on porous membranes and culture media is allowed to form an interface. This method preserves the gross architecture of the hippocampus for up to 2 weeks in culture.

Long Abstract: (400 words maximum)

The hippocampus, a component of the limbic system, plays important roles in long-term memory and spatial navigation [1]. Hippocampal neurons can modify the strength of their connections after brief periods of strong activation. This phenomenon, known as long-term potentiation (LTP) can last for hours or days and has become the best candidate mechanism for learning and memory [2]. In addition, the well defined anatomy and connectivity of the hippocampus [3] has made it a classical model system to study synaptic transmission and synaptic plasticity[4].

As our understanding of the physiology of hippocampal synapses grew and molecular players became identified, a need to manipulate synaptic proteins became imperative. Organotypic hippocampal cultures offer the possibility for easy gene manipulation and precise pharmacological intervention but maintain synaptic organization that is critical to understanding synapse function in a more naturalistic context than routine culture dissociated neurons methods.

Here we present a method to prepare and culture hippocampal slices that can be easily adapted to other brain regions. This method allows easy access to the slices for genetic manipulation using different approaches like viral infection [5,6] or biolistics [7]. In addition, slices can be easily recovered for biochemical assays [8], or transferred to microscopes for imaging [9] or electrophysiological experiments [10].

Protocol Text:

1) Before starting the preparation of hippocampal slices.

1.1) Prepare the tissue slicer by placing a piece of Teflon sheet and mounting a new blade.

1.2) Wipe the tissue culture (TC) hood with 70% ethanol and set the dissecting microscope inside. Sterilize the hood, microscope, tissue slicer and all dissecting instruments for 15 minutes with UV light.

1.3) Prepare six well TC plates. Add 750 µl slice culture media (SCM) per well and place cell culture inserts in each well. Make sure the membranes are thoroughly wet with no bubbles underneath. Place the plates in incubator at 35°C gassed with 5% CO_2 until needed.

1.4) Pour 50 ml low Na^+ ACSF (dissecting solution) into a 100ml beaker and place it on ice-salt mix. Bubble the low Na^+ ACSF with 5% CO_2 / 95% O_2 until color changes and ACSF forms a slurry mix of frozen and liquid solution (10-20 min).

1.5) Get a P5-P7 rat pup. Up to three pups can be used.

2) Hippocampal slices preparation.

2.1) Cut the head of the animal with sharp utility scissors. Cut the skin and expose the skull. Open the skull by cutting from side to side along the interaural line and then along the sagittal suture with small scissors. An optional cut from side to side in the front can be made to facilitate removing the bones and exposure of the brain. Scoop out the brain quickly with a rounded spoon micro spatula and place it in the slurry of dissecting solution to chill for ~ 1 minute. Pour ~10 ml of ice cold dissecting solution onto a 60 mm dish and transfer the brain from the beaker to the dish. The brain should be covered with dissecting solution.

2.2) Under the dissecting microscope: Place the brain and hold it at the midline with the dissecting forceps pressed to the bottom of the 60 mm dish. Use the hippocampus dissecting tool to separate the hemispheres leaving out the midbrain. The hippocampi are then exposed on each hemisphere. Then gently scoop the hippocampus out with the hippocampus dissecting tool. Use the dissecting needle to completely isolate the hippocampus and clean it as much as possible.

2.3) Using a snipped tip of a P1000 filter pipette tip, gently aspirate the hippocampus and transfer it to the Teflon sheet on the tissue slicer. Position the hippocampus on its concave side.

2.4) Align the hippocampi perpendicular to the blade to obtain coronal sections and drain excess of liquid.

2.5) Slice the hippocampi every 400 µm.

2.6) Pour ~10 ml cold SCM into a 60 mm dish and transfer sliced hippocampi from the slicer using another snipped P1000 filter tip and cold SCM. Avoid making bubbles.

2.7) With the help of an iris spatula and a straight spatula gently separate the slices from each other.

2.8) Separate well defined and undamaged slices from damaged slices.

3) Hippocampal slices culture

3.1) Bring the six-well plates with SCM and cell culture inserts from the incubator. With the help of another snipped P1000 filter tip, transfer individual slices onto the membrane. Place 4-5 slices per membrane. Be careful not to place the slices either close to the insert wall or close to each other. When necessary, use iris spatula to separate slices. Remove excess medium. Touch slices as little as possible once they are on the membrane.

3.2) Move plate back to incubator and culture at 35ºC and 5% CO_2.

3.3) Change SCM every 48-72 hours inside the TC hood by aspirating the SCM with a Pasteur pipette. Add 750 µl of fresh SCM. Make sure no bubbles are formed under the membrane.

4) Solutions

4.1) Low Na^+ ACSF - Dissecting Solution for slice cultures

To deionized and sterile H_2O add:

	For 500 ml	**For 1000 ml**	**Final Concentration**
$CaCl_2$ (1 M)	0.5 ml	1 ml	1 mM
D-Glucose	0.901 g	1.802 g	10 mM
KCl	0.149 g	0.298 g	4 mM
$MgCl_2$ (1 M)	2.5 ml	5 ml	5 mM
$NaHCO_3$	1.092 g	2.184 g	26 mM
Sucrose	40 g	80 g	234 mM
Phenol Red Solution 0.5% in DPBS	0.5 ml	1 ml	0.1% v/v

Mix ~30 min

Sterilize by passage through 0.22µm filter

Make 50 ml aliquots and store at 4 ºC no longer than 2 months.

4.2) Slice Culture Medium (SCM)

	For 500 ml	For 1000 ml	Final Concentration
MEM Eagle medium	4.2 g	8.4 g	8.4 g/l
Horse serum heat inactivated	100 ml	200 ml	20%
L-Glutamine (200 mM)	2.5 ml	5 ml	1 mM
$CaCl_2$ (1 M)	0.5 ml	1 ml	1 mM
$MgSO_4$ (1 M)	1 ml	2 ml	2 mM
Insulin (1 mg/ml), dissolved in HCl 0.01 N	0.5 ml	1 ml	1 mg/l
Ascorbic Acid, solution (25%)	0.024 ml	0.048 ml	
D-Glucose	1.16g	2.32g	13 mM
$NaHCO_3$	0.22g	0.44g	5.2 mM
Hepes	3.58g	7.16g	30 mM

Mix until thoroughly dissolved and bring to room temperature.

Adjust pH to 7.27-7.28 with 1 N NaOH

Measure osmolarity. Adjust to 320 mmol/kg with deionized and sterilized H_20. Expect to add approximately 25-40 ml. Check osmolarity again. .

***pH may change slightly while adjusting osmolarity, this is ok, it is more important that the osmolarity is in the correct range (317-323).

Sterilize by passage through 0.22 µm filter.

Make 20 ml aliquots and store for up to two-three weeks at 4ºC.

Representative Results:

Slices should look white under a dissecting scope without black spots and well defined and undamaged CA1, CA3, and Dentate gyrus regions. Bacterial contamination is easily seen as moving black specks in the medium or turbidity of the SCM. When placed under the microscope, the surface of the slice should look clean after 4 days in culture with clear and discernibly cell bodies. If no clear cell bodies are seen and much debris covers the surface after 4 days, then is not a healthy slice.

Discussion:

This method is based on the method first described by Stoppini et al. [11] and offers a rapid manner to culture hippocampal slices. The most important aspect of this protocol is to maintain slices sterile; therefore it is critical to use appropriate sterile techniques and to properly disinfect and sterilize all the material in contact with the tissue.

Placing the slices on a porous membrane warranties proper oxygenation and nutrition via a thin layer of SCM that is formed by capillarity. This method can be adapted to other brain regions providing that the density of the tissue allows proper oxygenation and nutrient penetration. Thus, tissue density limits this method to young tissue. For hippocampus, slices 300-400 μm thick from p6-p7 animals seem to give best results. This type of slices can rapidly be obtained with a tissue slicer diminishing the time the tissue is exposed to the air.

Importantly for those studying synaptic physiology, after a few days in culture, all the debris from dead cells has been removed, leaving a clean surface highly suitable for electrophysiological or imaging experiments. In addition, organotypic hippocampal slices continue developing normal connectivity comparable to acute slices [12]. However after 2 weeks in culture this normal connectivity disappears as neurons start forming too many connections that increases synaptic activity in the slice.

Acknowledgments: This work was funded by NINDS – NIH R01NS060756

Disclosures: I have nothing to disclose.

Table of specific reagents and equipment:

Tools	Company	Cat. Number
Cell culture inserts	Millipore	PICM03050
6 well plates	BD Falcon	353046
Tissue Slicer	Stoelting	51425/51415
Microscope	Olympus	SZX7-ILLD2-100
Hippocampus dissecting tool	F.S.T	10099-15
Large utility scissors. Perfection	F.S.T	37500-00/37000-00 Right/ Left handed
Iris Spatula	F.S.T	10093-13
Straight spatula	F.S.T	10094-13
Rounded spoon micro spatula	VWR	57949-039
Dissecting single cutting edge needle	Electron Microscopy Science	72946
Dissecting tweezers	Dummont	#2
Small dissecting scissors	F.S.T	14060-10
Teflon sheets	McMaster	8545K31

Reagents	Company	Cat. Number
MEM Eagle medium	Cellgro	50-019 PB
Horse serum heat inactivated	Invitrogen	26050-88
L-Glutamine (200 mM)	Invitrogen	25030081
$CaCl_2$ (1 M)	Sigma	C3881
$MgSO_4$ (1 M)	Sigma	M2773
Insulin (1 mg/ml), dissolved in HCl 0.01 N	Sigma	I0516

Ascorbic Acid, solution (25%)	Sigma	A4544
D-Glucose	Sigma	G5767
$NaHCO_3$	Sigma	S6014
Hepes	Sigma	H7523
Sucrose	Sigma	S5016
Phenol Red Solution 0.5% in DPBS	Sigma	P0290
KCl	Sigma	P3911
$MgCl_2$ (1 M)	Sigma	M9272

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