Video Article

Organotypic Hippocampal Slice Cultures

Ximena Opitz-Araya[1], Andres Barria[1]

[1]Department of Physiology and Biophysics, University of Washington School of Medicine

Correspondence to: Andres Barria at barria@u.washington.edu

URL: https://www.jove.com/video/2462
DOI: doi:10.3791/2462

Keywords: Neuroscience, Issue 48, Hippocampus, Hippocampal formation, Brain Slices, Organotypic Cultures, Synaptic Transmission, Synaptic Physiology

Date Published: 2/3/2011

Citation: Opitz-Araya, X., Barria, A. Organotypic Hippocampal Slice Cultures. *J. Vis. Exp.* (48), e2462, doi:10.3791/2462 (2011).

Abstract

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].

Video Link

The video component of this article can be found at https://www.jove.com/video/2462/

Protocol

1. Before Starting the Preparation of Hippocampal Slices.

1. Prepare the tissue slicer by placing a piece of Teflon sheet and mounting a new blade.
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.
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.
4. Pour 50 mL low Na^+ ACSF (dissecting solution) into a 100 mL 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).
5. Get a P5-P7 rat pup. Up to three pups can be used.

2. Hippocampal Slices Preparation.

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. 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.
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.
4. Align the hippocampi perpendicular to the blade to obtain coronal sections and drain excess of liquid.
5. Slice the hippocampi every 400 µm.

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.
7. With the help of an iris spatula and a straight spatula gently separate the slices from each other.
8. Separate well defined and undamaged slices from damaged slices.

3. Hippocampal Slices Culture

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.
2. Move plate back to incubator and culture at 35°C and 5% CO_2.
3. Change SCM every 48 hours inside the TC hood by aspirating the SCM with a Pasteur pipette. Add 750 µL of fresh pre-warmed SCM per well. Make sure no bubbles are formed under the membrane.

4. Solutions

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 °C no longer than 2 months.
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% w/v)	0.024 mL	0.048 mL	0.00125%
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_2O. 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 4°C.
   Gluatamine stock: prepare at a concentration of 200 mM and store at -20 °C in aliquots of 2.5 mL.
   Ascorbic acid stock: prepare at a concentration of 25% (w/v) and stored at -20 °C in aliquots of 100 µL.

5. 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.

Different serums sources can influence the quality of the slices. We recommend testing several batches first. If contamination is a recurrent problem, check incubator and tissue culture hood for possible sources of contamination. Proper use of sterile techniques during the whole procedure is essential.

The total time from decapitation to placing the slices on the membrane and in the incubator should be no longer than 1.5 hours. If the procedure takes too long, it will compromise the health of the slices.

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.

Disclosures

No conflicts of interest declared.

Acknowledgements

This work was funded by NINDS - NIH R01NS060756

References

1. Martin, S. J., Grimwood, P. D. & Morris, R. G. Synaptic plasticity and memory: an evaluation of the hypothesis. Annu Rev Neurosci 23, 649-711 (2000).
2. Bliss, T. V., Collingridge, G. L. & Morris, R. G. Introduction. Long-term potentiation and structure of the issue. Philos Trans R Soc Lond B Biol Sci 358, 607-611 (2003).
3. Shepherd, G. M. The synaptic organization of the brain. 5th edn, (Oxford University Press, 2004).
4. Malinow, R. & Tsien, R. W. Presynaptic enhancement shown by whole-cell recordings of long-term potentiation in hippocampal slices. Nature 346, 177-180 (1990).
5. Malinow, R. *et al.* Introduction of green fluorescent protein (GFP) into hippocampal neurons through viral infection. Cold Spring Harb Protoc 2010 (2010).
6. Shi, S., Hayashi, Y., Esteban, J. A. & Malinow, R. Subunit-specific rules governing AMPA receptor trafficking to synapses in hippocampal pyramidal neurons. Cell 105, 331-343 (2001).
7. Woods, G. & Zito, K. Preparation of gene gun bullets and biolistic transfection of neurons in slice culture. J Vis Exp (2008).
8. Esteban, J. A. *et al.* PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity. Nat Neurosci 6, 136-143 (2003).
9. Mainen, Z. F. *et al.* Two-photon imaging in living brain slices. Methods 18, 231-239, 181 (1999).
10. Barria, A. & Malinow, R. Subunit-specific NMDA receptor trafficking to synapses. Neuron 35, 345-353 (2002).
11. Stoppini, L., Buchs, P. A. & Muller, D. A simple method for organotypic cultures of nervous tissue. J Neurosci Methods 37, 173-182 (1991).
12. De Simoni, A., Griesinger, C. B. & Edwards, F. A. Development of rat CA1 neurones in acute versus organotypic slices: role of experience in synaptic morphology and activity. J Physiol 550, 135-147 (2003).

