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Protocol

Preparation of Slice Cultures from Rodent Hippocampus

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This protocol describes the preparation of hippocampal slice cultures from rat or mouse pups using sterile conditions that do not require the use of antibiotics or antimycotics. Combining very good optical and electrophysiological accessibility with a lifetime approaching that of the intact animal, many fundamental questions about synaptic plasticity and long-term dynamics of network connectivity can be addressed with this preparation.

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MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous material used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Animals (rat [P4–P6] or mouse [P5–P8] pups)
Appropriate anesthesia according to local guidelines
Dissection solution (sterile; 50 mL is sufficient for approximately 10 pups) <R>
Slice culture medium (sterile; 50 mL is sufficient for approximately 10 pups) <R>

Equipment

95% O_2/5% CO_2 regulator and tubing
Beaker (glass; 500-mL)
Cell Saver Tips (1000-µL) (BIOzym 693000)
Culture plates (six-well; e.g., Corning 3516 or Sarstedt 83.1839)
Dissection stereomicroscope with cold platform (e.g., ice-cold metal plate or glass Petri dish filled with ice, prewiped with 70% ethanol)
Filter paper or Whatman paper (sterile, 6-cm diameter; MACHEREY-NAGEL 431005)
Fine paintbrush (e.g., Ted Pella 11810/11812)
Hot bead sterilizer (e.g., Fine Science Tools 18000-45)
Incubator (37°C/5% CO_2 with rapid humidity recovery, copper chamber recommended; e.g., Heracell 150i/160i, Thermo Scientific)
Large shallow ice bucket containing ice scattered with NaCl

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Millicell Cell Culture Insert (30-mm, hydrophilic PTFEm [polytetrafluoroethylene], 0.4-µm; Millipore PICMORG50)
Paper towels (sterile)
Pasteur pipettes (glass, 9″, sterilized; e.g., Carl Roth 4518.1)
Pasteur pipettes (plastic disposable, sterile; e.g., Sarstedt 86.1171.010)
Pasteur pipettes with tips broken off (glass, sterilized)
Wear goggles and wrap pipettes before breaking to prevent injury.
Pipette bulbs (two, rubber, 2-mL; e.g., Sigma-Aldrich Z111597)
Pipettor (1000-µL; BIOzym 655070)
Razor blade (two-sided; Fine Science Tools 10050-00)
Small ice bucket containing ice scattered with NaCl
Spray bottle containing 70% ethanol
Stainless steel tray
Sterilized dissection tools
Bone curette (small, back thinned on sharpening stone; DO608R Lucas sharp spoon, Aesculap)
Coarse forceps (e.g., Fine Science Tools 11002-16)
Fine scissors (Carl Roth LT28.1)
Forceps (two, No. 5 Dumont [fine]; WPI 500342)
Large scissors (ET140R, Aesculap or Fine Science Tools)
Scalpel handle and blades (#3 and #10) (e.g., Fine Science Tools 10003-12/10010-00)
Small spatula or spoon
Tissue Chopper (McIlwain type 10180, Ted Pella)
Tissue culture dishes (60-mm, sterile; Sarstedt 83.1801)
Tissue culture hood
Tubes (sterile, 50-mL)

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METHOD

The preparation of slice cultures that are to be made and maintained without antibiotics requires stringent sterility be maintained at all times. Antibiotics affect a myriad of cellular properties (Amonn et al. 1978; Llobet et al. 2015); therefore, it is essential to avoid their use. Aseptic techniques must therefore be strictly observed at all steps and everything coming into contact with brain tissue must be sterile. For a culture to be maintained it must remain uncontaminated. Normally experiments should be performed within 6 wk of culture preparation, but slice cultures can be successfully maintained for more than a year.

Preparation

1. Put a 1-mL slice of culture medium in each well of the six-well plates. Using sterile forceps place one membrane insert into each well. Put plates into 37°C incubator.
   A mouse pup will yield approximately 10 to 15 slices, a rat pup approximately 15 to 20. If two slices are to be placed on each insert, then one six-well plate will hold 12 slice cultures.
2. Next to the tissue culture hood arrange the following.
   - A stainless steel tray with two 60-mm dishes per animal
     One dish should contain a sterile filter paper. Sit the tray in a shallow ice bucket containing ice sprinkled with NaCl. The dishes can be turned upside down and the lids used because the rims are lower.
   - Hot bead sterilizer and beaker filled with water for rinsing tools
   - Large scissors, fine scissors, coarse forceps, scalpel, and spatula arranged between sterile paper towels
   - Sterile paper towel for placing head
   - Container for body

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3. Inside the tissue culture hood arrange the following.
   - Tissue chopper set to cut 400-µm slices

     The blade must be cleaned of oil/wax before mounting.
   - Dissection stereomicroscope with ice-cold platform
   - Small bucket of NaCl-sprinkled ice holding a 50-mL tube containing dissection solution (~5 mL per animal), bubbled with 95% O_2/5% CO_2 through a sterile glass Pasteur pipette

     Place a sterile disposable pipette in the dissection solution. When the bubbled dissection solution has turned from magenta to red/orange in color it is ready to use.
   - 1000-µL pipettor with Cell Saver Tip
   - Sterile glass Pasteur pipette with bulb
   - Sterile transfer pipette, made by breaking the tip from a Pasteur pipette and mounting the bulb on the broken end
   - Scalpel, two fine forceps (#5 Dumont), bone curette, arranged on sterile paper towel
   - Sterile fine paint brush

4. Spray or wipe down the microscope, tissue chopper, ice bucket, and stainless steel tray with 70% ethanol. Spray the blade and stage (with mounted disks) of the tissue chopper and allow to air dry before beginning the procedure.

Dissection

Wear sterile gloves and a mask. Neurons are sensitive to ethanol, including fumes, so if using ethanol to sterilize surfaces, tools, etc., allow thorough drying to prevent tissue exposure.

5. Immediately before beginning the procedure, place 1–2 mL of the cold, bubbled dissection solution into one of the 60-mm dishes containing the filter paper (this can be done while pups are undergoing anesthesia). Place covered dish back on the chilled tray.
6. Anesthetize pups according to local regulations and decapitate using the large scissors.

   Do not put the head in the dissection solution as this will result in contamination of the slice cultures.
7. Place the head on a sterile paper towel and hold (without squeezing) between the fingers of one hand—it is important to note that this hand is now contaminated. With a scalpel, open the skin from the nose to the base of the skull without cutting through the skull. Pull back the skin with the fingers already holding the head, slide the scalpel under the skin on one side and cut downward through the ears, repeat on the other side, and the skin can now easily be held back leaving the skull exposed. Cut the skull with the fine scissors along the midline. Make small cuts at the most rostral end out toward the sides keeping the orientation of the scissors so that the blade closest to the brain is always the same. Put the scissors into the beaker of water. Take the coarse forceps and pull open/remove the skull. The flaps of skull can be held with the fingers holding the head if necessary. Again be sure to keep the orientation of the forceps constant to avoid contaminating the surface of the brain. Put the forceps into the beaker of water and remove the cover from the chilled 6-cm dish with filter paper. Take the spatula/spoon and slide under the brain flipping it out of the skull onto the chilled wetted filter paper.
8. Put the dish with the brain on an ice-cold platform under the dissection microscope in the hood. Drop some of the cold, bubbled dissection solution onto the brain. While the brain chills for ~1 min, quickly dry off the dissection tools and place the tips into the bead sterilizer for 10 sec to sterilize. Replace and/or clean contaminated glove(s) with 70% ethanol. Ensure gloves are dry before moving close to the tissue to avoid exposing the tissue to ethanol vapor.
9. Under the dissection microscope in the hood, dissect the hippocampi. Holding the cerebellum with one pair of the fine forceps, cut along the midline twice with the scalpel without severing the most caudal part of the hemispheres (Fig. 1A). Using the bone curette fold down one hemisphere, which will expose the hippocampus (Fig. 1B). Cut the ends of the hippocampus with the scalpel

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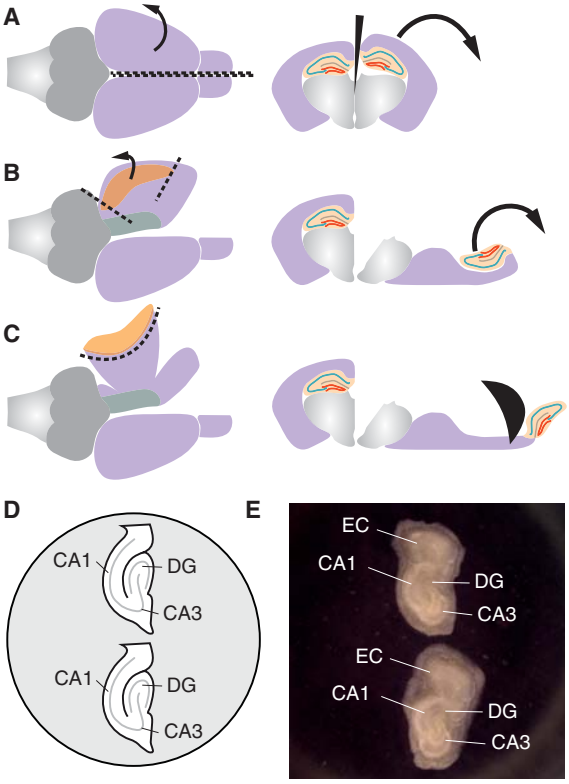

FIGURE 1. Dissecting the hippocampus. (*A*) Cut along the midline with a scalpel, using the cerebellum/brain stem as a handle. (*B*) Fold out the left hemisphere, exposing the hippocampus. Cut free the tips of the hippocampus. (*C*) Flip out the left hippocampus and cut through the entorhinal cortex to completely separate the left hippocampus from the brain. (*D*) Arrangement of slices on a cell culture insert for injection and electroporation. (*E*) Six-week-old Wistar rat hippocampal slice cultures. DG, dentate gyrus; EC entorhinal cortex; CAI and CA3, regions of the hippocampus proper.

and carefully flip out the hippocampus with the curette. Cut away most of the attached cortex (Fig. 1C). Repeat with the other hippocampus.

It is often helpful to pull away some of the meninges from the brain with the fine forceps before removing the hippocampi.

10. Transfer the hippocampi to the stage of the tissue chopper using the transfer pipette. Arrange the hippocampi perpendicular to the blade and remove all surrounding dissection solution with the glass Pasteur pipette. Cut 400-µm thick slices.

    The sliced hippocampus should remain on the platform. If the slices stick to the blade, there is probably too much dissection solution remaining on the stage.

11. Take the Teflon chopping disk off the stage and gently rinse the slices into one of the prechilled 60-mm dishes using sterile dissection solution. Quickly clean the chopping disk with 70% ethanol, wipe dry with a sterile paper towel, and replace. If necessary, gently tease apart any slices that are adhering to each other with fine forceps. Do not grasp the slices but slide the forceps between the slices. The slices should be cut through and come apart easily.

12. Transfer the undamaged slices showing nice morphology onto the prewarmed membrane inserts (from Step 1) using the Cell Saver Tip. Two slices are typically placed near the center of each membrane and oriented so that the CA1 regions are pointing to one side using a fine brush (Fig. 1D). This simplifies injection of recombinant adeno-associated virus and electroporation. Carefully suck away any liquid from the top of the membrane using the glass Pasteur pipette and put the slices into the incubator.

    If slices are to be transfected by gene gun, place three to five slices on each membrane and do not worry about the orientation. From the start of the dissection to placing the slices in the incubator should take 20–30 min per pup. It is more important not to damage the hippocampus and to not contaminate anything that will come in contact with the slices than it is to perform the procedure very quickly.

13. One or two days after making the cultures, change the medium by aspirating about two-thirds and replacing with 700–800 µL prewarmed fresh medium. Thereafter, change the medium

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twice per week (or at least every 5 d) for one to two slices per membrane and at least every 3 d for three to five slices per membrane. Allow 3 d for the slices to firmly adhere to the membrane.

Do not inject recombinant adeno-associated virus or electroporate the cultures within the first 2 d after preparation because they are likely to become detached and are then no longer usable.

RECIPES

Dissection Solution

Reagent	Final concentration	Amount (for 500 mL)
Sucrose (Fluka 84100)	248 mM	40 g
$NaHCO_3$ (Fluka 31437)	26 mM	1.09 g
D-glucose (Fluka 49152)	10 mM	0.9 g
KCl (1 M; Fluka 60129)	4 mM	2 mL
$MgCl_2$ (1 M; Fluka 63020)	5 mM	2.5 mL
$CaCl_2$ (1 M; Fluka 21114)	1 mM	0.5 mL
Phenol red (0.5%; Riedel-De Haen 32662)	0.001%	1 mL
Ultrapure H_2O	–	to 500 mL
Kynurenic acid (100 mM; Fluka 61260)	2 mM	1 mL in 50 mL dissection solution

After mixing all ingredients except the kynurenic acid, check the osmolality (it should be 310–320 mOsm/kg) and the color (it should be red-magenta, indicating a pH >8). Filter-sterilize (0.2 µm pore size) and store at 4°C in 50-mL aliquots. Prepare a 100 mM stock solution of kynurenic acid and store at −20°C in 1-mL aliquots. Just before use, thaw and vortex one aliquot of kynurenic acid and add to 50 mL of dissection solution.

Slice Culture Medium

Reagent	Final concentration	Amount (for 500 mL)
MEM (Sigma-Aldrich M7278)		394 mL
Heat-inactivated horse serum[a]	20%	100 mL
L-glutamine (200 mM; Gibco 25030-024)	1 mM	2.5 mL
Insulin (1 mg/mL; Sigma-Aldrich I6634)	0.01 mg/mL	0.5 mL
NaCl (5 M; Sigma-Aldrich S5150)		1.45 mL
$MgSO_4$ (1 M; Fluka 63126)	2 mM	1 mL
$CaCl_2$ (1 M; Fluka 21114)	1.44 mM	0.72 mL
Ascorbic acid (25%; Fluka 11140)	0.00125%	2.4 µL
D-glucose (Fluka 49152)	13 mM	1.16 g

[a]The serum is often a critical factor in slice quality and it is often necessary to test several batches (lots); three products that have been successfully used are Sigma-Aldrich H1138, Gibco 26050070, and Gibco 16050122. Gibco 16050122 must be heat-inactivated for 30 min at 55°C.

After mixing, filter-sterilize (0.2-µm pore size), and store at 4°C in 50-mL aliquots. (The solution should be orange-red [i.e., pH ~7.3] and osmolality should be ~320 mOsm/kg.)

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