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Cortical Slice Culture Protocol for Rodent Brain

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1. Prolonged Activity Deprivation Causes Pre- and Postsynaptic Compensatory Plasticity at Neocortical Excitatory Synapses
Derek L. Wise, Yasmin Escobedo-Lozoya, Vera Valakh, Berith Isaac, Emma Y. Gao, Aishwarya Bhonsle, Qian L. Lei, Xinyu Cheng, Samuel B. Greene, Stephen D. Van Hooser, Sacha B. Nelson
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2. Progressive Circuit Hyperexcitability in Mouse Neocortical Slice Cultures with Increasing Duration of Activity Silencing
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We use this protocol and it's working

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Abstract

This protocol details a method for preparing and culturing organotypic cortical slices from mouse neocortex, using an interface method such as that first described in Stoppini et al., 1991, emphasizing sterile techniques for long-term culture viability (weeks in vitro) without antibiotics. The resulting preparations maintain significant circuit architecture, exhibit spontaneous network activity, and remain amenable to experimental manipulation and longitudinal study. Cultures prepared using this protocol have proven suitable for investigating network development, homeostatic plasticity mechanisms, and the consequences of chronic perturbations such as activity deprivation. This includes modeling the emergence of maladaptive states characterized by progressive circuit hyperexcitability and alterations in synaptic structure (Wise, et al., eNeuro 2024a & 2024b). The model system generated by this protocol is compatible with diverse downstream analyses, including functional imaging (e.g., calcium dynamics), electrophysiology, and high-resolution structural or synaptic assessments (e.g., immunofluorescence, electron microscopy). The protocol covers brain dissection, slice preparation using a vibratome, plating on Millicell inserts, and culture maintenance.

Image Attribution

Yasmin Escobedo Lozoya, PhD

Materials

1. Equipment

- 1.1 CO₂ Incubator (HERAcell 150i, Thermo Scientific)
- 1.2 Falcon 6-well plates (#35046, Falcon)
- 1.3 Millicell membrane inserts (0.4 µm, #PICMORG50, Millipore/Merck)
- 1.4 Biological Safety Cabinet (Class II Type A/B)
- 1.5 Vibratome (Leica VT1000S) or Compresstome (Precisionary Instruments)
 - 1.5.1 If Using a compresstome, place 2% molecular biology grade low melting point agarose diluted in ACSF in a 37 C bath before starting. Place the cooling block on ice and prepare the specimen syringe.
- 1.6 Vibratome Specimen Plate, Bath Tray, and Blade holder (Leica)
- 1.7 Disposable Personna Double Edge Stainless Steel Blades or Reusable Ceramic blades (EMS, E7550-1-C)
- 1.8 Stainless Steel Trays (for sterilizing instruments)
- 1.9 Sterile Silicone Mat (#20325-00, Fine Science Tools)
- 1.10 Glass Pasteur Pipettes (sterile, 1 ml or similar) - Modification: Tip cut ~1 inch from end, bulb placed on wide end.
- 1.11 Filter Paper Circles (10 cm, sterile)
- 1.12 Kimwipes (small, sterile)
- 1.13 Paper Towels (sterile)
- 1.14 Stereomicroscope with illumination
- 1.15 Dissection Instruments (Autoclaved):
 - Medium forceps (e.g., Student Dumont #7, #91197-00, Fine Science Tools)
 - Fine forceps (e.g., Dumont #5CO, #11295-20, Fine Science Tools)
 - Curved Medium Forceps
 - Short Scalpel Handle (#7 solid 12 cm, #10007-12, Fine Science Tools)
 - Long Scalpel Handle
 - Medium Scissors
 - Fine rounded-edge scissors
 - Large Round and Flat end spatula
 - Medium Round and Flat end spatula
- 1.16 Glass Petri Dish (10 cm)
- 1.17 Glass Beaker (200 ml)
- 1.18 Fritted Glass Bubbler (Medium Bubble) or Aquarium Airstone
- 1.19 Allen Wrench (for vibratome blade holder)
- 1.20 Ice Bucket/Container
- 1.21 Carbogen Gas Tank (5% CO₂ / 95% O₂) with regulator and tubing
- 1.22 Parafilm

2. Reagents

- 2.1 Slice Culture Medium (SCM) - See Step 4 for preparation
- 2.2 Cutting and Dissection Medium (CM) - See Step 4 for preparation

2.3 Cyanoacrylate glue (e.g., Super Glue)

2.4 Ethanol (70% and 100%)

2.5 Ice

2.6 Optional: Isoflurane or Ketamine/Xylazine for anesthesia

2.7 CIDECON 1:125 solution or similar surface disinfectant

3. Sterilization of Tools and Materials

3.1 Autoclave all dissection instruments, silicone mat, glass petri dish, glass pipettes (if not pre-sterilized), paper towels, kimwipes, and filter paper within stainless steel trays sealed with aluminum foil or in appropriate autoclave bags. Allow to cool completely before use.

3.2 Sterilize vibratome specimen plate, bath tray, and blade holder according to manufacturer instructions (often involves ethanol wipes and/or autoclaving if compatible).

4. Preparation of Solutions

4.1 Cutting and Dissection Medium (CM):

- Composition [mM]: Choline Chloride 110, KCl 2.5, NaHCO₃ 25, d-Glucose 25, Ascorbic Acid 12, MgSO₄ 7, Sodium Pyruvate 3.1, NaH₂PO₄ 1.3, CaCl₂ 0.5.

- Prepare using sterile water and filter-sterilize (0.22 µm filter). Store appropriately.

- Before use: Prepare an ice-cold ("slushy") aliquot (e.g., 100 ml). Place in an ice bath. Bubble vigorously with carbogen (5% CO₂ / 95% O₂) for at least 15-20 minutes prior to and during dissection, ensuring the solution doesn't splash excessively. Cover with parafilm around the bubbler tubing.

4.2 Slice Culture Medium (SCM):

- Base: Minimum Essential Medium Eagle (MEM)

- Supplements: HEPES (30 mM, pH 7.5 final conc.), NaHCO₃ (5.2 mM final conc.), d-Glucose (12.9 mM final conc.), L-Ascorbate (0.5 mM final conc.), MgSO₄ (2 mM final conc.), Glutamine (1 mM final conc.), CaCl₂ (1 mM final conc.)

- Prepare using sterile water and reagents. Check/adjust pH to ~7.35-7.45 after bubbling with carbogen (if required for pH stability, typically done if NaHCO₃ is used as primary buffer). A

- Add: 20% Horse Serum, Insulin (1 µg/ml final conc.),

- Check osmolarity (~315-320 mOsm).

- Filter-sterilize (0.22 µm filter). Store at 4°C. Warm to 37°C before use.

5. Preparation of The Culture Plate

5.1 One hour before plating, place one Millicell insert into each well of a 6-well plate.

5.2 Add 0.75 - 1 ml of pre-warmed (37°C) complete SCM to each well, adding it around the insert, not directly into it. Ensure the membrane is wetted.

5.3 Place the prepared plate into the 37°C, 5% CO₂ incubator to equilibrate.

Preparation for Dissection and Slicing

- 1 Bench Setup
 - 1.1 Wear clean gloves. Clean the work surface (Biological Safety Cabinet) with disinfectant.
 - 1.2 Arrange the benchtop with the dissecting scope, vibratome or compresstome, ice bucket with CM slushy (bubbling with carbogen), stainless steel tray with sterile instruments on ice (or organized sterile), glass petri dish with filter paper on ice, and silicone mat.
 - 1.3 Fill the vibratome ice tray with ice/water slurry.
 - 1.4 Fill the vibratome buffer tray with ice-cold, carbogenated CM. Place the bubbler stone in a corner to maintain oxygenation without obscuring view.
 - 1.5 Carefully mount a sterile vibratome blade onto the blade holder, ensuring the edge is parallel. Wipe blade with 100% Ethanol and allow to air dry briefly just before use if desired (standard practice for RNase removal, optional here).
 - 1.6 Have cut-tip sterile glass Pasteur pipettes ready.
 - 1.7 Optional: Wear a face mask to minimize contamination.
- 2 Dissection and Slicing (Protocol optimized for P7 or older mice; adjustments may be needed for different ages)
 - 2.1 Brain Dissection (Perform quickly; can process 2 animals sequentially)
 - 2.2 Anesthetize the animal appropriately (e.g., isoflurane or ketamine/xylazine injection, follow institutional guidelines). Confirm deep anesthesia.
 - 2.3 Decapitate the animal using large scissors.

- 2.4 Working on a clean surface (e.g., silicone mat), use small scissors to make a midline incision in the scalp from neck towards the nose. Peel back the skin to expose the skull.
- 2.5 Score skull with scalpel down center line. Carefully cut the skull along the midline using fine scissors. Make transverse cuts behind the olfactory bulbs and anterior to the cerebellum. Gently lift the skull plates away using forceps, avoiding pressure on the brain. Do another cut to join the foramen magnum with the lateral cut across the brainstem.
- 2.6 Use a small spatula to gently scoop the brain out and immediately immerse it in the beaker of ice-cold CM slushy.
- 2.7 Repeat for the second animal if applicable. Change gloves.
- 2.8 Transfer the brain(s) from the beaker to the petri dish containing filter paper on ice using the larger spatula. Add a small amount of slushy CM.
- 3 Brain Preparation for Slicing
 - 3.1 Orient the first brain dorsal side up.
 - 3.2 Use a scalpel to make a coronal cut to remove the cerebellum.
 - 3.3 Make a second coronal cut to remove the anterior-most ~2 mm (olfactory bulbs and frontal cortex - adjust distance based on animal age and target region).
 - 3.4 Apply a small drop of cyanoacrylate glue to the vibratome specimen plate.
 - 3.5 Using the curved spatula, carefully lift the brain block, blot the posterior (cut) surface gently on a sterile kimwipe to remove excess moisture.
 - 3.6 Immediately place the posterior surface onto the glue on the specimen plate. Ensure the brain adheres securely.
 - 3.7 Repeat for the second brain if applicable, placing it adjacent to the first.

3.8 Quickly place the specimen plate with mounted brain(s) into the vibratome buffer tray filled with ice-cold, carbogenated CM. If using a compresstome, glue the brain to the specimen holder and fill the specimen syringe with warm low-melting point agarose solution. Cool with the cooling block until it gels.

4 Vibratome Slicing

4.1 Ensure the brain is fully submerged in the cold, oxygenated CM. Use the bubbler to keep the medium oxygenated and prevent bubbles from obscuring the view.

4.2 Using fine forceps under the stereomicroscope, gently remove any remaining meninges from the cortical surface.

4.3 Set vibratome parameters (e.g., slice thickness: 300 μ m; speed: low-medium, e.g., 1-3; amplitude/vibration: medium-high, e.g., 8-10 – optimize based on tissue age and vibratome model).

4.4 Begin slicing. Collect healthy-looking cortical slices using a cut-tip glass Pasteur pipette. Avoid slices that are damaged, folded, or contain significant non-cortical tissue.

4.5 Transfer collected slices immediately into a temporary holding container (e.g., a small petri dish or well plate) filled with fresh, ice-cold, carbogenated CM on ice.

Plating Slice

5 Transferring Slices to Inserts

5.1 Bring the 6-well plate containing inserts and equilibrated SCM into the Biological Safety Cabinet.

5.2 Using a sterile cut-tip glass Pasteur pipette, carefully pick up one cortical slice along with a minimal amount of CM.

5.3 Position the pipette tip just above the center of a Millicell membrane insert. Gently expel the slice with the drop of medium onto the membrane surface. The slice should unfold and lie flat. Avoid transferring excess liquid.

- 5.4 If the slice is folded, very gently try to unfold it using the pipette tip or sterile forceps with minimal manipulation. If it cannot be easily unfolded, discard it.
- 5.5 Place 1-2 slices per insert, ensuring they do not touch each other.
- 5.6 Using a P1000 pipette with a filter tip, carefully aspirate any excess medium pooling on top of the insert membrane, leaving the slices moist but not submerged.
- 5.7 Repeat for all slices and inserts.
- 5.8 Return the plate to the incubator (Initially 37°C, 5% CO₂ for 1-2 days, then optionally move to 35°C for longer-term culture).

Feeding the Cultures

- 6 Media Changes
- 6.1 Culture medium (SCM) should be changed every 2-3 days. Perform changes in a sterile Biological Safety Cabinet.
- 6.2 Pre-warm the required volume of fresh SCM to 37°C (or 35°C if using lower temperature).
- 6.3 Carefully remove the plate from the incubator.
- 6.4 Using a sterile pipette (e.g., P1000 or serological pipette), aspirate the old medium from around the insert in each well. Avoid disturbing the insert or slices.
- 6.5 Gently add 0.75 - 1 ml of fresh, pre-warmed SCM to each well, again adding it to the side of the insert.
- 6.6 Return the plate to the incubator.

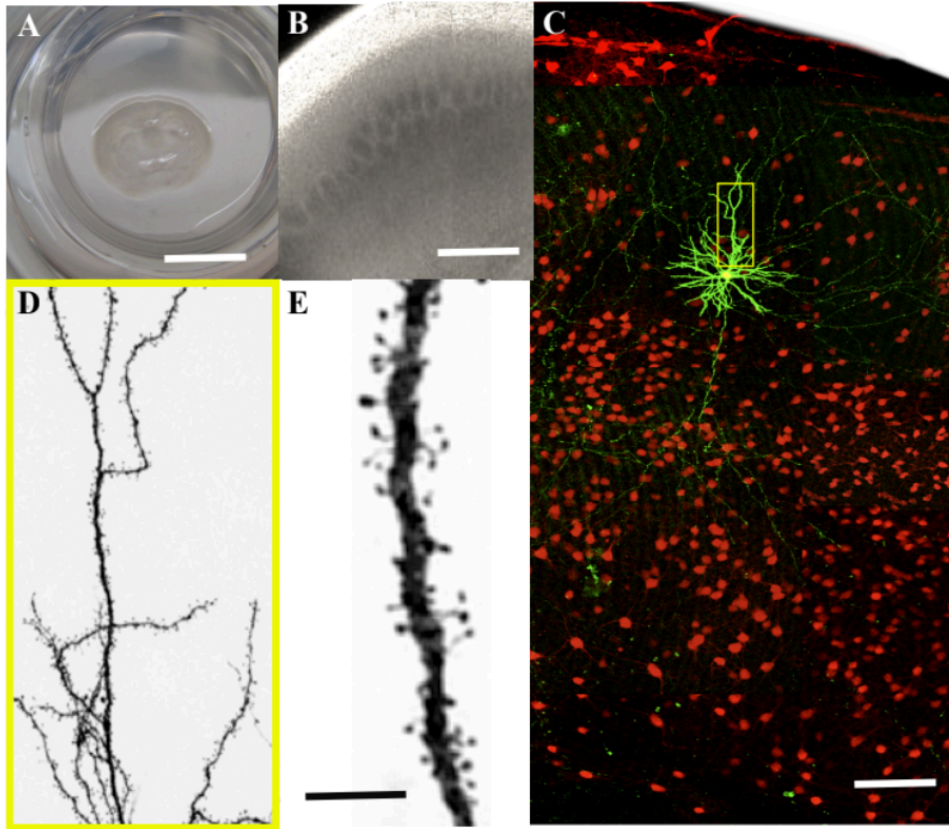


Clean Up

- 7 Post-Procedure Cleaning
 - 7.1 Dispose of all sharps (blades, needles) in appropriate sharps containers.
 - 7.2 Dispose of animal carcasses according to institutional guidelines.
 - 7.3 Clean the vibratome, benchtop, and all non-disposable tools thoroughly according to standard laboratory procedures.
 - 7.4 Prepare used instruments for autoclaving.

Expected Results

8



A. Coronal slice culture including cortex (left, top); B. 4× brightfield image showing cortical layers including barrels in layer 4 (left, bottom); C. Confocal fluorescent image of culture with filled pyramidal neuron (green) at P17/DIV 10 (right). Scale bar, 100 μm . D. Detail of dendrite in C, presented in grayscale for clarity of illustration. E. Detail of spines in the apical dendritic tree of the neuron in C and D Scale bar 25 μm .

Protocol references

Stoppini L, Buchs PA, Muller D. A simple method for organotypic cultures of nervous tissue. *J Neurosci Methods*. 1991;37:173–182. doi: 10.1016/0165-0270(91)90128-m. [[DOI](#)] [[PubMed](#)] [[Google Scholar](#)]