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# Acute human brain slice preparation for ex vivo electrophysiology and imaging

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Jaeyoung Yoon<sup>1</sup>

<sup>1</sup>Boston Children's Hospital, Harvard Medical School



Jaeyoung Yoon

Boston Children's Hospital, Harvard Medical School

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We use this protocol and it's working



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## Abstract

This protocol describes the procedure for preparing acute human brain slices from neocortical tissue, to be used for patch clamp electrophysiology experiments. The size, shape, properties, and scarcity of human brain tissue demand specialized slicing methods for optimal results. Appropriate methods will ensure that neuronal anatomy is properly preserved across all cortical layers, and slices remain in healthy, physiological condition suitable for patch clamp experiments for several days or up to a week after resection.

## Summary

- 1 This protocol describes the procedure for preparing acute human brain slices from resected cortical tissue, to be used for ex vivo patch clamp electrophysiology experiments. Characteristics of resected human cortical tissue such as its size and shape, as well as its scarcity, demand specialized slicing methods for optimal results. Appropriate methods will ensure that neuronal anatomy is preserved across all cortical layers, and that slices remain in healthy, physiological condition suitable for patch clamp experiments for several days or up to a week after resection.

**Steps 2-6** describe the solution preparation and tissue collection process prior to slicing. **Steps 7-10** describe the slicing procedure, particularly with respect to methods for preparing the tissue for optimal quality and yield. **Steps 11-12** describe how to maintain and examine slices after slicing.

## Solutions

### 2 Cutting solution for tissue transport and slicing

- 2.1 Prepare the cutting solution according to the following final concentration (leaving room for **Step 2.2**):

(in H<sub>2</sub>O) 165 mM sucrose, 20 mM HEPES, 25 mM NaHCO<sub>3</sub>, 2.5 mM KCl, 1.25 mM NaH<sub>2</sub>PO<sub>4</sub>, 20 mM D-glucose, 5 mM sodium ascorbate, 3 mM sodium pyruvate, 0.5 mM CaCl<sub>2</sub>, 7 mM MgCl<sub>2</sub>. (pH ~7.3 with NaOH)

The composition can be adapted according to the specific experimental needs and preferences, without changes in slice quality. Some other working examples include, but are not limited to, the following:

i) (in H<sub>2</sub>O) 75 mM sucrose, 75 mM NaCl, 25 mM NaHCO<sub>3</sub>, 2.5 mM KCl, 1.25 mM NaH<sub>2</sub>PO<sub>4</sub>, 20 mM D-glucose, 5 mM sodium ascorbate, 3 mM sodium pyruvate, 0.5 mM CaCl<sub>2</sub>, 7 mM MgCl<sub>2</sub>.

ii) (in H<sub>2</sub>O) 110 mM choline chloride, 26 mM NaHCO<sub>3</sub>, 2.5 mM KCl, 1.25 mM NaH<sub>2</sub>PO<sub>4</sub>, 10 mM D-glucose, 11.6 mM sodium ascorbate, 3.1 mM sodium pyruvate, 0.5 mM CaCl<sub>2</sub>, 7 mM MgCl<sub>2</sub>.

iii) (in H<sub>2</sub>O) 75 mM sucrose, 87 mM NaCl, 25 mM NaHCO<sub>3</sub>, 2.5 mM KCl, 1.25 mM NaH<sub>2</sub>PO<sub>4</sub>, 10 mM D-glucose, 0.5 mM CaCl<sub>2</sub>, 3 mM MgCl<sub>2</sub>.

iv) (in H<sub>2</sub>O) 94 mM N-methyl-D-glucamine, 20 mM HEPES, 25 mM NaHCO<sub>3</sub>, 2.5 mM KCl, 1.25 mM NaH<sub>2</sub>PO<sub>4</sub>, 20 mM D-glucose, 5 mM sodium ascorbate, 3 mM sodium pyruvate, 12 mM N-acetylcysteine, 2 mM thiourea, 0.5 mM CaCl<sub>2</sub>, 10 mM MgCl<sub>2</sub>. (pH ~7.3 with HCl; 10 min recovery in at ~36 °C in the same cutting solution before switching to a specific incubation solution; see **Step 4.1.iii** below)

2.2 Adjust pH to ~7.3 if applicable (as stated above), then adjust volume with H<sub>2</sub>O to arrive at the final concentration described in **Step 2.1**. The osmolality of the final solution will be ~300 mOsm/kg with the composition stated in **Step 2.1**. The osmolality of the cutting solution does not necessarily need to be precise as the cutting solution will be partially frozen in subsequent steps.

### 3 **Recording solution for electrophysiology experiments (artificial cerebrospinal fluid, aCSF)**

3.1 Prepare the recording solution according to the following final concentration:

(in H<sub>2</sub>O) 125 mM NaCl, 25 mM NaHCO<sub>3</sub>, 3 mM KCl, 1.25 mM NaH<sub>2</sub>PO<sub>4</sub>, 10 mM D-glucose, 1 mM sodium ascorbate, 3 mM sodium pyruvate, 1.2 mM MgCl<sub>2</sub>, 1.2 mM CaCl<sub>2</sub>.

The composition can be adapted according to the specific experimental needs and preferences. The osmolality of the final solution will be ~300 mOsm/kg with the composition stated above.

### 4 **Incubation solution for slice recovery and storage** *(Optional)*

Note: aCSF identical to the recording solution can be used in place of the incubation solution, with no change in slice viability. The use of a dedicated incubation solution is thus entirely optional. Acute human brain slices remained healthy in either condition typically for ~48-72 h following resection, or up to 132 h, with patched cells exhibiting normal physiology and intact morphology.

4.1 Prepare the incubation solution according to the following final concentration (leaving room for **Step 4.2**):

(in H<sub>2</sub>O) 90 mM NaCl, 20 mM HEPES, 25 mM NaHCO<sub>3</sub>, 2.5 mM KCl, 1.25 mM NaH<sub>2</sub>PO<sub>4</sub>, 20 mM D-glucose, 5 mM sodium ascorbate, 3 mM sodium pyruvate, 1 mM

\*

\*



CaCl<sub>2</sub>, 4 mM MgCl<sub>2</sub>.

The use of a separate incubation solution is optional, and slices can be maintained in the same aCSF as described in **Step 3.1** above without changes in slice quality or longevity. Similarly, the composition of the incubation solution can be adapted according to the specific experimental needs and preferences. Some other working examples include, but are not limited to, the following:

i) (in H<sub>2</sub>O) 75 mM sucrose, 75 mM NaCl, 25 mM NaHCO<sub>3</sub>, 2.5 mM KCl, 1.25 mM NaH<sub>2</sub>PO<sub>4</sub>, 20 mM D-glucose, 5 mM sodium ascorbate, 3 mM sodium pyruvate, 0.5 mM CaCl<sub>2</sub>, 7 mM MgCl<sub>2</sub>.

ii) (in H<sub>2</sub>O) 75 mM sucrose, 87 mM NaCl, 25 mM NaHCO<sub>3</sub>, 2.5 mM KCl, 1.25 mM NaH<sub>2</sub>PO<sub>4</sub>, 10 mM D-glucose, 0.5 mM CaCl<sub>2</sub>, 3 mM MgCl<sub>2</sub>.

iii) (in H<sub>2</sub>O) 94 mM NaCl, 20 mM HEPES, 25 mM NaHCO<sub>3</sub>, 2.5 mM KCl, 1.25 mM NaH<sub>2</sub>PO<sub>4</sub>, 20 mM D-glucose, 5 mM sodium ascorbate, 3 mM sodium pyruvate, 12 mM N-acetylcysteine, 2 mM thiourea, 1 mM CaCl<sub>2</sub>, 2 mM MgCl<sub>2</sub>. (pH to ~7.3 with NaOH; to be used specifically with the cutting solution in **Step 2.1.iv** above)

- 4.2 Adjust pH to ~7.3 if applicable (as stated above), then adjust volume with H<sub>2</sub>O to arrive at the final concentration described in **Step 4.1**. The osmolality of the final solution will be ~300 mOsm/kg with the composition stated in **Step 4.1**.

\*

## Transport

### 5 Preparing to collect resected tissue

- 5.1 Have the following materials ready in advance:
- 1) Bottles for cutting solution (e.g. 2× 500 mL glass media bottles)
  - 2) Insulated cooler box (any commercially available product)
  - 3) Ice packs, or similar (any commercially available product)
  - 4) Carbogen (95% O<sub>2</sub>, 5% CO<sub>2</sub>) tank, with accessories:
    - Gas regulator
    - Tubings, aeration stones, and roller clamps, for aerating solutions
    - Gas cylinder wrench (if necessary)
    - Gas cylinder cart, for transport
  - 5) (*Optional*) miscellaneous items, as needed (e.g. laboratory wipes, parafilm, etc.)

- 5.2 On the day of the resection, partly freeze the cutting solution to a slush, in quantity as needed (e.g. 2× 400 mL, in 2× 500 mL bottles).

The cutting solution should remain partly frozen throughout all steps, including tissue transport and slicing, for optimal slice quality.

- 5.3 Place the bottles containing the cutting solution into an insulated container suitable for maintaining the cutting solution in cold temperature (e.g. cooler box filled with ice packs). Aeration of the cutting solution can start at a later point closer to the time of actual tissue collection.

- 5.4 Arrive at the operating room with the cutting solution (in the insulated container) and the carbogen tank, assembled beforehand with the parts necessary for aerating the cutting solution.

- 5.5 Start aerating the cutting solution with carbogen sufficiently prior to (e.g. 5 min in advance) when the resected tissue is anticipated.

## 6 Collecting the tissue



- 6.1 Upon resection, place the tissue immediately into the ice-cold, aerated cutting solution, and return to the laboratory while aerating continuously.



The tissue must stay in the cutting solution as much as possible in all steps throughout transport and slicing, unless it is absolutely necessary to take it out of the solution (e.g. for pathology). Minimize the amount of time that tissue is exposed outside of the cutting solution. Transient exposure to air is tolerable.

The cutting solution must be ice-cold and continuously aerated with carbogen, and can be replaced as per needed; for example, if the cutting solution holding the tissue is about to thaw completely, it should be replaced with a partly frozen solution that had been stored separately and aerated before transferring the tissue.

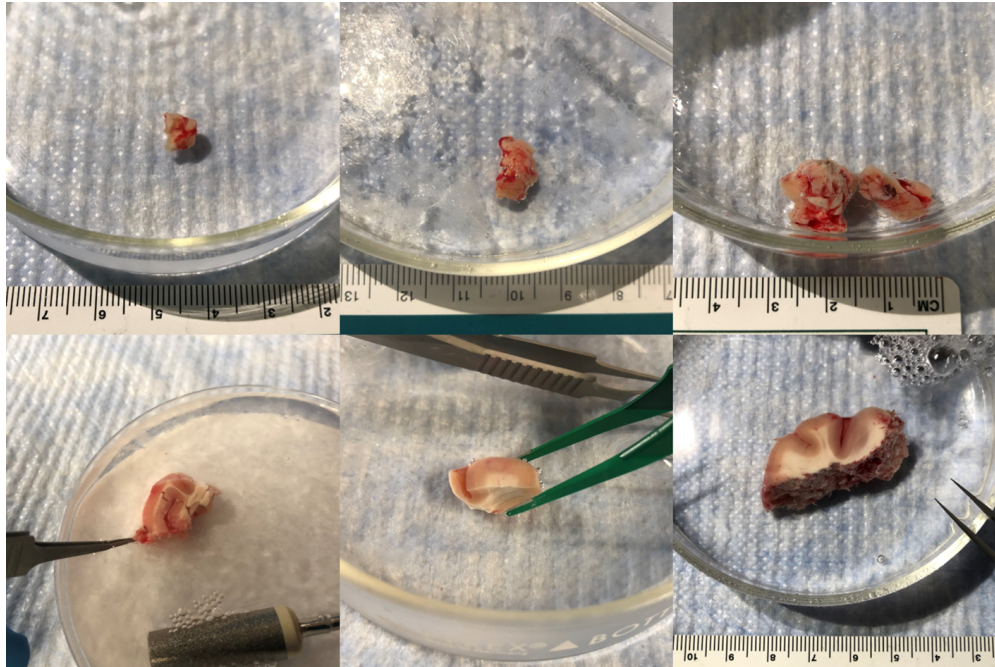
So long as it is kept in an aerated, ice-cold cutting solution, the tissue can stay in healthy condition, and subsequently sliced, up to several hours; such as when slicing a larger tissue.

## Acute brain slice preparation

### 7 Dividing the tissue for optimal yield *(Optional but strongly recommended)*



#### 7.1



**Figure 1.** Examples of resected cortical tissue. All rulers are metric (cm).

Resected tissue can vary considerably in size, shape, and curvature, as well as vasculature, damage during the resection process, and many other factors (**Fig. 1**). Depending on these specifics, it can often be advantageous to divide the tissue into smaller parts, such that the resulting parts of tissue can each be sliced at different angles that are most appropriate for each specific part (**Fig. 2**). For example, if the resected tissue contains multiple gyri, it may be reasonable to separate individual gyri for optimal quality and yield. Similarly, a gyrus can also be divided into smaller parts to maximize the volume of tissue sliced in proper angle, according to curvature. Even for very small tissue, trimming to obtain the ideal slicing angle is almost always necessary. This step is especially critical when the tissue is too small or complicated such that it is difficult to perform, as cortical volume that can yield good slices in correct orientation will be limited with such tissue, hence requiring more precision.



**Figure 2.** (Left and middle) Resected cortical tissue, viewed from two different angles. (Right) The arachnoid mater and the pia mater are removed from the tissue, and the tissue is further divided into smaller parts for optimal slice orientation and yield (see **Step 8** for details).

**Steps 7-9** are critical in order to ensure that all cortical layers are properly in plane such that neuronal anatomy is preserved, and to maximize yield for healthy slices suitable for electrophysiology experiments.

**Steps 7-9** can be performed in no particular order, repeating as necessary. The procedure should depend on the specific shape of the cortical tissue; see **Step 8** for a more detailed description. If the pia mater is to be removed, it can be easier to do it first before dividing the tissue; i.e. in the order of **Step 8 - Step 7 - Step 9**.

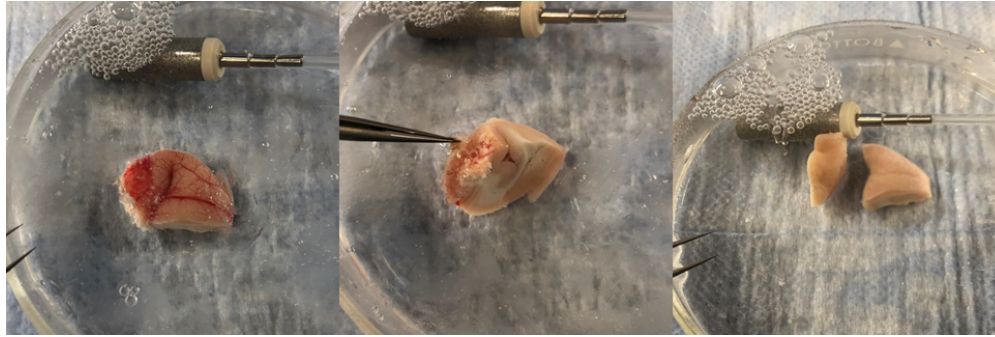
**Steps 7-10** must be performed in aerated, ice-cold cutting solution, refreshing the cutting solution as necessary.

A **Video** of the procedure is available online ([https://flosfor.github.io/human\\_brain\\_slicing](https://flosfor.github.io/human_brain_slicing)).

## 8 **Pia mater removal** (*Optional but recommended*)



### 8.1



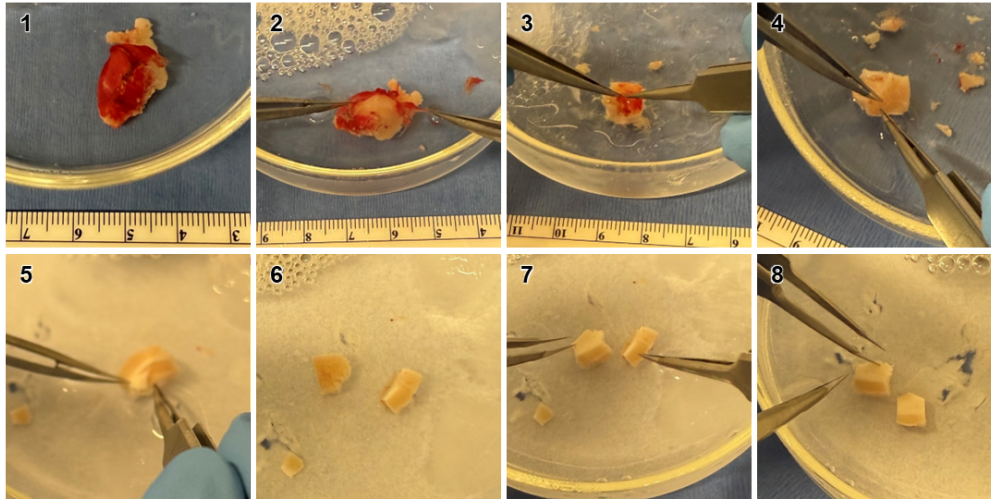
**Figure 3.** Example tissue before and after pia mater removal. (Left and middle) original tissue, from two different viewing angles. (Right) same tissue, after dividing along the sulcus and removing the pia mater. Note undamaged cortical surface, and indentation where thicker blood vessels were present. Tissue was later divided further into smaller parts for slicing.

Removal of the pia mater can be optional but recommended for optimal slicing, as the thick pia mater of humans can impede slicing much more compared to rodent brain slicing. If performed, it must be done without damaging the cortical surface (**Fig. 3**). If the pia mater is not removed, the slicing direction can later be adjusted accordingly for better results (see **Step 9**).

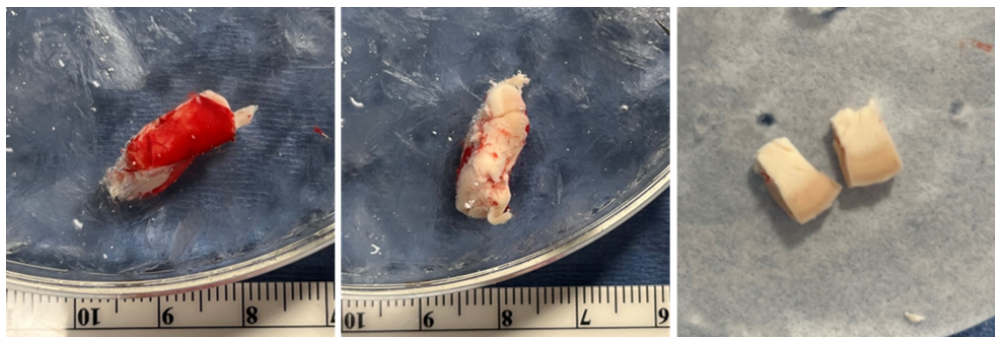
To remove the pia mater, carefully pinch and peel off the pia mater (along with the arachnoid mater, as applicable) using two forceps (e.g. Dumont #5, #3, etc.; per preference) (**Fig. 4**). Work slowly and carefully, taking caution not to tear off the cortex itself along with the pia mater, or otherwise damage parts of the cortex that could be included in slices. Start working from areas that will not be important in the final slices, such as the periphery of the tissue or the white matter (e.g. if intended for patch clamp experiments from neocortical neurons). Similarly, the tissue can be held at these less important parts while removing the pia mater. Repeat as necessary, until the pia mater is completely removed from the cortical tissue block.

Refer to the **Video** for further reference ([https://flosfor.github.io/human\\_brain\\_slicing](https://flosfor.github.io/human_brain_slicing)).

Excess tissue not important for the slices can also be trimmed with a scalpel (e.g. #10), razor blade, or scissors (e.g. iris or Metzenbaum), which is recommended for structural stability and/or convenience (**Fig. 5**).



**Figure 4.** Another example tissue before and after pia mater removal, followed by dividing it into smaller pieces for optimal slicing angle and yield. See **Video** for an example with a different tissue throughout the entire procedure.



**Figure 5.** Another example tissue, before (Left and Middle) and after (Right) the pia mater was removed and the tissue was divided for orientation. Note different shades between the supragranular and the infragranular layers in the final picture, indicating good angle (see also **Fig. 6**). The white matter can be trimmed further from the smaller blocks if it is of no interest.

## 9 Orienting and mounting the tissue for slicing



- 9.1 Cut off one side of the cortical tissue block with a scalpel or a razor blade, which will be the bottom side (i.e. the base of the tissue to be mounted) that will be glued in **Step 9.2**.



When making the base cut, it is important to choose the correct angle according to the cortical plane, although some fine adjustments can be made at the slicing step using an orienting specimen disc. In addition, it is advantageous to choose the side

opposite from the part that would produce better slices, since some volume of tissue near the glued part at the bottom will be lost. Cut decisively in a single quick motion, as cutting hesitantly can easily produce a more uneven base or a different angle than intended due to the tissue being pushed against the blade. Settling the tissue on top of a filter paper (while still submerged in cutting solution in a petri dish) can be helpful while cutting, due to added friction.

The cross section viewed from the side (i.e. orthogonal to the plane of slices) can give a visual clue in choosing the correct angle; however, it should be interpreted with caution when the curvature of the tissue changes towards the sides, i.e. is not uniform across the tissue. Another visual clue is the top-down view of the tissue (i.e. normal to the plane of slices); the pial surface should be orthogonal to the bottom at the final mounting position.

With the correct angle, the supragranular and the infragranular layers should be visibly distinguishable by eye from having different shades (**Fig. 5 & Fig. 6**). Incorrect angle would result in a more smeared appearance with a less distinguishable border between the supragranular and infragranular layers, and relatively thicker-appearing layers. Note that this only applies to the appearance of the actual slices (or tissue at an equivalent angle), hence it is not relevant if there is additional tissue on top before the level of actual slices.

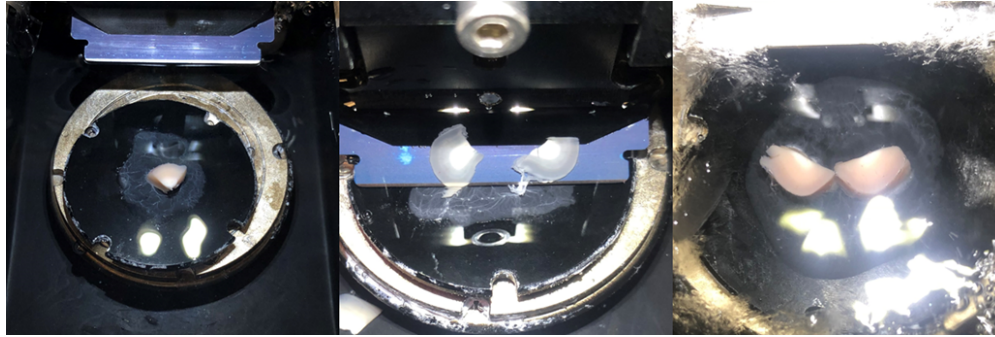
- 9.2 Mount the cortical tissue block(s) prepared through **Steps 7-9** using cyanoacrylate glue (e.g. Loctite 406), on an orienting specimen disc (e.g. Leica 14048142068) which will allow further fine adjustment of the slicing angle. Using antimagnetic tools are advised for mounting the tissue, as the base of the orienting specimen disc is magnetic and can cause conventional tools to be attracted abruptly, potentially damaging the tissue.



Tissue block(s) could be mounted either separately or together, depending on the similarity of the slicing angle required for each block. Each block should be prepared for optimal slicing orientation, according to **Steps 7-9**.

- 9.3 *(Optional)* If the pia mater were not removed or only partly removed, the tissue block can be oriented such that the slicing direction is orthogonal to the pial surface, to encourage uninterrupted slicing through the pia mater. Alternatively, the slicing direction can run from the white matter towards the pia mater, instead of from the pial surface towards the white matter which can be more effectively used for slicing tissue after the removal of pia mater (**Fig. 6**).





**Figure 6.** Example slicing strategies using different approach directions. (Left) from cortical surface to white matter. (Middle) approximately along the cortical surface, for the part of the slice where orientation is expected to be ideal. (Right) from white matter to cortical surface. Note well-defined boundaries between the supragranular and infragranular layers in all pictures, which are indicators of optimal slicing angle. Note also the fine adjustment of the slicing angles using the orienting specimen disc.

## 10 Slicing



### 10.1 Slice with a vibratome (e.g. Leica VT1200S).



Use standard procedures and precautions for acute brain slice preparations, such as minimizing mechanical disturbance, maintaining the cutting solution partly frozen, or in choosing the slicing parameters.

Below are example slicing parameters, which can be adapted per preference or specific experimental needs:

- Slicing speed (blade feed rate): 0.1 – 0.4 mm/s.\*
- Lateral movement (vibration amplitude): 1.0 – 1.5 mm (e.g. 1.2 mm)
- Vibration frequency: 85 Hz (native setting for Leica VT1200S)
- Slice thickness: 300  $\mu$ m (or as needed)

\* Note: in general, human brain tissue can be sliced much faster than mouse or rat brain slices without compromising slice quality. As such, it could be advantageous to employ a faster feed rate in order to minimize total slicing time, especially when the tissue is large. We typically used 0.2 mm/s, but also occasionally 0.1 or 0.4 mm/s, when more caution or speed was desired, respectively.

## Electrophysiology

### 11 Slice recovery and maintenance

11.1 Place the slices in aCSF at ~36 °C for recovery. If a different incubation solution were prepared (**Step 4**), it could be used for recovery and storage instead of the recording aCSF. In either case, the solution must always be continuously aerated with carbogen (95% O<sub>2</sub>, 5% CO<sub>2</sub>) throughout all procedures. Note: follow the additional instructions in **Step 2.1.iv** when using an NMDG-based cutting solution.



11.2 Transfer the slices, or the solution containing the slices, to room temperature after spending at least 30 min in ~36 °C.



Longer recovery times are neither required nor detrimental to the slices, although it is recommended not to leave the slices at warmer temperature for excessive periods for maximal slice longevity. Shorter recovery times are not recommended as they can compromise slice quality.

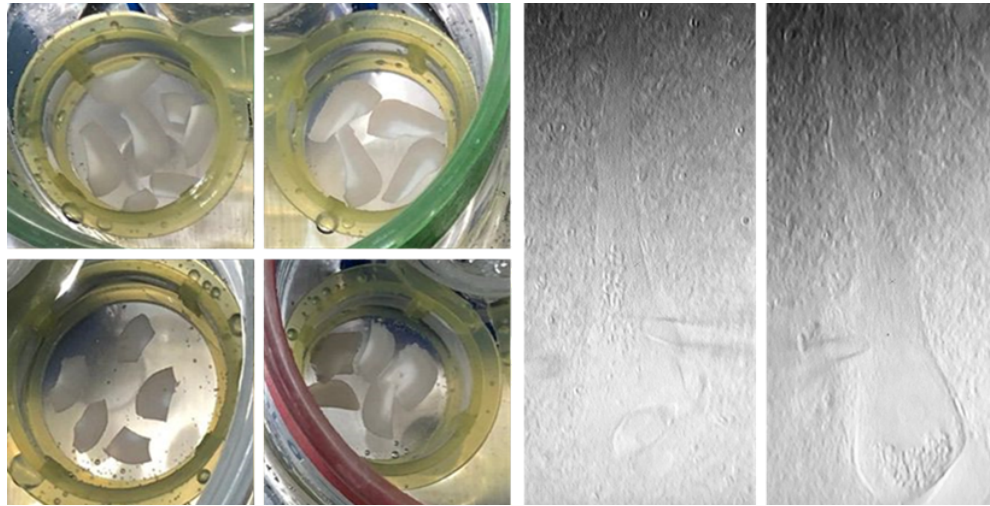
11.3 Replace the aCSF (or the incubation solution) periodically with a fresh solution (e.g. every 8 h).

Make sure to have the replacement solutions ready beforehand, such that they would be aerated sufficiently before moving the slices, and that no temperature shocks are introduced.

Slice containers must be covered at all times except when transferring slices in order to minimize evaporation. If in doubt, measure the osmolality of the solution and compare it to when it was freshly made; this is strictly a troubleshooting suggestion, and not normally required.

## 12 Patch clamp experiments

12.1



**Figure 7.** Example slices and cells. Note also lipofuscin aggregates on the somata of pyramidal neurons, which are visual indicators of patient age and not normally seen from rodent neurons.

Examine the slices visually under the microscope by inspecting the pial surface for any damage, and by following the apical dendrites from the soma towards the cortical surface (**Fig. 7**). Ideally, the apical dendrites should run in parallel to the plane of the slice, and be traceable up to the apical tuft until reaching the very edge of the cortical surface. With correct slicing angle, neuronal anatomy can be preserved even for deeper layer neurons that span  $> 2$  mm.

- 12.2 When prepared and maintained properly, acute human brain slices can last healthy and suitable for patch clamp experiments easily up to 48–72 h after resection, or even longer. In our conditions, neurons remained in good, physiological state up to 132 h in one example, or 120 h in another example; both using normal cutting and recording aCSF as described earlier in this protocol, without requiring a dedicated recovery or incubation solution.

## Protocol references

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Cho, E., Kwon, J., Lee, G., Shin, J., Lee, H., Lee, S. H., Chung, C. K., Yoon, J. & Ho, W. K. (2024). Net synaptic drive of fast-spiking interneurons is inverted towards inhibition in human FCD I epilepsy. *Nature Communications*, 15(1), 6683. DOI: 10.1038/s41467-024-51065-7