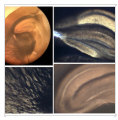

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In devel.

Protocol for obtaining rodent brain slices for electrophysiological recordings or neuroanatomical studies

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ABSTRACT

Patch clamp recording performed *in vitro* using brain slice preparations is a standard technique used in cellular biophysics and neurophysiology to study the electrical activity of neurons. In particular, our research group is interested in obtaining patch clamp recordings from neurons in the CA1, CA3, and dentate gyrus regions of the hippocampal formation to investigate how the excitability of neurons change during development and aging. To carry out these experiments, we must first dissect out the brain and obtain slices, all while keeping the brain healthy so that the neurons survive and can later be recorded. Here we outline our procedures for anesthetizing, perfusing, dissecting out the brain, and finally obtaining slices. This protocol can also be used as a teaching tool to train students in the handling and dissection of rodents, and the preparation of brain tissue. The slices obtained can also be used for neuroanatomical studies or in training students to identify different brain structures. Our goals in sharing this protocol are to be transparent about our scientific methodology and to help other researchers performing similar experiments.

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PROTOCOL STATUS

In development

We are still developing and optimizing this protocol.

MATERIALS TEXT

- anesthesia (mix of ketamine and xylazine)
- 1mL plastic syringe for injecting anesthesia
- 5mL (mouse) / 20 mL (rat) syringe for injecting perfusion solution
- perfusion solution (sucrose)
- artificial cerebrospinal fluid (Krebs)
- glass beaker (100 mL)
- parafilm “M” (Bemis)
- metal or plastic dissection tray
- curved tip atraumatic forceps
- mosquito hemostatic forceps
- 4.5 inch stainless steel sharp scissors
- 5.5 inch stainless steel sharp scissors
- small spoon (Fisherbrand Spoonula Lab Spoon)
- ice and bucket
- fine tip paint brush (Rodin Serie S-9700)
- filter paper (Whatman Grade 1 circles, 90mm)
- small glass petri dish (5cm)
- large glass petri dish (9cm) with attached Vibratome base
- slice incubation chamber or small plastic petri dish (5.5cm)
- stainless double edge razor blade (Dorco ST300)
- Vibratome (Series 1000)

- gas (carbogen 5%)
- cyanoacrylate glue ('Super Glue' or 'Krazy Glue')
- red sharps disposal container
- yellow plastic bag for biological waste

BEFORE STARTING

Prepare perfusion solution:

Reagent	Concentration (mM)	Quantity (to prepare 500 mL)
sucrose	234	40 g
NaHCO3	28	1.17 g
dextrose	7	631.0 mg
pyruvate	4.54	200.0 mg
ascorbic acid	0.28	24.6 mg
KCl (stock 500 mM)	25	2.50 mL
MgCl2 (stock 500 mM)	7	7.00 mL
NaH2PO4 (stock 500 mM)	1.44	1.44 mL
CaCl2 (oxigenate; stock 500 mM)	0.4	0.40 mL

Prepare Krebs solution:

Reagent	Concentration (mM)	Quantity (to prepare 1 L)
NaCl	126	7.360 g
dextrose	10	1.802 g
NaHCO3	26	2.180 g
thiourea	0.2	15.0 mg
ascorbic acid	0.2	35.2 mg
KCl (stock 500 mM)	2.5	5.0 mL
MgCl2 (stock 500 mM)	1.3	2.6 mL
NaH2PO4 (stock 500 mM)	1.2	2.4 mL
CaCl2 (oxigenate; stock 500 mM)	1.0	2.0 mL

Composition of the artificial cerebrospinal fluid (Krebs solution), including the concentration of each reagent and the quantities needed to make a total of 1 L solution.

Preparation for anesthesia and dissection

1 **Prepare and check all materials and equipment:** Before beginning the anesthesia and dissection protocol, verify that all the necessary materials are available and in good condition (Fig. 1). The perfusion and Krebs solutions should have been prepared previously and must not be more than 7 days old. Even if the solutions are less than 7 days old but visible particulates can be seen or the solutions appear cloudy, they should not be used and new solutions should be prepared before anesthetizing the animal. The anesthesia (ketamine and xylazine mix) should also be checked by verifying that it has not passed the expiration date and there is no coloration of the liquid which could indicate oxidation. Also verify that all the dissection instruments are clean, undamaged, and laid out for easy access (Fig. 1B). Also confirm the Vibratome is in working condition.

Position the large glass petri dish with glued attachment block in the Vibratome, and break a new razor blade in half and fix it in place (Fig. 1F,G). Place the other half of the blade together with a folder piece of filter paper on top of a small glass petri dish on ice (Fig. 1E). This will later be used to cut the brain before placing it in the Vibratome for slicing. Place fine tip paint brushes and forceps at the side of the Vibratome to be used when transferring brain slices from the dish to incubation chamber. Put other items, such as the cyanoacrylate glue and syringe with perfusion solution, on ice (Fig. 1C,D).

It is important that all these materials are set up before anesthetizing the animal so that the dissection and brain removal can occur as quickly and smoothly as possible.

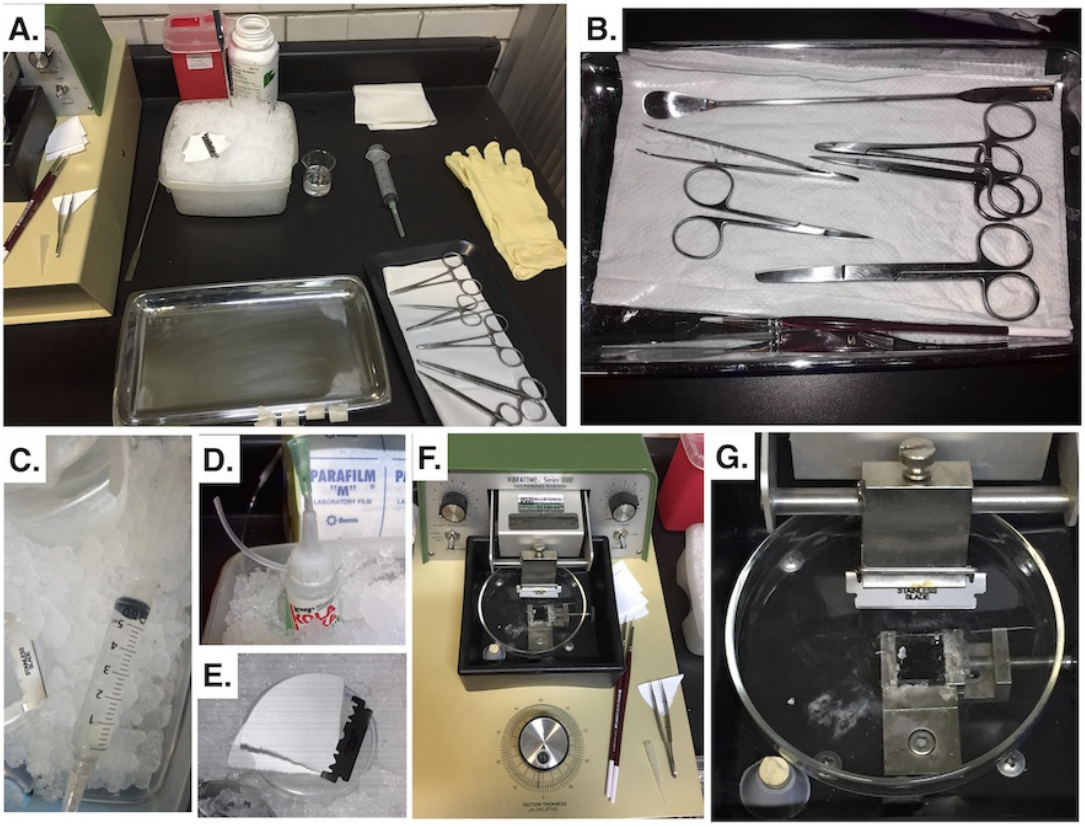


Figure 1: Dissection area and setup.

2 **Cool and oxygenate solutions:** Pour around 80 mL of perfusion (sucrose) solution into a glass beaker and cover the top of the beaker with parafilm (Fig. 2D). Place the beaker in the freezer for approximately 10-15 minutes, until the solution is partially but not completely frozen (consistency similar to a slushie). Pour around 60 mL of Krebs solution into the incubation chamber (Fig. 2E), and place the end of the tube running from the gas tank into the solution and turn the gas on to oxygenate. You should see small bubbles continuously produced in the solution (not shown)

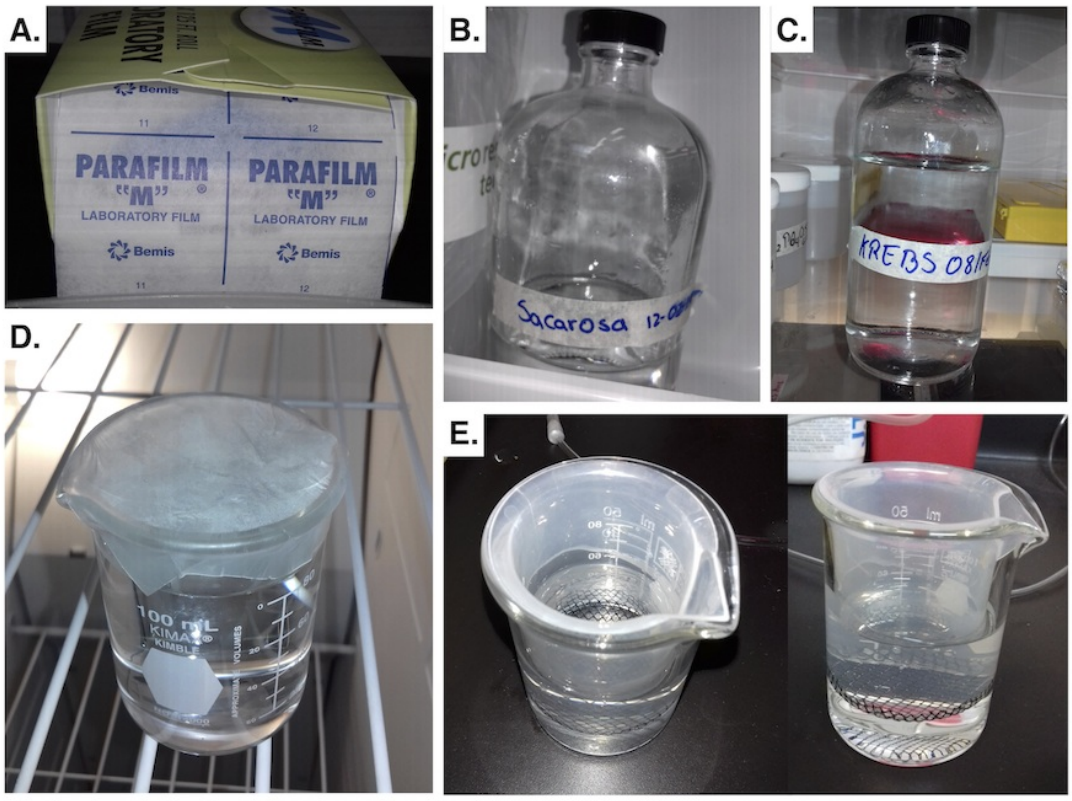


Figure 2: Solutions used for perfusion and slicing of the brain.

Anesthesia

3 The anesthesia used is a mixture of ketamine (85 mg/kg) and xylazine (15 mg/kg). The initial dosis is 1 unit (0.01 mL) for mice or 5 units (0.05 mL) for large rats. Additional doses of 1-2 units each are applied every 5-10 minutes as necessary. The final total dosis will depend of the weight of the animal.

Anethesia is applied by intraperitoneal injection, as follows. Remove the animal from its cage, holding it with one hand by the tail. Still holding the tail, place the animal on a flat surface. (If it feels more comfortable, place a small cloth over the animal, particularly covering the eyes and mouth area to calm the animal and avoid any biting.) With the other hand, gently but firmly grab the animal by the extra skin on the back of the neck just below the ears to immobilize it. Turn the animal ventral side up and inject the anesthesia into the peritoneal cavity (between the abdominal midline and the leg) at an angle of 30°(Fig. 3). Do this procedure as quickly as possible to minimize stress and discomfort for the animal. When done, place the animal back in its cage and allow the anesthesia to take effect (approx. 5-10 minutes)

Figure 3: Intraperitoneal injection to anesthesize the animal before dissection.

After anesthesia is applied, motor behavior, respiratory rhythm, and responses to noxious stimuli, such as foot or tail pinch with atraumatic forceps, are monitored. Following the first dose of anesthesia, the majority of gross motor behavior should cease with around 5-10 minutes. The animal can then be removed from the cage and laid out on the dissection tray (but not restrained). Additional doses of anesthesia can be applied as needed. The animal is considered to be deeply anesthetized when motor behavior has ceased, respiratory rhythm is slow and regular, and the animal does not show any reflex responses to noxious stimuli. Only after deep anesthesia is verified is the animal restrained on the dissection tray and the procedure can begin.

Dissection

4 **Position and restrain the animal for dissection:** Once the animal is completely anesthetized, position it ventral side up in the dissecting tray and fix each of the limbs to the tray with masking tape (Fig. 4).

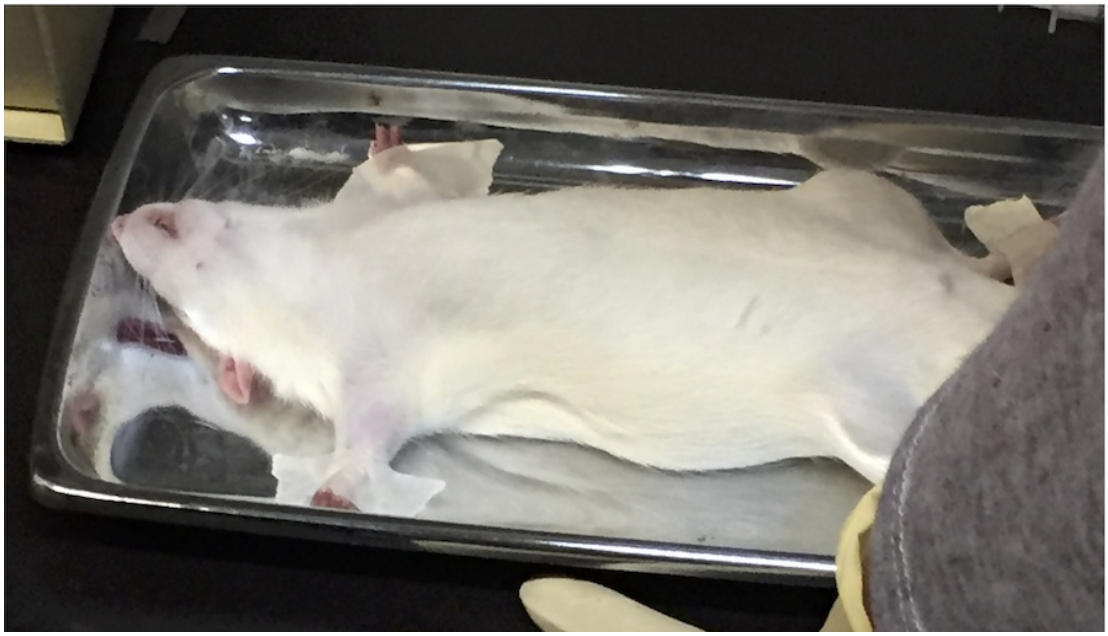


Figure 4: Anesthetized animal positioned and limbs fixed in the dissecting tray.

5 **Expose the thoracic wall:** Use the hemostatic forceps to clamp and pull up on the fur and skin in the center of the thorax (Fig. 5A). Use the xiphoid process as a point of reference. Cut away the fur and skin to reveal the tissue of the thoracic wall (Fig. 5B).

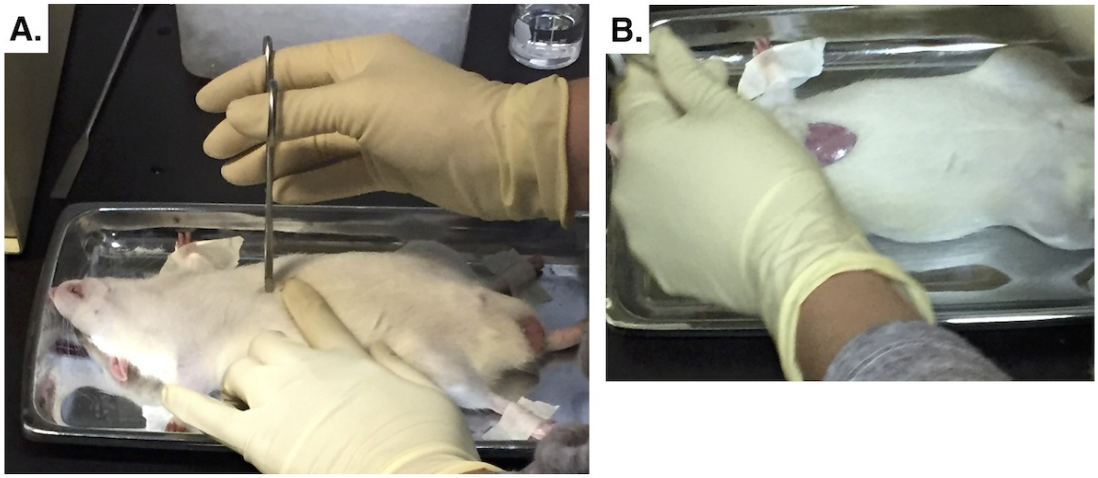


Figure 5: Exposing the thoracic wall.

6 **Open the thoracic cavity:** With the hemostatic forceps in one hand, clamp the xiphoid process. Take the 4.5 inch scissors in the other hand and cut on either side of the thoracic cavity to open it up (Fig. 6A). Then, continue to cut on either side of the thorax, moving anteriorly, to fully open the thoracic cavity (Fig. 6B). When finished, the heart should be visible and accessible (Fig. 6C,D). Once the heart is exposed, use the scissors to make a small incision in the right atrium (not shown).

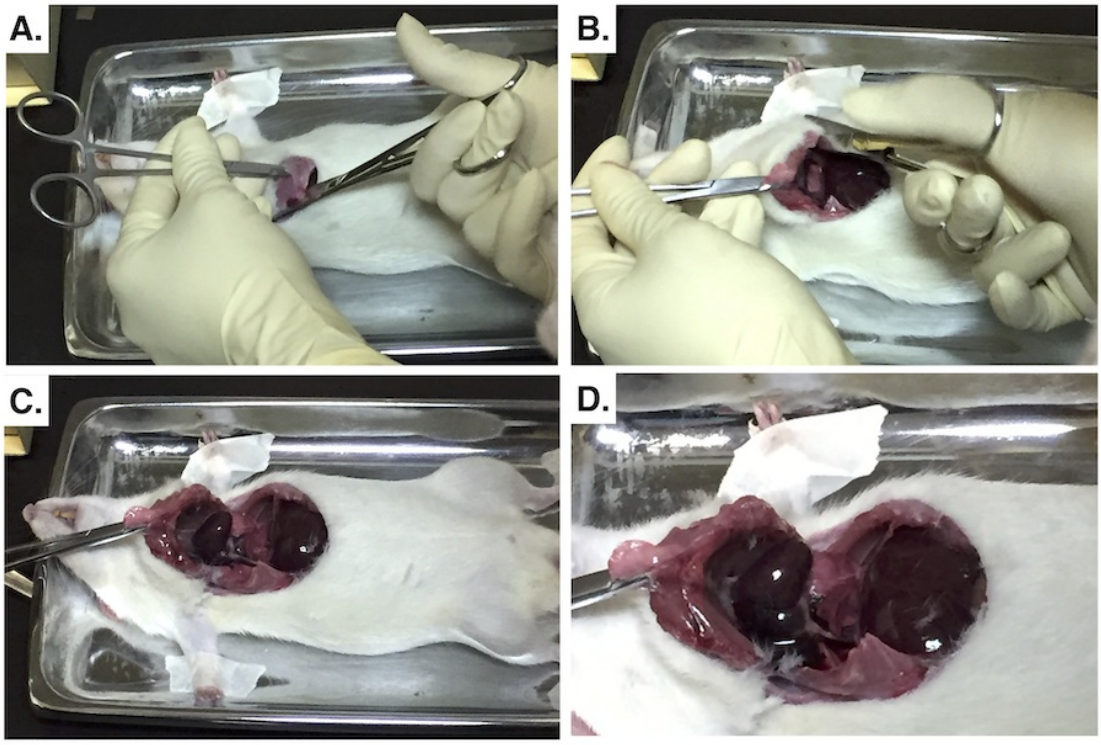


Figure 6: Opening up the thoracic cavity to expose the heart.

Perfusion

7 **Insert the needle into the heart to begin perfusion:** Take the 20 mL syringe (or 5 mL for mouse) filled with perfusion solution and carefully insert the needle into the left ventricle of the heart (Fig. 7). Be sure not to completely puncture the heart, i.e. do not not pass the needle through to the other side.

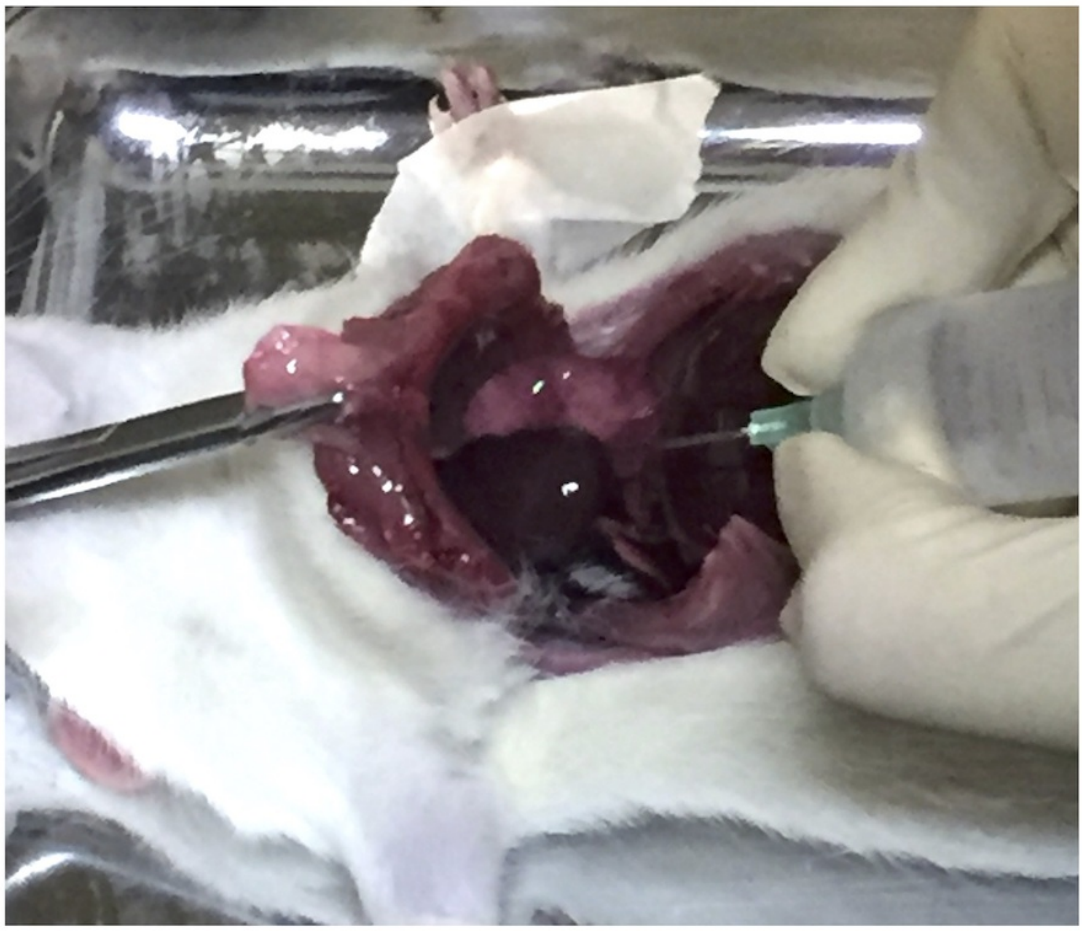


Figure 7: Inserting the needle into the heart to begin perfusion.

8 **Perfuse the animal:** Slowly inject the perfusion solution into the left ventricle in a pulsatile manner, roughly in sync with the cardiac rhythm. Blood will gradually flow out of the animal as it gets replaced with the perfusion solution (Fig. 8). To confirm that perfusion was done correctly, check the color of the eyes: they should be whitish by the end of perfusion (not shown).

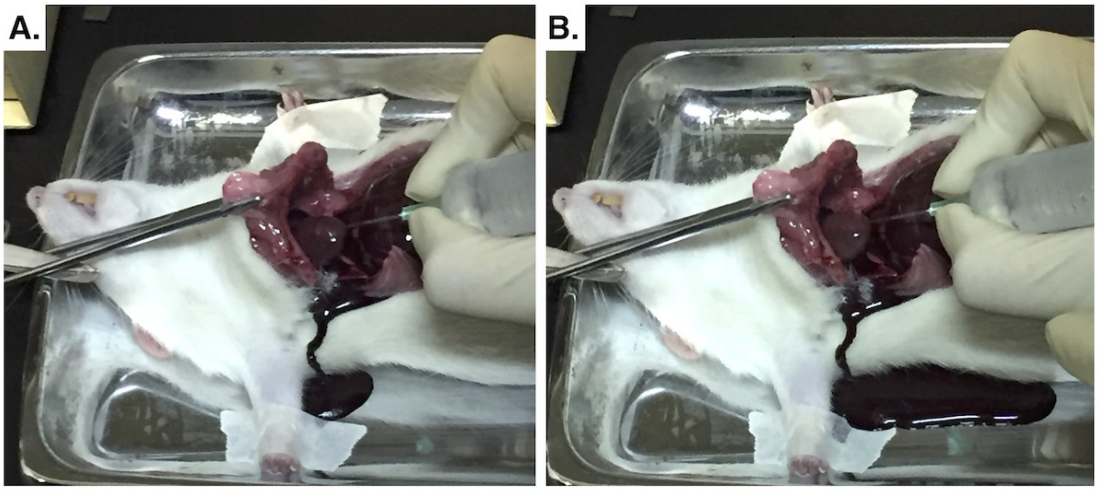


Figure 8: Perfusion, just beginning (A.) and near the end of the process (B.).

Sacrifice and brain removal

9 **Sacrifice the animal:** Once the animal has been completely perfused, use the 5.5 inch scissors to cut at the base of the cranium above the first cervical vertebra and decapitate the animal (Fig. 9).

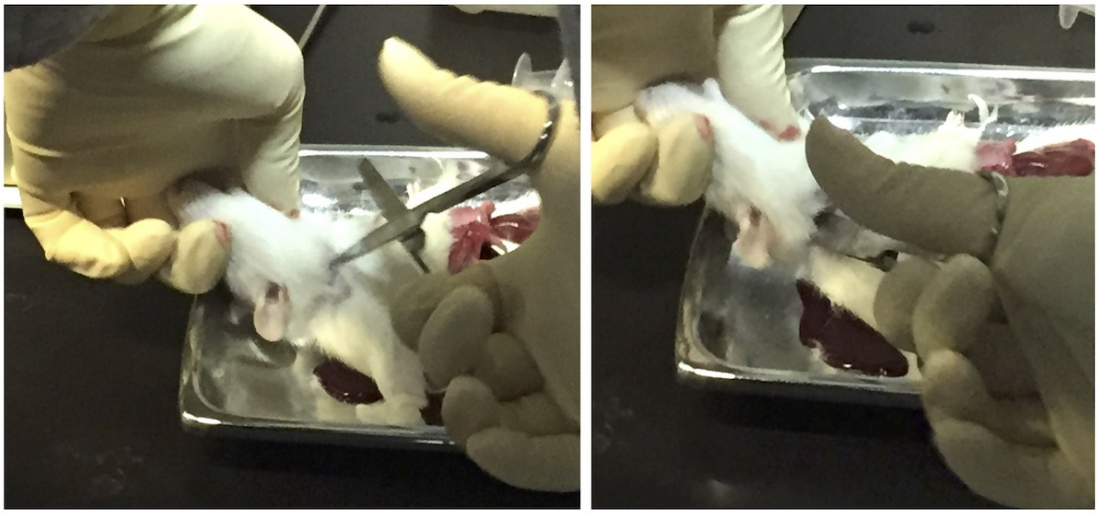


Figure 9: Decapitation of the animal.

10 **Expose the cranium:** Use the 5.5 inch scissors to carefully cut away the fur and skin to expose the cranium (Fig. 10).

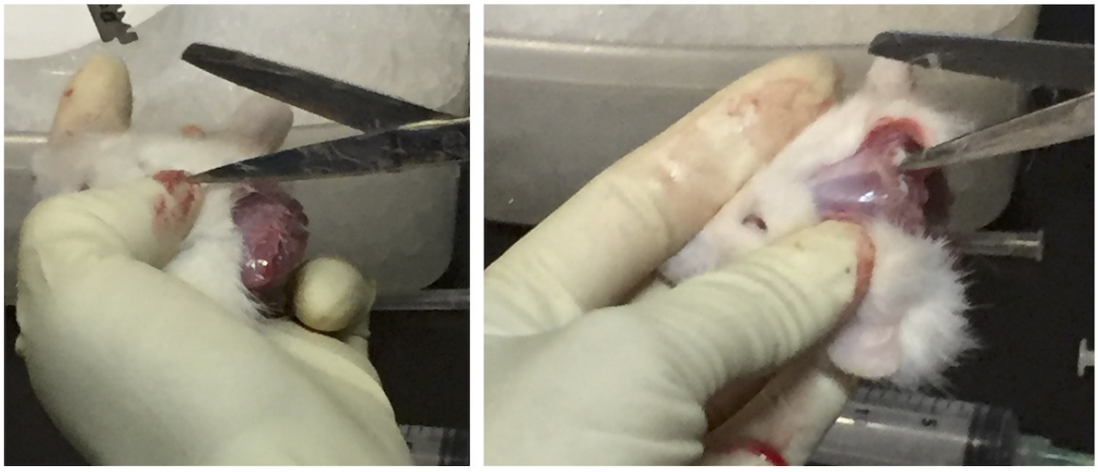


Figure 10: Exposing the cranium.

11 **Expose the brain:** Once the cranium is exposed, use the 4.5 inch scissors to cut from the occipital to temporal area on the left side and then perform a similar cut on the right side. Next, perform a horizontal cut at the most anterior part of the cranium. Finally, cut down the midline in the posterior to anterior direction. Lift up slightly on the scissors while cutting to avoid damaging the brain. Once these cuts have been made, gently peel away the cranial bones to expose the brain (Fig. 11; not all cuts are shown).

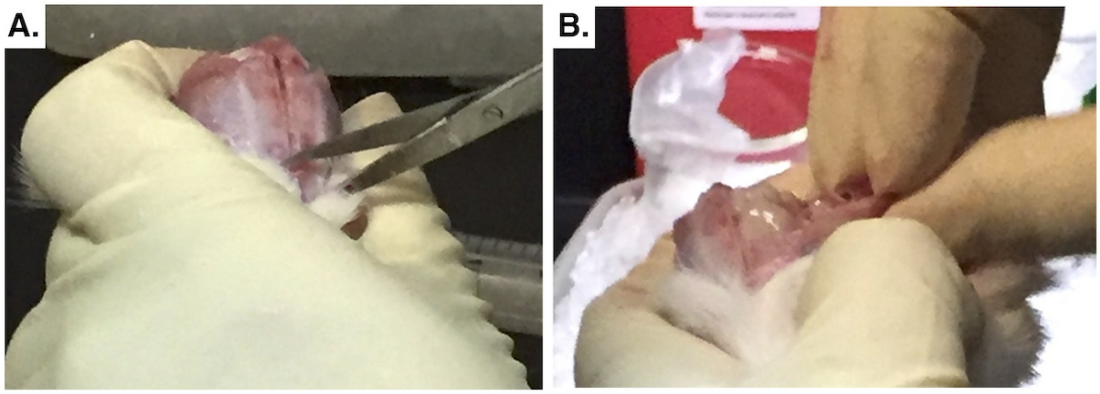
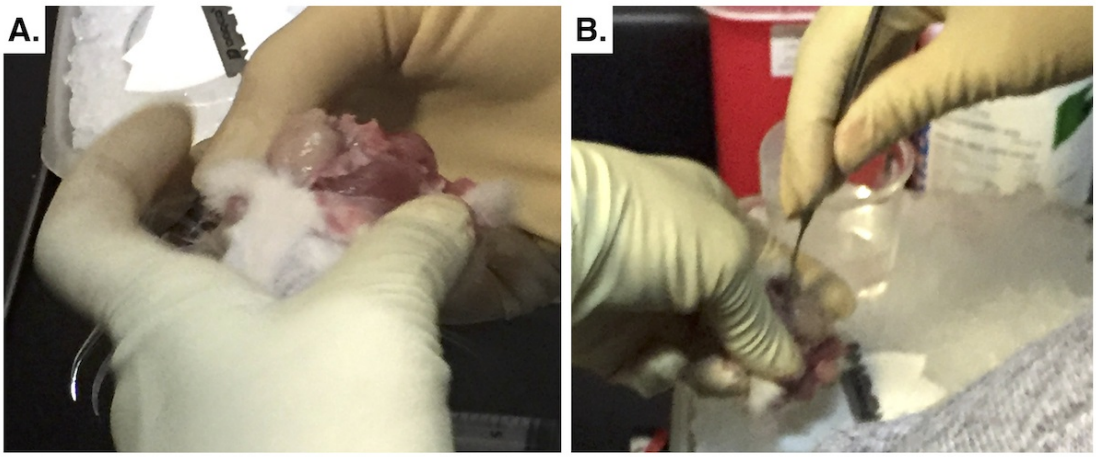


Figure 11: Exposing the brain.

12 **Remove the brain:** Carefully peel back and pick away the cranial bones to access the brain (Fig. 12A). Then, using the small spoon, gently scoop the brain out of the cranium and on to the filter paper located in the small glass petri dish on ice (Fig. 12B).



Brain slicing

13 **Cut the brain to prepare for slicing:** Once the brain has been removed fromt he cranium, use a sharp razor blade to carefully cut away the cerebellum and brainstem from the rest of the brain (Fig. 13A). Then, cut the brain down the midline to separate the two hemispheres. (Fig. 13B).

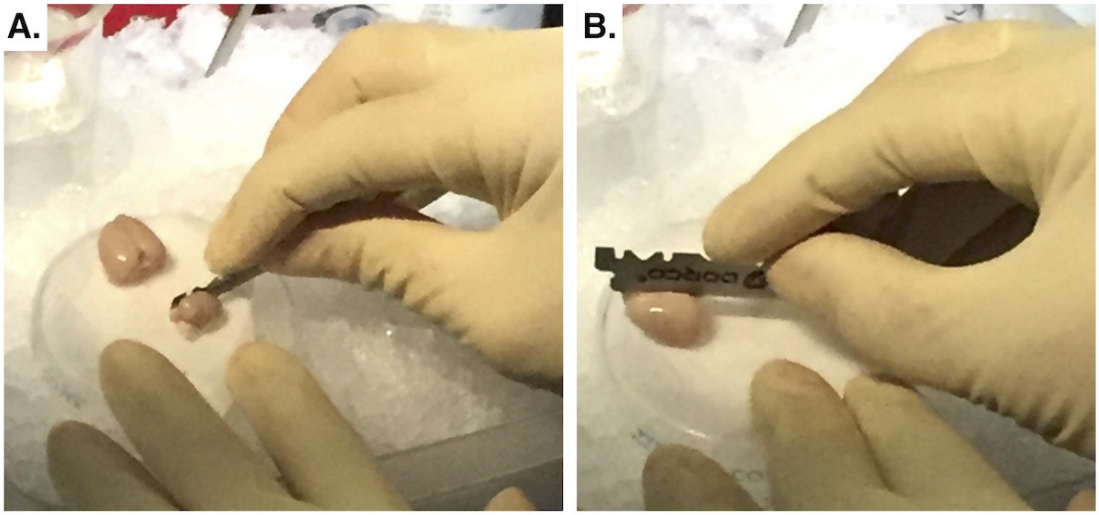


Figure 13: Cutting away the cerebellum (A.), and then cutting the brain in half (B.).

14 **Glue the brain hemispheres in the Vibratome petri dish for slicing:** Place two drops of cyanoacrylate glue in the glass petri dish, one closer to the left side of the blade and the other on the right side. One drop should be slightly anterior with respect to the other. Gently pick up one of the brain hemispheres and place it on one of the drops of glue with the cut side (midline) down and the anterior-posterior axis perpendicular to the blade (Fig. 14 and Fig. 15A.). This orientation will give us sagital slices. Repeat this process to glue the second brain hemisphere. Once both hemispheres are fixed in place, any extra glue can be removed using a small piece of filter paper.

Figure 14: Gluing the two halves of the brain in the petri dish for slicing.

15 **Run the Vibratome to obtain multiple brain slices:** Fill the petri dish with chilled Krebs solution (Fig. 15A). Place the tube running from the gas tank into the dish to oxygenate the brain. Set the Vibratome to cut brain slices 200-300 microns thick at the lowest velocity and power. After each slice is cut, move the blade up 50 microns and return it to its original position; this ensures the blade will not touch the brain as it moves backwards. Once in its original position, move the blade back down 50 microns and then down the additional 200-300 microns to cut the next slice. Repeat this process until most of the brain tissue has been sliced. While some slices may fragment, this procedure should result in 6-7 good quality sagital slices from each brain hemisphere (Fig. 15B).

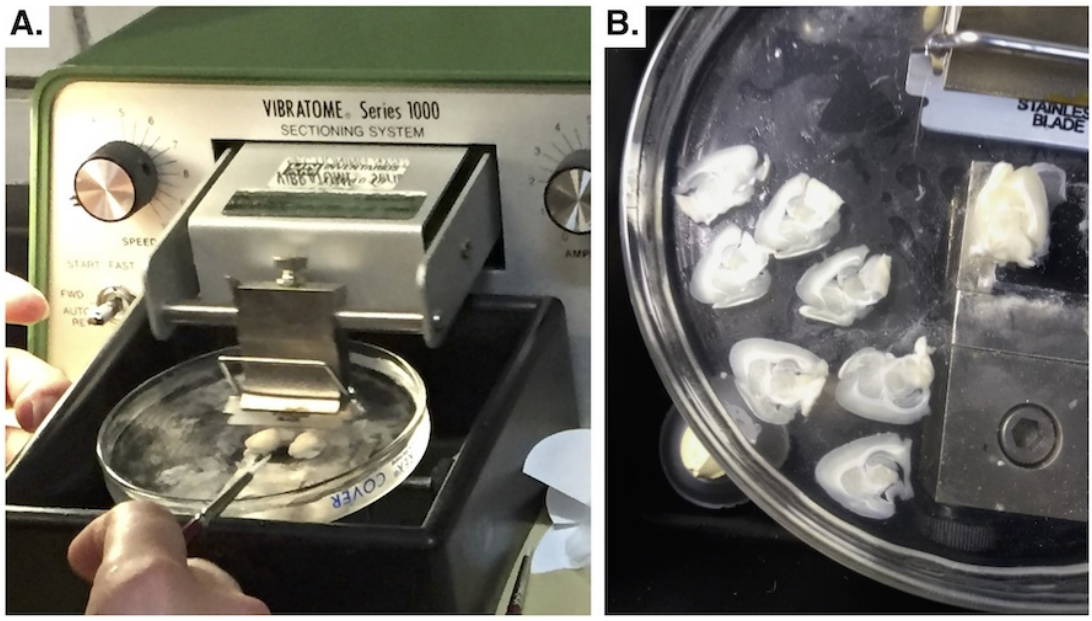


Figure 15: Obtaining sagital brain slices.

16 **Transfer the slices to the incubation chamber or petri dish:** Use a fine-tip paint brush to transfer the slices from the Vibratome dish (Fig. 16A) to the incubating chamber if doing electrophysiology recordings (Fig. 16B), or to a small petri dish if looking at anatomy (Fig. 16C).

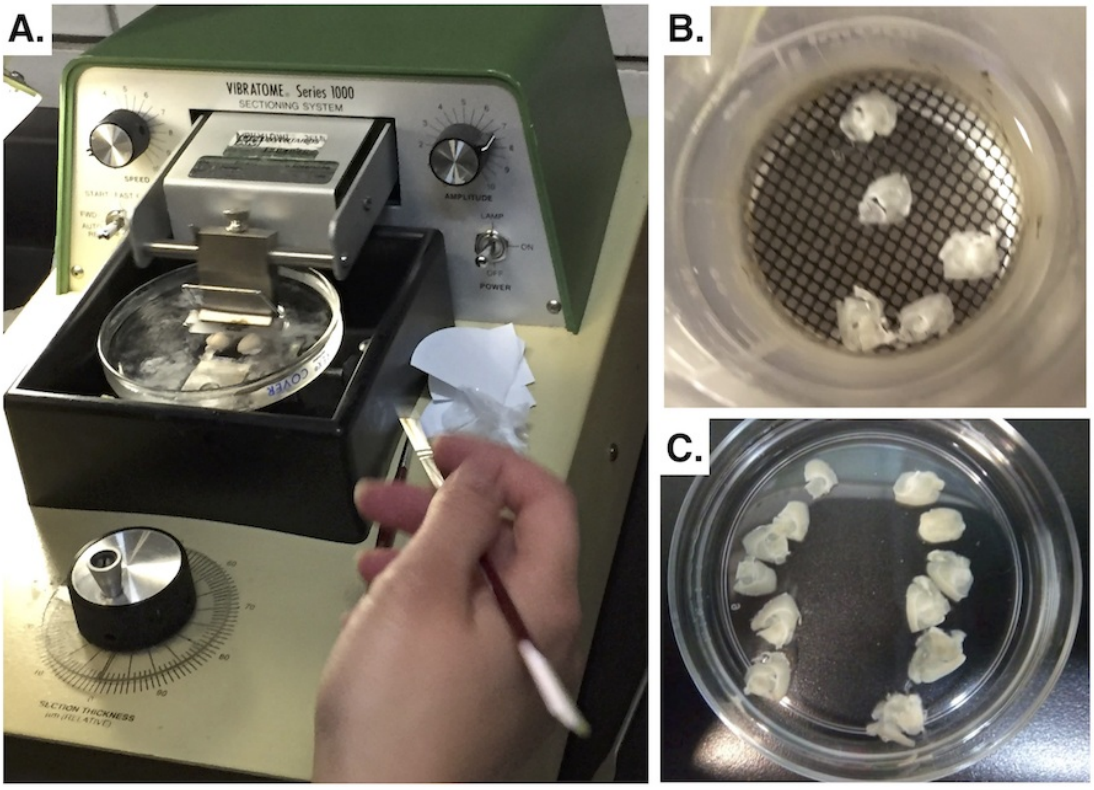


Figure 16: Transferring the slices from the Vibratome to the incubating chamber (B.) or petri dish (C.).

Visualize slices

17 Transfer slices to the light microscope: If using the slices for neuroanatomy instead of electrophysiological recordings, the petri dish containing the brain slices can be placed under a light microscope for visualization (Fig. 17).

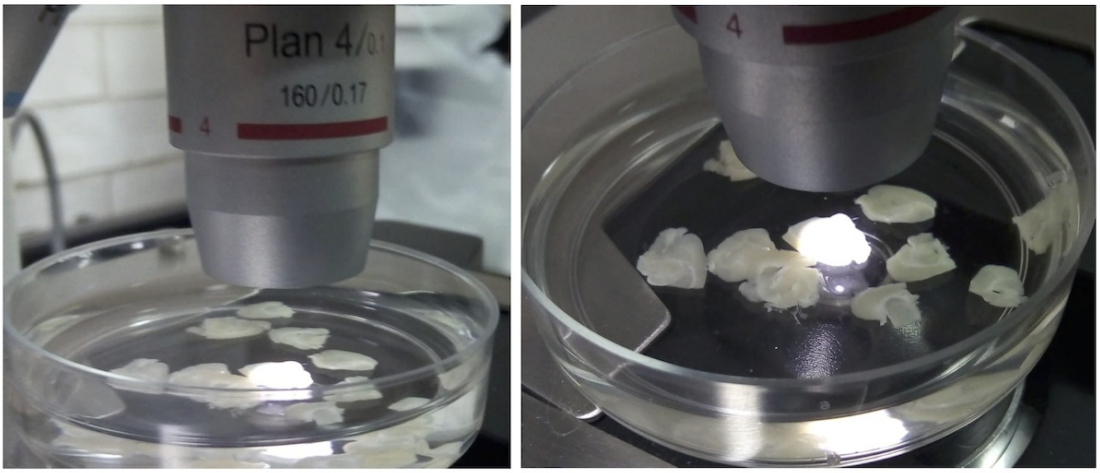


Figure 17: Slices placed under a light microsope with a 4x objective.

18 **Visualize the slices under the microscope:** Slices can be visualized under the light microscope at various magnifications and contrasts to reveal structures such as the hippocampus (Fig. 18B,D,) and striatum (Fig. 18C).

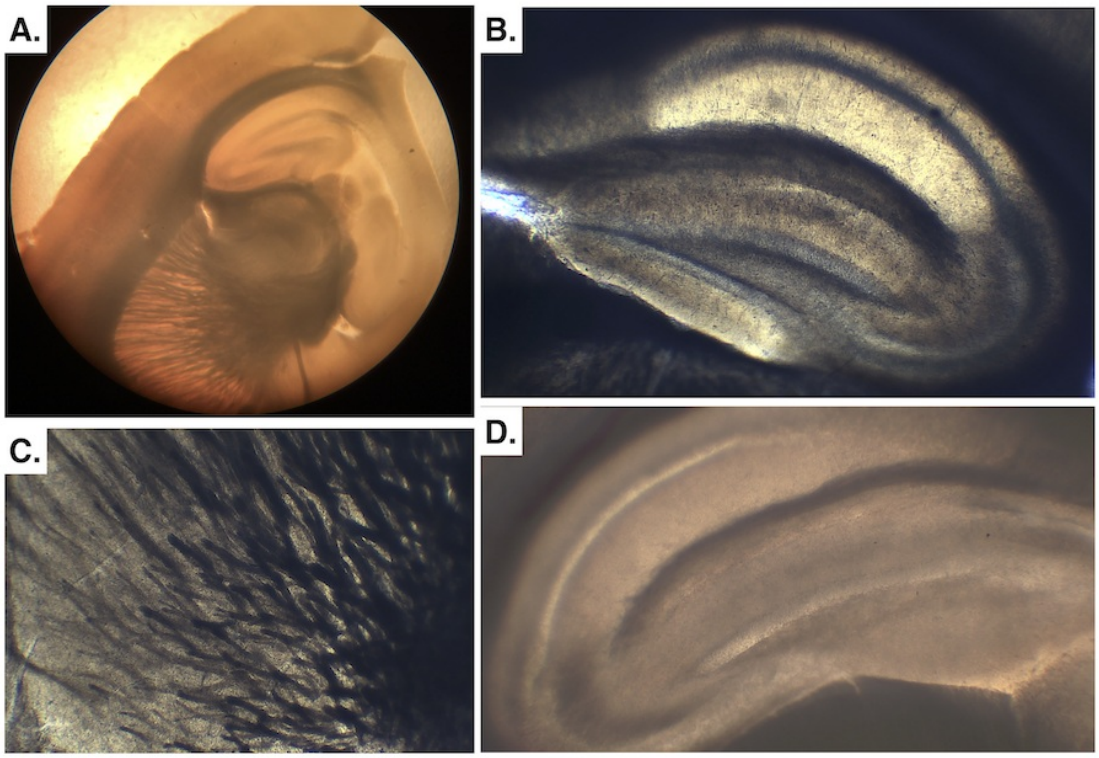


Figure 18: Visualizing sagital brain slices under the microsope.

Clean up

19 **Clean up and properly dispose of biological waste:** Once the procedure is complete, clean all materials and equipment, place all sharps in the red sharps disposal container, and wrap the biological waste in newspaper and place it in the yellow biological waste bag for pickup. The work area should be wiped down with ethanol.