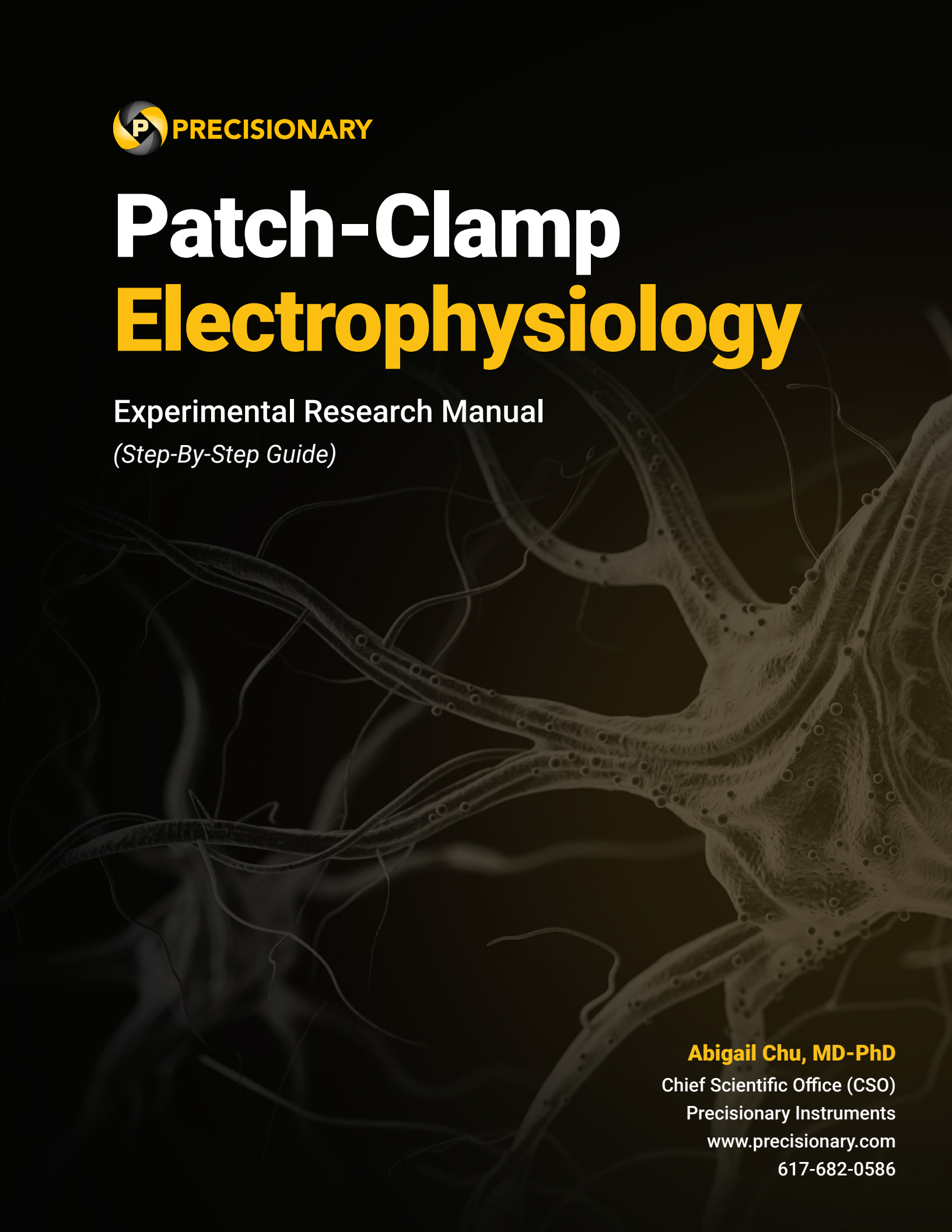
PRECISIONARY

Patch-Clamp Electrophysiology

Experimental Research Manual

(Step-By-Step Guide)



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Goals of this Experimental Guide

Patch-clamp electrophysiology is an essential technique in neuroscience and cellular physiology for studying the electrical properties of cells. This guide aims to provide researchers with a comprehensive understanding of patch-clamp techniques, applications, and experimental protocols. As illustrated in Figure 1, which shows live hippocampal neurons from a mouse acute brain slice, our goal is to help you achieve beautiful, healthy acute brain slices like the plump, round neurons highlighted as ideal candidates for patch-clamp recording.

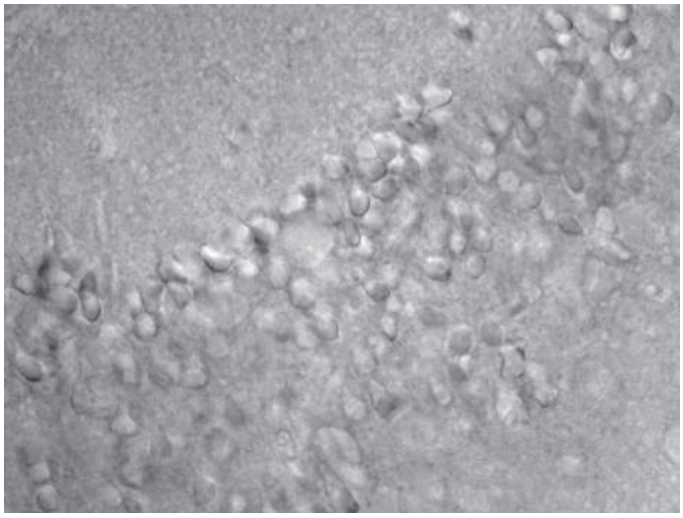

Figure 1. A camera image shows live hippocampal neurons from a mouse acute brain slice, where the plump, round neurons are highlighted as ideal candidates for patch-clamp recording. One of our goals in this research guide is to help you get beautiful, healthy acute brain slices for patching!

What is Patch-Clamp Electrophysiology and Why is it Important?

In the realm of cellular and neuroscience research, scientists have developed various methods to understand how cells communicate and function. Patch-clamp electrophysiology is one such method. It allows researchers to measure the electrical currents of individual cells, providing insights into how cells, particularly neurons, respond to different stimuli and interact with their environment.

While other techniques like calcium imaging or voltage-sensitive dyes have contributed to our understanding of cellular activity, they often lack the

precision and direct measurement capabilities of patch-clamp electrophysiology. This method bridges the gap between traditional electrophysiological recordings and the complex behaviors of cells in a living organism.

As outlined in Figure 2, the general workflow for patch-clamp electrophysiology begins with the preparation of acute brain slices, followed by key steps like transferring the slices to a recording chamber with oxygenated artificial cerebrospinal fluid (ACSF), identifying target neurons, and using micromanipulators to position glass pipettes for cell attachment. This process allows researchers to establish a high-resistance seal with the cell membrane and record the electrical activity of neurons in real-time. This method not only enhances our understanding of cellular mechanisms but also reduces the need for extensive animal models by gathering detailed data from individual cells or small cell groups.

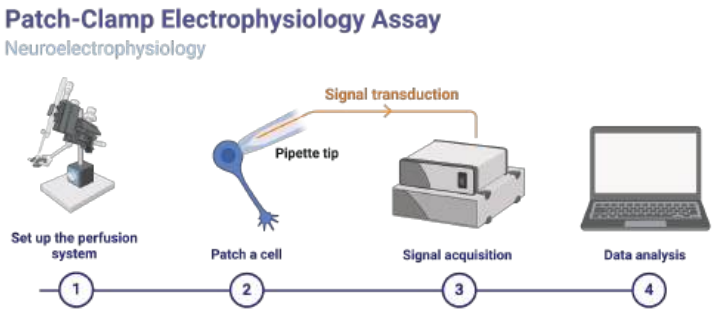

Figure 2. The figure outlines the general workflow for patch-clamp electrophysiology following the preparation of acute brain slices. Key steps include transferring the brain slices to a recording chamber filled with oxygenated artificial cerebrospinal fluid (ACSF), identifying target neurons under a microscope, using micromanipulators to position glass pipettes for cell attachment, establishing a high-resistance seal with the cell membrane, and recording the electrical activity of the neuron to study its physiological properties.

Who This Guide Can Help

This guide is designed to assist a wide range of individuals involved in cellular and neuroscience research, including:

- **New Researchers**: If you're just starting to work with patch-clamp electrophysiology, this guide will walk you through the step-by-step process of setting up and conducting your first experiments.
- **Experienced Scientists**: For those with previous experience, this guide offers troubleshooting tips and advanced techniques to refine and enhance the quality of your patch-clamp procedures.
- **Research Mentors**: If you're responsible for training others in patch-clamp techniques, this manual serves as a valuable teaching resource to ensure your trainees develop a strong foundation in this critical method.
- **Curious Minds**: Anyone interested in deepening their understanding of patch-clamp electrophysiology techniques and applications will find this guide useful, regardless of their expertise level in cellular and neuroscience research.

As illustrated in Figure 3, this guide is crafted to be a comprehensive resource for individuals at all levels of expertise. Whether you're a beginner, an experienced scientist, a mentor, or simply someone eager to expand your knowledge, this guide aims to provide valuable insights and practical guidance to support your research endeavors and enhance your understanding of this powerful technique.

Figure 3. Is this you? This guide serves as a comprehensive resource for individuals at all levels of expertise in patch-clamp electrophysiology, offering valuable insights and practical guidance. Whether you are a beginner, an experienced scientist, a mentor, or simply someone eager to expand your knowledge, this guide is designed to support your research endeavors and enhance your understanding of this powerful technique.

Contact Precisionary Instruments for Any Additional Experimental Support

For further assistance, feel free to reach out to Precisionary Instruments — a leading provider of vibratome equipment and accessories essential for making acute brain slices for patch-clamp electrophysiology. Whether you have technical inquiries, need troubleshooting guidance, or require customized solutions, our expert team is here to support you at every stage of your patch-clamp experiments. Through this guide, we aim to empower researchers with the knowledge and tools necessary to leverage the full potential of patch-clamp electrophysiology in advancing cellular and neuroscience research.

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Let's begin!

Gross Anatomic Location of Mouse Brain Structures

Understanding the gross anatomic location of brain structures is essential for effectively working with acute brain slices for patch-clamp electrophysiology. This section provides a simplified overview of the anatomy of the mouse brain, highlighting key regions and their functions.

Mouse Brain Anatomy

In mice, the brain is divided into several major regions, each responsible for different functions. Here are several mouse brain regions commonly studied for patch-clamp electrophysiology:

- **Cerebrum**: The largest part of the brain, responsible for voluntary actions, sensory perception, and cognitive functions. It is divided into two hemispheres.
- **Cerebellum**: Located at the back of the brain, the cerebellum is involved in motor control and coordination.
- **Brainstem**: Comprising the midbrain, pons, and medulla oblongata, the brainstem controls basic life functions such as breathing, heart rate, and blood pressure.
- **Hippocampus**: A crucial part of the brain for learning and memory, located in the medial temporal lobe.
- **Thalamus**: Acts as a relay station, processing and transmitting sensory information to the appropriate areas of the cerebrum.
- **Hypothalamus**: Regulates various autonomic functions, including hunger, thirst, and temperature control.

Key Differences and Similarities

While there are significant anatomical differences between mouse and human brains, such as size and complexity, mice serve as valuable models for studying brain function and diseases. Mice share fundamental similarities in brain structure and neurophysiological processes with humans, making them indispensable in neuroscience research.

By understanding the gross anatomy of the mouse brain (Figures 4-6), researchers can more effectively prepare and utilize brain slices for patch-clamp electrophysiology, leading to advancements in our knowledge of neural circuits and brain function.

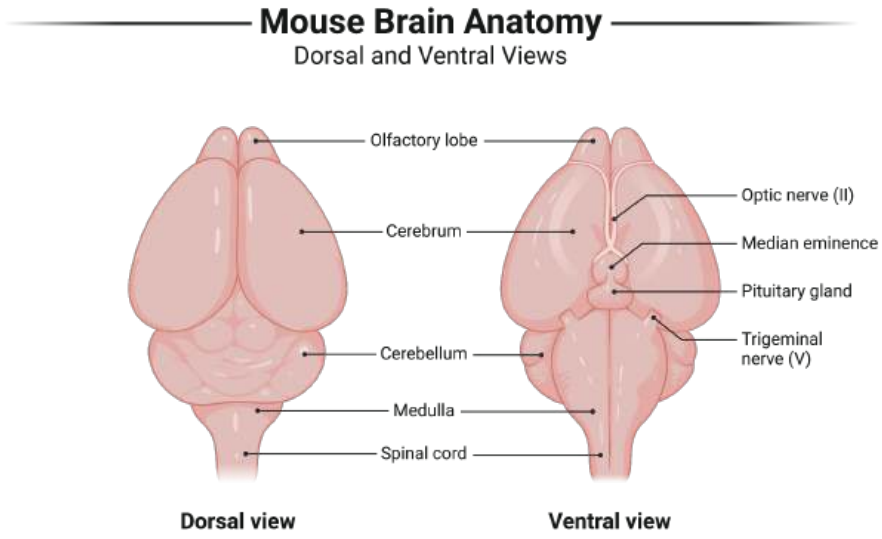


Figure 4. This figure illustrates the mouse brain from both dorsal and ventral perspectives, highlighting key anatomical structures. The dorsal view reveals the cerebrum, divided into hemispheres, along with the cerebellum at the posterior end and the olfactory bulbs at the anterior. The ventral view showcases the brainstem, including the midbrain, pons, and medulla oblongata, as well as the hypothalamus and optic chiasm.

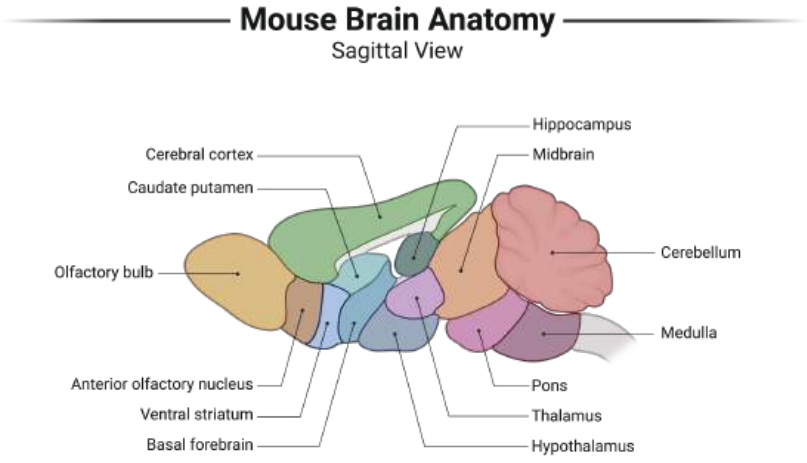


Figure 5. A sagittal cross section of the mouse brain, providing a detailed view of internal anatomical structures. Key regions include the cerebrum, cerebellum, and brainstem, along with the hippocampus, thalamus, and hypothalamus, which are crucial for various cognitive, motor, and regulatory functions. The image highlights the spatial relationships between these regions, offering insights into the brain's complex organization.

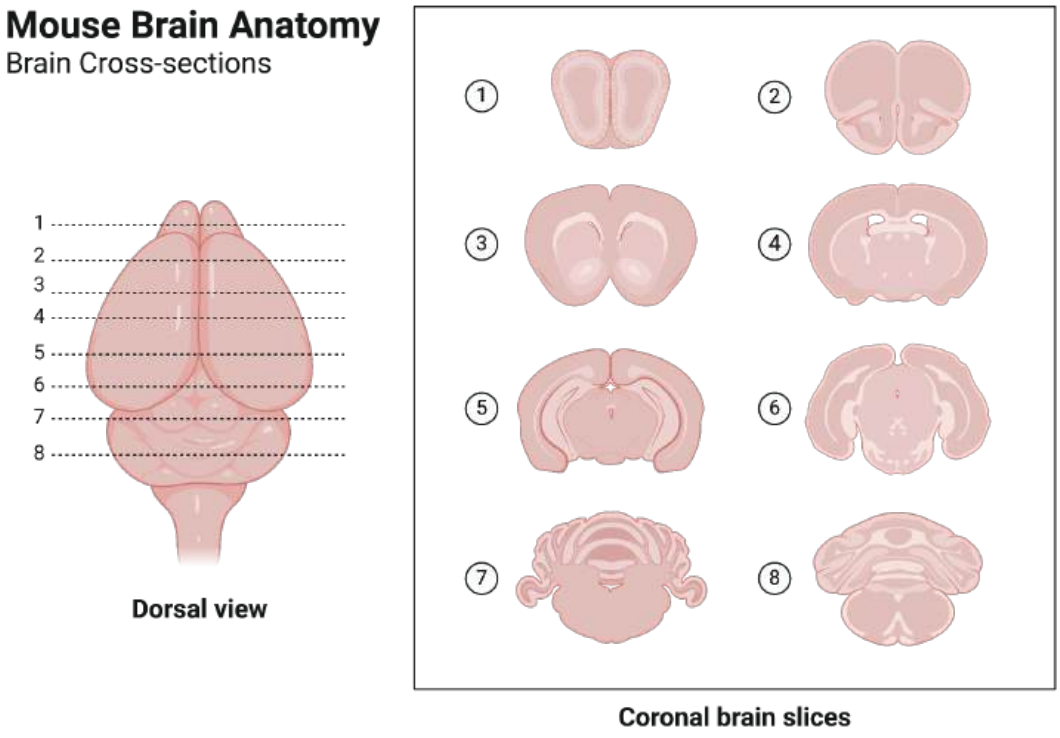


Figure 6. Coronal sections of the mouse brain, displaying detailed anatomical features at different levels. Key structures such as the cortex, hippocampus, thalamus, and ventricles are visible, providing insights into the spatial organization and functional areas within the brain. These sections highlight the brain's symmetry and the relative positioning of important neural regions.

Human Brain Anatomy

The human brain is a highly complex organ, essential for daily life and overall health (Figure 7). Unlike animal models where whole brain slices are often used, patch-clamp studies in humans typically utilize small biopsies or resections obtained directly from the operating room (OR). These samples are crucial for understanding human brain physiology and pathophysiology.

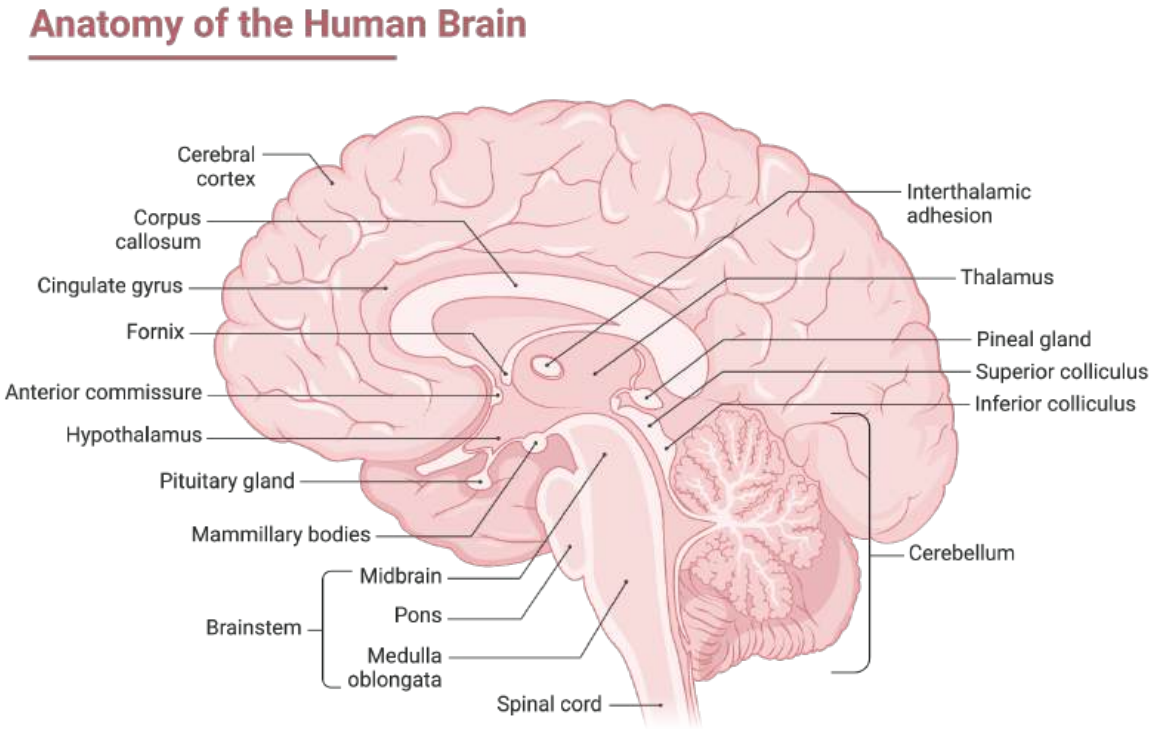


Figure 7. Sagittal cross section of the human brain, illustrating the major anatomical structures. Key regions include the cerebrum, cerebellum, brainstem, and corpus callosum, along with the thalamus, hypothalamus, and hippocampus. This view highlights the organization and connectivity of these regions, emphasizing their roles in cognitive functions, motor control, and regulation of vital processes.

Using Human Brain Tissue in Patch-Clamp Studies

In patch-clamp electrophysiology, small biopsies or resections of the human brain, typically obtained directly from the OR during surgical procedures, are used. These samples allow researchers to study the electrical properties of human neurons in detail. The direct use of human brain tissue provides invaluable insights into the specific characteristics of human neural circuits, which can differ significantly from those of animal models.

By understanding the detailed anatomy of the human brain and utilizing small, precise tissue samples, researchers can better investigate and develop treatments for neurological diseases and disorders.

This approach bridges the gap between laboratory research and clinical applications, enhancing our ability to translate findings into real-world medical advancements.

Understanding the gross anatomical differences between mouse and human brains is crucial for translational research, where findings from animal models like mice are extrapolated to human physiology and pathology. While mice serve as valuable models for studying brain biology and disease, researchers must consider species-specific anatomical variations when interpreting experimental results and designing therapeutic interventions. Recognizing these differences ensures that insights gained from mouse studies are accurately applied to human health, enhancing the relevance and effectiveness of neurological research.

Essential Materials and Reagents for Patch-Clamp Electrophysiology

In conducting patch-clamp electrophysiology experiments, it's imperative to have the appropriate materials and reagents ready for use. Below are categorized lists of essential equipment, lab accessories, and reagents required for this procedure:

Equipment

- Patch-clamp amplifier
- Micromanipulators
- Inverted microscope
- Vibration isolation table
- Data acquisition system
- Vibrating microtome (Compresstome® vibratome)

Lab Accessories

- Micropipettes and pipette puller
- Recording chamber
- Perfusion system
- Faraday cage
- Headstage holder

Reagents

- Intracellular solution
- Extracellular (bath) solution
  - Cutting solution
  - Recording/incubation solution
- Agarose for brain slicing
- Enzyme solutions (e.g., trypsin) for cell dissociation
- Seal-enhancing substances

Having these materials and reagents prepared and organized will streamline your patch-clamp experiments and ensure accurate and reliable results.

Solutions & Recipes for Patch-Clamp Electrophysiology

This section provides recipes and instructions for making solutions for patch-clamp electrophysiology experiments.

External Solutions

External solutions are designed to mimic the ionic composition of the physiological fluid that the plasma membrane is exposed to in vivo or in culture. The 'extracellular solution' (ECS) is used as the bathing solution and primarily consists of sodium chloride. The pH and osmolarity of these solutions are critical for obtaining good seals, often more so than the ionic composition.

Table 1: Example of Typical Extracellular Solution (ECS) Used in Patch Clamping a Mammalian Cell

Chemical	ECS Concentration (mM)
Na+	126
K+	6
Mg2+	2.5
Ca2+	1.2
Cl-	125
GTP	0
ATP	0
HEPES	10
Glucose	11
Sucrose	67

Typical pH and Osmolarity of External Solution:

- pH: Typically set using NaOH for ECS (around 7.4) and KOH for ICS (around 7.2)
- Osmolarity: ECS is typically around 300-310 mOsm, and ICS is around 290-300 mOsm

It is important to oxygenate the external solution (ACSF) with 95% O2 / 5% CO2, a process commonly referred to as "carbogen."
External solutions are usually made in large batches and then divided to create "cutting solution" and "recording/incubation solution."

Cutting Solution:

- Used for cutting acute brain slices
- Typically low calcium (approximately 0.1 mM Ca) and high magnesium (approximately 3 mM Mg)

Recording/Incubation Solution:

- Used in the perfusion chamber and during the post-cutting incubation period
- Typically 2 mM Ca and 1 mM Mg

→ Why Low Calcium and High Magnesium During Brain Slicing? Low calcium and high magnesium are used during brain slicing to reduce the excitability of neurons and protect them from excitotoxicity. This creates a more stable environment for cutting and helps maintain the integrity of the brain slices for subsequent electrophysiological recordings.

Internal Solutions

The 'intracellular solution' (ICS) used to fill pipettes for whole-cell and outside-out excised patch recording contains a high concentration of potassium. This composition is essential for mimicking the intracellular environment and ensuring accurate recordings of cellular electrical activity.

Table 2: Example of Typical Intracellular Solution (ICS) Used in Patch Clamp Electrophysiology

Chemical	ICS Concentration (mM)
Na+	5
K+	147
Mg2+	1.2
Ca2+	0
Cl-	150
GTP	0.1
ATP	5
HEPES	20
Glucose	11
Sucrose	0

When preparing ICS, it's crucial to carefully monitor the pH, as the inclusion of chemicals such as EGTA for quenching calcium ions can make the solution very acidic. The desired pH for ICS is slightly more acidic, around 7.2 to 7.3, to match the intracellular conditions.

Tips for Making and Storing Internal Solution for Patch-Clamping:

1. **Accurate pH Adjustment**:
   - Always measure the pH after all components have dissolved.
   - Use KOH to adjust the pH to the desired range (7.2 – 7.3).
   - Be cautious when adding strong bases or acids, as small volumes can cause significant pH shifts.
2. **Preparation and Sterilization**:
   - Prepare solutions using high-purity reagents and ultrapure water to avoid contamination.
   - Sterilize the solution by filtering it through a 0.22 µm filter to remove particulates and potential microbial contaminants.
3. **Storage**:
   - Store internal solutions in small aliquots to minimize freeze-thaw cycles, which can degrade components.
   - Keep the aliquots at -20°C for long-term storage and thaw only as needed.
   - Label aliquots clearly with the date of preparation and the composition.
4. **Avoiding Precipitation**:
   - Ensure all salts are fully dissolved to prevent precipitation, which can block pipettes and affect recordings.
   - Vortex the solution thoroughly after adding each component.
5. **Handling Calcium Chelators**:
   - When using calcium chelators like EGTA or BAPTA, add them slowly to the solution while stirring continuously to prevent localized precipitation.
   - Ensure complete dissolution before adjusting the pH.

By following these guidelines, you can prepare and store internal solutions that are stable, reliable, and suitable for high-quality patch-clamp electrophysiology experiments.

Final Tips and Hints for Making External and Internal Solutions for Patch-Clamping

When preparing solutions for patch-clamp electrophysiology, a few key factors can make a significant difference in the success of your experiments. Here are some friendly, easy-to-understand tips and hints to keep in mind:

Importance of Osmolarity

Osmolarity is crucial but often underrated. Both the absolute osmolarity and the difference between solutions on either side of the membrane are critical for success. Differences in osmolarity can affect cell volume and exert osmotic force on the membrane. If the osmolarity difference is too large, cells can die. Always ensure your solutions have compatible osmolarity levels.

Practical Solution Preparation

- **Stock Solutions**: It's practical to keep a stock bathing solution at tenfold strength. This way, you can prepare the exact quantity you need on the day of your experiment. This approach saves time and ensures consistency.
- **Omitting Calcium and Glucose in Stock Solutions**: Calcium and glucose are often omitted from stock solutions. This prevents the precipitation of calcium salts and the growth of microorganisms. Add these components fresh on the day of the experiment.
- **Intracellular Solutions**: ICS often contains perishable chemicals like ATP. Make these solutions in batches and aliquot them into small portions. Keep these aliquots frozen and thaw only as needed to maintain their effectiveness.

Additional Tips Helpful Hints

- **Accurate pH Adjustment**: Always adjust the pH after all components are dissolved. Use a pH meter and add NaOH or KOH carefully to avoid large pH shifts.
- **Sterilization**: Filter solutions through a 0.22 µm filter to remove particulates and potential contaminants. This step is especially important for intracellular solutions.
- **Labeling**: Clearly label your solutions with the date of preparation and their components. This practice helps you keep track of solution freshness and composition.
- **Avoiding Precipitation**: Ensure all salts are fully dissolved to prevent blockages in pipettes and disruptions in recordings. Vortex thoroughly after adding each component.
- **Storage**: Store solutions in appropriate conditions. External solutions can often be kept at room temperature if used within a short period, while intracellular solutions should be frozen in aliquots to preserve their integrity.
- **Voltage Clamp Experiments**: If the evoked responses at the calyx are too large, try adding 2 mM QX314 (N-(2,6-dimethylphenylcarbamoylmethyl) triethylammonium chloride) to the internal solution.
- **Sealing with CsF Internal**: The CsF internal allows for very easy sealing. If fluoride does not interfere with your experiments, try using the CsF internal instead of Cs-gluconate for patching.
- **Preventing Precipitation in ACSF**: Bubble the 1X ACSF first before adding Ca2+ to prevent precipitation. You'll get precipitation when your solution looks “cloudy” upon adding Ca2+.
- **Preparing and Storing CaCl2**: Prep a 1 M CaCl2 solution and keep it in the 4°C fridge. You can use this stock each time when adding Ca2+ to your 1X ACSF.
- **Buying MgCl2**: The 1 M MgCl2 solution can be purchased directly from Sigma.

By following these practical tips and helpful hints, you'll ensure your external and internal solutions are prepared correctly, leading to more successful and reliable patch-clamp experiments.

Agarose Solution

Ingredients:

- 1.5 g low-melting point agarose powder
- 100 ml HBSS/HEPES buffer

Instructions:

1. Measure out 1.5 grams of low-melting point agarose powder using a laboratory balance.
2. In a suitable container, such as a glass beaker or flask, pour 100 ml of HBSS/HEPES buffer.
3. Add the measured agarose powder to the HBSS/HEPES buffer.
4. Microwave the mixture in short intervals, stirring occasionally, until the agarose powder is completely dissolved. Be cautious not to overheat the solution.
5. Once the agarose is fully dissolved, allow the solution to cool slightly before use in lung tissue processing.

Note: This recipe yields 100 ml of 2% agarose solution, suitable for processing multiple lung samples. Adjust the quantities accordingly if a smaller or larger volume is required.

Alternative Method using Agarose Tablets (Precisionary Instruments):

- Instead of low-melting point agarose powder, use agarose tablets provided by Precisionary Instruments.
- Each tablet is 0.5 grams, so use three tablets for a total of 1.5 grams to achieve a 2% agarose concentration.
- Mix the agarose tablets with 75 ml of HBSS/HEPES buffer (25 ml per tablet) to yield a 2% agarose solution.
- Follow the same procedure as described above for dissolving the agarose tablets in the buffer solution using the microwave.

Note: The use of agarose tablets provides a convenient and precise method for preparing agarose solution, ensuring consistent results in lung tissue processing. Adjust the volume of buffer solution accordingly to achieve the desired agarose concentration. Here is a visual guide for making agarose solution using tablets:

70% Ethanol

Ingredients:

- 70 ml absolute ethanol
- 30 ml distilled water

Instructions:

1. Measure out 70 ml of absolute ethanol using a graduated cylinder or measuring beaker.
2. Measure out 30 ml of distilled water using a separate graduated cylinder or measuring beaker.
3. Combine the measured amounts of absolute ethanol and distilled water in a clean container, such as a glass bottle or flask.
4. Mix the ethanol and water thoroughly by gently swirling or stirring the solution with a clean stir rod or spatula.
5. Once mixed, the solution will be 70% ethanol. Label the container appropriately with the concentration and contents.
6. Store the 70% ethanol solution in a tightly sealed container at room temperature. Ensure that the container is properly labeled and stored away from direct sunlight or heat sources.

Note: Always prepare ethanol solutions in a well-ventilated area and avoid inhaling the vapors. Use caution when handling ethanol as it is flammable. Dispose of any unused or expired ethanol solution according to local regulations for hazardous waste disposal.

Making Acute Brain Slices from Mouse

Now that we've gained a quick understanding of mouse and human brain anatomy, let's delve into the process of preparing acute brain slices. We'll start with the mouse model, as it is the most commonly used mammalian model in basic neuroscience research.

Preparing for Slicing Mouse Brain Slices

1. **Prepare Solutions**:
   - Make the cutting solution (0.1 mM Ca2+/3 mM Mg2+).
   - Make the recording solution (2 mM Ca2+/1 mM Mg2+).

2. **Measure Solutions**:
   - For each brain, you will need approximately 250 mL of cutting solution.
   - Pour some of the cutting solution into the recording chamber to cover just the bottom. This amount of solution will freeze and keep the chamber cold by acting like an ice cube (See Figure 3).
3. **Chill Solutions and Equipment**:
   - Freeze the cutting solution and slice chamber at -20°C.
   - Chill the orienting disk at 4°C.
4. **Prepare the Slice Area**:
   - Set up your slicing area with all necessary tools and equipment (See Figures 4-5).
5. **Prepare for Slicing**:
   - Once you are almost ready to begin, take the cutting solution out of the freezer and bubble it with carbogen (95% O2/5% CO2) for approximately 5 minutes before you start slicing.

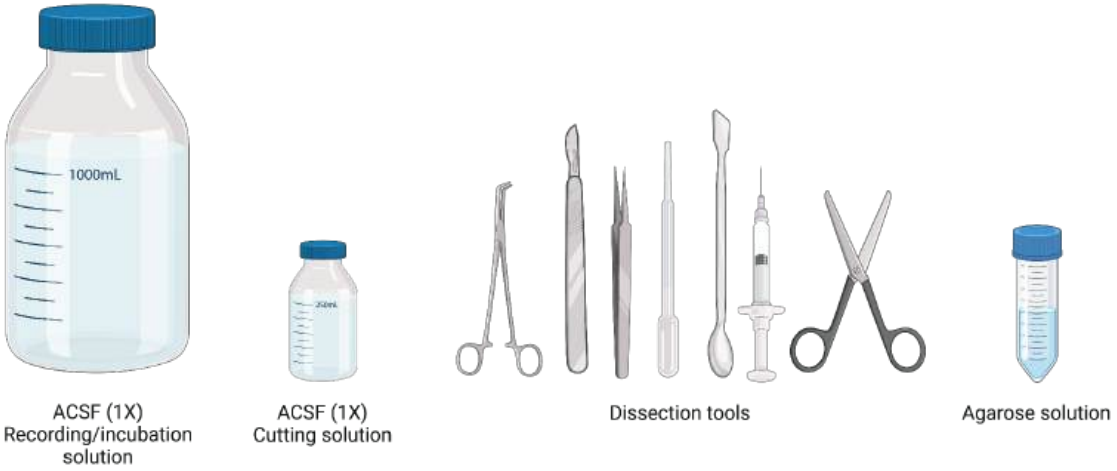


Figure 8. Preparing the Slice Area and Dissection Tools. Ensure all solutions are bubbled with carbogen (95% O_2/5% CO_2) before slicing, and fill the incubation chamber with cutting solution containing 0.1 mM Ca^{2+} and 3 mM Mg^{2+}. Set up the slicing area with the necessary tools and equipment as shown. A close-up view highlights essential dissection tools, including fine forceps, razor blades, and a transfer pipette for moving slices. Additionally, a syringe with a bent needle tip, critical for detaching brainstem slices from the cerebellum, is detailed.

Dissection of the Mouse Brain for Slicing

Follow these step-by-step instructions to dissect the mouse brain for slicing:

1. **Anesthetize the Animal**: Ensure the animal is fully anesthetized by confirming the lack of response to a tail-pinch and toe-pinch.
2. **Decapitate the Head**: Make an incision down the top of the skull and pull back the skin to expose the skull/cranium.
3. **Open the Skull**: Cut down the midline of the skull, keeping close to the bone to avoid damaging the underlying tissue. Then, make horizontal cuts along the bregma and lambda lines on each side. Peel back the skull pieces to expose the brain.
4. **First Cut**: Make the first cut between the brainstem and spinal cord. The yellow arrows in Figure 6 show where this cut is made, separating the brainstem from the spinal cord.
5. **Second and Third Cuts**: Make the second cut between the cortex and cerebellum. The third cut is near the anterior part of the cortex and olfactory bulbs.
6. **Remove the Cortex**: Using fine forceps, carefully remove the cortex, leaving the cerebellum and brainstem intact.

Note: During the brain dissection process, you can isolate and dissect specific brain regions required for your patch-clamp experiments. Additionally, further dissect the extracted brain tissue to optimize the orientation for cutting.

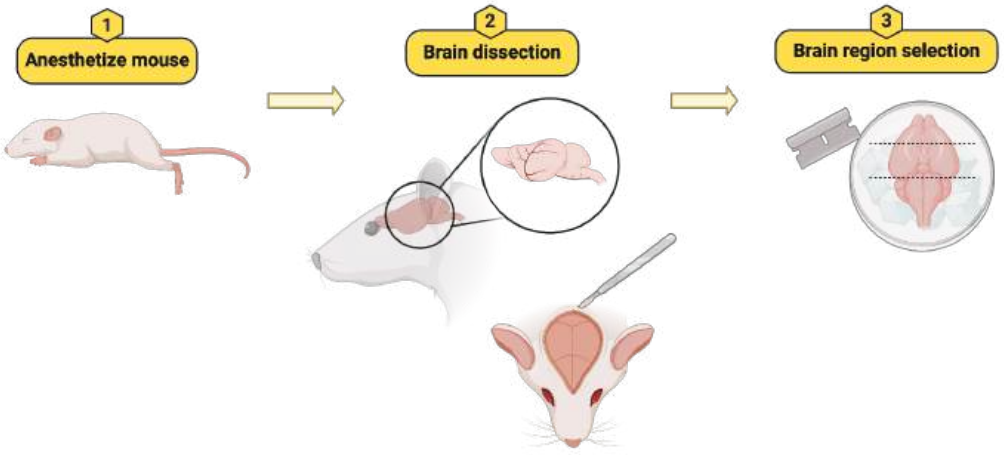


Figure 9. Step-by-step dissection of the mouse brain for slicing, starting with anesthetization, followed by decapitation and opening of the skull to expose the brain. Key cuts are made between the brainstem and spinal cord, the cortex and cerebellum, and near the anterior cortex. The cortex is carefully removed, leaving the cerebellum and brainstem intact. Yellow arrows in Figure 6 indicate the precise locations of the cuts. This process allows for the isolation and further dissection of specific brain regions for patch-clamp experiments.

Sectioning Acute Brain Slices on the Compresstome Vibratome

Embedding Brain Tissue into a Specimen Tube: A Step-by-Step Guide

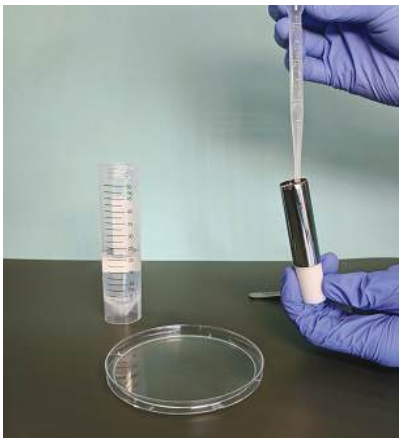
Before embedding the brain tissue into a specimen tube, it's essential to properly prepare both your sample and tools to ensure successful mounting and preservation. The following steps will guide you through the process to achieve optimal results.

1. **Chill the Block:** Start by placing the chilling block in an ice bath or freezer for at least 1 hour before use. It's important that the block is well-chilled before embedding your tissue sample.
2. **Prepare Your Tissue Sample:** Cut your tissue sample so it fits neatly inside the specimen tube. Then, squeeze a small amount of super glue onto the base of the specimen tube, covering an area roughly the size of your tissue. Be careful to avoid any glue spilling down the sides of the plunger; if it does, the metal casing might get stuck. If this happens, soaking the tube in acetone can help dissolve the glue.

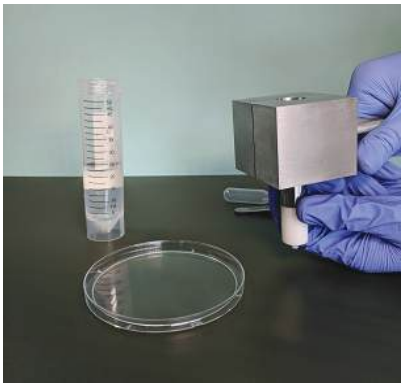
3. **Position the Tissue:** Use forceps to carefully place the tissue onto the glued base of the specimen tube. Allow the glue to cure fully. If needed, cut a flat surface on the tissue to ensure it adheres securely to the tube.

4. **Insert the Tissue:** Gently withdraw the specimen tube base downward until the tissue sample is fully enclosed within the tube.

5. **Add Agarose:** Pipette agarose into the tube, making sure the sample is completely covered. Tap the side of the tube to remove any bubbles, or use a pipette to suction them out.



6. **Solidify the Sample:** Finally, place the pre-chilled chilling block over the specimen tube to cool the entire sample and solidify the agarose. This should take about 30 seconds to 1 minute, depending on the size of your sample.

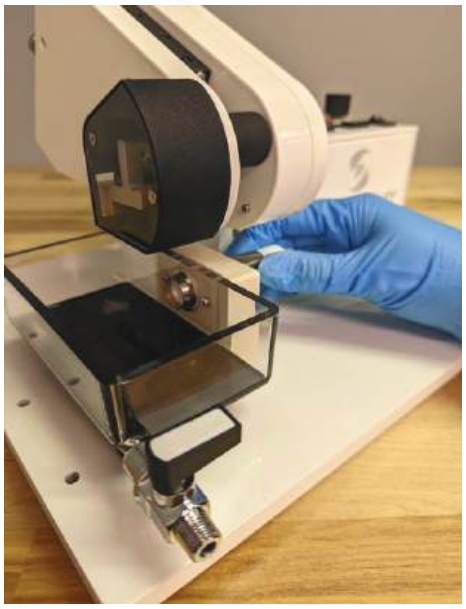


Cutting Acute Brain Slices on the Compresstome Vibratome

After embedding your tissue, the next step is to prepare your Compresstome Vibratome for precise brain slicing. Follow these instructions to ensure accurate and consistent sectioning.

1. **Prepare the Control Box:** Ensure the sliding control box is pushed back away from the buffer tray. To do this, press down on the lever on the right side of the box, slide the control box back until it locks in place, and then remove the lever.

2. **Insert the Specimen Tube:** Insert the tapered end of the specimen tube into the buffer tray. Push the tube fully in until the stopper ring meets the adapter base of the buffer tray, which will prevent further insertion, ensuring proper positioning for sectioning.



3. **Position the Control Box:** Once the specimen tube is removed, bring the sliding control box back in front of the buffer tray by pressing the same lever on the right and sliding the box towards you. Ensure the micrometer is touching the back of the specimen tube before starting the sectioning process. You can adjust the micrometer using the 'Fast Forward' and 'Fast Reverse' functions on the control box.

4. **Attach the Blade Holder:** Slide the prepared blade holder onto the axial bar of the vibrating unit and lock it in place using the included Allen key. The blade should face the control box and the specimen tube.

5. **Set Up and Power On:** Plug one end of the power cord into the VF-510-0Z machine and the other into a standard power outlet. Turn the power on, and use the control box to set the slice thickness, sectioning speed, and oscillation frequency to meet your experimental requirements.

6. **Begin Sectioning:** To achieve optimal slice consistency, start cutting approximately 50-100 microns higher than your target thickness. Begin sectioning in continuous mode, gradually reducing the thickness in smaller increments until the desired thickness is reached.
   Example 1: For a desired slice thickness of 20 µm, start sectioning at 120 µm and reduce in increments (110 µm, 100 µm, 90 µm, etc.) until reaching 20 µm.
   Example 2: For a desired slice thickness of 250 µm, start sectioning at 300 µm and reduce in increments (290 µm, 280 µm, 270 µm, etc.) until reaching 250 µm.

Continue slicing with the VF-510-0Z, adjusting the sectioning speed and frequency as necessary to maintain precision.

Collecting and Incubating Acute Brain Slices

After cutting acute brain slices, it is crucial to incubate them at 37°C or physiological temperature immediately. This step is essential because maintaining the brain slices at physiological temperature helps preserve their cellular integrity and function, ensuring that they remain viable and responsive for subsequent experiments, such as patch-clamp recordings.

To prepare for this, prewarm your incubation chamber filled with artificial cerebrospinal fluid (ACSF) by placing it in a hot water bath set to 37°C. After the brain slices are cut, use a plastic transfer pipette with one end cut off to create a larger opening, and gently transfer each slice into the prewarmed incubation chamber.

Typical incubation times range from 30 to 60 minutes at 37°C. After this incubation period, remove the chamber containing the slices and proceed with your patch-clamp recording experiments to ensure optimal results.

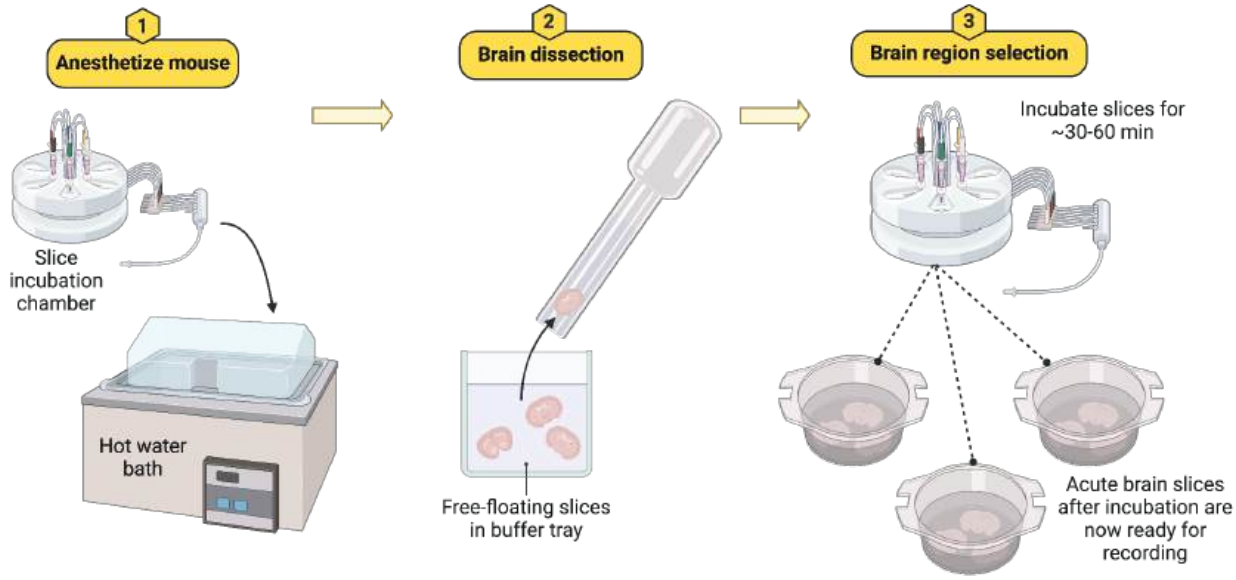


Figure 10. Acute brain slices are incubated at 37°C immediately after cutting to preserve cellular integrity and function. The slices are gently transferred into a prewarmed incubation chamber filled with ACSF, using a modified plastic pipette. After a 30-60 minute incubation period, the slices are ready for patch-clamp recording experiments.

Recording from Acute Brain Slices

The purpose of this section is to guide you through the actual procedure of making a gigaseal on a cell membrane and establishing a patch-clamp configuration. We'll assume your setup is complete and operational.

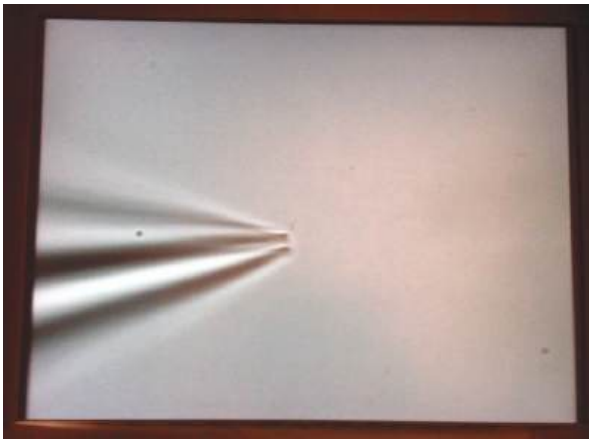
Making Recording Pipettes

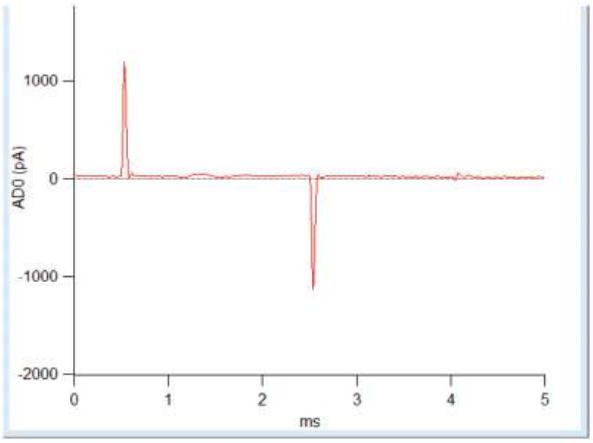
1. Prepare the Pipettes:
   - Quick Tip: The patch pipette should be made and used immediately to reduce tip contamination and ensure good sealing properties.
   - Fire-Polishing: The critical step is fire-polishing the tip, which cleans it and improves seal quality. Pipettes can be pulled and stored in dust-free batches, so they only need fire-polishing and filling just before use.
   - Mount the Pipette: After preparing and mounting the pipette in its holder, position the tip above the bathing solution, directly over the preparation.

Lowering the Pipette Towards the Target Cell

1. Apply Pressure:
   - Prevent Contamination: Before lowering the pipette into the bathing solution, apply slight pressure on the pipette fluid to blow away any contaminants. Contaminants often gather at the fluid-air interface, so maintain pressure before crossing this boundary.
   - Watch the Pressure: Use about 10 cm of water pressure. Too much pressure can blow away your cells!
2. Guide the Pipette:
   - Practice Makes Perfect: Carefully guide the pipette towards the target cell. This step requires practice, and breaking some pipettes while learning is normal.
   - Three-Dimensional Awareness: Be aware of the three-dimensional movement of the manipulator. Know where the focal point of the microscope is situated.
   - Approach the Cell: When approaching the cell, position the focal point between the cell and the pipette.

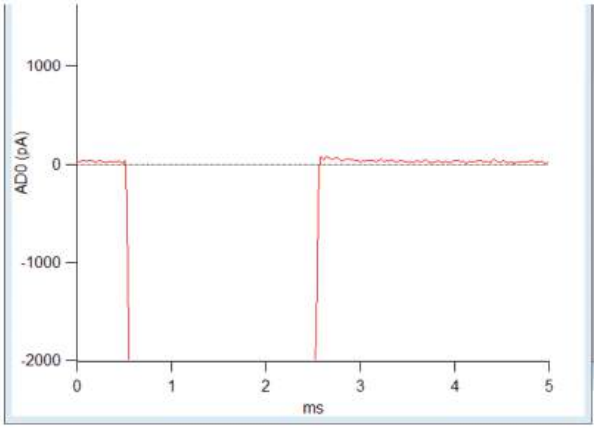
The following figure shows the correct technique for lowering the pipette towards the target cell. Notice the positioning and the slight pressure applied to avoid contaminants.





The recording pipette has not yet been immersed in the external solution, so the screen should display a baseline or near-zero current with no significant electrical activity. The resistance will be high, and there will be minimal noise, indicating that the pipette tip is still in the air and not in contact with the solution.

Locate your pipette under the microscope objective. Carefully adjust the focus until the pipette tip is clearly visible. Slowly lower the pipette using the micromanipulator controls, positioning it just above the surface of the brain slice. Ensure that the pipette tip remains in clear view and is precisely aligned with the targeted area on the slice before proceeding to the next step.

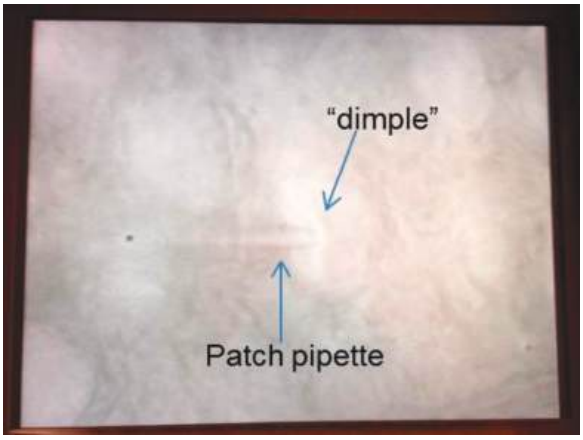


Once the pipette is placed into the bath, the screen should display a stable baseline current, reflecting the electrical properties of the external solution. The resistance reading in this example shows approximately 2.5 MΩ, indicating proper contact between the pipette and the solution. At this point, it's important to correct for the liquid junction potential (LJP) to ensure accurate measurements during your recordings.

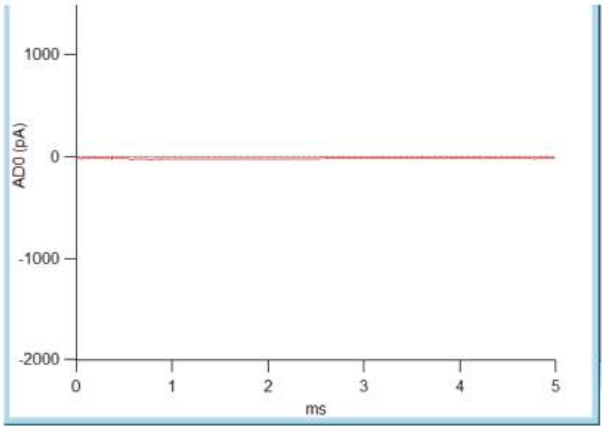
Making the Gigaseal

1. Final Positioning:
    - Contact: The pipette tip should make complete contact with the cell membrane without stressing it. Aim for a spot on the cell that faces the pipette tip straight on.
    - Baseline Nulling: Position the pipette tip near the cell using the fine micromanipulator. Perform final baseline nulling using the amplifier's offset knob.
2. Seal Formation:
    - Monitor Approach: Ideally, monitor the final approach on the microscope and the oscillating test pulse simultaneously.
    - Near-Contact Indication: Near-contact is indicated by a dip in the test pulse response due to increased resistance.
    - Remove Pressure: Remove the pipette pressure, causing a significant drop in the test pulse response. Apply a little suction—usually by mouth—to achieve a gigaseal. A successful seal is indicated by a near absence of current response to a test pulse. The resistance should be more than 1 GΩ for most whole-cell recording applications and even higher for single-channel recording.

Helpful Figure: The following figure illustrates the steps to achieve a gigaseal, including baseline nulling and monitoring the test pulse response.



Apply a small amount of positive pressure to the recording pipette and slowly lower it towards the principal cell. As the pipette approaches the cell surface, observe for a small "dimple" or



When the pipette has successfully gigasealed the cell, the electronics will display a sudden and significant increase in resistance, typically reaching values in the GΩ range. The baseline

indentation where the pipette makes contact with the cell membrane.
Once this dimple is visible, promptly release the positive pressure, allowing the cell membrane to adhere to the pipette tip and form a high-resistance gigaseal. current will stabilize, and the noise level will decrease, indicating a tight seal between the pipette and the cell membrane. This high-resistance gigaseal is essential for accurate recording of the cell's electrical activity.

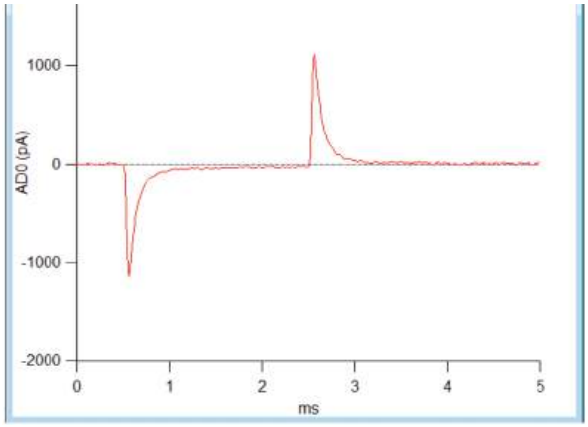
Establishing the Type of Patch-Clamp Recording

Once you have successfully formed a gigaseal and ensured the cell's stability, the next step is to establish the specific type of patch-clamp recording suited to your experimental needs. Different configurations, such as whole-cell recording, perforated patch recording, and single-channel recording, each have unique requirements and procedures. This section will guide you through the process of transitioning to and optimizing these various patch-clamp techniques.

Whole-Cell Recording

1. Gigaseal Formation: Once you have a gigaseal, break the patch of membrane under the pipette tip while maintaining the seal resistance. This allows the pipette solution to contact the cytoplasm.
2. Breaking the Patch:
    - Suction Pulse: Apply a suction pulse through the pipette pressure tubing by mouth. The amount and type of suction depend on the pipette size, cell type, and setup.
    - Zap Function: Alternatively, send a large current pulse through the pipette using the 'zap' function on some patch clamp amplifiers.

Helpful Figure: The following figure shows the steps to transition from gigaseal formation to whole-cell recording, including the use of suction or zap function.



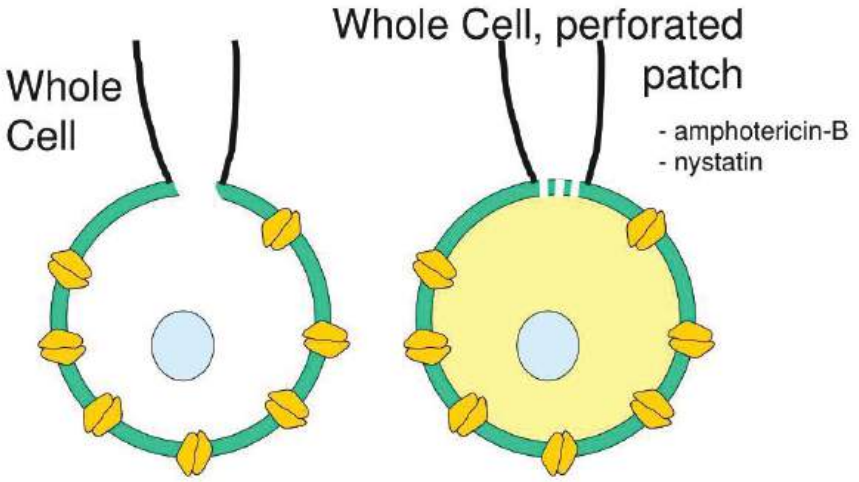
After breaking into the cell using gentle, quick suction on the recording pipette, the electronics will show a sudden drop in resistance, typically down to a lower value consistent with the cell's membrane resistance. The baseline current will adjust to a new level, and you'll observe a change in the noise pattern, indicating that you are now recording from the interior of the cell. This marks the transition to whole-cell configuration, allowing for the measurement of intracellular electrical activity.
You can now begin recording!!

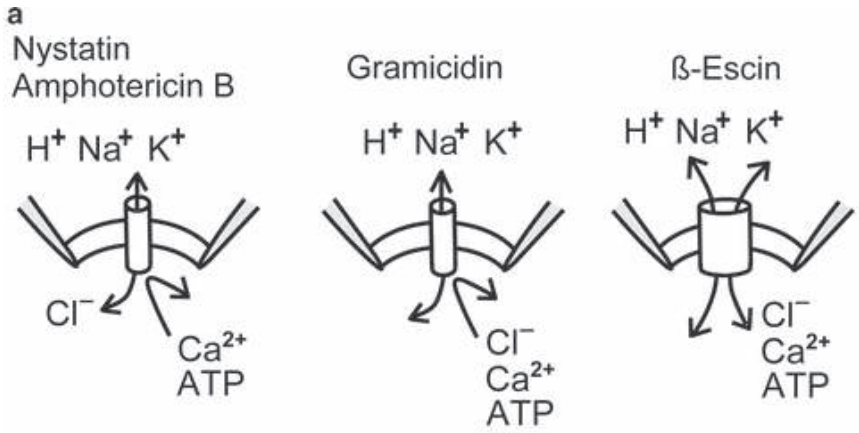
Perforated Patch Recording

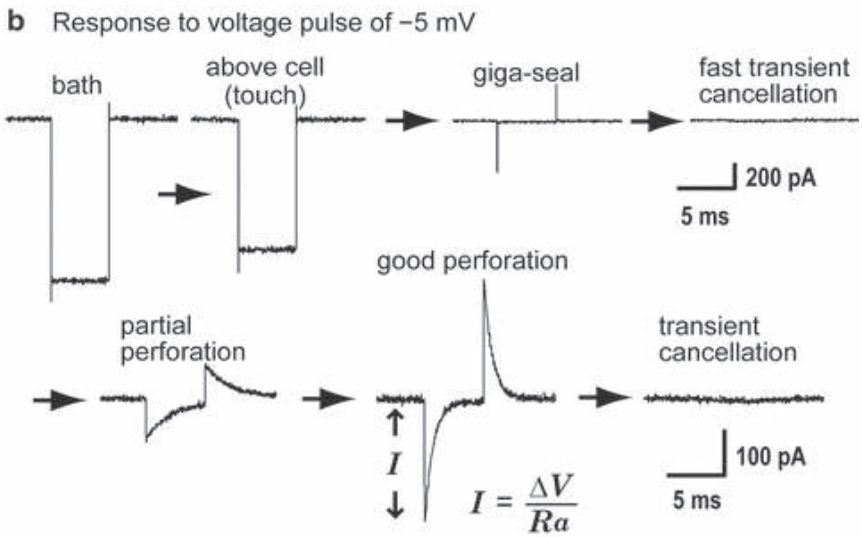
1. Principle: Perforated patch clamping uses antifungal drugs like nystatin or amphotericin B dissolved in ethanol or DMSO and added to the intracellular solution.
2. Preparing the Pipette:
    - Filling the Pipette: Fill the pipette tip with normal intracellular solution using suction from a large syringe connected to the back of the pipette with tubing. Then, fill the pipette with the intracellular solution containing the perforating agent (5–20 µg/ml from a 1000-fold stock).
3. Gigaseal Formation: Form the gigaseal as usual, but leave the patch intact and monitor the current response to a test pulse. After a few minutes, the cell capacitance should manifest, with a decreasing access resistance indicating a progressively faster capacitive transient. The experiment can begin after about 10 minutes when the capacitive transient stabilizes.

Helpful Figure: The following figure illustrates the setup for perforated patch recording, including the use of antifungal agents and monitoring the current response.

The configurations of the patch clamp technique





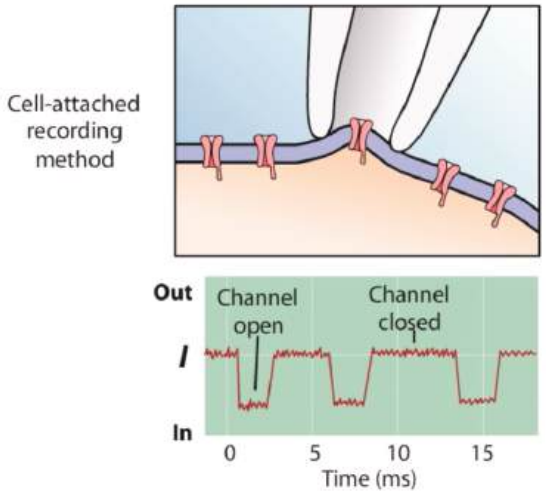


Overview of Cell Perforation. (a) Shows the size and selectivity of commonly used perforating agents. (b) Demonstrates the changes in electrical current in response to a -5 mV voltage pulse during two key stages: first, sealing the patch pipette to the cell to create a high-resistance (GΩ) seal (illustrated in the upper drawing), and then perforating the patch membrane to allow for stable recordings (shown in the lower traces). These recordings were made using an acutely isolated hippocampal neuron, illustrating the transition from sealing to successful cell perforation.

Single Ion Channel Recordings

1. Noise Control: Single-channel recordings require significantly higher sensitivity. Ensure external noise is undetectable.
2. Recording: Single-channel activity appears as instantaneous jumps between current levels, reflecting the stochastic nature of channel opening and closing.

Helpful Figure: The following figure shows a comparison between single-channel and whole-cell recordings, highlighting the differences in sensitivity and noise control.



By following these detailed steps and using the helpful figures, you can successfully establish various patch-clamp configurations and conduct high-quality recordings from acute brain slices. Happy recording!

What to Include in Your Ephys Logbook

It's good practice to keep a record of the standard parameters of each cell being patched. Here's a checklist of what should appear in your laboratory logbook for each cell:

1. **$R_{pipette}$ (Pipette Resistance)**: Record with the pipette in the bath but not on the cell.
2. **R_{seal} (Seal Resistance)**: Note after gigaseal formation.
3. **E_m (Membrane Potential)**: Measure by briefly switching to current clamp mode (I = 0) after the patch is disrupted.
4. **R_{series} and C_m (Series Resistance and Cell Capacitance)**: Record after capacitive transient cancellation.
5. **R_m and R_{leak} (Membrane Resistance and Leak)**: Measure from the ohmic component of the current response to a test pulse.
6. **Percentage R_{series} Compensation (if used)**: Note the percentage if using series resistance compensation.

Sample Logbook Entry:

Date	[Insert Date]
Cell ID	[Insert Cell ID]
Experiment	[Insert Experiment Type]

Parameter	Value
Rpipette	___ MΩ (Pipette in bath)
Rseal	___ GΩ (After gigaseal formation)
Em (Membrane Potential)	___ mV
Rseries	___ MΩ
Cm (Cell Capacitance)	___ pF
Rm (Membrane Resistance)	___ MΩ
Rleak	___ MΩ
% Rseries Compensation	___%

Notes	
- Observations on cell health and behavior.	
- Any deviations from standard protocol.	
- Environmental conditions (temperature, humidity, etc.).	

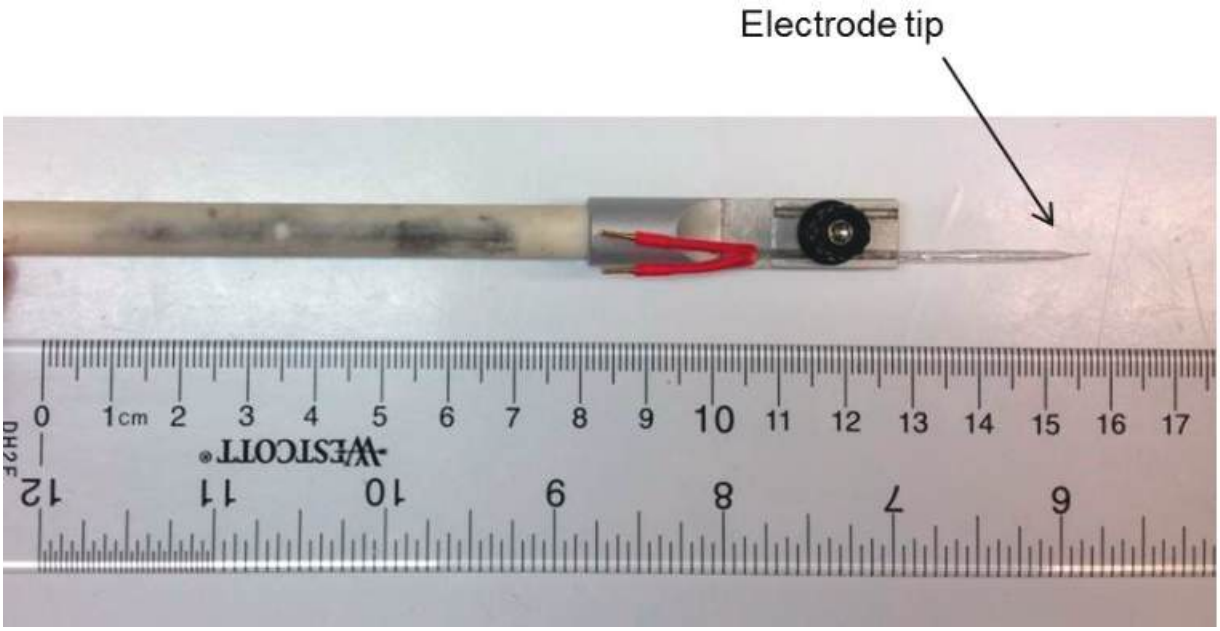
Setup Diagram	
[Insert Diagram/Sketch of Setup]	

Making Bipolar Electrodes for Stimulation

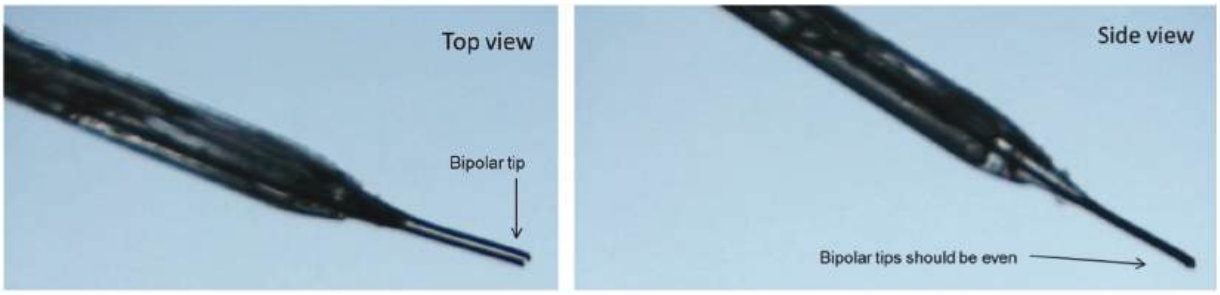
For patch-clamp electrophysiology, stimulating specific fibers or areas often requires the use of a bipolar electrode. While you can purchase commercial electrodes, making your own can be more effective and tailored to your specific needs. Here's a general guide to making a bipolar electrode:

1. **Use Theta Glass as the Electrode Holder**:
   - Theta glass is ideal for making bipolar electrodes due to its dual-channel structure.
2. **Pull the Theta Glass**:
   - Use a pipette puller to pull the theta glass as you would for a recording pipette. Adjust the length to fit onto the electrode holder.
   - **Tip Adjustment**: Break the tip of the pulled theta glass so that the tapered end is somewhat blunt. This helps in positioning the stimulation electrodes correctly.
3. **Select and Thread Tungsten Wire Electrodes**:
   - Choose two tungsten wire electrodes for their durability and conductive properties.
   - Thread each tungsten electrode through one of the theta glass chambers.
4. **Apply Glue**:
   - Apply glue to the opposite end of the theta glass and allow it to travel down to the tip. This secures the tungsten wires in place.
   - **Drying**: Allow the electrode to dry overnight to ensure the glue sets properly.
5. **Adjust the Electrode Tips**:
   - Once dried, bend the bipolar electrode tips so that both are parallel. This configuration ensures consistent stimulation.
   - **Tip Cutting**: Cut the tips with scissors so that they are even. This creates a uniform stimulation field.

Helpful Figure: The following figure shows the process of making a bipolar electrode, from pulling the theta glass to threading and securing the tungsten wires. Here's what the bipolar electrode looks like in its holder:



Here's a higher magnification view of the bipolar electrode. Top view (left) and side view (right). Note that the electrode tips should be parallel and even.



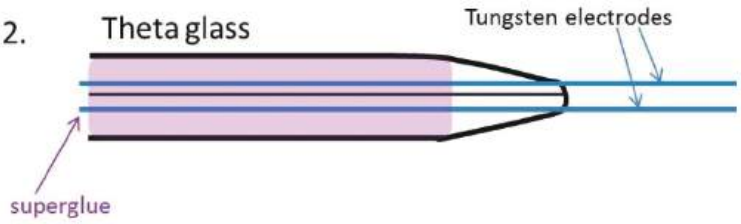
Steps for Making a Bipolar Electrode:

1. **Prepare the Electrode Holder**: Use theta glass as the electrode holder.
2. **Pull the Theta Glass**: Use a pipette puller to pull the theta glass as you would for a recording pipette. Adjust the length to fit your electrode holder.
3. **Blunt the Tip**: Break the tip of the pulled theta glass so that the tapered end is somewhat blunt.
4. **Prepare the Tungsten Wires**: Select two tungsten wire electrodes and thread each one through a separate chamber of the theta glass.

5. **Apply Glue**: Apply glue to the opposite end of the glass, allowing it to flow down to the tip where the wires are. Let the electrode dry overnight.
6. **Final Adjustments**: Once dry, bend the tip of the bipolar electrode so that both wires are parallel. Use scissors to trim the tips evenly.

1.

2.



By following these steps, you can create a reliable and effective bipolar electrode for your patch-clamp experiments. Custom-made electrodes can often provide better performance and adaptability compared to commercially bought ones. Happy experimenting!

Troubleshooting Patch-Clamp Electrophysiology

Even experienced researchers can encounter challenges in patch-clamp electrophysiology. Here are some commonly asked questions and their solutions to help you troubleshoot your experiments.

Q1: Why am I having trouble forming a gigaseal?

There are several factors that can affect gigaseal formation. First, ensure that your pipettes are clean and fire-polished, as dirty or rough pipette tips can prevent good seals. Maintaining slight positive pressure when lowering the pipette into the bathing solution helps keep contaminants away from the tip. Additionally, the cell membrane surface must be clean and undamaged; healthy cells with a debris-free surface are ideal. Finally, always use freshly prepared, properly buffered solutions, as contaminated or improperly mixed solutions can interfere with seal formation.

Q2: What should I do if my cell membrane keeps breaking during whole-cell recording?

If the cell membrane breaks frequently, consider adjusting the suction pressure—gentle suction is key, as too much force can rupture the cell. Ensure your pipette has an appropriate resistance; very high or very low resistance can cause issues. Checking the health of your cells is also important, as stressed or unhealthy cells are more prone to breaking. Lastly, make sure your solutions are at the correct temperature and osmolarity to prevent osmotic shock to the cells.

Q3: Why is my series resistance (Rseries) too high?

High series resistance can affect the quality of your recordings. To reduce it, use pipettes with larger tip openings if appropriate for your cell type, and ensure you have a high-quality gigaseal, as poor seals can contribute to higher resistance. Additionally, make use of series resistance compensation on your amplifier to correct for high resistance during recordings.

Q4: My recordings are noisy. What can I do to reduce the noise?

Noise in recordings can come from various sources. Ensure that all equipment is properly grounded and shielded from external electrical noise. Use clean, well-polished pipettes to reduce noise and make sure your solutions are of high purity to minimize noise from ionic impurities. Placing your setup on a vibration isolation table can also help reduce mechanical noise.

Q5: What should I do if the cell's membrane potential (Em) is unstable?

An unstable membrane potential can be due to several factors. First, ensure the cells are healthy and not under stress. Keep the bathing solution at a stable temperature to prevent fluctuations, and make sure the pipette is stable and properly positioned on the cell membrane. Additionally, check the osmolarity of your solutions, as mismatched osmolarity can cause instability.

Q6: How can I improve the quality of my single-channel recordings?

Single-channel recordings require precise control and setup. Minimize noise by using high-quality, well-shielded equipment and maintaining a clean environment. Aim for gigaseals with resistance much higher than for whole-cell recordings, and ensure your setup is mechanically stable to prevent movement artifacts. Using high-purity reagents and freshly prepared solutions can also reduce noise.

By addressing these common issues with targeted troubleshooting steps, you can improve the quality and reliability of your patch-clamp electrophysiology experiments. Happy experimenting!

More Questions? Contact Us!

If you have any further questions, require additional experimental support, seek references, protocols, or referrals related to patch-clamp electrophysiology or any other aspect of neuroscience research, do not hesitate to reach out to us at Precisionary Instruments. Our dedicated team of experts is here to assist you every step of the way.

You can contact us via:

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Whether you need troubleshooting assistance, personalized guidance, or customized solutions for your research needs, we are committed to providing you with the support and resources necessary to ensure your success. Don't hesitate to get in touch with us, and let us help you advance your research endeavors.