NeuroNexus

Acute Penetrating Arrays

Surgical Guide and Electrophysiology

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Introduction

This comprehensive technical document provides crucial information on animal surgery in biomedical research settings. It encompasses key topics including pre-operative procedures, anesthesia administration, analgesia protocols, aseptic/sterile techniques, surgical procedures, incision closure methods, and post-operative care. It is imperative to adhere to the guidelines established by the Institutional Animal Care and Use Committee (IACUC), following the standard procedures. For further details, please consult the IACUC approved policies of your university.

Principles of Animal Surgery

To familiarize yourself with the principles of rodent surgery, please read the following articles:

1) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3376945/

2) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2587003/

Reagents

Experimental animals

- Mouse, rat, hamster, rabbit, cat, bat, bird, dog, ferret, guinea pig, swine, ruminant, non-human primate, etc.

Anesthetic drug

The recommended anesthesia protocols for intraperitoneal injection in animal research are as follows:

Intraperitoneal Injection:

- Xylazine: 10 mg/kg for both rats and mice.
- Ketamine: For rats, the recommended dose is 50-100 mg/kg. For mice, the recommended dose is 80 mg/kg.

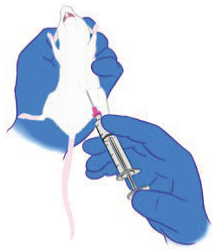
Local Anesthetics:

- Lidocaine 1% can be used as a local anesthetic.

Inhaled Anesthetics with Isoflurane Setup:

- For mice: Induction dose of 2-3%, followed by a maintenance dose of 1-2%.
- For rats, rabbits, guinea pigs, and ferrets: Induction dose of 3-5%, followed by a maintenance dose of 2-3%.

It is important to note that using expired anesthetics or euthanasia agents is never acceptable. Please ensure adherence to proper dosing and safety guidelines when administering anesthesia to animals in research settings.



Sterilization of surgery site (for chronic surgery)

70% ethanol and iodine-based wash (Betadine) are commonly used for sterilization and cleaning in various medical and laboratory procedures.

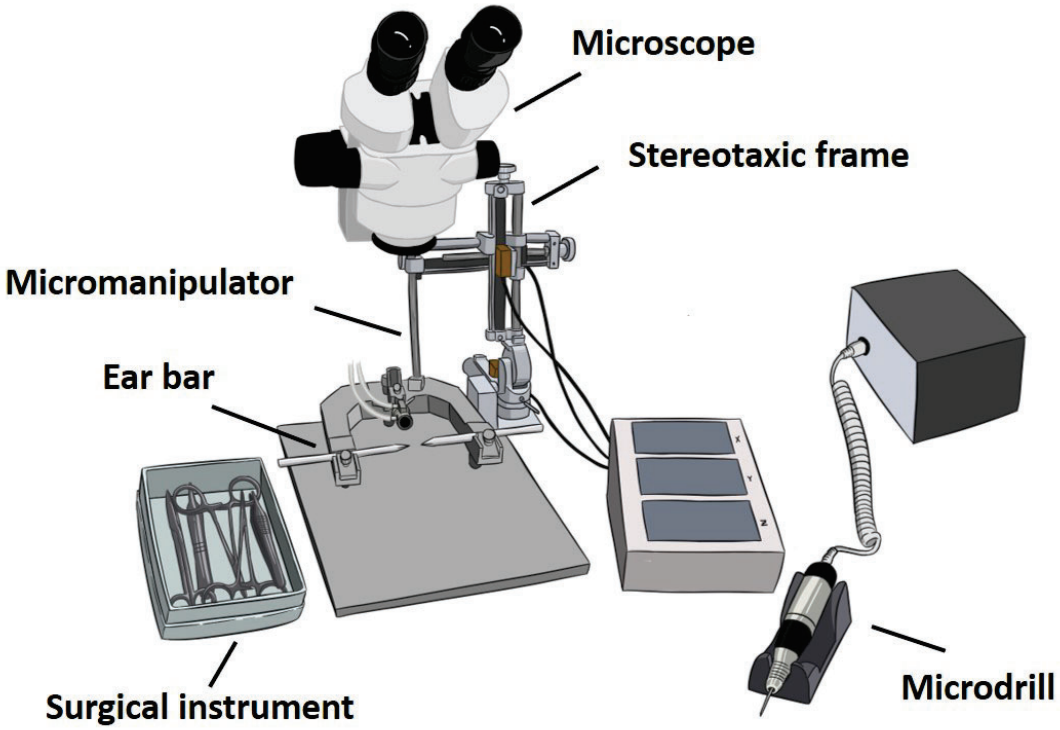
Fluid support

- Sterile saline

Cyanoacrylate glue (VetBond tissue adhesive)

Eye lubricant (Puralube ointment)

Equipment



Stereotaxic frame, auxiliary ear bar, and micromanipulator
Wide-field dissecting microscope
Cold light source
Insertion tools
Heating therapy pump and pad
Micromotor high-speed drill with appropriate burrs
Bone screws
Surgery instruments:

During the surgical procedure, various surgical instruments and materials are commonly utilized:

- Scissors: Employed for cutting tissues and sutures.
- Scalpel: A precise cutting tool used for making incisions.
- Surgical needle with suture: Used for stitching and closing incisions or wounds.

- Hemostat: A clamp-like instrument used to control bleeding by compressing blood vessels.
- Forceps: Utilized for grasping and manipulating tissues or objects during surgery.
- Tuberculin syringe with needle: A syringe typically used for precise and small-volume injections.
- Cotton swab: Used for applying or removing substances during the surgical procedure.
- Absorbent surgical sponge triangle: A triangular sponge used for absorbing fluids or maintaining a clear surgical field.
- Gel foam: A sterile sponge-like material used to aid in hemostasis and control bleeding.

It is crucial to ensure that all surgical materials are within their expiration date. Expired suture, bandage material, and surgical gloves should be avoided, as they may compromise the safety and effectiveness of the surgery. Always follow established guidelines and protocols regarding the use of surgical instruments and materials to ensure optimal outcomes for the surgical procedure and the well-being of the animals involved.

General Considerations

Monitoring

- To ensure the safety and well-being of the animal during anesthesia, it is crucial to monitor vital parameters and prevent excessive depression of cardiac and respiratory functions while maintaining an appropriate level of anesthesia. While specialized equipment can offer more precise measurements, the following parameters can be observed in an anesthetized mouse without specialized tools:
- Respiratory rate and pattern: Observe the mouse's breathing to ensure it remains within a normal range and exhibits a regular pattern.
- Mucous membrane color: Check the color of mucous membranes, such as the gums or inner eyelids, to assess oxygenation levels. A normal pink color indicates proper oxygenation.
- Body temperature: Monitor the mouse's body temperature to ensure it stays within a normal range, as anesthesia can impact thermoregulation. Maintaining an appropriate body temperature is essential for the animal's comfort and overall well-being.

- Oxygen saturation and heart rate: If available, use a pulse oximeter to non-invasively measure oxygen saturation and heart rate. This provides valuable information about the animal's cardiovascular and respiratory status.
- Blood pressure: While specialized equipment is typically needed for accurate blood pressure monitoring, changes in perfusion and circulation can sometimes be observed indirectly by assessing mucous membrane color, capillary refill time, and overall vitality.

Regularly monitoring these parameters during anesthesia helps detect any deviations from the normal range and allows for timely intervention if necessary. It is important to refer to relevant guidelines and protocols for specific recommendations on monitoring and maintaining anesthesia levels for your research animals.

Heat support

During anesthesia, all species are vulnerable to the risks of hypotension (low blood pressure) and hypothermia (low body temperature). To mitigate these risks, it is recommended to use circulating water blankets for temperature regulation. Please note that electric heating pads should be avoided as they pose a safety hazard.

Regardless of the chosen heat source, it is crucial never to place animals directly on the heat surface. Using a barrier, such as a towel or a layer of insulating material, between the animal and the heat source is essential to prevent thermal injury.

By employing circulating water blankets and adhering to proper precautions, you can help maintain the animal's body temperature and minimize the risk of hypotension and hypothermia during anesthesia. It is advisable to consult specific guidelines and protocols applicable to your research and species to ensure appropriate temperature management.

Fluid support

In situations where prolonged anesthesia is anticipated or when dealing with animals that are ill, aged, or debilitated, considering the administration of warm subcutaneous (SQ) or intraperitoneal (IP) fluids is worthwhile. This approach can help maintain the animal's body temperature and provide additional support during the anesthesia event.

The administration of warm fluids serves to counteract hypothermia and contributes to maintaining a stable body temperature throughout the procedure. The choice between subcutaneous

or intraperitoneal routes depends on the specific needs of the animal and the nature of the procedure being performed.

It is crucial to emphasize that the administration of fluids should adhere to established guidelines and protocols. Proper aseptic technique and accurate dosing must be followed to ensure the safety and well-being of the animal.

Pre-Surgery Steps

- Turn on the data acquisition system.
- Access Radiens Allego™, the electrophysiological software.
- Customize the settings by selecting the appropriate port, SmartLink headstage, and electrode. Refer to the manual for detailed instructions on electrode and headstage selection at the following link: https://nnx.mcoutput.com/1376106/Content/Home-Radiens.htm.
- Connect the probe securely to the SmartLink headstage, ensuring a stable connection at the cable end.
- Open the Impedance tab in Allego and measure the 1 kHz impedance before implantation. For guidance on measuring impedance using SmartBox Pro, consult the Allego manual.
- If the experiment is chronic, follow the serialization section as outlined in the relevant documentation.
- By adhering to these pre-surgery steps, you can ensure the proper setup of the data acquisition system, correct configuration of Radiens Allego software, and accurate measurement of probe impedance before initiating the implantation procedure.

Surgery Tips

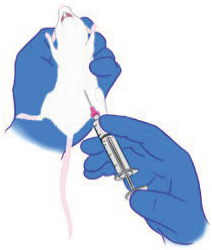
- For chronic studies, it's crucial to maintain sterility by using sterile surgical tools to minimize the risk of infection.
- During the procedure, periodically apply saline to the surgical site to prevent thermal damage caused by drilling and remove any blood present.

- When using the Micro Drill, make sure to use appropriate sterile burrs. Choose a tip diameter of 0.7 mm for mice, 0.9 mm for rats, and a 2 mm carbide burr for primates.
- Select the size of the craniotomy based on your study's specific needs. For electrophysiology-only studies, a small craniotomy may be sufficient, while imaging studies like optogenetics or 2-photon imaging may require a larger craniotomy.
- Create at least two additional holes for bone screws, which will be used to attach ground and reference wires.
- Before electrode insertion, remove the dura. Note that this step is not necessary for mice.
- For precise and controlled electrode insertion, utilize a microscope and either a manual or automatic manipulator.

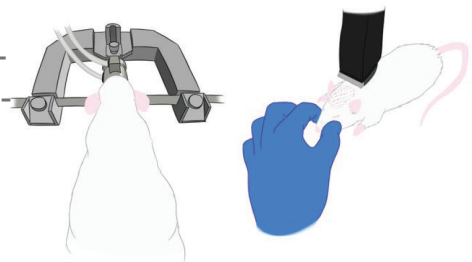
By following these surgery tips, you can enhance the sterility of the procedure, minimize thermal damage, choose appropriate tools, customize the craniotomy size, ensure proper grounding, and perform accurate electrode insertion using the right equipment.

General Surgical Steps

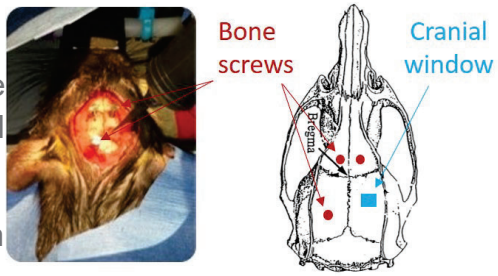
- Administer anesthesia to the animal using a combination of xylazine (rat and mouse: 10 mg/kg) and ketamine (rat: 50-100 mg/kg, mouse: 80 mg/kg) via intraperitoneal (IP) injection or through isoflurane inhalation (4% in 100% O2). Sustain anesthesia during the surgery with isoflurane (rat: 1-2.5%, mouse: 0.8-1.25%) and ensure ventilation with a blend of oxygen and medical air (50:50).
- Secure the threads of the screw to the bone by applying a small drop of VetBond adhesive.
- Evaluate the depth of anesthesia during the surgery by conducting a toe pinch reflex test.



- Trim the fur in the surgical area to maintain a clean working environment.
- Intubate the animal and secure it in a stereotaxic frame for stability during the procedure.

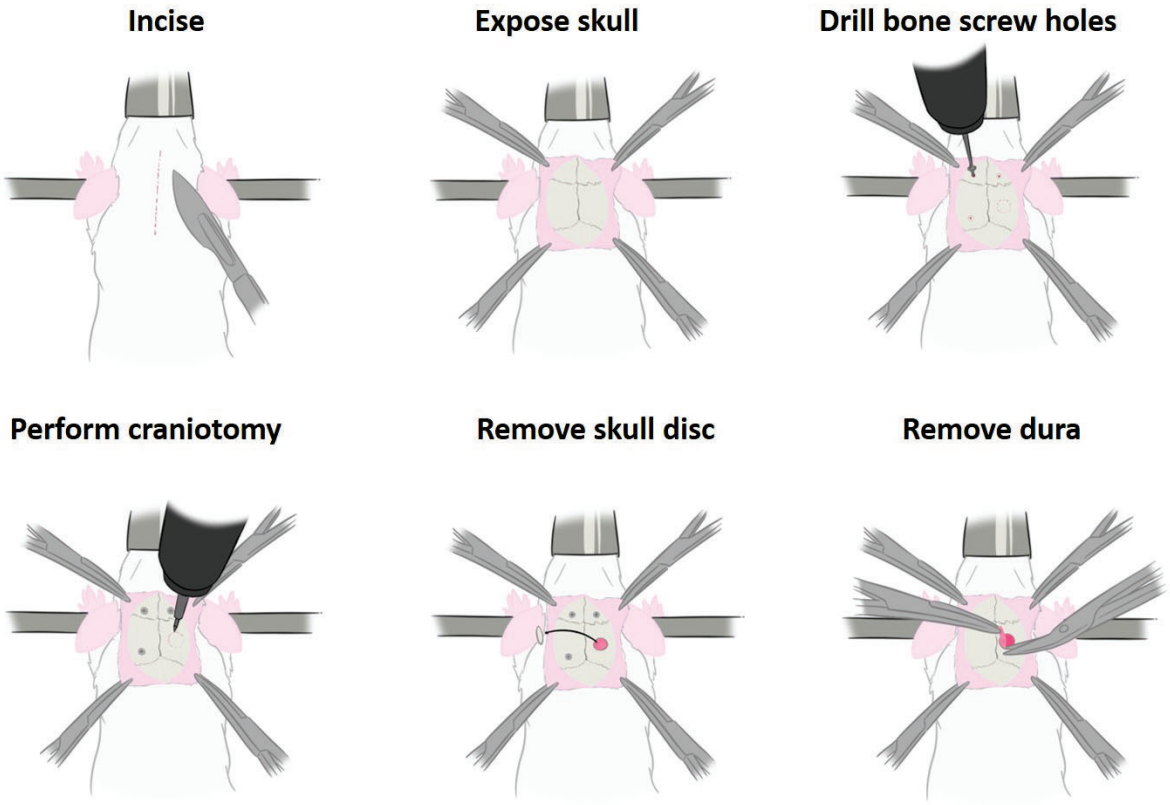


- Apply artificial tear ointment to the rodent's eyes to prevent drying.
- Optionally, administer dexamethasone sodium phosphate (2 mg/kg) before surgery to reduce cerebellar edema.
- Sterilize the skin at the surgical site in a chronic setup by applying betadine and 70% alcohol.
- Make a midline sagittal incision along the scalp to expose the skull covering the desired location.
- Use a cotton-tip applicator to delicately remove the periosteum from the skull and cleanse the skull surface with saline.
- Apply a thin layer of VetBond adhesive to dry the skull surface, enhancing adhesion and grip for the dental cement head cap.
- Use a surgical marker to mark the position of three screws and the implant site.



- Drill two or three small holes (1 mm diameter) above the implantation site and one over the contralateral site for use as ground and reference points.
- Carefully insert screws (mouse: 4 mm long, 0.86 mm diameter) into the drilled holes, ensuring not to penetrate the dura.
- Secure the threads of the screws to the bone with a small drop of VetBond adhesive.
- Perform a craniotomy above the brain region of interest using a high-speed drill. Note: For electrophysiology-only studies, a small craniotomy is recommended; for imaging studies like optogenetics or 2-photon imaging, a larger craniotomy is preferred.
- While drilling, regularly flush the surgical window with saline to minimize heat buildup and remove blood and bone debris.
- Thin the edges of the craniotomy until the underlying pial vasculature becomes visible, taking care not to damage the brain tissue.
- Use forceps to gently separate the bone flap from the skull, avoiding protrusion into the brain tissue.

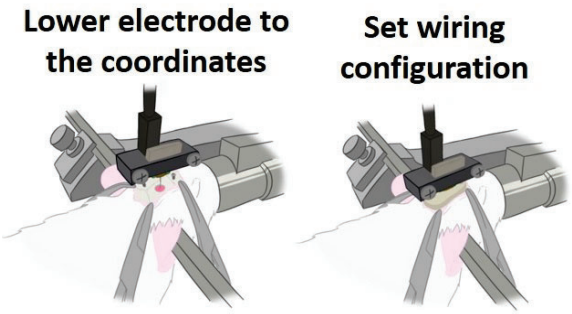
- Grasp adjacent corners of the loosened bone flap and slowly peel it away from the underlying dura mater.
- Remove the dura before electrode insertion and control any bleeding using gel foam soaked with sterile saline. Note: This step is not necessary for mice.



You are now prepared to proceed with the electrode implantation.

Surgical Procedures for Penetrating Electrodes

- Lower the probe to the surface of the cortex.
- Insert the probe using either an automatic or manual micromanipulator, advancing at a rate of 1 mm/min until the desired depth is reached. Note: It is advisable to choose varying step sizes at different depths to monitor neural spiking and pinpoint positions conducive to effective activity recording.



- Fill the craniotomy with saline to ensure a stable environment.
- Securely wrap your ground and reference wires around the designated bone screws, following the wiring configuration outlined in this protocol.

Wiring Configuration

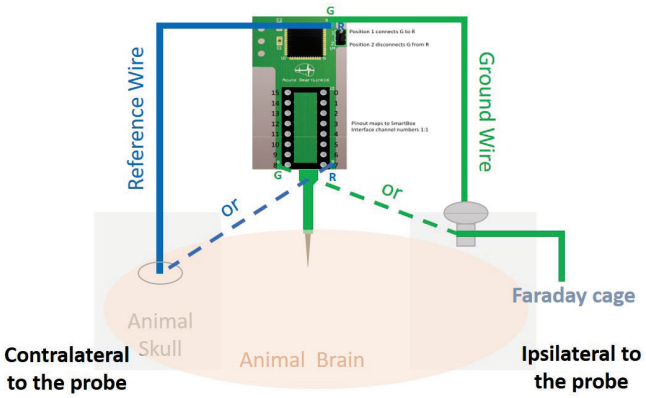
Proper wiring and grounding are essential for obtaining clean and usable signals, maximizing the performance of NeuroNexus probes. This protocol outlines effective strategies for referencing and grounding probes. While the theory is straightforward, practical implementation can be complex. NeuroNexus probes offer multiple wiring options for optimal flexibility. It's crucial to understand these options before placing an animal on the stereotaxic frame, allowing quick adjustments if needed. NeuroNexus has designed probe wiring to accommodate diverse experiments. This section details wiring setups for 16, 32, and 64-channel electrodes. For more information, contact us at support@NeuroNexus.com or visit our website.

Acute 16-channel Electrode

For 16-channel acute electrodes that lack reference and ground wires, unlike chronic microelectrode arrays, you can choose to solder reference and ground wires to the headstage. The following steps outline the process:

Add a separate reference electrode

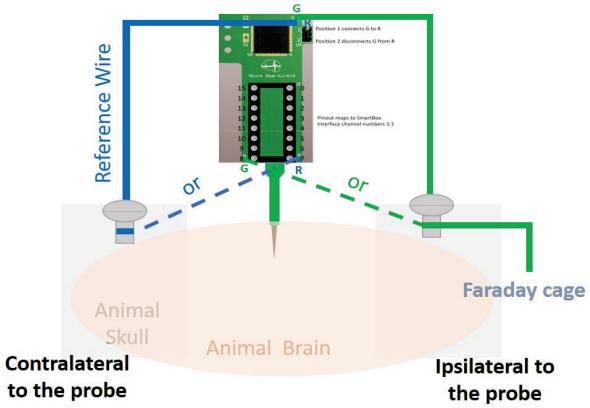
- Perform a craniotomy on the opposite side of the probe.
- Place an Ag/AgCl or stainless steel Reference Wire into the saline-filled craniotomy.
- Connect the Reference Wire to the designated reference holes on the top or bottom of the headstage, as illustrated in the figure.
- Affix a distinct wire as a Ground Wire to the ground hole on the top or bottom of the headstage.



- Establish a connection between the Ground Wire and a bone screw located anywhere on the animal's skull.

Not adding a separate reference electrode

As an alternative, you have the option not to include a separate reference electrode. Follow these steps for this choice:



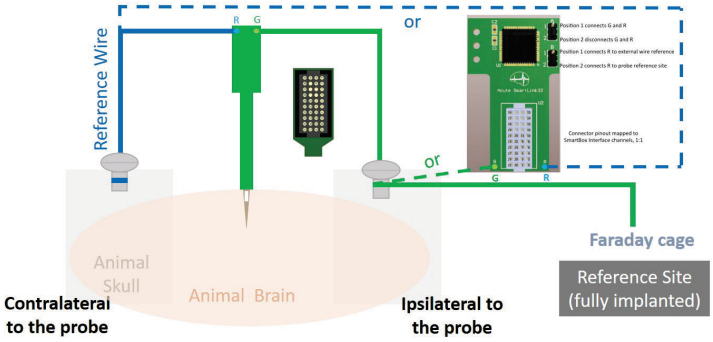
- Fasten an Ag/AgCl or stainless steel Reference Wire to the reference holes on the top or bottom of the headstage.
- Directly link the Reference Wire to the nearest bone screw adjacent to the implanted probe.
- Attach a distinct wire as a Ground Wire to the ground hole on the top or bottom of the headstage.
- Establish a connection between the Ground Wire and any bone screw on the animal's skull.

Acute 32-channel Electrode

For a 32-channel electrode with acute characteristics, you have various options for reference and ground wiring configurations, contingent on the presence of an internal reference site on the probe:

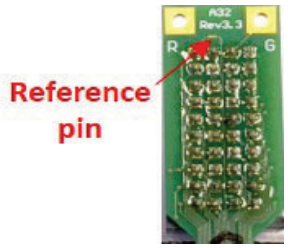
Using the internal reference site

- If the probe features an internal reference site, connect a wire from the A32 connector or A32 headstage to the bone screw. This establishes a reference connection.
- Attach a distinct wire as a Ground Wire to the ground hole on the top of the probe connector or the bottom of the headstage. Connect this wire to the bone screw on the animal skull to ensure proper grounding.

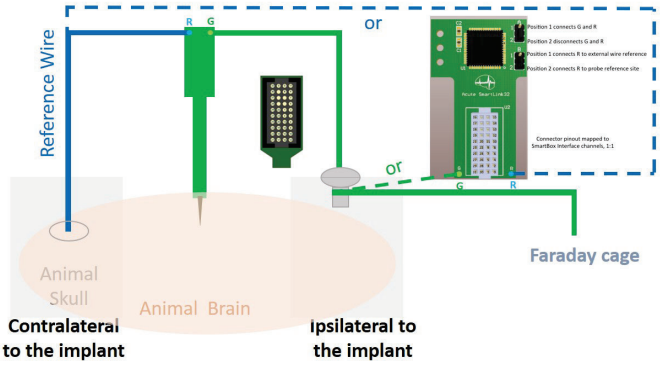


Disabling the internal reference site

- Locate the reference pin in the A32 connector, positioned above the second column of pins, indicating an exposed horizontal trace.
- To deactivate the reference site, meticulously cut the reference trace using a razor or a similar tool.

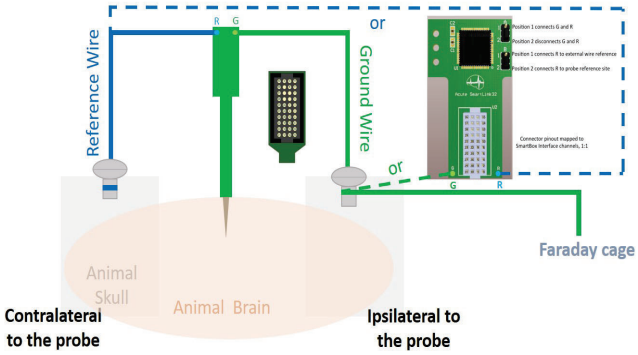


Adding a reference electrode



- If the probe lacks a reference site or after deactivating it, establish a small craniotomy near the implant side.
- Insert an Ag/AgCl or stainless steel Reference Wire into the saline-filled craniotomy. Connect this wire to the reference holes on the top of the probe connector or the bottom of the headstage.
- Attach a distinct wire as a Ground Wire to the ground hole on the top of the probe connector or the bottom of the headstage. Connect this wire to the bone screw anywhere on the animal skull.

Simple setup



- After deactivating the reference site or if your electrode lacks one, link an Ag/AgCl or stainless steel Reference Wire from the Reference holes on the top or bottom of the headstage directly to the bone screw closest to the implantation site.
- Attach a separate wire as a Ground Wire to the Ground hole on the top or bottom of the headstage. Connect this wire to any bone screw on the animal skull.
- These wiring configurations will ensure proper reference and grounding for your 32-channel acute electrode setup.

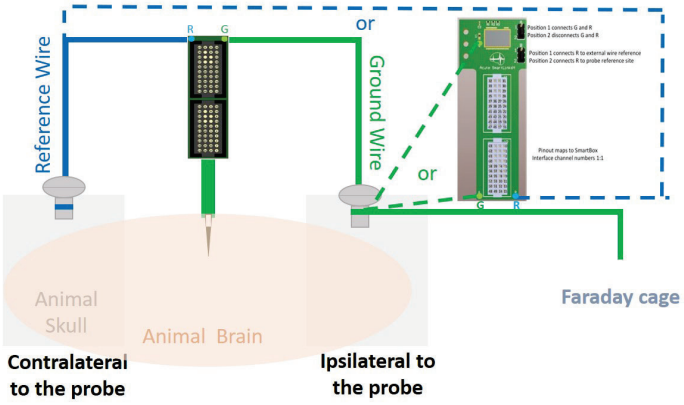
Acute 64-channel Electrode

Some acute probes with 64 or more channels come equipped with a reference site on the probe shank, allowing researchers to customize the reference and ground wiring configuration for their

specific application.

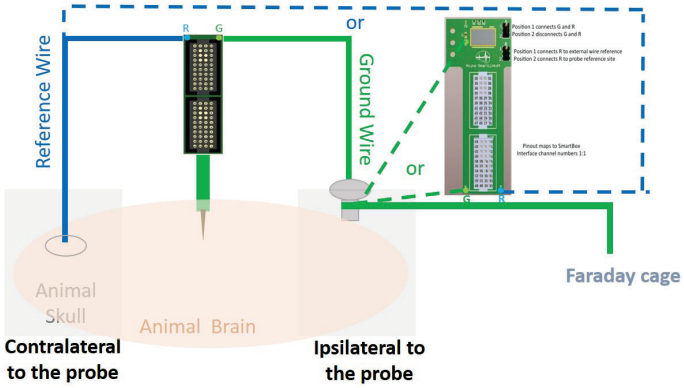
Use a Reference Site

If the 64-channel probe includes a Reference Site, connect a wire from the A64 connector or A64 headstage to the bone screw on the contralateral site of the implant. Attach a separate wire as a ground wire to the ground hole on the top of the probe connector or the bottom of the headstage, and then connect it to the bone screw on the ipsilateral site of the implant and the Faraday cage.



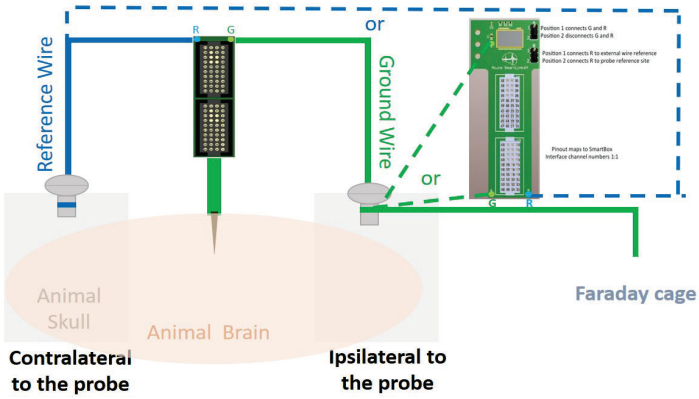
Add a Reference Electrode

If your electrode lacks a reference site on the probe, create a small craniotomy on the skull and affix a wire from the A64 connector or A64 headstage to the bone screw. Attach a separate wire as a Ground Wire to the Ground hole on the top of the probe connector or the bottom of the headstage, and then link it to the bone screw on the ipsilateral site of the implant and the Faraday cage.



Simple Setup

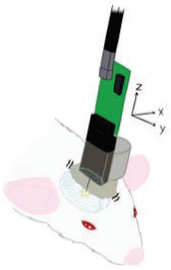
If your electrode lacks a Reference Site on the probe, connect a wire from the A64 connector or A64 headstage to the bone screw on the contralateral site of the implant. Attach a separate wire as a Ground Wire to the Ground hole on the top of the probe connector or the bottom of the headstage, and then link it to the bone screw on the ipsilateral site of the implant and the Faraday cage.

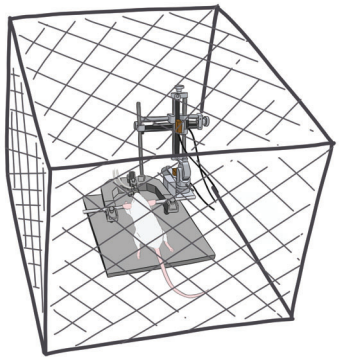


Steps After Surgery

After concluding the surgery and preparing the animal for the electrophysiology experiment, you can proceed with the following steps:

Place the animal, secured in the stereotaxic frame, inside the Faraday cage for electromagnetic shielding against external noise.





Connect the probe connector to the headstage, ensuring a secure and reliable connection.

Immediately after electrode insertion, record impedance to verify the electrode's integrity and its ability to capture neural activity.

Determine a name and saving path for your recording data through the settings tab in your electrophysiology software. Choose a descriptive naming convention for organized data management.

Begin streaming neural signals by selecting the Monitor tab from the Dashboard in your software to monitor real-time neural activity.

Utilize the noise removal section in your software to eliminate potential system noise, enhancing signal quality.

Apply filters to the streaming data to monitor specific frequency ranges of interest. Access the signal processing tab in your software's Dashboard and follow the SmartBox Pro/Allego manual instructions for filter application.

Open the electrode tab from the Dashboard to add or remove specific recording sites, allowing focus on particular regions of interest during the experiment.

Track real-time recording to observe neural signals as they are acquired, ensuring the desired signals are being recorded.

Check signal metrics in real-time to assess the quality of recorded signals, including measures such as signal-to-noise ratio, amplitude, or waveform characteristics.

Initiate the recording of neural activity once satisfied with the setup and signal quality, following the software's instructions.

After completing the recording session, review and replay the saved data to analyze captured neural activity. Utilize the playback functionality in your software for detailed examination and extraction of relevant information from the recorded data.

Following these steps will facilitate the effective conduct of your electrophysiology experiment, ensuring accurate recording and analysis of neural signals.

Recommendations for Reducing Noise in Ephys Setup

To further enhance the performance of your electrophysiology setup and reduce noise, we recommend implementing the following measures:

Proper Grounding: Ensure correct grounding of your electrophysiology setup to prevent ground loops between instruments. Connect all equipment to the same mains ground using a power strip linked to a single wall socket. This helps maintain a consistent voltage level, minimizing 50 Hz or 60 Hz ground noise.

Grounded Metal Plate: Place a heavy, grounded metal plate down the Faraday cage, connecting it to the main electrical socket. Ground the Faraday cage, microscope, stereotaxic frame, manip-

ulator, and other equipment to the metal plate using heavy gauge wires. This containment minimizes ambient noise in the recording setup.

Check Faraday Cage and SmartBox Pro Grounding: Ensure proper grounding of the Faraday cage and SmartBox Pro, connecting them effectively to the main electrical ground.

Additional Stereotaxic Frame Grounding: If the animal is under the stereotaxic frame during recording, add extra alligator clips from the frame to the Faraday cage for additional grounding.

Camera and Light Deactivation: Turn off cameras or lights on the microscope during recording to prevent introduced noise.

Microscope Grounding: Check the microscope's grounding to prevent radiation and electrical noise pickup; ensure it is properly grounded.

Turn Off Nearby High-Power Devices: Power down high-power machines or devices close to the recording setup to prevent electromagnetic interference.

Minimize Other Device Interference: Unplug unnecessary devices and equipment from main outlets to minimize interference.

Additional Grounding with Needle: Consider adding an extra needle as a probe grounding wire to the animal's nuchal musculature, attaching it to the Faraday cage with alligator clips for added grounding.

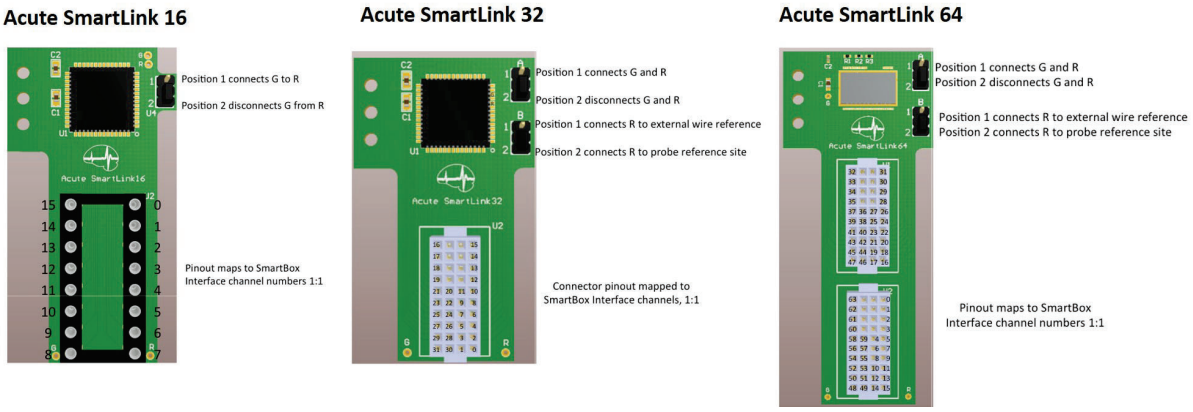
Address Thermal Noise: If using the recording setup continuously, check for thermal noise in the headstage. Allow the headstage to cool down if thermal noise is detected.

If the above steps do not effectively remove noise, you may adjust the ground and referencing on the Smartlink acute headstage. The jumpers (A and B) on the headstage allow for customization of referencing and grounding levels. Refer to the documentation or contact support for specific instructions on adjusting the jumpers.

Please remember that each setup is unique, and achieving the desired noise reduction may require a combination of methods. For additional guidance, feel free to contact support@NeuroNexus.com.

The Smartlink Acute Headstages are meticulously crafted with jumpers to provide users with enhanced flexibility in obtaining the most pristine signal. Jumper A is spe-

cifically associated with headstage referencing and grounding levels, while Jumper B is designated for referencing at the probe level.



- Section A, position 2, represents the default setup intended to achieve optimal noise levels. In this configuration, as previously explained, it is necessary to affix two separate bone screws to the skull and connect the reference and ground wires to them individually.
- Regarding Section B, certain electrodes feature a sizable reference site on the probe shank. In most instances, users prefer placing it in position 2 to utilize the probe reference. However, should they opt for an external wire reference, they must switch the jumper on Section B from 2 to position 1. Otherwise, there is no need to adjust the jumper on Section B.

Cleaning Procedure

If you want to reuse the probe after withdrawal from tissue following an experiment, it's important to follow a protocol to ensure that the probe is properly cleaned and sterilized. Here are some steps you can follow:

- After withdrawal from tissue after an experiment, immediately rinse in a beaker of distilled water to remove any excess tissue or debris from the probe.

- Soak the probe (only the shank; avoid soaking the PCB) in a proteindissolving detergent (Tergazyme) or enzyme such as contact lens solution
- or diluted surgical instrument detergent for up to 4 hours to remove any residual biological material.
- Rinse the probe in deionized water and then repeatedly immerse and extract or gently stir it inside the beaker to remove any detergent residue.
- use, isopropyl alcohol (e.g. 70% IPA) for cleaning AFTER the protein dissolving procedure. CAUTION: Without first dissolving the residual tissue from the probe, alcohol could cause protein to stick to the electrode sites.
- Store probes in their shipping box. Keep the record that came with the probes. This will help you identify the probe designs in the future.
- DO NOT use ultrasonic cleaners on NeuroNexus probes as this may cause damage.
- DO NOT autoclave either NeuroNexus probes as this may cause damage.