NeuroNexus

ECoG Electrode 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.

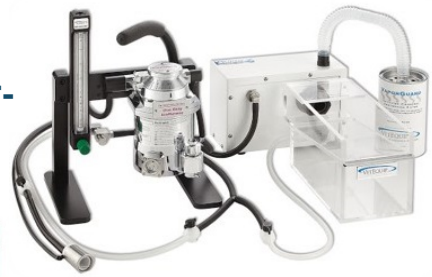
Inhaled Anesthetics with Isoflurane Setup:

- 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.



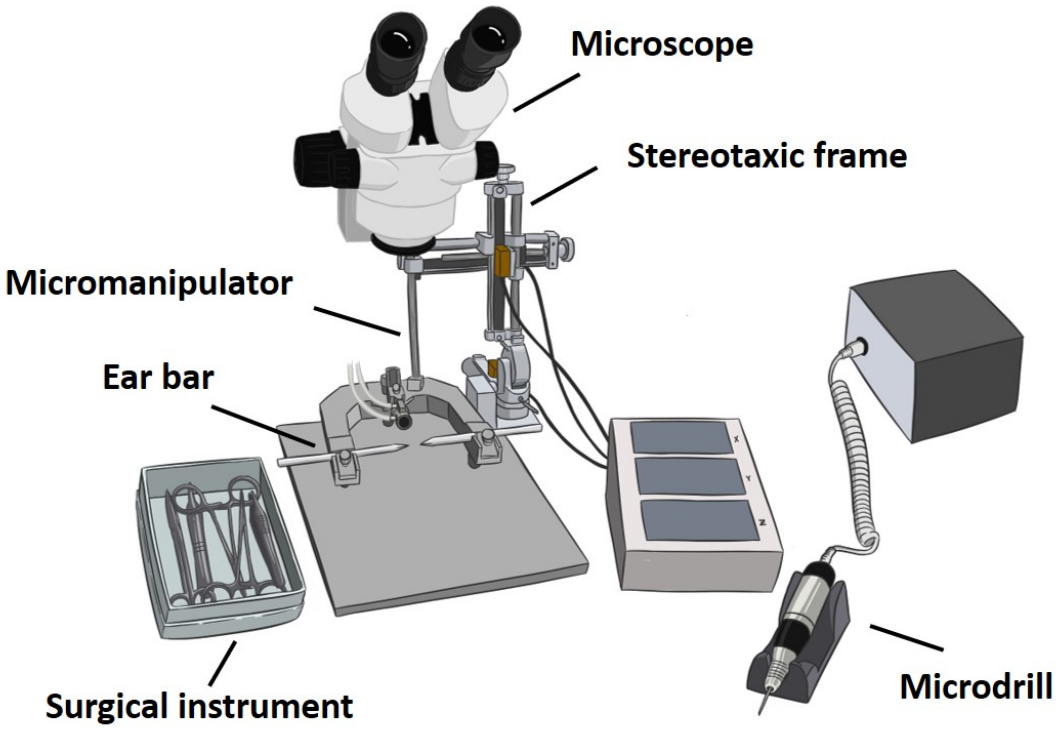
Fluid support

- 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

Acclimation (chronic surgery)

- A one-week period is recommended to prevent stress-induced diseases in rodents, pigs, cats, dogs, and ruminants.

- Fasting (chronic surgery)
- Not required for rodents due to their inability to vomit. Essential for guinea pigs, cats, dogs, ruminants, and non-human primates.
- Do not restrict access to water.

Eye protection (chronic surgery)

- In rodents and guinea pigs, eyes remain open under anesthesia, which can lead to corneal drying and trauma. To prevent this, apply ophthalmic ointment.

Monitoring

Monitor the animal under anesthesia to avoid excessive depression of cardiac and respiratory functions or insufficient anesthesia. Parameters that can be monitored in an anesthetized mouse without specialized equipment include:

- Respiratory rate and pattern
- Mucous membrane color
- Body temperature
- Oxygen saturation and heart rate
- Blood pressure

Regularly monitoring these parameters helps ensure the well-being of the animal and allows for timely intervention if needed.

Heat support

- All species are susceptible to the risks of hypotension (low blood pressure) and hypothermia (low body temperature) during anesthesia. To mitigate these risks, it is recommended to use circulating water blankets for temperature regulation. It is important to note that electric heating pads should not be used, as they pose a safety hazard.
- Regardless of the heat source chosen, 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 following 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

- Consider administering warm subcutaneous (SQ) or intraperitoneal (IP) fluids, especially during prolonged anesthesia or for animals that are ill, aged, or debilitated.

Recovery (chronic surgery)

- Continue monitoring animals until they are fully recovered.
- Allow them to recover on paper towels and provide a heat source if needed.

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.

Surgical Sterilization

For chronic studies, it is essential to sterilize the surgical tools.

Note: Microelectrode array, insertion tool, vector array probe holder, absorption surgical sponge triangle, and gel foam require ETO or VHP sterilization. The remaining tools can be steam sterilized (autoclaved). Please keep in mind that when placing your probe order, you can request ETO sterilization for your micro-electrodes.

High pressure/temperature (autoclave)

- The effectiveness of autoclaving must be verified by a steam integrator strip placed inside the surgical pack. It is recommended to seal the pack with autoclave tape as a second indicator.
- Autoclaving utilizes steam at high heat and pressure, which must penetrate the pack to achieve sterilization for surgical tools.
- Do not autoclave the microelectrode array, insertion tool, vector array probe holder, absorption surgical sponge triangle, or gel foam.
- Avoid using aluminum foil or wax paper as a wrapping pack, as steam cannot penetrate these materials.
- The standard exposure time in an autoclave is typically 20 minutes at 121°C (250°F).

  Note: Do not use this process for the microelectrode array.

Dry bead sterilization (dry heat)

- This method is designed to sterilize the tips of surgical instruments between multiple surgeries.
  - Note: Instruments must undergo full sterilization by another method between separate surgical sessions.
- The sterilizer must be turned on for at least 20 minutes before the sterilization procedure to achieve the appropriate temperature.

  All biological debris (e.g., blood, tissue) must be removed using alcohol before placing the instruments into the sterilizer machine.
  - Note: Immediately after removing the instruments from the sterilizer, the tips will be very hot. Let them cool down for 5 minutes to avoid burning the

animal.

- Note: Only the tips of the instruments are sterilized, and the handles are considered to be contaminated. Spray 70% alcohol on the handles.

Ethylene oxide (ETO) gas sterilization

ETO (Ethylene Oxide) is designed for items that cannot withstand high temperatures. Microelectrode array, insertion tool, vector array probe holder, absorption surgical sponge triangle, and gel foam need to be sterilized using ETO.

The four essential parameters for ETO are:

- Gas concentration: 450 - 1200 mg/L
- Temperature: 37 - 63°C
- Relative humidity: 40% - 80%; water molecules carry ETO to reactive sites
- Exposure time: 6 - 12 hours; these parameters influence the effectiveness of ETO sterilization.

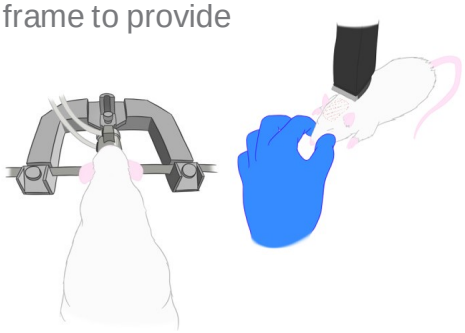
Vaporized hydrogen peroxidze (VHP) sterilization

- Also known as hydrogen peroxide gas sterilization, VHP (Vaporized Hydrogen Peroxide) is a low-temperature sterilization process commonly used to sterilize heat-sensitive devices. Microelectrode array, insertion tool, vector array probe holder, absorption surgical sponge triangle, and gel foam need to be sterilized using VHP.
- A sterilization cycle typically lasts for 3 hours and requires less time than ETO sterilization.
- The hydrogen peroxide sterilization process involves H2O2 vapor filling the sterilizer chamber, contacting and sterilizing exposed device surfaces.
- VHP gas vaporizes hydrogen peroxide, which is maintained at a constant concentration while catalytically transforming to oxygen and water in the return air. The process is "dry" because it prevents condensation of the peroxide/water vapors.
- Incubator components, including the CO2 sensor and HEPA components, can remain inside during sterilization with VHP. There is no standard set of conditions for VHP.

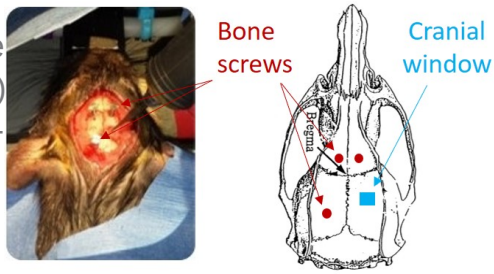
General Surgical Steps

- To anesthetize the animal, administer a cocktail of xylazine (10 mg/kg for rats and mice) and ketamine (50-100 mg/kg for rats, 80 mg/kg for mice) via intraperitoneal (IP) injection or using isoflurane inhalation (4% in 100% O2). Maintain anesthesia during the surgical procedure with isoflurane at the appropriate concentration (1-2.5% for rats, 0.8-1.25% for mice), and provide ventilation using a mixture of oxygen and medical air (50:50). Ensure that the animal remains adequately anesthetized throughout the surgery.
- Assess the depth of anesthesia throughout the surgery by performing a toe pinch reflex test.

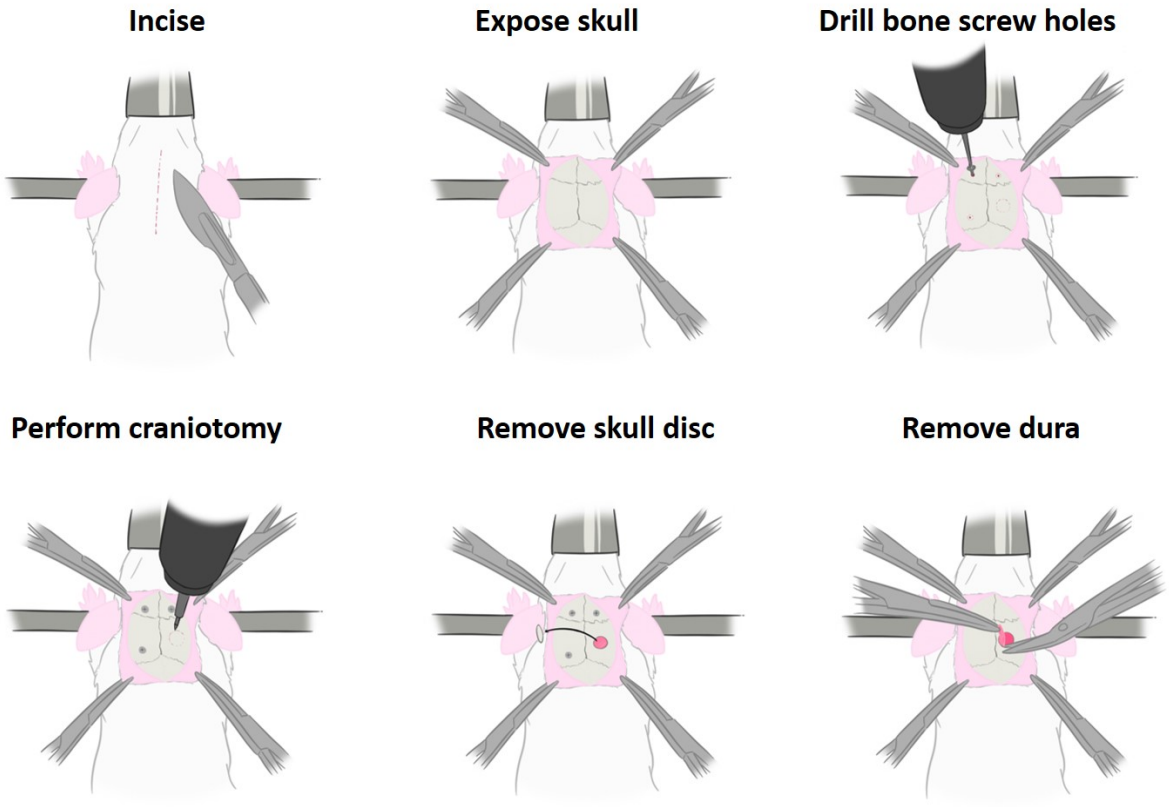
- Shave the surgical area to ensure a clean working environment.
- Intubate the animal and secure it in a stereotaxic frame to provide stability during the procedure.



- Apply artificial tear ointment to the rodent's eyes to prevent drying.
- Optionally, inject dexamethasone sodium phosphate (2 mg/kg) before surgery to reduce cerebellar edema.
- Sterilize the skin of 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 remove the periosteum from the skull and clean the skull surface with saline.
- Apply a thin layer of VetBond adhesive to dry the surface of the skull for improved adhesion and grip of 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; two above the implantation site and one over the contralateral site of implant) for stainless steel screws to be used later as a ground and reference.



- Advance screws (mouse: 4 mm long, 0.86 mm diameter) into drilled holes, taking special care not to advance the screws beyond the point of contact with the dura.
- Secure the threads of the screws to the bone with a small drop of VetBond. Perform a craniotomy above the brain region of interest using a high-speed drill.
    - Note 1: If you are planning to do electrophysiology only, make a small craniotomy; for imaging studies like optogenetics or 2-photon imaging, a larger craniotomy is desired.
    - Note 2: During drilling, regularly flush the surgical window with saline to reduce heat buildup and remove blood and bone debris.
    - Thin the edges of the craniotomy until the underlying pial vasculature becomes visible, being cautious not to damage the brain tissue.
    - Use forceps to gently separate the bone flap from the skull, taking care not to protrude 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.

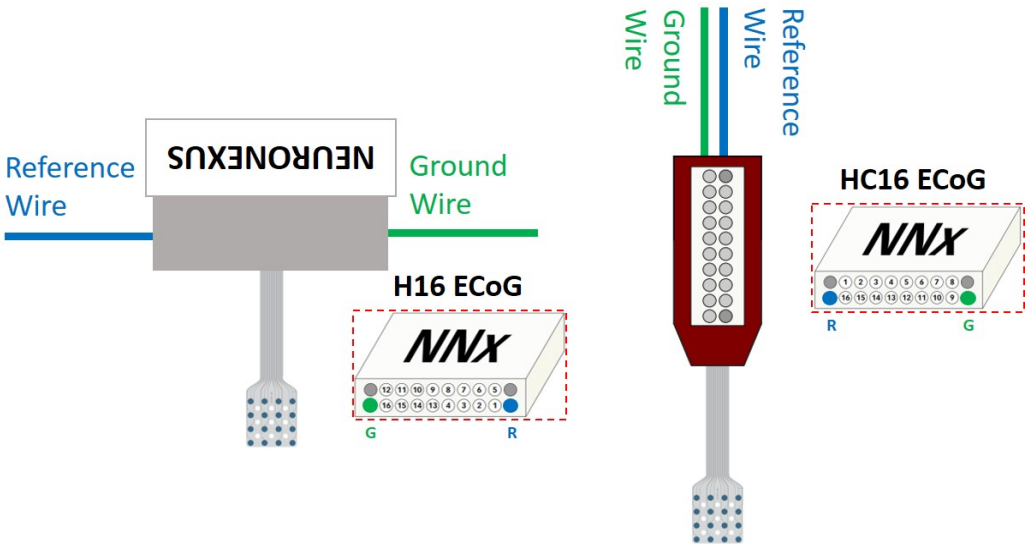
Surgical Procedures for ECoG Electrodes

- Place the probe on top of the dura mater to avoid causing any damage to the cortex.
- Apply octyl cyanoacrylate tissue adhesive on the edge of the microelectrode array to fix it on the pia surface.
- Cover your electrode with autologous connective tissue to seal the dura opening.
- Fix the micro connector at the end of the microelectrode array ribbon cable to the skull, using octyl cyanoacrylate (as an adhesive layer over the skull) and dental acrylic.
- Wrap your ground and reference wires around the bone screws (check the wiring configuration section of this protocol).
- Apply dental cement around the screws and the wrap to create a head cap.

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.

ECoG16-channel Electrode

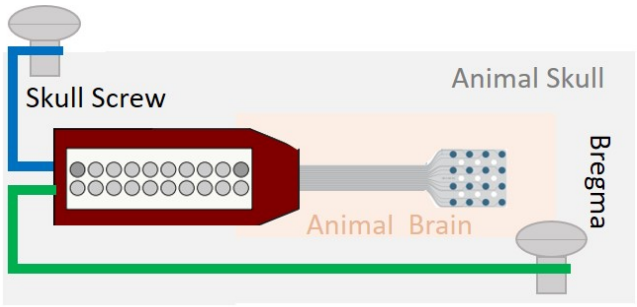


The top view displays ECoG probes with different packaging for H16 and HC16 ECoG. **It's important to note that all recording sites face upward.**

Here's an additional note: Strip a small length of insulation from the ends of the Reference and Ground Wires before implantation.

During the implantation procedure, attach the Reference and Ground Wires separately to the bone screws previously placed. The Ground Wire should be wrapped around the bone screw over the front side of the implantation site, while the Reference wire should be wrapped over the bone screws on the backside of the implantation site.

Note: In this configuration, all the sites are facing upward and are not in contact with the brain surface.

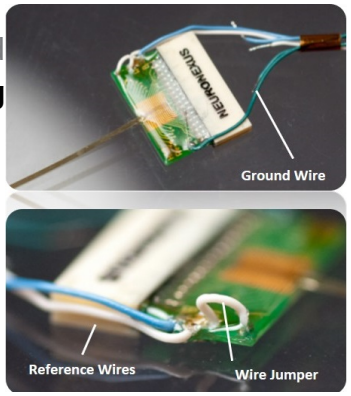


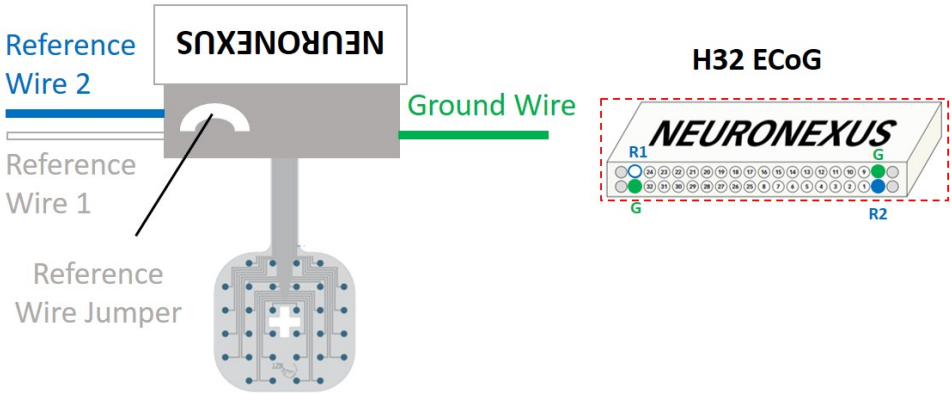
ECoG 32-channel Electrode

ECoG probes with 32 channels come with different packaging. Here, we are presenting H32 and HC32 ECoG packaging.

H32 ECoG packaging

The Reference Wire Jumper allows researchers to customize the Reference and Ground Wiring configuration for their application. **Note that all recording sites face up.**

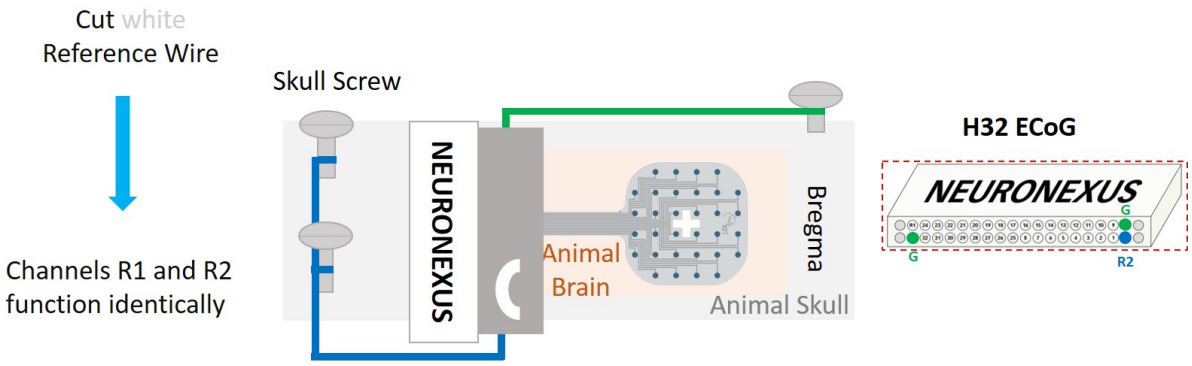




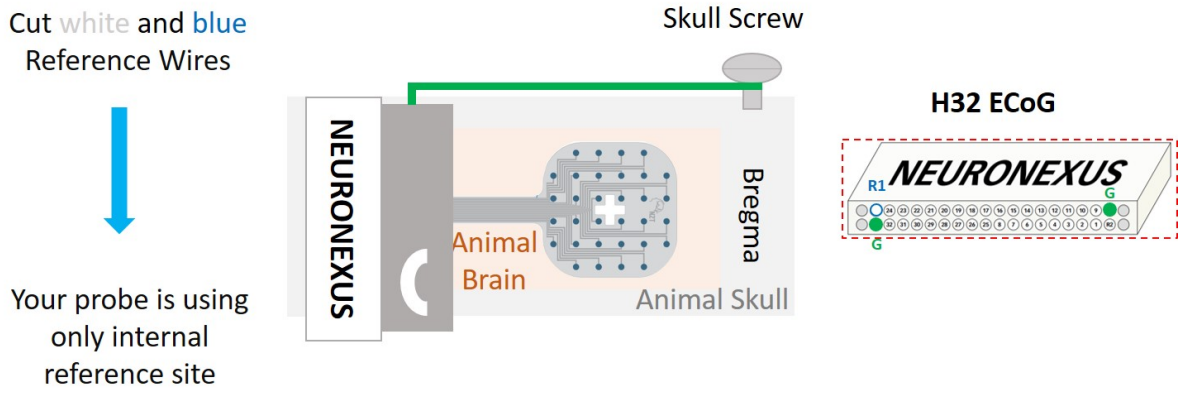
Models for H32 ECoG Probe

Internal reference site model

If the probe has an internal reference site and you wish to include an external reference, cut the white Reference Wire while connecting the blue wire to the back side of the implantation site. **Note: In this configuration, all the sites are facing up and are not in contact with the brain surface.**

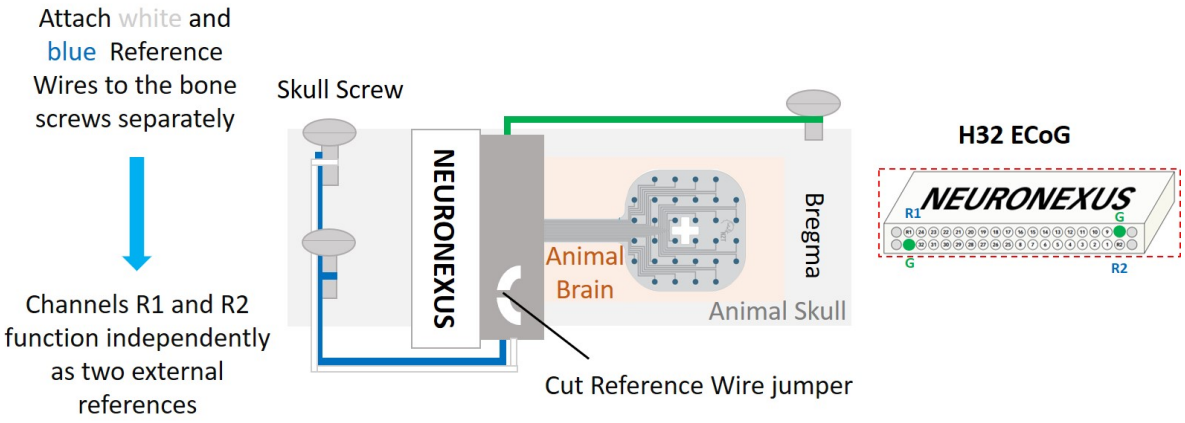


If you choose not to have an external reference, cut the blue Reference Wire while connecting the ground wire to the front side of the implantation site. **Note: In this configuration, all the sites are facing up and are not in contact with the brain surface.**



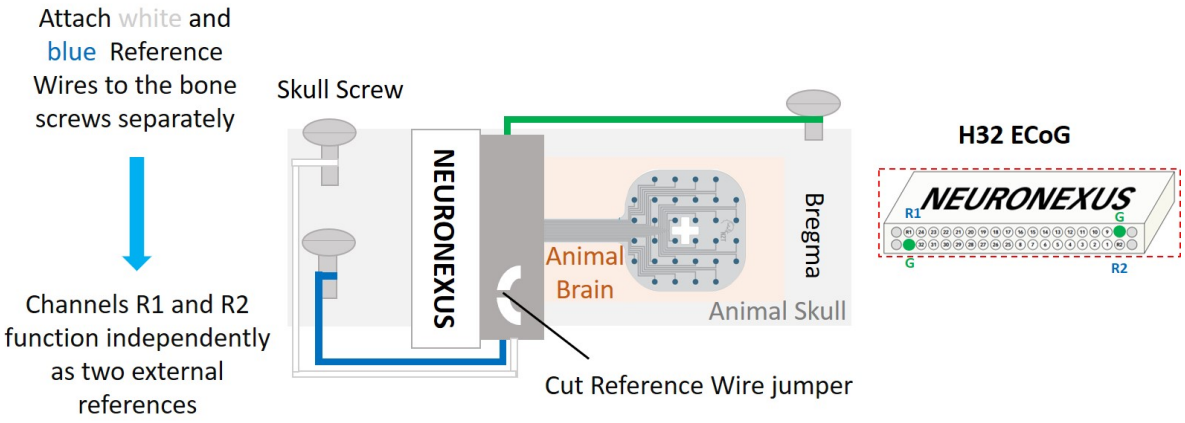
One external reference model

Cut the Wire Reference Jumper, tie both Reference Wires together, and attach them to the bone screws on the backside of the implantation site. **Note: In this configuration, all the sites are facing up and are not in contact with the brain surface.**



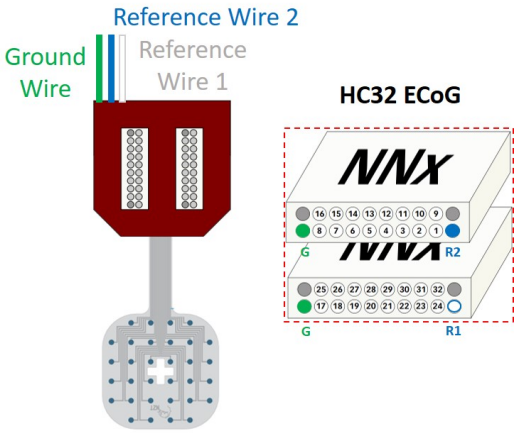
Two external references model

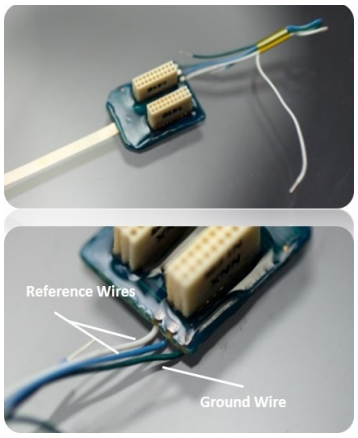
Cut the Wire Reference Jumper, and attach each Reference Wire to the bone screws on the backside of the implantation site. **Note: In this configuration, all the sites are facing up and are not in contact with the brain surface.**



HC32 ECoG packaging

Below is a top view of HC32 ECoG packaging. **Note that all recording sites face up.**





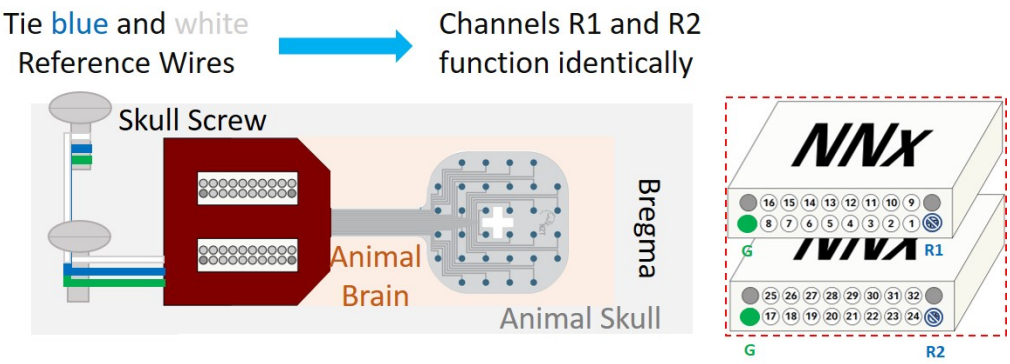
Note: Strip a small length of insulation from the ends of the Reference and Ground Wires before implantation.

Models for HC32 ECoG Probe

Some of the 32-channel electrodes come with a Reference Site on the flexible probe shank.

Simplest model

The simplest wire configuration is to tie the Ground and Reference Wires together and attach them to one bone screw on the front side of the implantation site. **Note: In this configuration, all the recording sites are facing up and not in contact with the brain surface.**

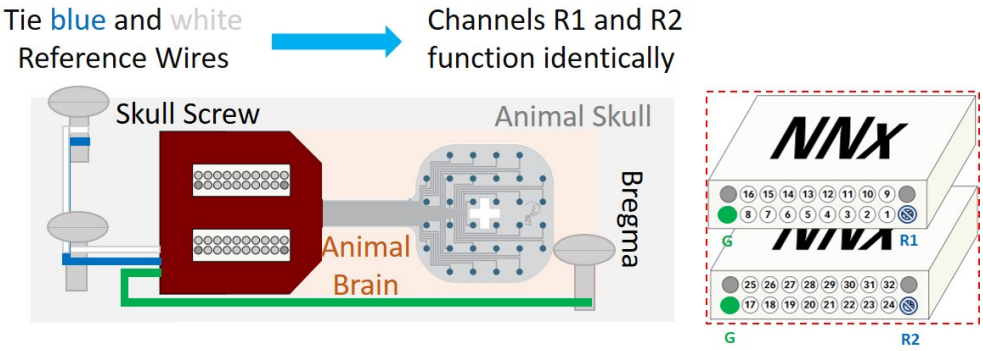


However, the following setups might be considered according to the experiment.

Internal reference site model

- Tie blue and white reference wires

If your electrode has an internal reference site, to use it, tie Reference Wires together and attach them to the bone screw on the backside of the implantation site. **Note: In this configuration, all the sites are facing up and not in contact with the brain surface.**

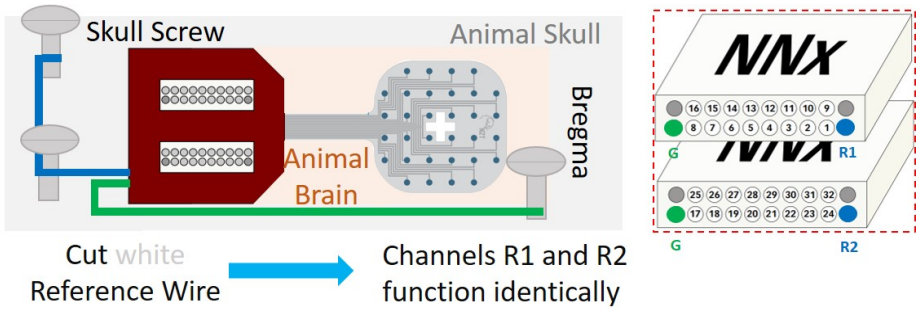


- Cut blue reference wires

Connect the white Reference Wire to the reference port on your headstage (if it has one). The probe reference now feeds into the reference channel on the headstage.

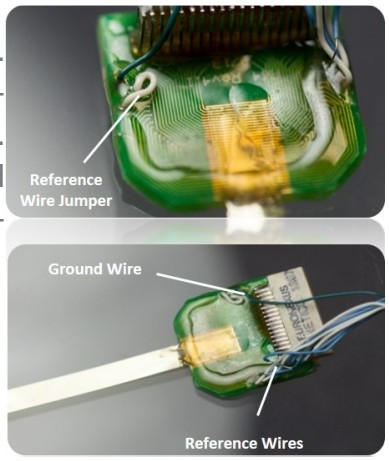
External reference model

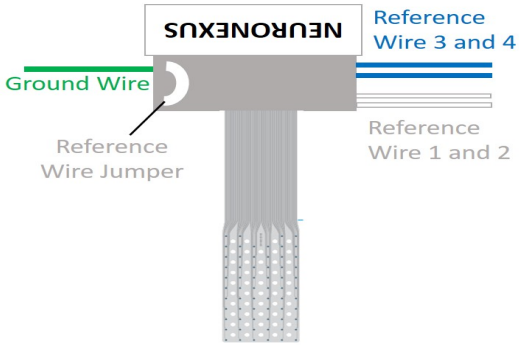
Cut the white Reference Wire. Connect the blue Reference Wires to the bone screws on the backside of the implantation site, while the Ground Wire should be wrapped around the screw on the front side of the implantation site. **Note: In this configuration, all the sites are facing up and not in contact with the brain surface.**

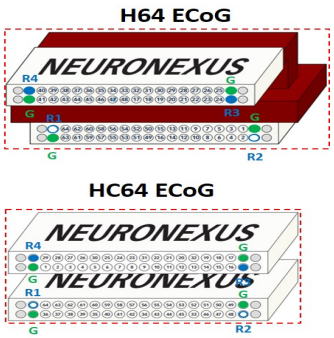


ECoG 64-channel Electrode

ECoG probes with 64 channels function similarly to those with 32 channels. They come with a Reference Wire Jumper, four References (two for the electrode in white, and two for the headstage in blue), and one Ground Wire. These components allow researchers to customize the Reference and Ground Wiring configuration for their specific application. Note that all recording sites face up.







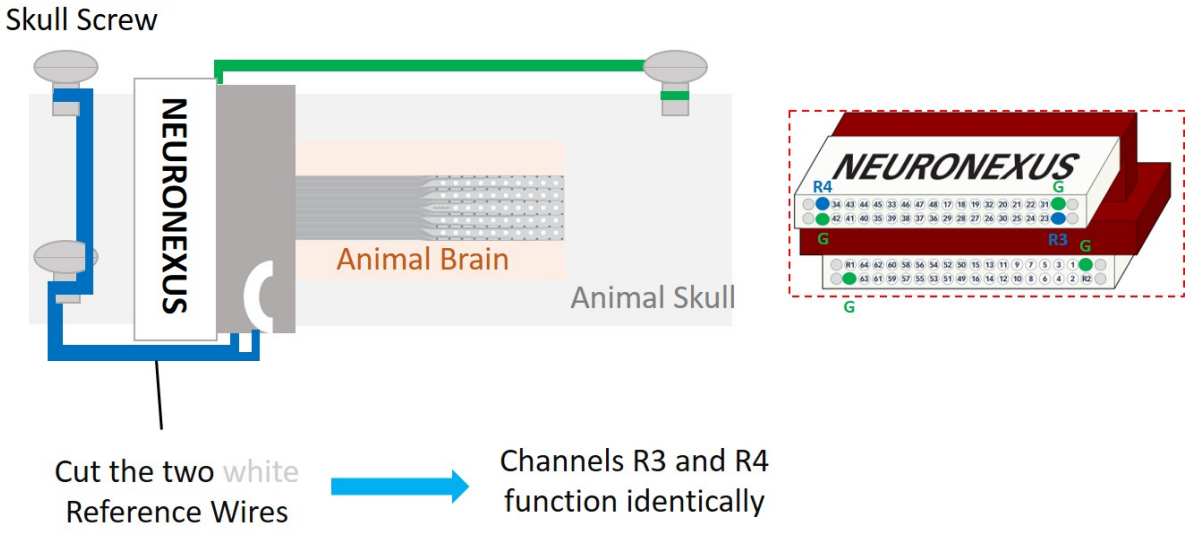
Note: Before implantation, it is important to strip a small length of insulation from the ends of the Reference and Ground Wires.

Models for 64-channel Chronic Probe

Some of the 64-channel ECoG electrodes come with an internal Reference Site.

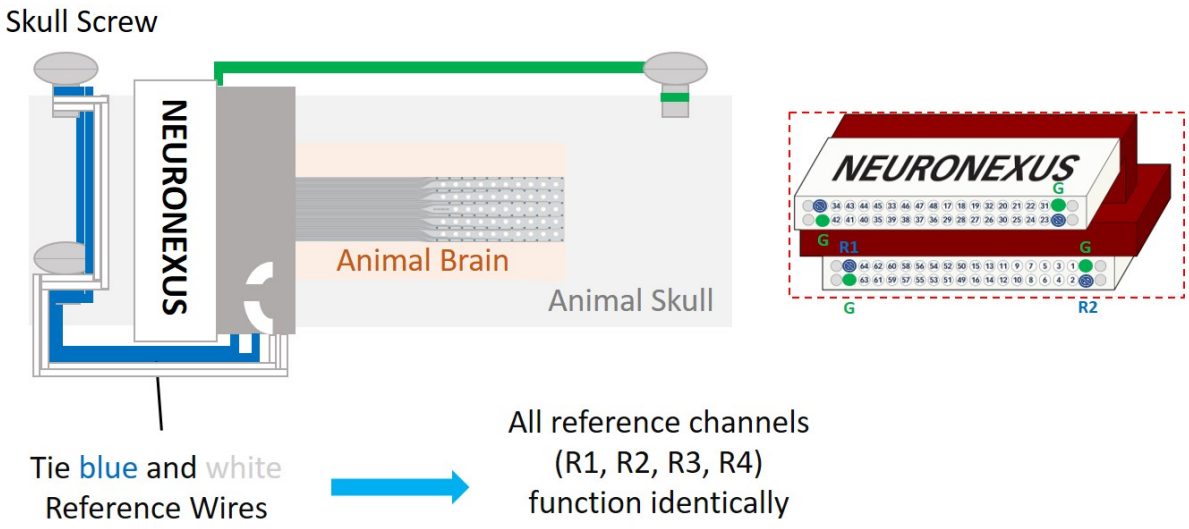
Use reference site model

To utilize the internal reference site, cut the white Reference Wire, while the blue Reference Wires are wrapped around the screws on the backside of the implantation site. **Note: In this configuration, all the sites are facing up and not in contact with the brain surface.**



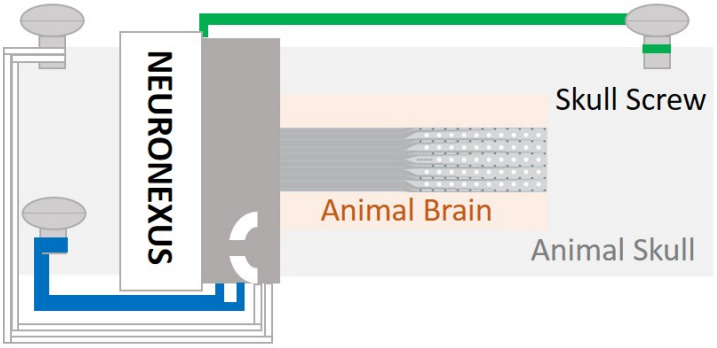
One external reference model

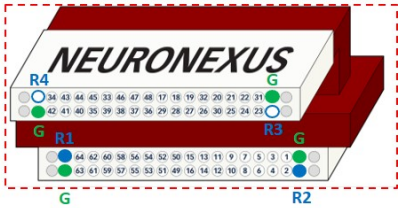
If you want to merge the electrode and headstage reference, cut the Reference Wire Jumper and tie the blue and white Reference Wires. Connect both to the bone screws on the backside of the implantation site, while the Ground Wire should be wrapped around the screw on the front side of the implantation site. **Note: In this configuration, all the sites are facing up and not in contact with the brain surface.**



Two external reference model

Cut the Reference Wire Jumper, and attach each Reference Wire separately to different bone screws on the backside of the implantation site, while the Ground Wire should be wrapped around the screw on the front side of the implantation site. **Note: In this configuration, all the sites are facing up and not in contact with the brain surface.**





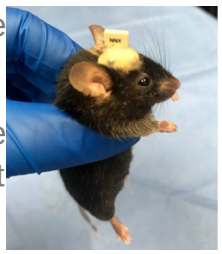
Separate blue and white Reference Wires → R1 and R2 work separately than R3 and R4

Steps After Surgery

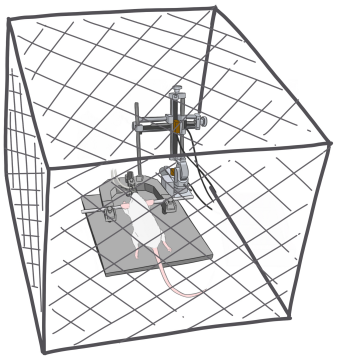
After surgery (chronic applications):

- Apply triple antibiotic along the edge of the dental cement.
- Administer a subcutaneous injection of buprenorphine (mouse: 0.1 mg/kg) or Ketoprofen (rat: 5 mg/kg).
- Place the animal on a heating pad to aid in recovery from anesthesia. Note: After surgery, the animal should be individually housed with nesting material.
    - Note: Electrophysiology recording may be performed on anesthetized or freely behaving animals.

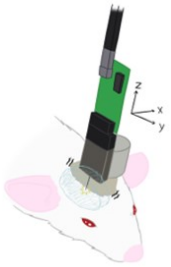
Here is an example of the 16-channel chronic probe implanted into the left side of the mouse brain after 1 week of recovery from the surgery.



When the animal is ready for electrophysiology experiments, place the animal with or without a stereotaxic frame in the Faraday cage and start your electrophysiology experiment as follows:



- Connect the probe connector to the headstage.
- Record impedance immediately after electrode insertion to ensure the electrode is intact and capable of picking up neural activity.
- Determine a name and saving path for your recording data from Allego settings.
- Start streaming. Open the Monitor tab from the Dashboard.
- Remove possible noise in your system using the guidelines below.
- Apply filters: To monitor your streaming data in the desired frequencies, from the Dashboard, click on the Signal processing tab and add filters as explained in the SmartBox Pro/Allego manual.
- Open the Electrode tab from the Dashboard to add or remove recording sites.
- Track real-time recording.
- Check signal metrics in real-time.
- Start recording.
- After recording, check and replay the saved data.



Recommendations for Reducing Noise in Electrophysiology Setup

One of the most common noise issues is 50 Hz (Europe) or 60 Hz (US) 'ground' noise, which can be mitigated by appropriately grounding the electrophysiology setup to prevent ground loops between instruments. Higher frequency noise may stem from sources such as the computer, monitor, room lights, digital oscilloscope, or instrument power supplies.

It is crucial to connect all equipment to the same ground, as different sockets may not have precisely the same voltage level. Begin by connecting a power strip to a single wall socket to ground all equipment.

To reduce noise, experiment with each suggested method individually or in combination to achieve the desired noise level. While these methods can yield a clean signal with minimal noise, every setup is unique and may encounter distinct noise issues. If needed, contact support@neuronexus.com for further assistance.

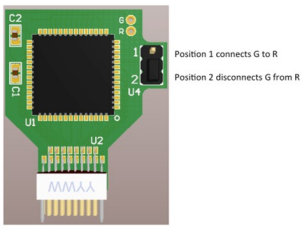
Using a heavy, grounded metal plate at the bottom of the Faraday cage can help contain ambient noise in the recording setup. Connect the Faraday cage, microscope, stereotaxic frame, manipulator, etc., to the ground metal plate using heavy gauge wires. Attach a single wire from the plate to the best possible main ground.

Check the grounding of the Faraday cage and SmartBox Pro. If the animal is in the stereotaxic frame during recording, add extra alligator clips from the stereotaxic frame to the Faraday cage. Turn off the camera or light on top of the microscope and check the microscope's grounding. Additionally, turn off any high-power machines close to your recording setup, unplug all other devices from the main outlets, and add an extra needle as a probe grounding wire to the nuchal musculature, attaching it to the Faraday cage using alligator clips.

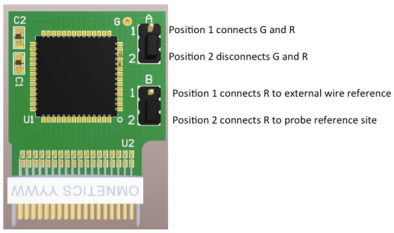
Verify if thermal noise from the headstage is present, especially if the recording setup has been in continuous use for an entire day.

If noise persists despite these measures, consider changing the ground and referencing on the headstage. Smartlink headstages are designed with jumpers (Jumper A for headstage referencing and grounding level, Jumper B for referencing at the probe level) to provide users with flexibility in achieving the cleanest possible signal.

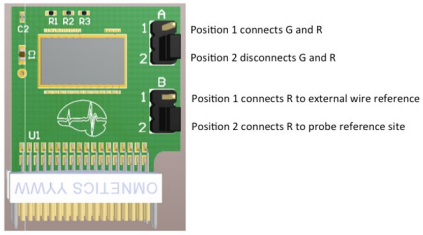
Chronic SmartLink 16



Chronic SmartLink 32



Chronic SmartLink 64



- Jumper A in position 2 represents the default configuration designed to provide the lowest noise level. In this setup, as previously mentioned, it is necessary to affix two separate bone screws to the skull and connect the reference and ground wires to them individually.
- For Jumper B, many customers opt for position 2 to utilize electrodes equipped with a substantial reference site on the probe shank. If an external reference is preferred, switch Jumper B from position 2 to position 1.

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.