NeuroNexus

# EEG Electrode Arrays

## Surgical Guide and Electrophysiology

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# Table of Contents

# 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.

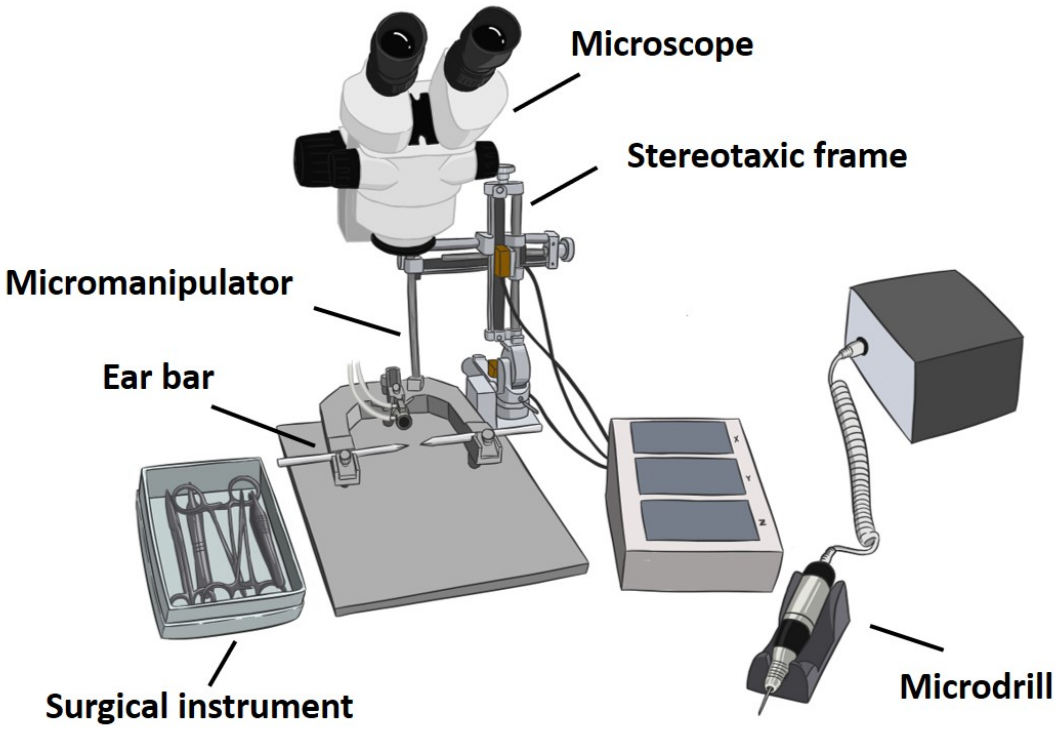
## Fluid support

- 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.

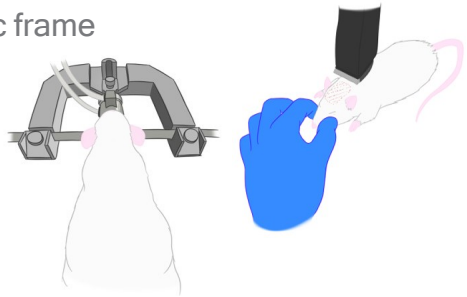
## Vaporized hydrogen peroxidze (VHP) sterilization

- 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.

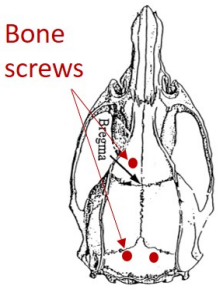
# General Surgical Steps

- Anesthetize the animal with a combination of xylazine (10 mg/kg for both rats and mice) and ketamine (50-100 mg/kg for rats, 80 mg/kg for mice) administered through intraperitoneal injection or inhalation of isoflurane (4% in 100% O2). Throughout the surgical procedure, sustain anesthesia using isoflurane (1-2.5% for rats, 0.8-1.25% for mice) and ensure ventilation with a mixture of oxygen and medical air (50:50).
- Utilize the toe pinch reflex to assess the depth of anesthesia throughout the surgery.

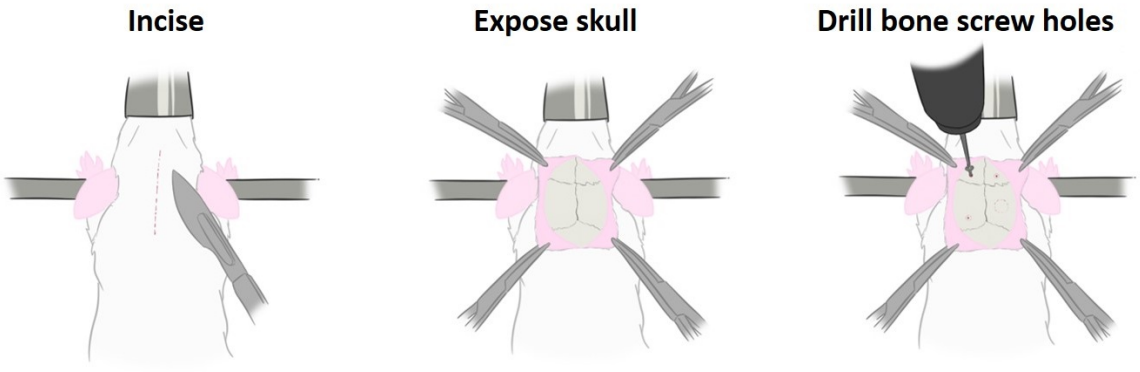
- Trim the fur in the surgical area to maintain a clean working environment.
- Intubate the animal and secure it in a stereotaxic frame



- 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 with betadine and 70% alcohol.
- Make a midline sagittal incision along the scalp to expose the skull covering the area of interest.
- Use a cotton-tip applicator to remove the periosteum from the skull. Apply saline for skull cleaning.
- Apply a thin layer of VetBond adhesive to the dry surface of the skull before drilling to provide a supportive grip for a 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 the implant for stainless steel screws, which will be used later as a ground and reference.

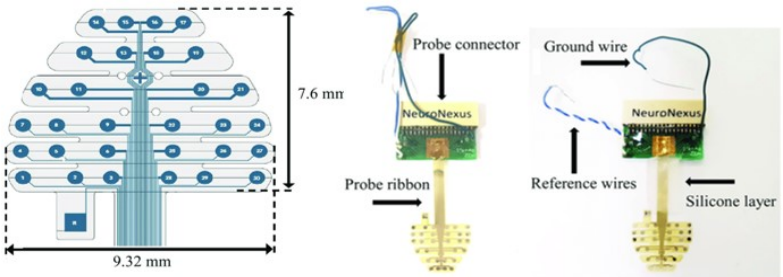



- Advance screws (mouse: 4 mm long, 0.86 mm diameter) into the drilled holes, taking special care not to advance the screws beyond the point of contact with the dura.
- Secure the threads of the screws to the bone with a small drop of VetBond.

  Right now, you are ready to implant the electrode.

# Surgical Procedures for EEG Electrodes

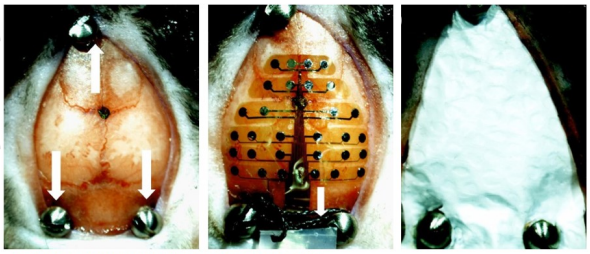
Here is an example of a 30-contact micro-electrode array with a thickness of 20 µm. To enhance the reusability of the surface array, NeuroNexus recommends adding a layer of silicone to the probe ribbon and allowing it to dry overnight.




Jonak et al, 2018, Frontiers in Integrative Neuroscience

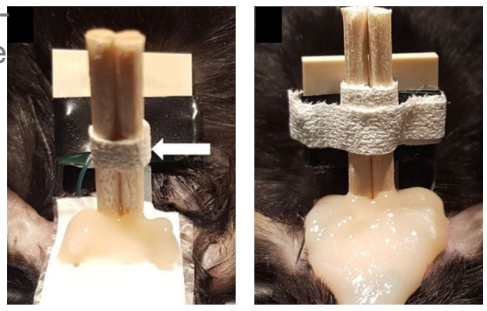
- Place the sterile probe flat as a "sheet" on the surface of the skull. Apply sterilized saline on top of the probe to facilitate adherence to the skull reference and allow it to dry. Use 4-0

silk tie to secure the probe ribbon cable



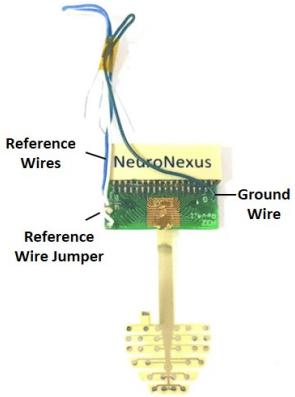
Jonak et al, 2018, Frontiers in Integrative Neuroscience

- Wrap your ground and reference wires around the bone screws (refer to the wiring configuration section).
- Place Teflon and then Saran Wrap on top of the probe to protect the microelectrode array.
- Make small holes to fit screws.
- Apply dental cement around the back screws to secure the ribbon cable.
- To secure the implant, add two pieces of a cotton-tip applicator (2 cm in length) that are secured with medical tape (white arrow), and place them between the two back screws.
- Using dental cement if your objective is to record from a behaving animal, secure the attached cotton-tip applicators to the probe connector using waterproof medical tape. This will serve as an anchoring "post" for the probe connector.
- Apply a final layer of dental cement to create a head cap.

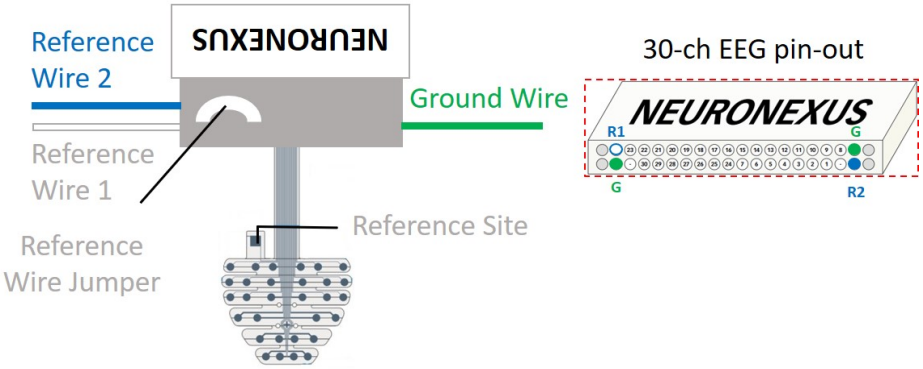


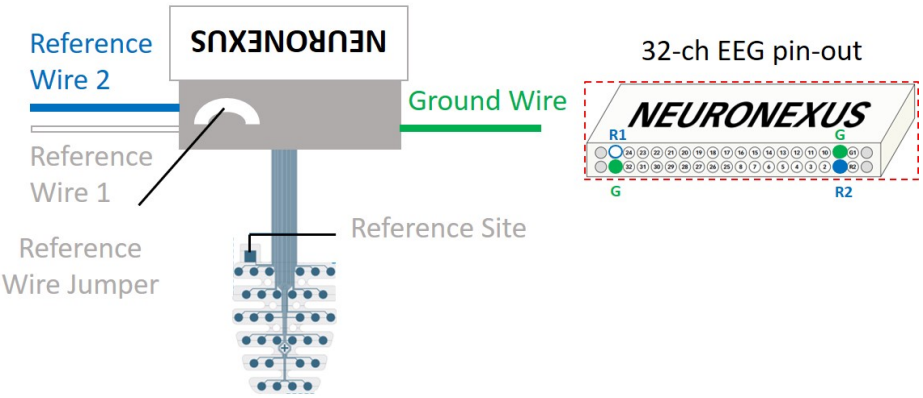
# 32-ch EEG Electrode Wiring Configuration

EEG probes come with a Reference Wire Jumper, allowing researchers to customize the Reference and Ground Wiring configuration for their application. **It's important to note that all recording sites face up.**



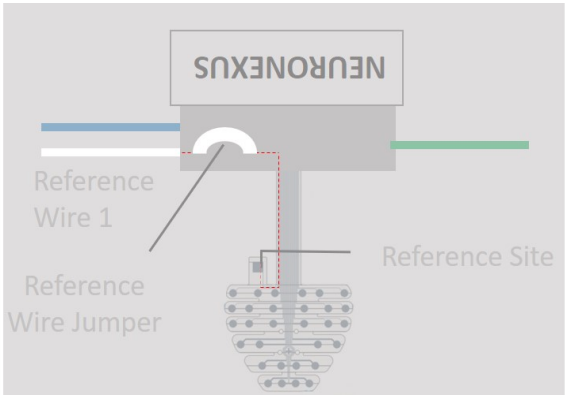

Note: Before implantation, strip a small length of insulation from the ends of Reference and Ground Wires.



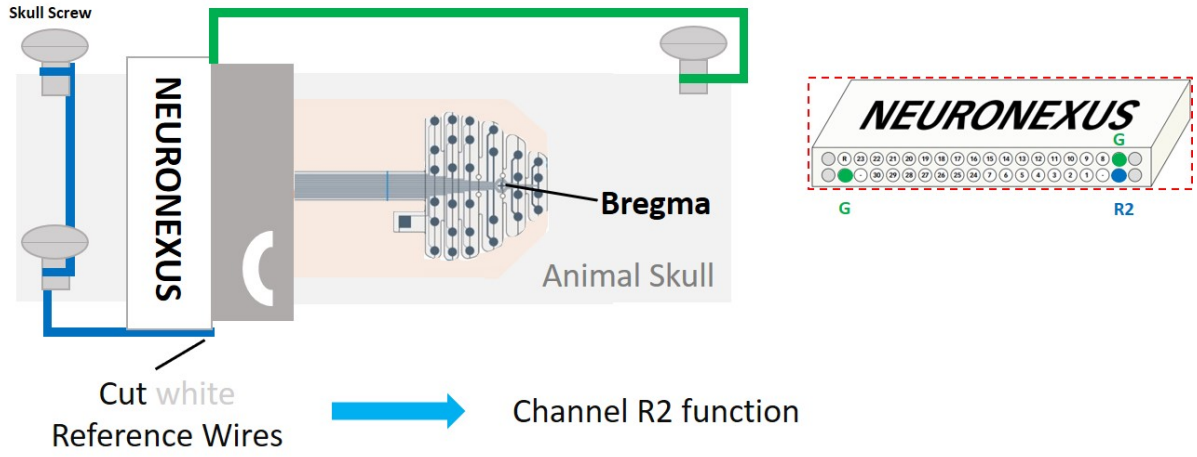




The Reference Wire Jumper and the white Reference Wire 1 are connected to the Reference Site featured in EEG electrode array designs, and they are wired to channel R1 on the Omnetics connector.

Cut the white Reference Wire while connecting the blue wire to the back side of the implant.




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

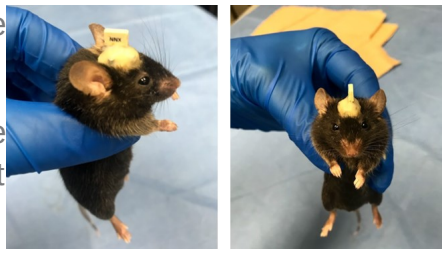



# Steps After Surgery

After surgery (chronic applications):

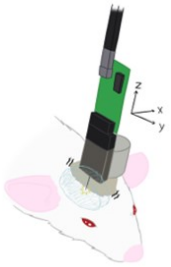
- Apply triple antibiotic along the edge of the dental cement.
- Administer a subcutaneous injection of buprenorphine (mouse: 0.1 mg/kg) or Ketoprofen (rat: 5 mg/kg).
- Place the animal on a heating pad to aid in recovery from anesthesia. Note: After surgery, the animal should be individually housed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

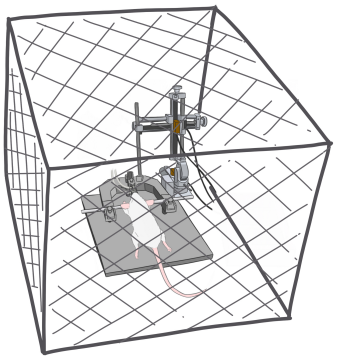
Here is an example of the 16-channel chronic probe implanted into the left side of the mouse brain after 1 week of recovery from the surgery.



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.

# Reduce Noise in EEG Setup

One common issue in electrophysiology setups is the presence of 50 Hz (Europe) or 60 Hz (US) 'ground' noise, which can be mitigated by ensuring proper grounding to avoid ground loops between instruments. Higher frequency noise may originate from various sources such as computers, monitors, room lights, digital oscilloscopes, or instrument power supplies.

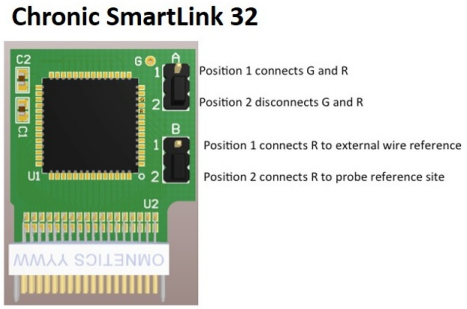
It is crucial to connect all equipment to the same ground, as different sockets may have varying voltage levels. Start by connecting a power strip to a single wall socket to ground all equipment. To further reduce noise, experiment with the suggested methods individually or in combination to achieve the desired noise level. While these methods generally result in a clean signal with low noise, each setup is unique, and for further assistance, contact support@neuronexus.com.

To contain ambient noise in the recording setup, use a heavy, grounded metal plate on the bottom of the Faraday cage. 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 if the Faraday cage and SmartBox Pro are grounded. If the animal is in the stereotaxic frame during recording, add extra alligator clips from the stereotaxic frame to the Faraday cage.

Additional steps include turning off the camera or light on top of the microscope, checking the microscope's earth connection, turning off any high-power machines near the recording setup, and unplugging all other devices except from the main outlets. Adding an extra needle as a probe grounding wire to the nuchal musculature and attaching it to the Faraday cage with alligator clips can further help.

Check for thermal noise from the headstage, especially if the recording setup has been used continuously for an entire day. If noise persists, consider changing the ground and referencing on the headstage.

Smartlink headstages are equipped with jumpers for enhanced flexibility in obtaining a clean signal. Jumper A is related to the headstage referencing and grounding level, while jumper B is for referencing at the probe level.




- Jumper A, set to position 2, is the default configuration designed to provide the lowest noise level. In this configuration, it is necessary to add two separate bone screws to the skull and attach the reference and ground wires to them individually.
- For jumper B, many users opt for position 2, especially when using electrodes equipped with a large reference site on the probe shank. If you prefer to use an external reference, switch jumper B from position 2 to position 1. This adjustment allows you to customize the referencing setup based on your specific preferences and experimental requirements.

# 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.