NeuroNexus

Chronic Penetrating Arrays

Surgical Guide and Electrophysiology

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Introduction

This comprehensive technical document provides crucial information on animal surgery in bio-medical research settings. It encompasses key topics including pre-operative procedures, anes-thesia 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.

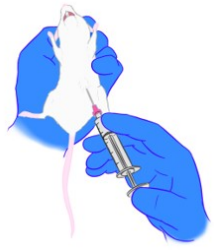
Local Anesthetics:

- 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.

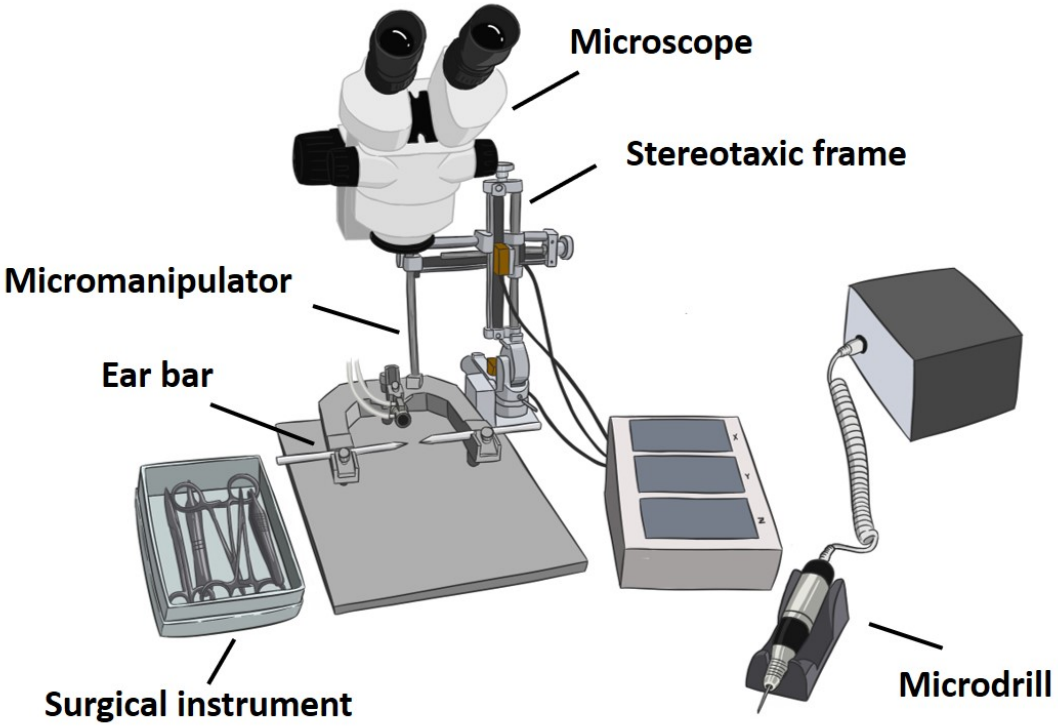
Fluid support

- 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 microelectrodes.

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

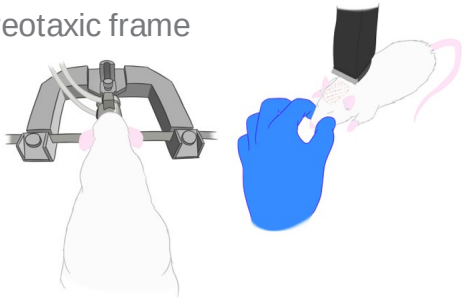
- Also known as hydrogen peroxide gas sterilization, VHP (Vaporized Hydrogen Peroxide) is a low-temperature sterilization process commonly used to sterilize heat-sensitive devices. Microelectrode array, insertion tool, vector array probe holder, absorption surgical sponge triangle, and gel foam need 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

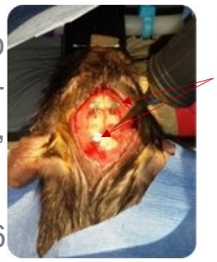
- Anesthetize the animal with a combination of xylazine (10 mg/kg for both rats and mice) and ketamine (50-100 mg/kg for rats, 80 mg/kg for mice) administered through intraperitoneal injection or inhalation of isoflurane (4% in 100% O2). Throughout the surgical procedure, sustain anesthesia using isoflurane (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.

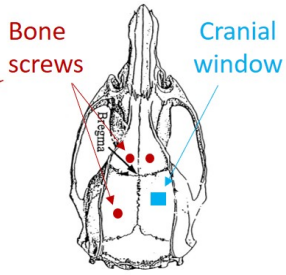
- Trim the fur in the surgical area to maintain a clean working environment.
- Intubate the animal and secure it in a stereotaxic frame
- Apply artificial tear ointment to the rodent's eye to prevent drying.
- Optionally, inject dexamethasone sodium phosphate (2 mg/kg) before surgery to reduce cerebellar edema.



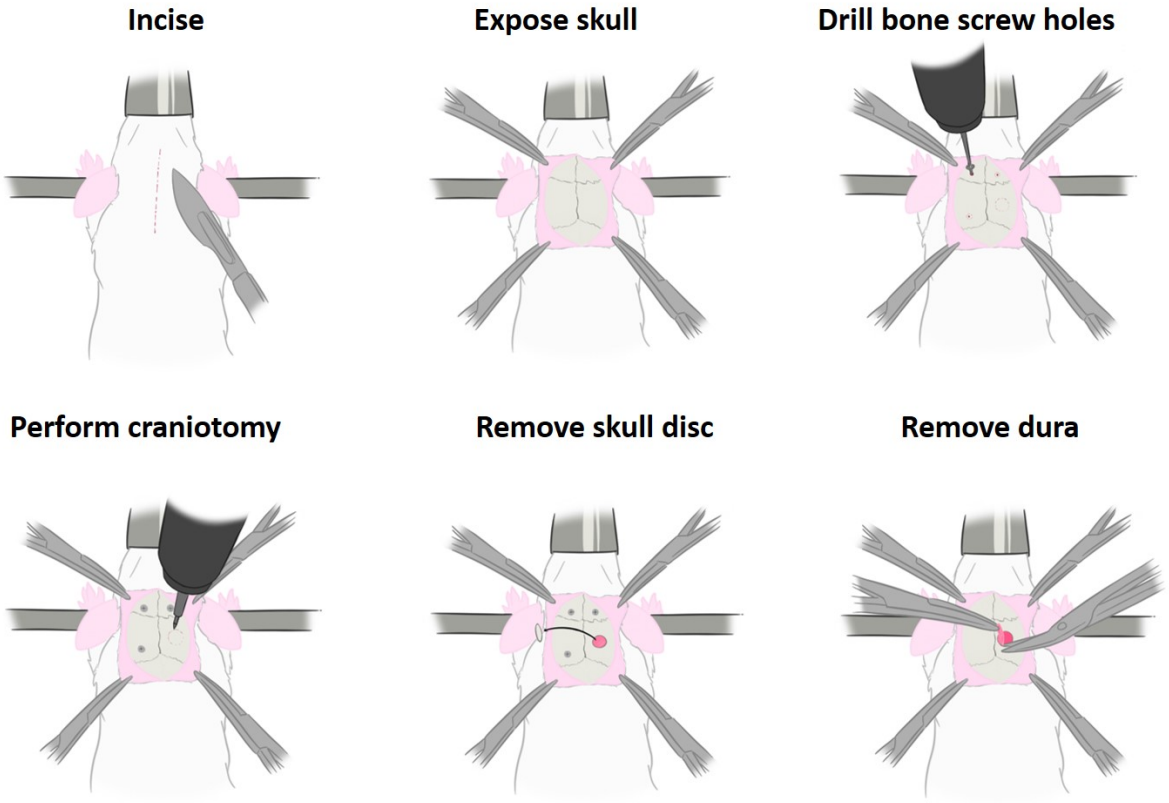
- Sterilize the skin of the surgical site in a chronic setup with betadine and 70% alcohol.
- Make a midline sagittal incision along the scalp to expose the skull, covering the area of interest.
- Use a cotton-tip applicator to remove the periosteum from the skull, applying saline for skull cleaning.
- Apply a thin layer of VetBond adhesive to dry the 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 over both motor cortices and one over the contralateral site of the implant for stainless steel screws, which will be used later as a ground and reference.





- Carefully 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 dab of VetBond.



1.

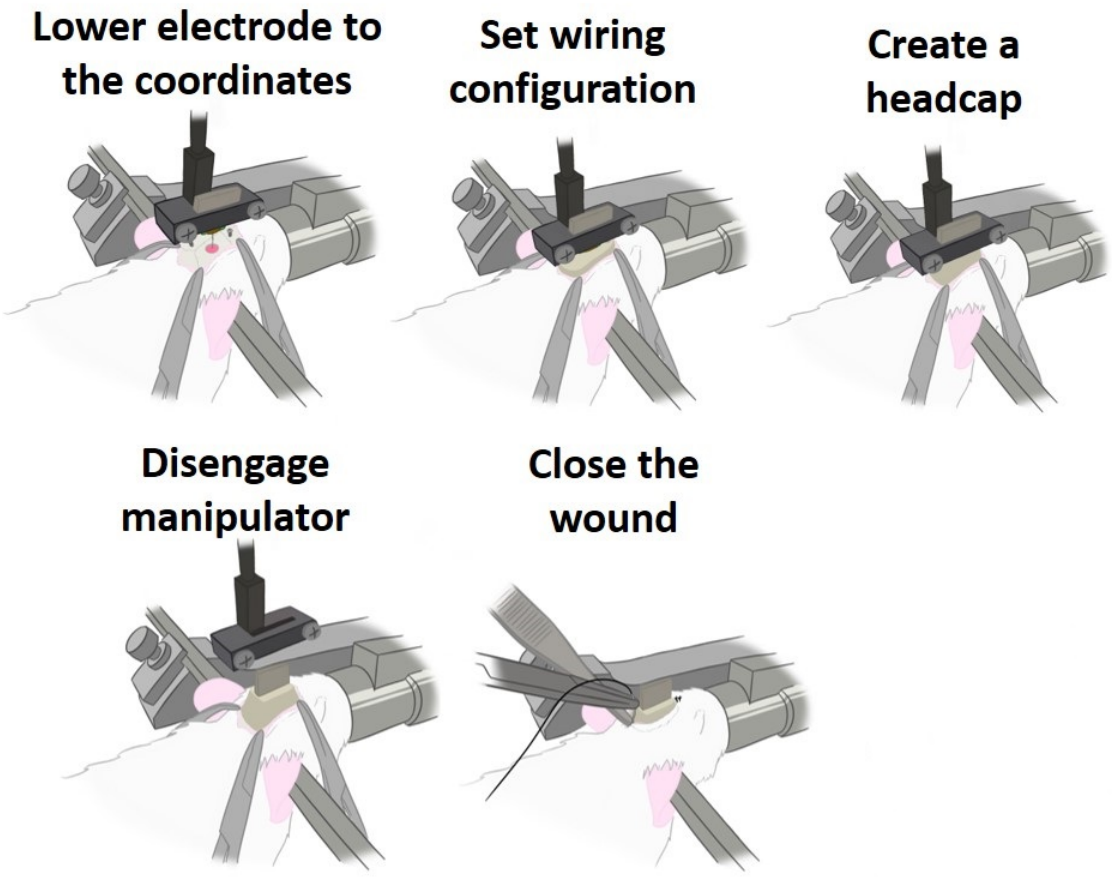
- Perform a craniotomy above the brain region of interest using a high-speed drill.
    - Note 1: If you are conducting electrophysiology (ephys), aim for a small craniotomy; for imaging studies like optogenetics or 2-photon imaging, a larger craniotomy is

desired.

- Note 2: During drilling, regularly flush the window with saline to reduce heat buildup and remove blood and bone shavings.

- Thin the edges of the craniotomy until the underlying pial vasculature becomes visible. Continue very carefully until the bone begins to craze. Then, use forceps to gently separate the bone flap from the skull without protruding 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 bleeding using gel foam soaked with sterile saline. Note: This step is not necessary for mice.
- Now, you are ready to implant the electrode.

Surgical Procedures for Penetrating Probes



- Lower the probe to the surface of the cortex.
- Insert the probe using an automatic or manual micromanipulator to the desired depth at a rate of 1 mm/min.
    - Note: To monitor neural spiking throughout the insertion and locate a position where activity could be recorded, different step sizes at different depths could be selected.
- Fill the craniotomy with silicon elastomer (Kwik-Cast, Kwik-Sil) and allow it to dry.
- Secure the probe to the skull using dental cement.

- 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 skull to create a robust head cap.
- Suture the surgery site and apply non-prescription tri-antibiotic cream.
- Inject 0.9% saline subcutaneously at the end of the surgery.

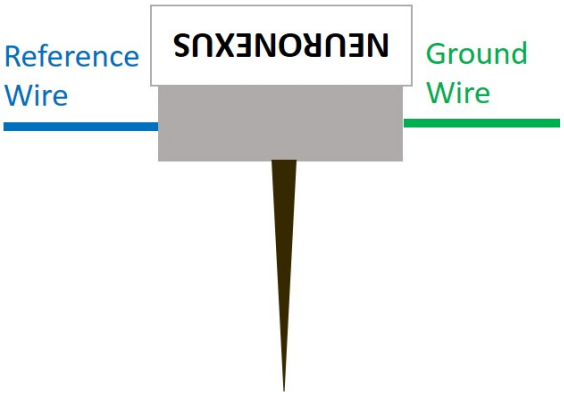
Wiring Configuration

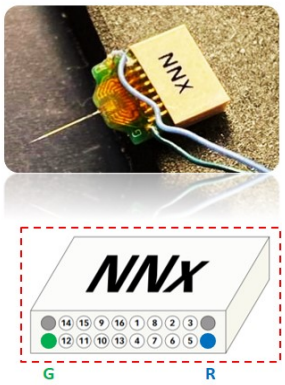
Proper wiring and grounding are essential for obtaining clean and usable signals, maximizing the performance of NeuroNexus probes. This protocol outlines effective strategies for referencing and grounding probes. While the theory is straightforward, practical implementation can be complex. NeuroNexus 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.

Chronic 16-channel Electrode

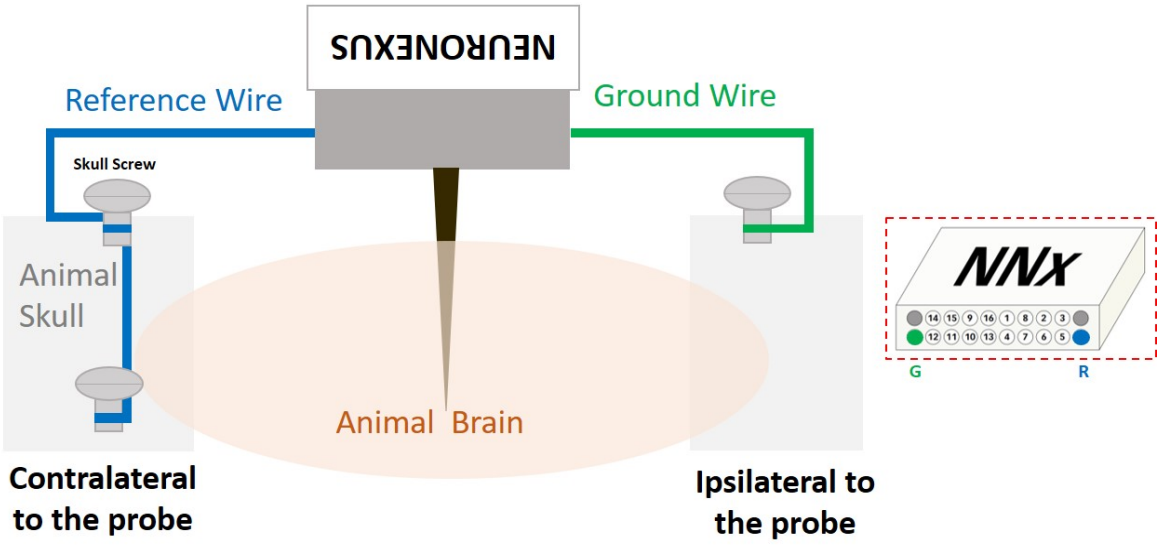
Chronic probes with 16 channels include a Ground Wire and a Reference Wire.

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



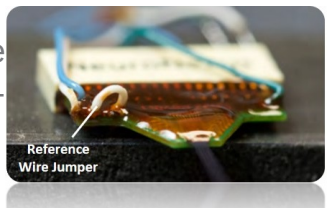


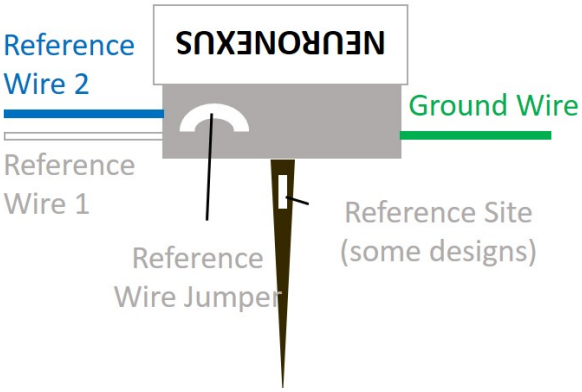
Attach the Ground and Reference Wires individually to the bone screws that were placed during the surgery. The Ground Wire should be wrapped around the bone screw on the side ipsilateral to the probe. The Reference Wire should be wrapped around the bone screws above the side contralateral to the probe.

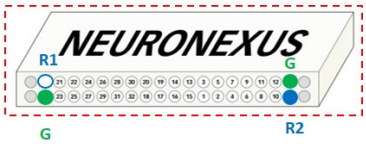


Chronic 32-channel Electrode

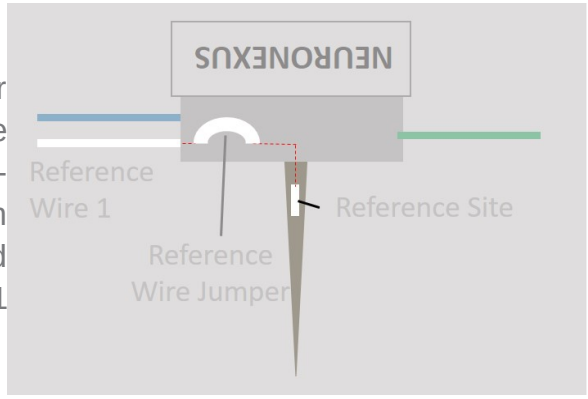
Chronic probes with 32 or more channels are equipped with a Reference Wire Jumper, allowing researchers to tailor the Reference and Ground Wiring configuration to suit their specific application.







The Reference Wire Jumper and the white Reference Wire 1 are linked to the reference site present in certain electrode array designs, and they are wired to channel R1 on the probe package.



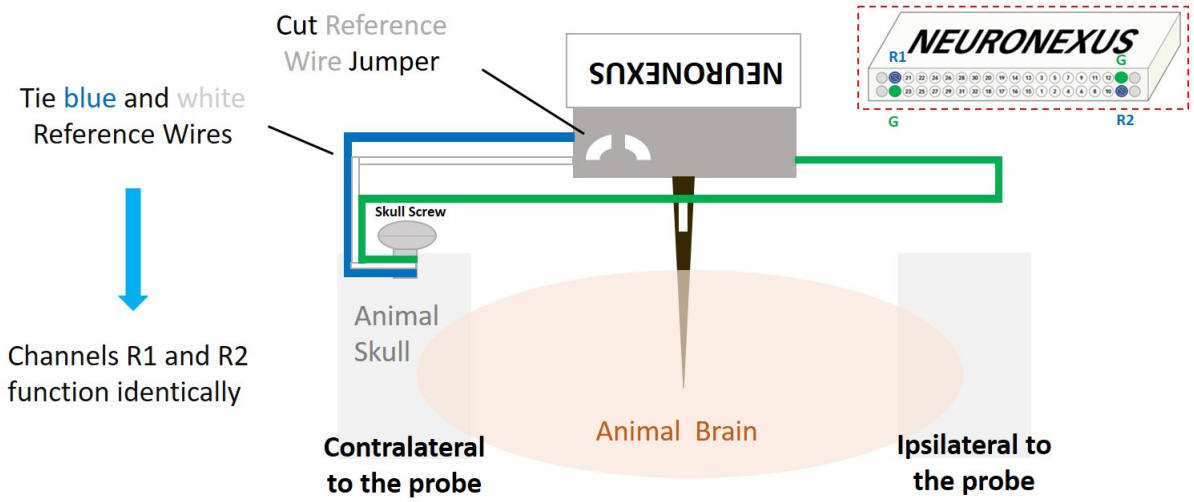
Note: Before implantation, strip a small length of insulation from the ends of Reference and Ground Wires.

Models for 32-channel Chronic Probe

Some 32-channel electrodes feature a reference site on the silicon probe shank.

32-channel chronic probe with reference site

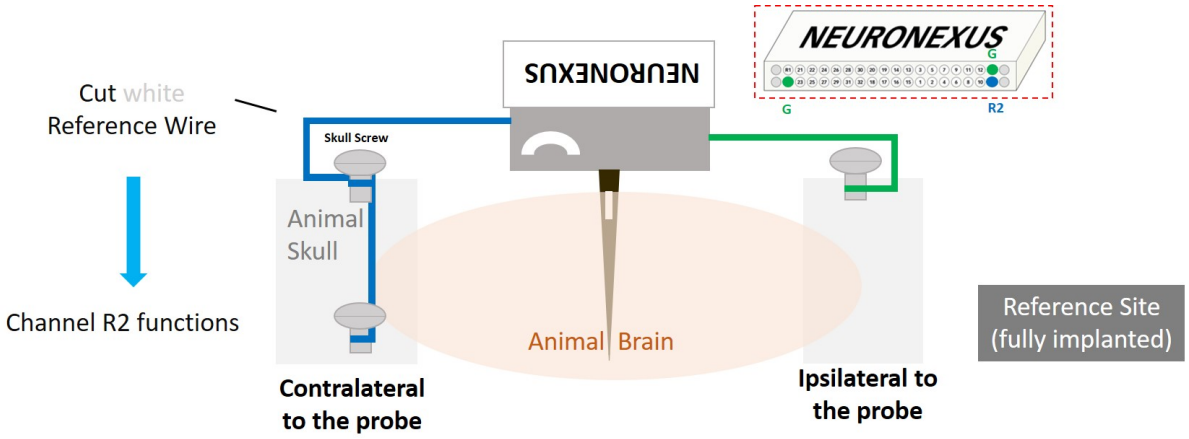
The most straightforward wire configuration involves bundling the Ground and Reference wires together and securing them to a single bone screw contralateral to the probe.



Nevertheless, the following configurations may be contemplated based on the experiment.

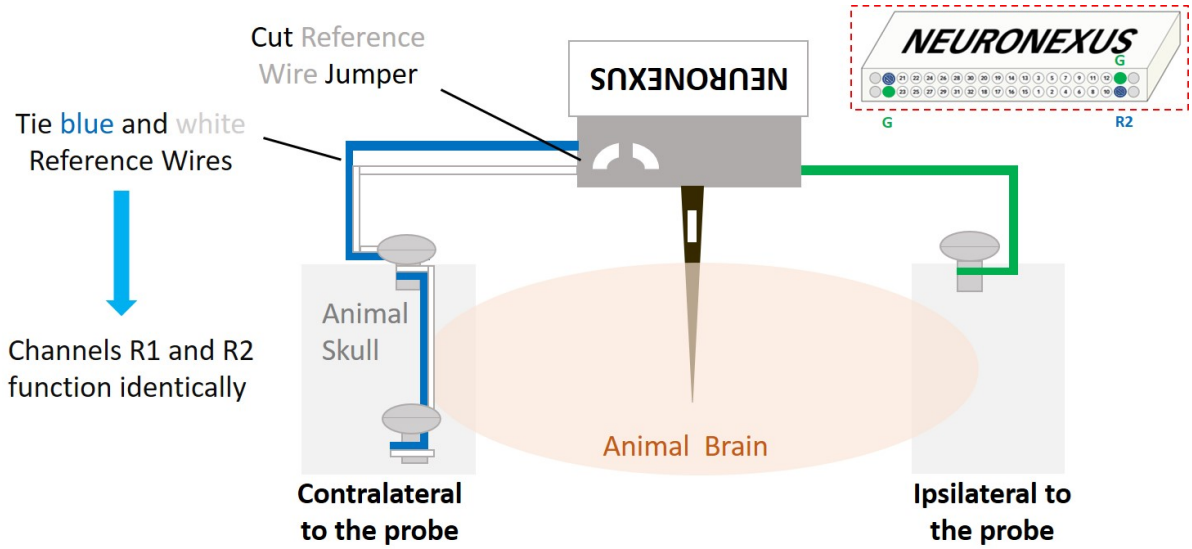
Internal reference site model

Cut the white Reference Wire and connect the blue wire to the screws contralateral to the probe.



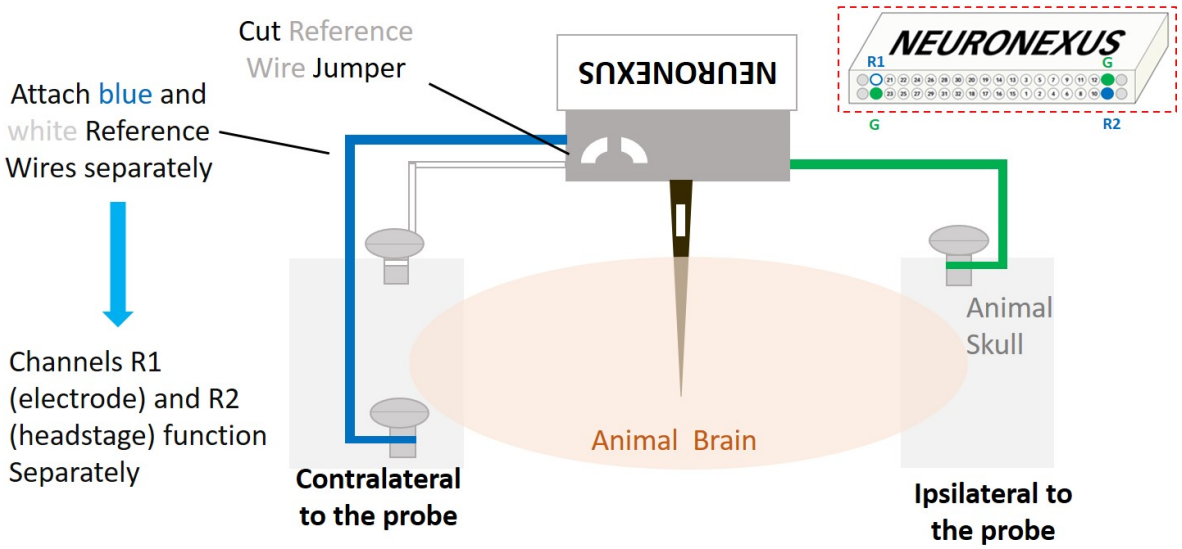
One external reference model

Cut the Reference Wire Jumper and connect both Reference Wires to the screws contralateral to the probe, while the Ground wire should be wrapped around the screw ipsilateral to the probe.



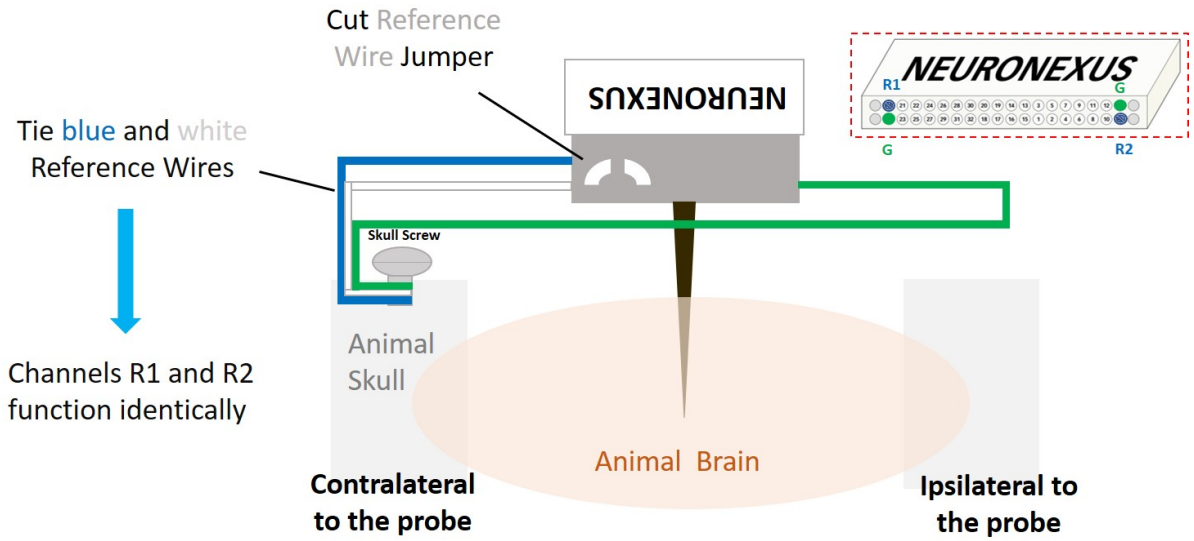
Two external references model

Cut the Reference Wire Jumper and affix each Reference Wire individually to different bone screws contralateral to the probe, while the Ground wire should be wrapped around the screw ipsilateral to the probe.



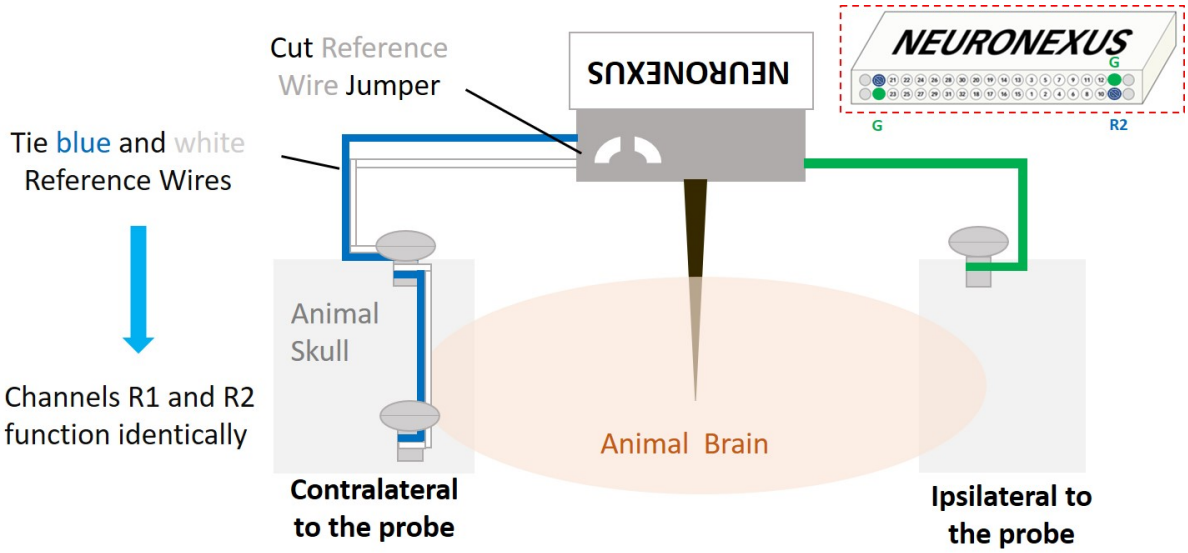
32-channel chronic probe without reference site

The simplest wire configuration is to bundle the Ground and Reference Wires together and fasten them to one bone screw contralateral to the probe.



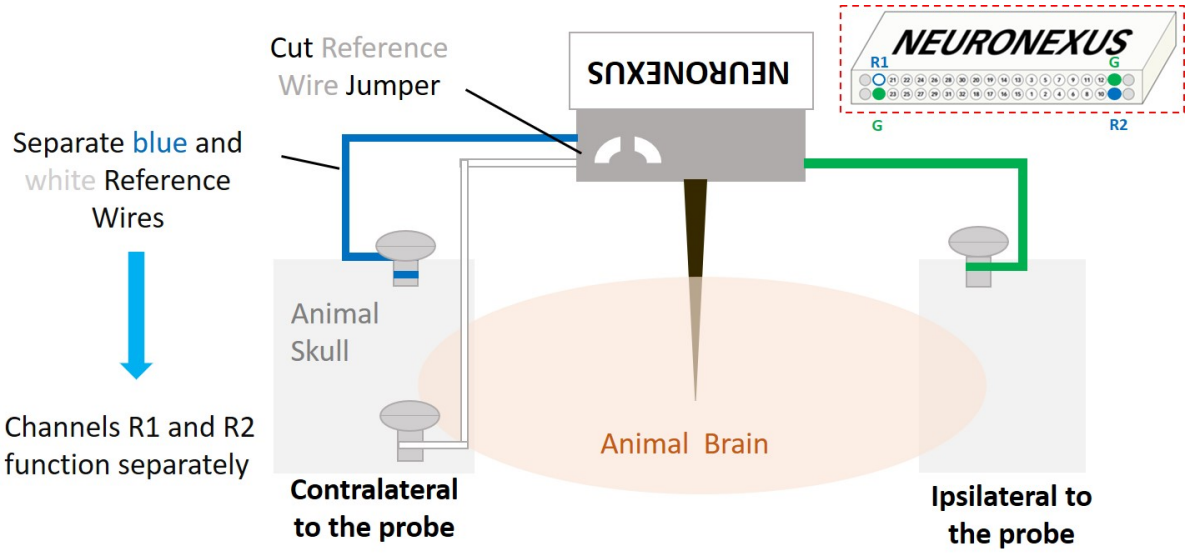
One external reference model

Cut the Reference Wire Jumper, combine the blue and white Reference Wires, and connect both to the bone screws contralateral to the probe. The Ground wire should be wrapped around the screw ipsilateral to the probe.



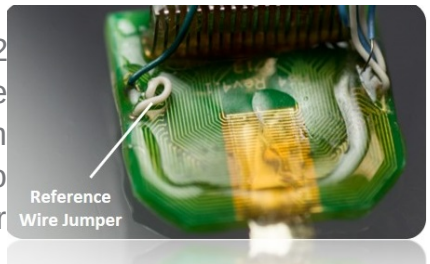
Two external references model

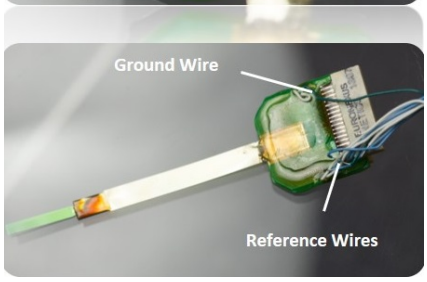
Cut the Reference Wire Jumper and attach each Reference Wire to the bone screws contralateral to the probe.

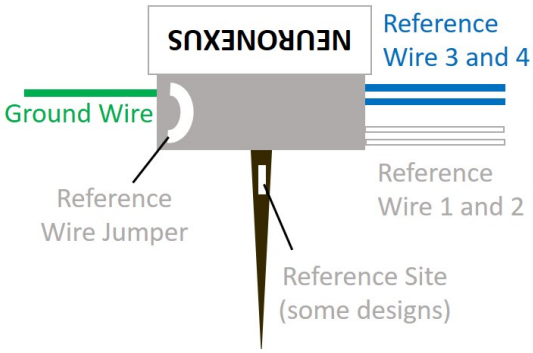


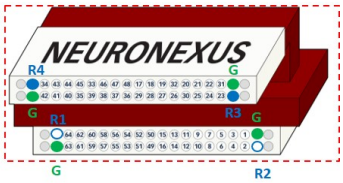
Chronic 64-channel electrode

Chronic probes with 64 channels operate similarly to those with 32 channels. They include a Reference Wire Jumper, 4 Reference wires (two for the electrode in white, and two for the headstage in blue), and 1 Ground Wire. These components allow researchers to tailor the Reference and Ground wiring configuration to suit their specific application.

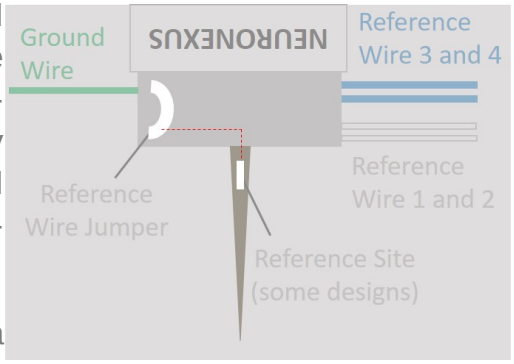


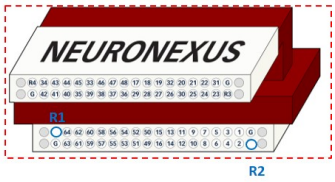






The Reference Wire Jumper and both white Reference Wires are linked to the Reference Site featured in certain electrode array designs. They are wired to channel R1 and R2 on the Omnetics connector.





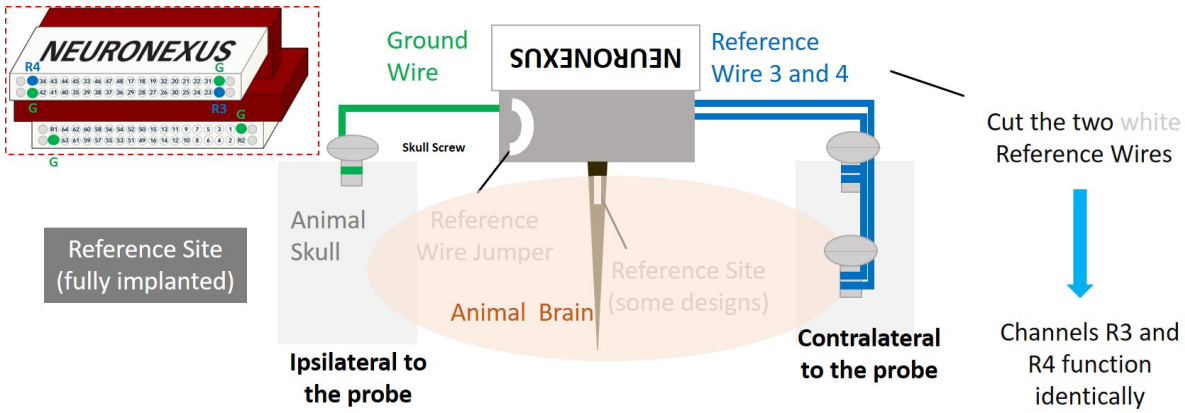
Note: Before implantation, strip a small length of insulation from the ends of Reference and Ground Wires.

Models for 64-channel Chronic Probe

Some 64-channel electrodes feature a Reference Site on the silicon probe shank.

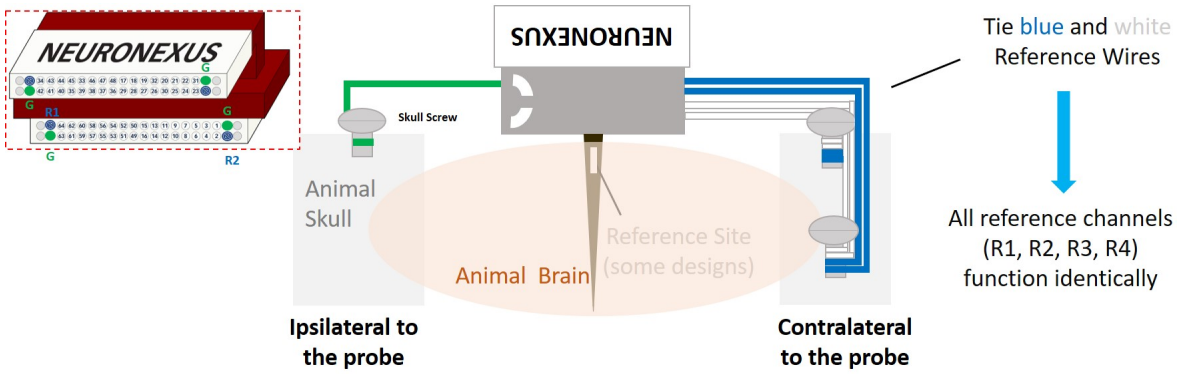
Internal reference site model

To utilize the Reference Site, cut the white Reference Wire, while connecting the blue Reference Wires to the screws contralateral to the probe.



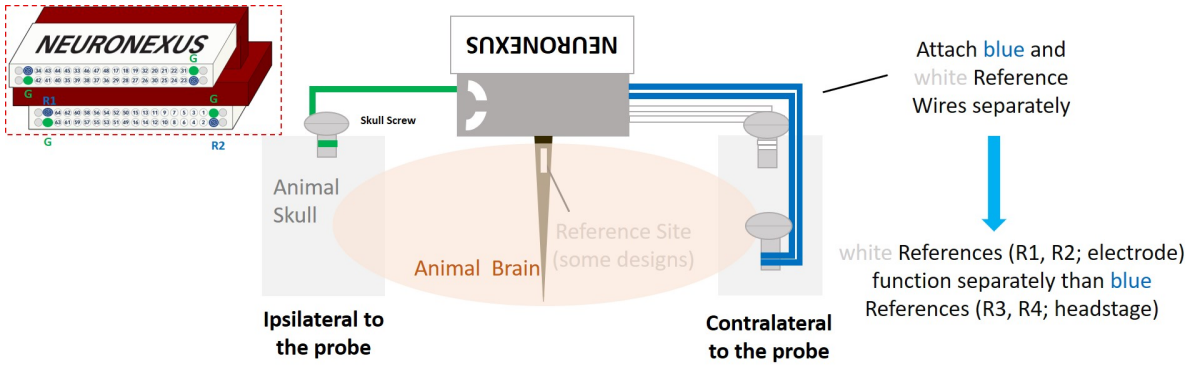
One external reference model

If you wish to combine the electrode and headstage references, cut the Reference Wire Jumper. Tie together the blue and white Reference Wires and connect both to the bone screws on the contralateral side of the probe. Meanwhile, wrap the Ground wire around the screw on the ipsilateral side to the probe.



Two external references model

Attach each Reference Wire separately to different bone screws contralateral to the probe, while the Ground wire should be wrapped around the screw ipsilateral to the probe.

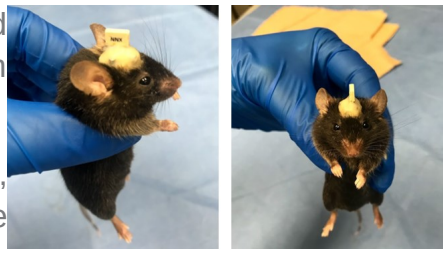


Steps After Surgery

After surgery (chronic applications):

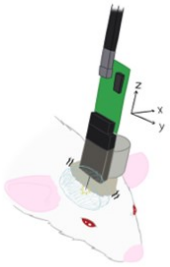
- Apply triple antibiotic along the edge of the dental cement.
- Administer a subcutaneous injection of buprenorphine (mouse: 0.1 mg/kg) or Ketoprofen (rat: 5 mg/kg).
- Place the animal on a heating pad to aid in recovery from anesthesia. Note: After surgery, the animal should be individually housed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

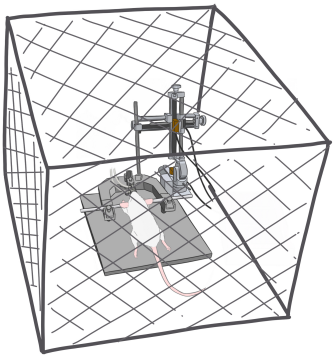
Here is an example of the 16-channel chronic probe implanted into the left side of the mouse brain after 1 week of recovery from the surgery.



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.

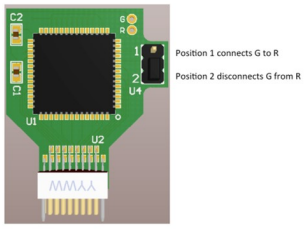
Using a heavy, grounded metal plate at the bottom of the Faraday cage can help contain ambient noise in the recording setup. Connect the Faraday cage, microscope, stereotaxic frame, manipulator, etc., to the ground metal plate using heavy gauge wires. Attach a single wire from the plate to the best possible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

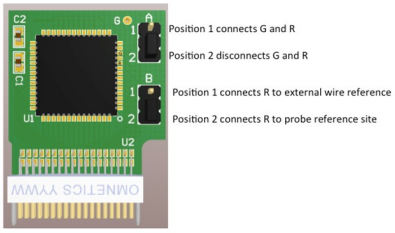
Verify if thermal noise from the headstage is present, especially if the recording setup has been in continuous use for an entire day.

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.

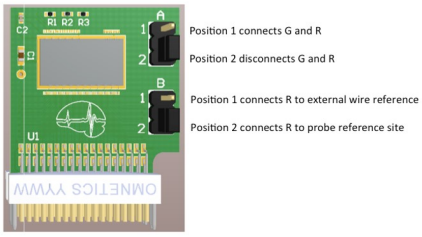
Chronic SmartLink 16



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.