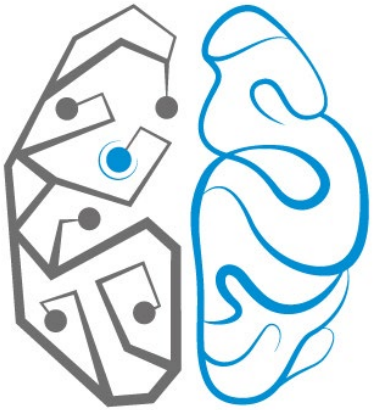

NeuroLux

Surgical guide for unilateral and bilateral devices

March 24, 2022

NeuroLux

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IACUC Disclaimer

The following documentation outlines NeuroLux's recommended materials and procedures for carrying out successful device implantation surgeries. It is not a comprehensive surgical training guide and does not provide researchers with the proper certifications required to handle rodents in a laboratory setting.

All researchers who plan to carry out the following procedures must first receive the proper training and authorization from their IACUC and/or other relevant organizations.

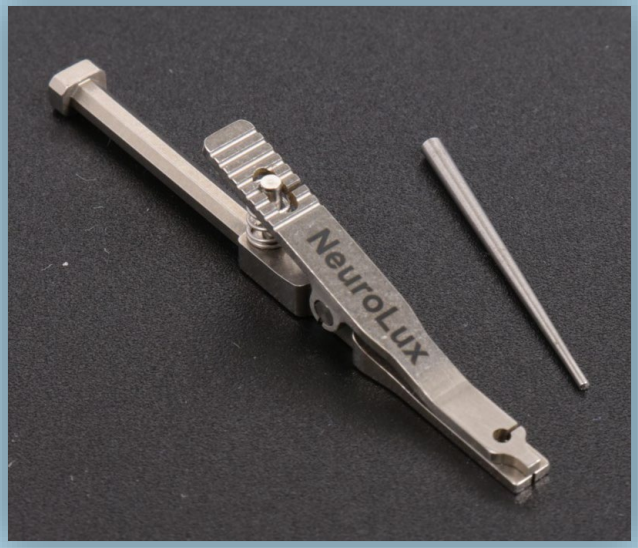
Best Regards,

NeuroLux

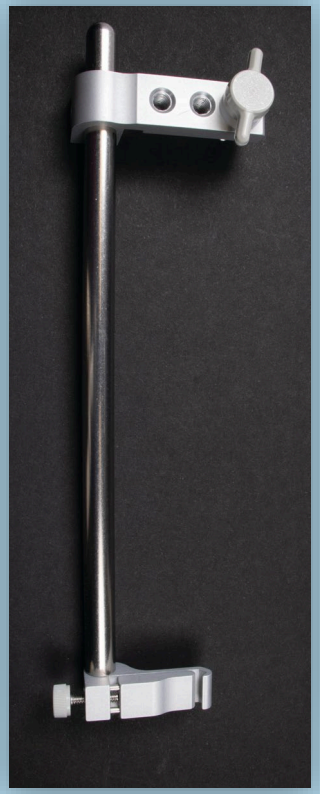
NeuroLux Surgical Package

The NeuroLux surgical package has three primary components, each of which play an integral role in successful device implantation surgeries.

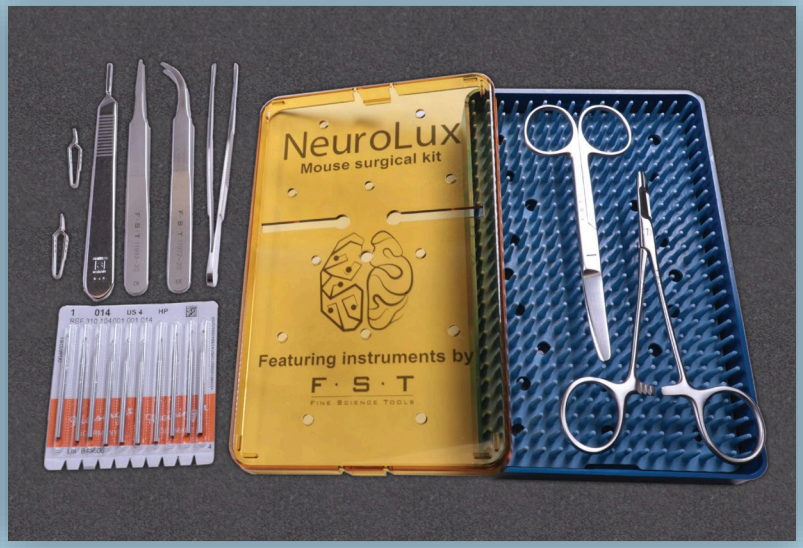
SURGICAL CLIP



STEREOTAXIC ADAPTOR



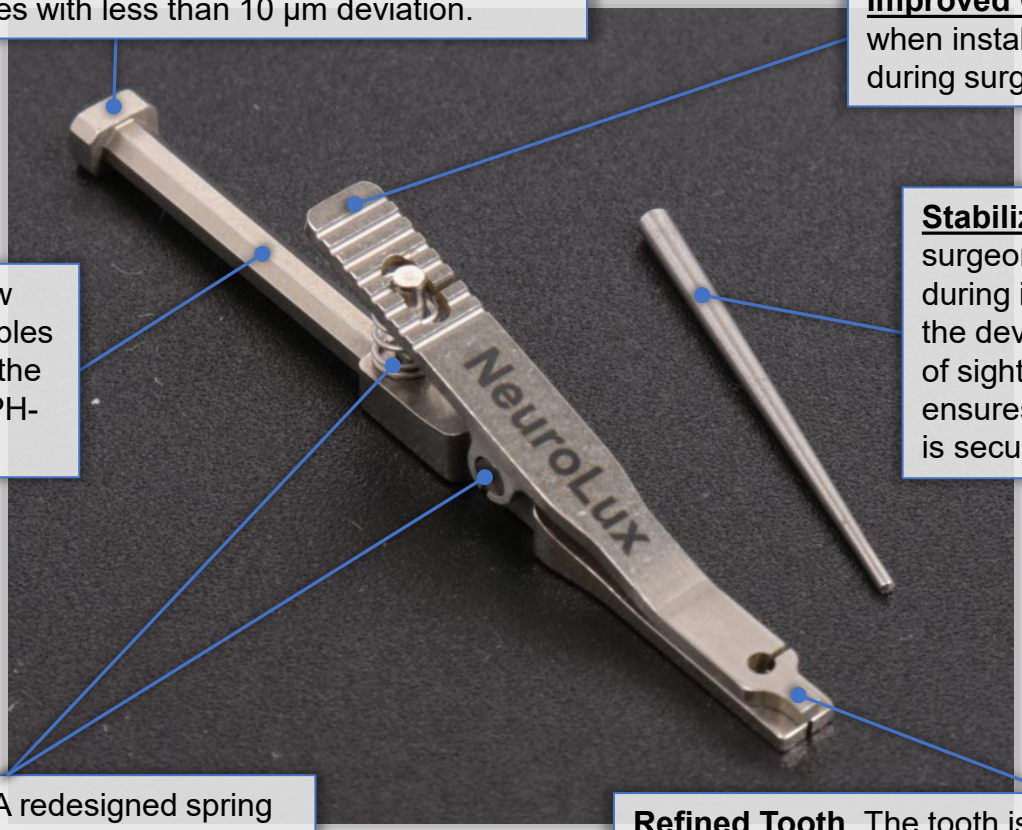
SURGICAL KIT



NeuroLux Surgical Clip

Endcap The integrated end cap allows users to remove and replace our surgical clip from the PH-300 stereotaxic adaptor and consistently reach the same dorsal/ventral and medial/lateral coordinates with less than 10 µm deviation.

Improved Grip Grooves provide grip when installing and releasing the probe during surgery.



Stabilizing Pin The tapered pin allows surgeons to have full view of the probe during implantation by neatly securing the device body above the surgical line of sight. The conical nature of the pin ensures easy removal once the probe is secured to the skull.

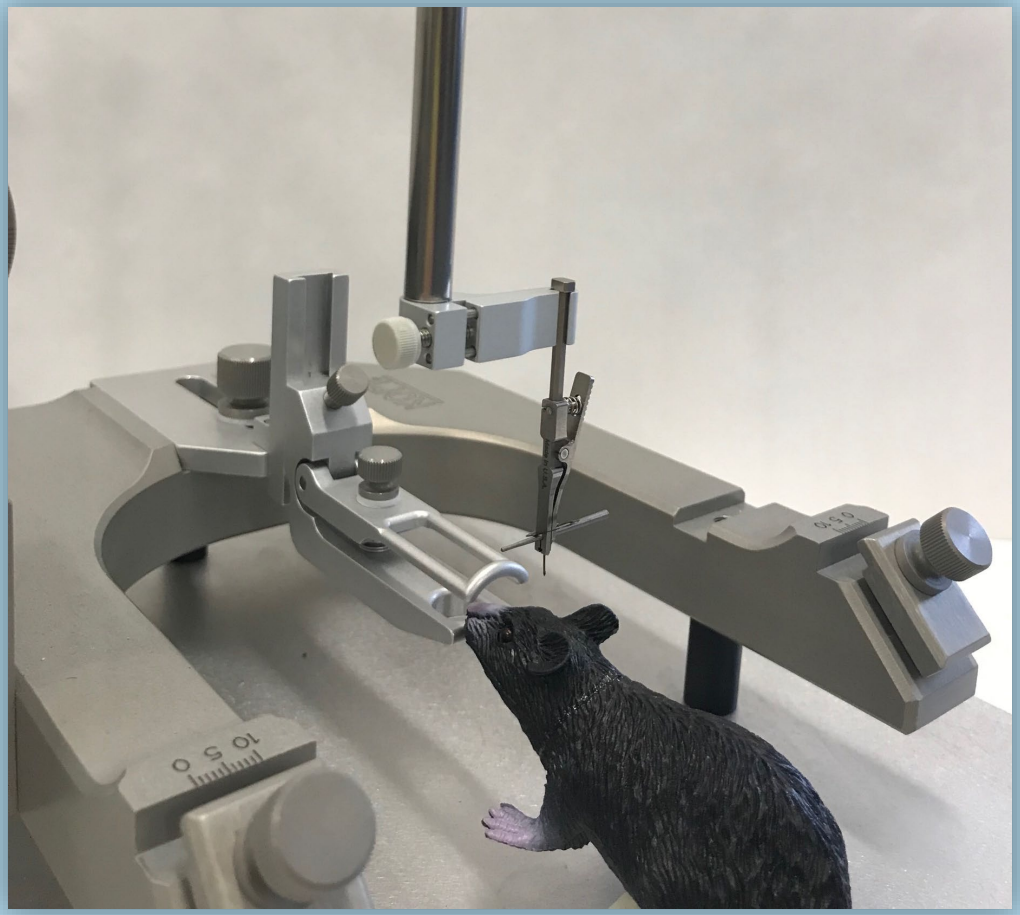
Octagonal Shaft Our new octagonal rod design enables precise 90° placement of the surgical clip within ASI's PH-300 stereotaxic adaptor.

Spring and Pivot A redesigned spring and pivot point provide optimal tension on the probe, which allows for smooth mounting and release of the probe from the clip once inserted into the brain.

Refined Tooth The tooth is perfectly shaped to align and hold our optogenetic probes in a vertical plane and resists bending of the probe in lateral directions with less than 0.8° of deviation. The notches on the side allow our flat forceps to hold the flag against the surgical clip during release, preventing probe movement in the brain.

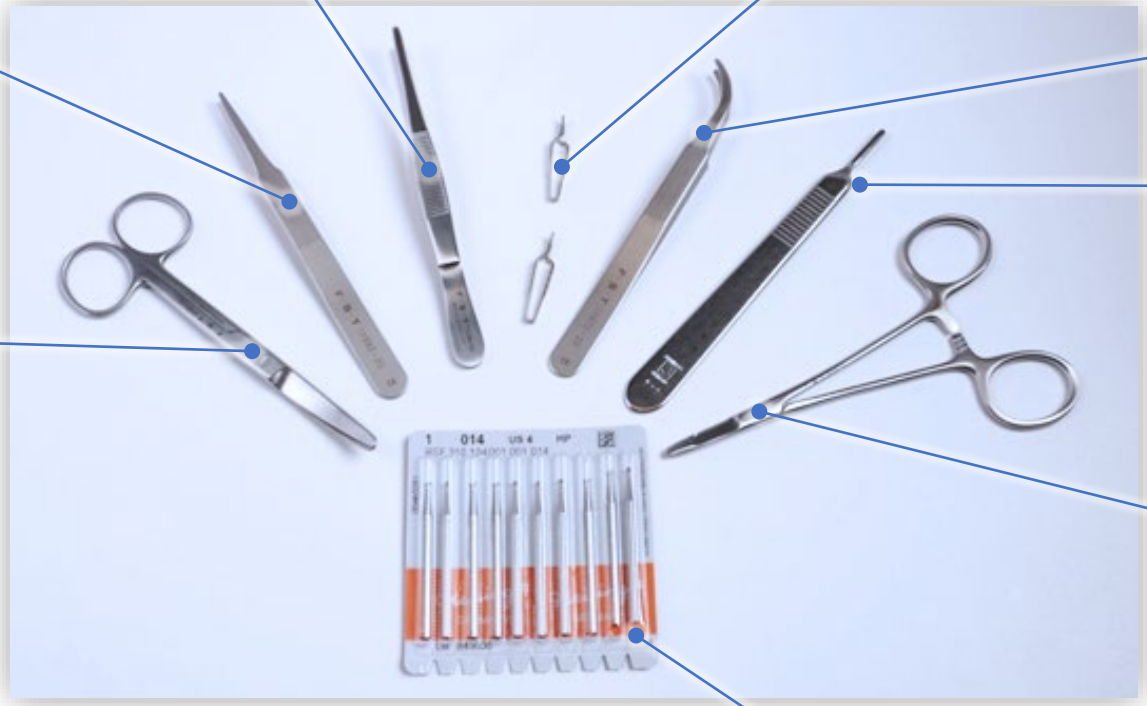
Stereotaxic Adaptor

NeuroLux's custom surgical clip is specifically designed to integrate with the PH-300 stereotaxic adaptor created by ASI Instruments. The PH-300 provides an offset design that increases a surgeon's visibility during device implantation. The clamping mechanism holds our surgical clip at a perfect right angle, allowing precise orientation of the µLED relative to the targeted brain region.



NeuroLux Surgical Kit

Having the correct set of tools is imperative for a successful implantation surgery. The NeuroLux and FST colaborative surgical kit provides users with the optimal set of tools to carry out all surgeries discussed in the following slides. Each tool is outlined below:



NeuroLux

Supplemental Surgical Materials

The following is a list of supplemental materials that we found to significantly ease the implantation process. While these materials are not mandatory, they are referenced in the surgical procedure. Obtaining the following materials before beginning a surgery is extremely beneficial:

- **#10 and/or #11 Scalpel blades**
- **27 g 1” needles**
- **Alcohol wipes**
- **Iodine swabs**
- **Cling wrap**
- **Sterile surgical gloves**
- **Fine tipped marker**
- **Cotton swabs**
- **Loctite superglue 454**
- **Loctite 7452 accelerator**

Wireless Brain Device Overview

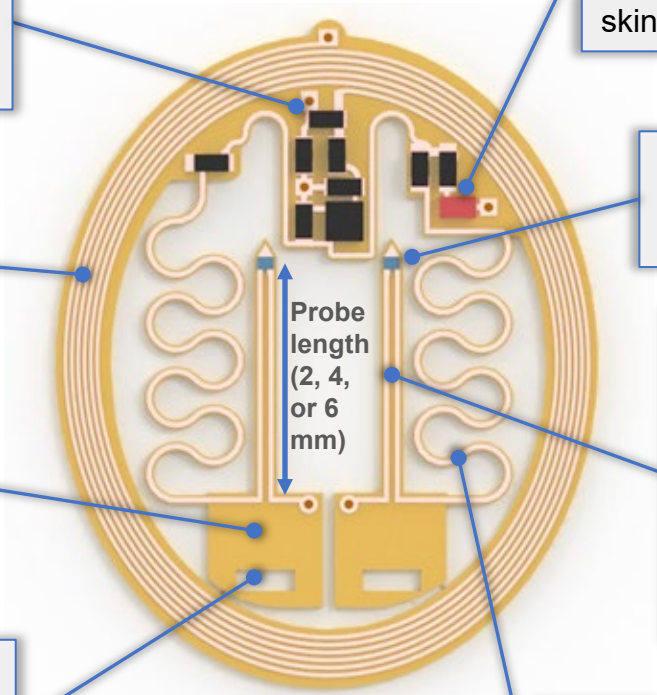
NeuroLux

Electronic circuit These components convert the wireless power from the coil into direct current that turns on the µLEDs.

Coil Allows wireless power harvesting from your enclosure antenna.

Flag Allows the surgeon to grab the probe and maneuver it in place within the surgical clip.

Flag hole Allows alligator tooth on the surgical clip to clamp the flag and secure it in a vertical plane for stereotaxic insertion into the brain.



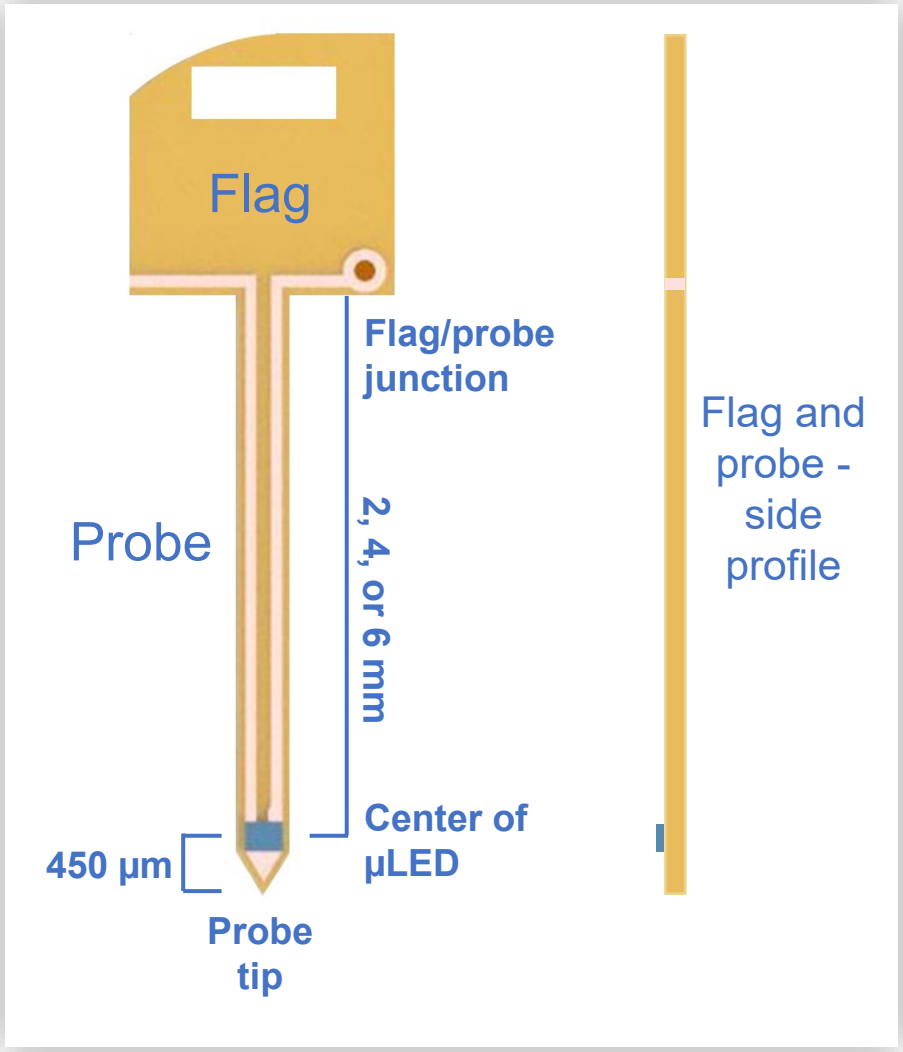
Red indicator LED The red rectangle is a separate LED that turns on when the device is powered and the µLED(s) are on. It is generally visible through the animal's skin to confirm device operation.

µLED The implanted µLED activates opsins deep in the brain to modulate cellular activity.

Probe These allow the µLEDs to be implanted deep in the brain. The triangle tip helps navigate through brain tissue to reduce damage. *Note that probe length is defined as the distance from the center of the µLED to the edge of the flag, as illustrated here*.

Serpentine These curved copper traces provide power from the electronics to the µLEDs. They are stretchable and flexible to allow mounting the probe in the surgical clip and maneuverability around the skull for placement.

Implantable Probe Overview

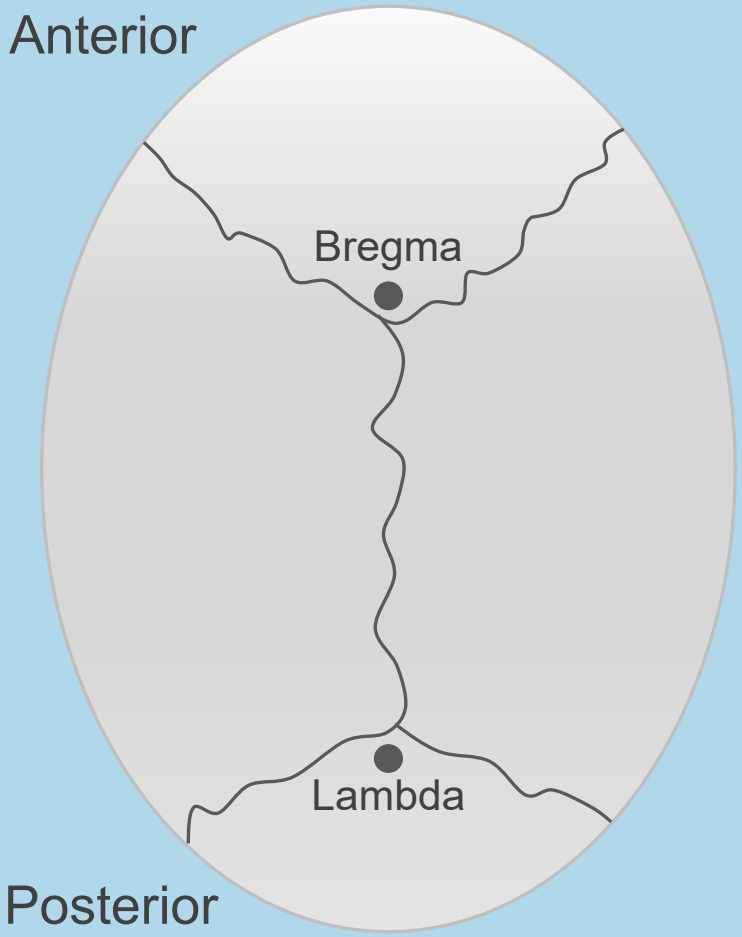


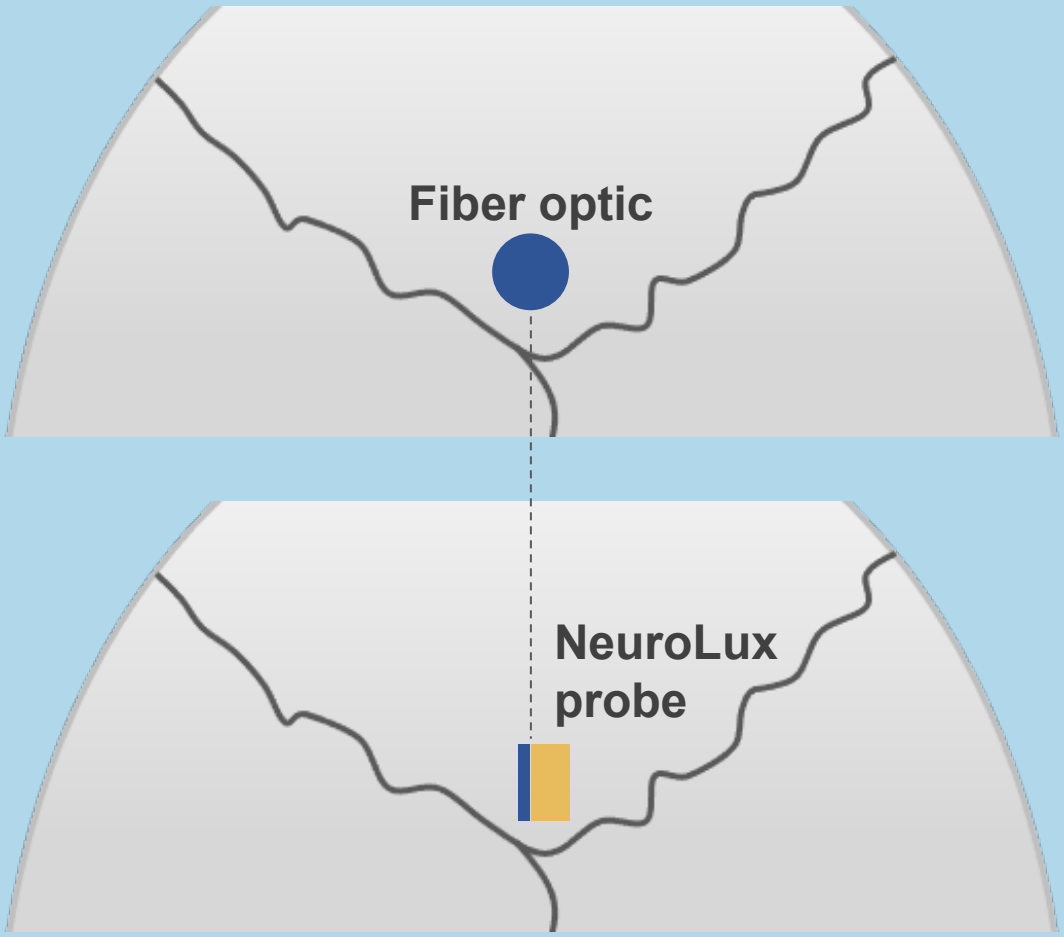
NeuroLux's probe length (2, 4, or 6 mm) is measured from the junction of the flag and probe to the center of the µLED, as shown in the figure to the left. There is an additional 450 µm from the center of the µLED to the tip of the probe which helps minimize brain tissue damage during implantation.

Probe dimensions:
Length = 2, 4, or 6 mm to center of µLED
Width = 400 µm
Depth (thickness) = 200 µm
µLED depth (thickness) = 60 µm

How to take bregma with NeuroLux probes

With traditional fiber optics, Bregma is generally taken at the center of the fiber. For NeuroLux probes, most of our customers offset the probe slightly and center the junction of the probe and µLED over their defined Bregma. This can be accomplished by using the front face of the triangular tip as that junction, as the µLED resides ~450 µm above the tip.



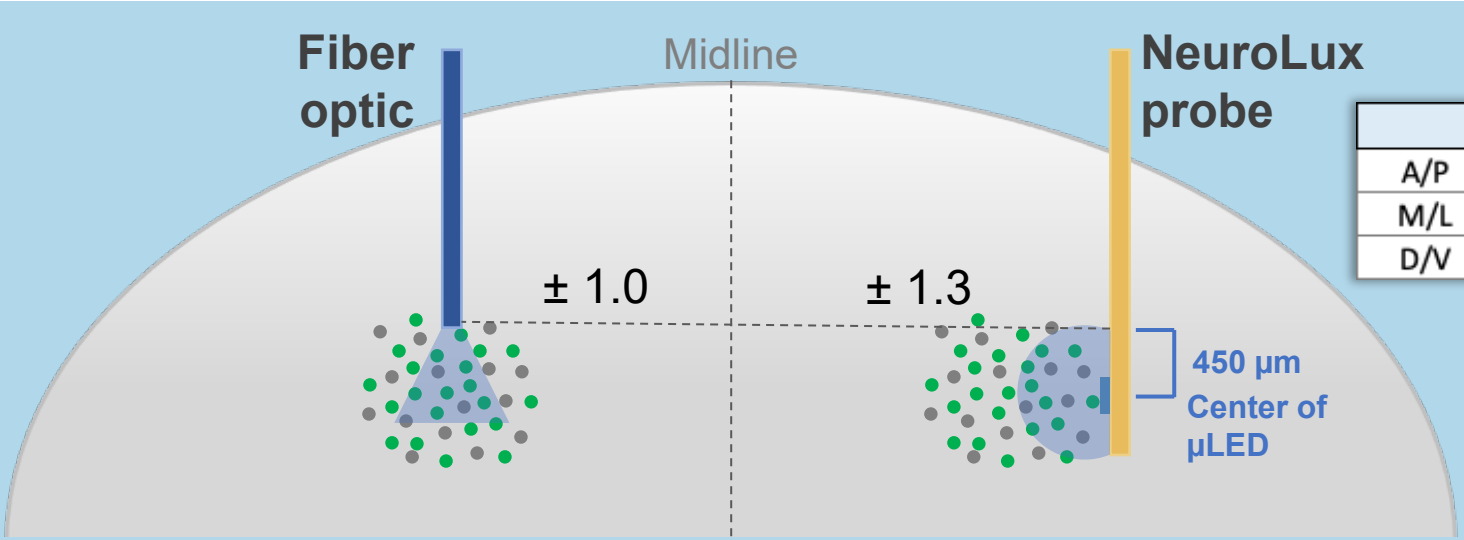


Stereotaxic Coordinate Adjustments

Lab personnel who are experienced with optogenetic surgeries often maintain a list of proven stereotaxic coordinates for their studied brain regions. When transitioning to the wireless NeuroLux system, these coordinates must be adjusted to account for lateral light delivery from the μLED on our probes, versus vertical light delivery from fiber optics. This difference is illustrated in the figure below.

NeuroLux probes have a single μLED at the end, the center of which resides ~450 μm above the probe tip. To account for this extra probe length, we recommend implanting the probes 300 - 450 μm more ventral than you would a fiber optic. To account for lateral light delivery from the μLED, we also recommend shifting your stereotaxic coordinates ~300 μm more lateral that you would implant a fiber optic. By shifting both the medial/lateral and dorsal/ventral coordinates, you will achieve optimal light delivery to your target brain region for improved behavioral results.

If your coordinates are far lateral, please contact NeuroLux Support for tips on implantation.



	Fiber optic	NeuroLux probe
A/P	-3.0	-3.0
M/L	+/- 1.0	+/- 1.3
D/V	-4.0	-4.45

Surgical Preparation Overview

The following are common surgical preparations for all implant procedures. Always follow your specific IACUC guidelines and the procedures approved on your animal protocol.

STERILIZE INSTRUMENTS

Instruments should be sterilized using an autoclave prior to surgery. Between surgeries and following cleaning, a hot bead sterilizer can be used to re-sterilize the tips of instruments.

SHAVE SURGICAL SITE

The surgical site is shaved directly after anesthesia induction. Hold the anesthetized mice in one hand, which is used to stretch the skin taunt to improve shaving. The other hand is used to shave the surgical site with an electric razor. Shaving should be done away from the stereotaxic frame to keep the surgical area clean and free of excess hair.

BETADINE-ALCOHOL WASH

The surgical site must be disinfected with a series of betadine-alcohol scrubs. First, betadine is applied to the shaved site until it is saturated. An alcohol wipe is then used to remove excess betadine. This process is repeated for a total of three cycles.

PAIN MANAGEMENT

Per IACUC's documentation of rodent pain management, mice should be given meloxicam subcutaneously prior to any incisions.

ANESTHESIA INDUCTION

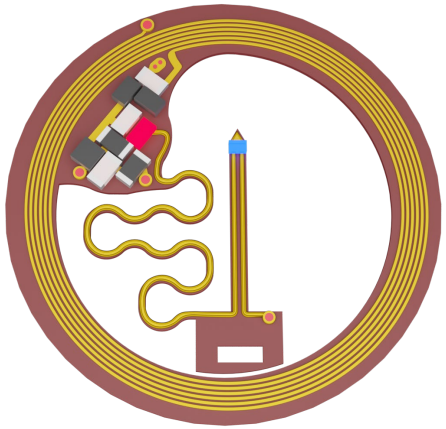
Mice are placed in an induction chamber for ~5 minutes at 2.5-3% (vol/vol) isoflurane until the animal is fully anesthetized and respiration rate drops to ~1 breath per second.

LOCAL SODIUM CHANNEL BLOCKERS

Local anesthetics are routinely injected subcutaneously or intradermally around the surgical incision sites. Refer to your IACUC documentation for specific local anesthetic requirements.

NeuroLux

Unilateral device surgical procedure



Unilateral Procedure | Shave and clean scalp

Figure 1 | Steps 1, 2 & 3

Step 1 | Position the mouse on the stereotaxic frame with a warming pad underneath it and supply a constant flow of ~2% isoflurane.

Step 2 | Apply Puralube to the eyes to prevent drying of the cornea during surgery.

Step 3 | Shave the back of the anesthetized mouse's head from behind the ears up to the back of the eyes.

Figure 2 | Step 4

Step 4 | Scrub the exposed skin with betadine solution followed by 70% (vol/vol) ethanol. Repeat wash process three times.

Unilateral Procedure | Scalp incision

Figure 3 | Steps 5 & 6

Step 5 | Make a small scalpel incision starting behind the eyes and ending in front of the ears.

Step 6 | Use two bulldog clamps to hold the incision open during surgery.

Figure 4 | Step 7

Step 7 | Use a cotton swab to remove the membrane layer on top of the skull. Start at the midline and push the membrane laterally towards the edge of the skull. *Note the lack of reflection on the skull after the membrane was removed in Figure 4 vs. Figure 3 when it was intact*.

Unilateral Procedure | Locate brain coordinates

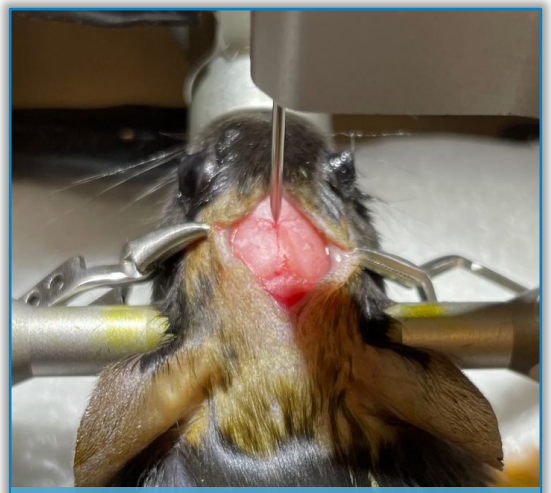


Figure 5 | Steps 8, 9 & 10

Step 8 | Use a syringe needle or other sharp point to locate bregma and record the stereotaxic coordinates.

Step 9 | Locate lambda and record the stereotaxic coordinates.

Step 10 | Compare the D/V coordinates of bregma and lambda to ensure that the skull is flat. If the coordinates differ by > 100 µm, reposition the skull in the stereotaxic frame.

! CRITICAL STEP | Refer to ”Flat skull procedure” for additional details.

Figure 6 | Step 11

Figure 7 | Step 12

Step 11 | Locate the desired coordinates and mark the location using a marker or pencil.

Step 12 | Use a stereotaxic drill and make a hole at the marked location.

Unilateral Procedure | Insert probe into brain

Figure 8 | Steps 13, 14 & 15

Step 13 | Load the device into the surgical clip, then insert surgical clip into the stereotaxic arm at the desired angle. Refer to “Method for inserting probe into the surgical clip” for additional details.

Step 14 | Find the desired coordinates using Bregma, then move the probe to the center of the drilled hole.

Step 15 | Slowly lower the probe into the drilled hole until the desired depth is reached. Make sure probe does not bend, which would indicate that it is hitting skull bone fragments. Retract the probe and clean or redrill the burr hole as needed until probe inserts cleanly.

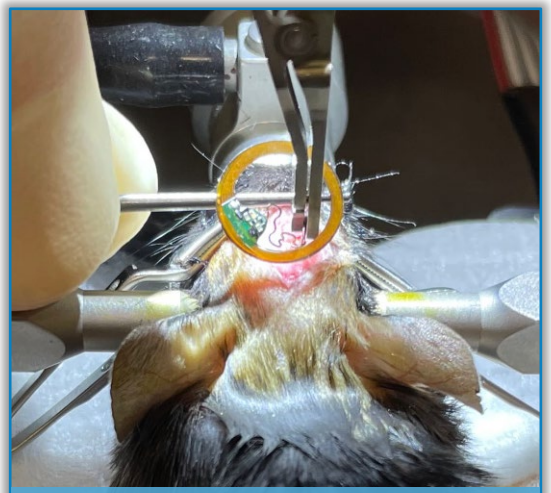


Figure 9 | Step 17

Step 16 | Secure probe to skull using Loctite 454, followed by accelerator to quickly harden it.

! CRITICAL STEP | Refer to ”How to secure implanted probe to the skull” for additional details.

Step 17 | Remove stabilizing pin from the surgical clip and allow device body to drop towards skull.

Step 18 | Apply a second layer of Loctite 454 around the probe to fully secure it to the skull (not shown).

Unilateral Procedure | Remove the surgical clip

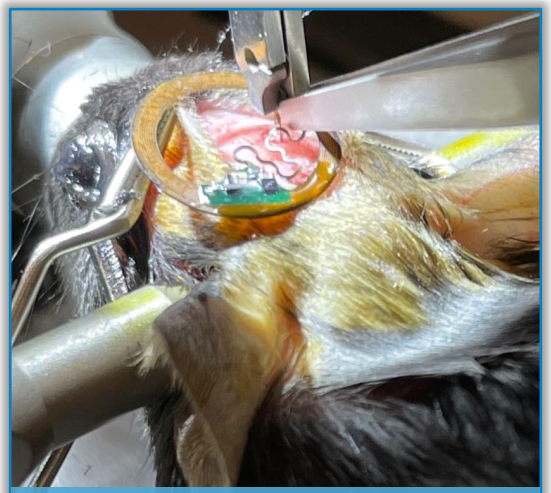


Figure 10 | Step 19

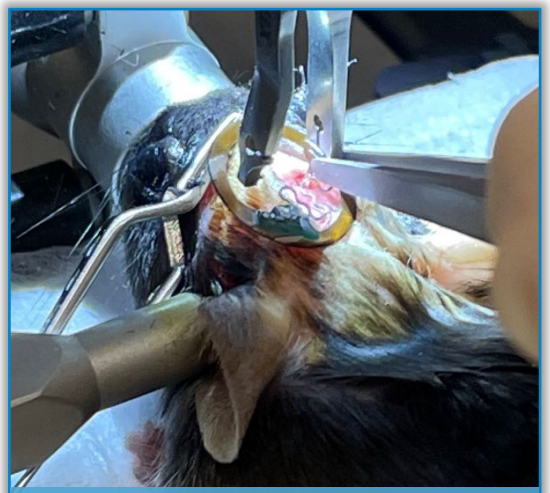


Figure 11 | Step 20

Step 19 | Use forceps to stabilize the flag by securing it the surgical clip.

Step 20 | Slowly open the surgical clip while maintaining pressure on the flag to prevent it from moving.

! CRITICAL STEP | Refer to ”Method for releasing probe from surgical clip” for additional details.

Figure 12 | Step 21

Figure 13 | Step 22

Step 21 | Remove the forceps while the surgical clip remains open.

Step 22 | Retract the surgical clip vertically and remove it from the stereotaxic arm.

Unilateral Procedure | Secure flag to skull

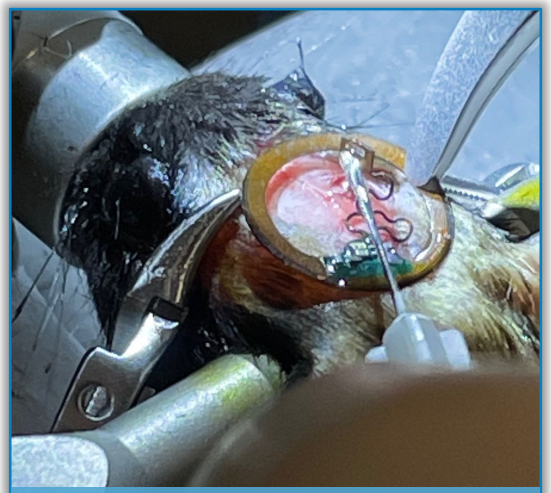


Figure 14 | Step 23

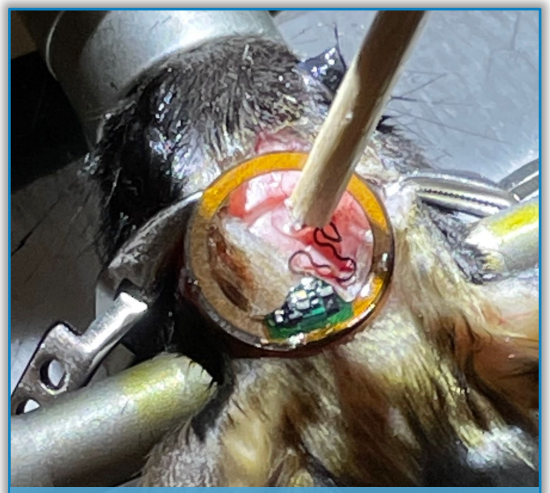


Figure 15 | Steps 24 & 25

Step 23 | Use a needle head to apply Loctite 454 to the flag that will be folded down to the skull.

Step 24 | Use a blunt wooden stick to fold the flag down until it touches the skull.

Step 25 | Use an insulin syringe to apply super glue activator to the flag.

Figure 16 | Step 26

Figure 17 | Step 27

Step 26 | Add more superglue to stabilize the flag and cover exposed corners, as needed.

Step 27 | Use blunt forceps to tuck the device under the skin, and serrated forceps to manipulate the skin around the device. Secure the device to skull using Loctite if needed.

Unilateral Procedure | Close scalp incision

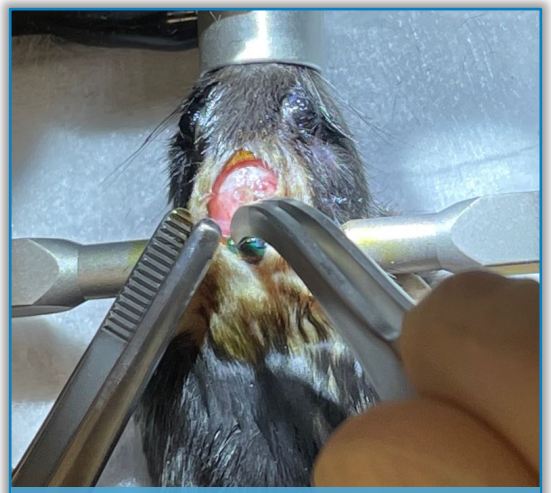


Figure 18 | Step 28

Figure 19 | Step 29

Step 28 | Use serrated forceps to pull the skin completely over the device.

Step 29 | Close the incision with sutures. Be careful not to pull the skin too tight or it will promote scratching, which could open the wound and compromise the device if damaged.

SURGICAL TIPS

SCALP INCISION

Do not cut the scalp between the animal's eyes. If you suture this area incorrectly, it can prevent them from blinking.

LOCAL SODIUM CHANNEL BLOCKERS

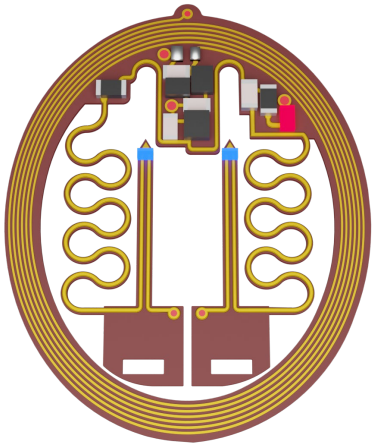
Local anesthetics are routinely injected subcutaneously or intradermal around the surgical incision sites. Refer to your IACUC documentation for specific local anesthetic requirements.

SUTURE TECHNIQUE

When making the first suture knot, ensure that the two sides of the incision just touch each other. Pulling the first suture knot too tightly can create a ridge along the incision line that may inhibit the healing process of the wound. This can also irritate the animal and promote scratching, which can tear the skin and sutures and damage the underlying device. Subsequent suture knots can be tightened over the first knot to lock it in place.

NeuroLux

Bilateral device surgical procedure



Bilateral Procedure | Shave and clean scalp

Figure 1 | Steps 1, 2 & 3

Step 1 | Position the mouse on the stereotaxic frame with a warming pad underneath the mouse and a constant flow of ~2% isoflurane.

Step 2 | Apply Puralube to the eyes to prevent drying of the cornea during surgery.

Step 3 | Shave the back of the anesthetized mouse's head from behind the ears up to the back of the eyes.

Figure 2 | Step 4

Step 4 | Scrub the exposed skin with betadine solution followed by 70% (vol/vol) ethanol. Repeat wash process three times.

Bilateral Procedure | Scalp incision

Figure 3 | Steps 5 & 6

Step 5 | Make a small scalpel incision starting behind the eyes and ending in front of the ears.

Step 6 | Use two bulldog clamps to hold the incision open during surgery.

Figure 4 | Step 7

Step 7 | Use a cotton swab to remove the membrane layer on top of the skull. Start at the midline and push the membrane laterally towards the edge of the skull. *Note the lack of reflection on the skull after the membrane was removed in Figure 4 vs. Figure 3 when it was intact*.

Bilateral Procedure | Locate brain coordinates

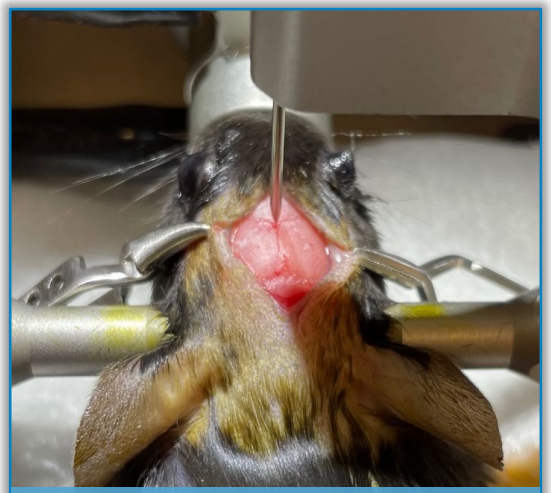


Figure 5 | Steps 8, 9 & 10

Step 8 | Use a syringe needle or other sharp point to locate bregma and record the stereotaxic coordinates.

Step 9 | Locate lambda and record the stereotaxic coordinates.

Step 10 | Compare the D/V coordinates of bregma and lambda to ensure that the skull is flat. If the coordinates differ by > 100 µm, reposition the skull in the stereotaxic frame.

! CRITICAL STEP | Refer to ”Flat skull procedure” for additional details.

Figure 6 | Step 11

Figure 7 | Step 12

Step 11 | Locate the desired coordinates and mark the locations using a marker or pencil.

Step 12 | Use a stereotaxic drill and make two symmetrical holes at the marked locations.

Bilateral Procedure | Pre-stretch serpentines

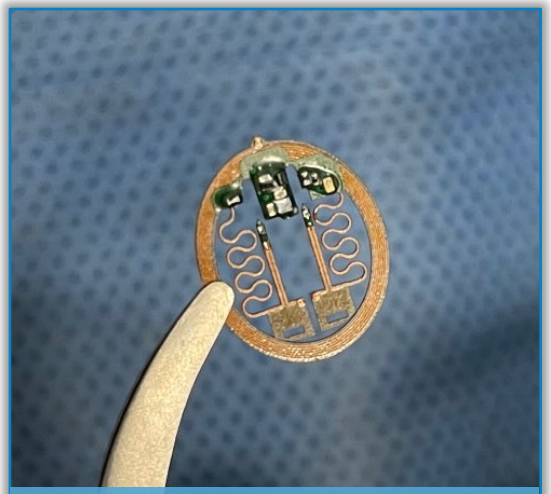


Figure 8 | Step 13

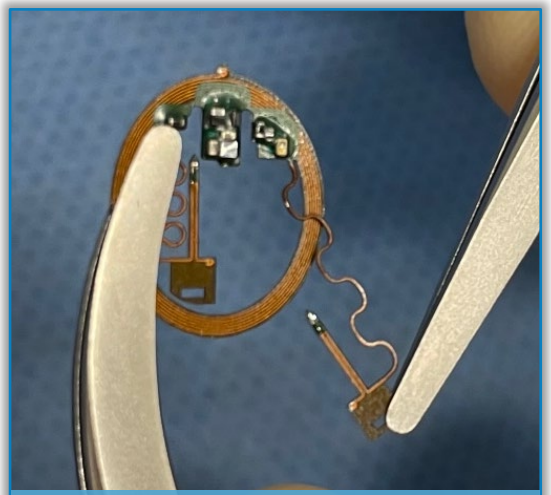


Figure 9 | Step 14

Step 13 | Hold the coil of the device using the curved pair of smooth forceps.

Step 14 | Pre-stretch one of the the serpentines using the straight, smooth forceps.

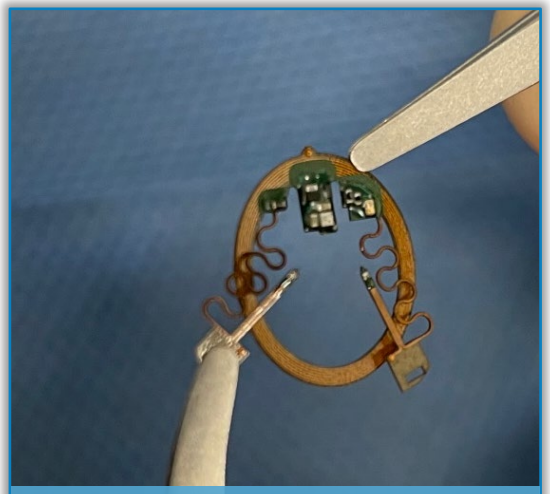


Figure 10 | Steps 15 & 16

Step 15 | Hold the coil of the device using a pair of smooth forceps.

Step 16 | Pre-stretch the other serpentine using the other pair of smooth forceps.

Bilateral Procedure | Insert probe into brain

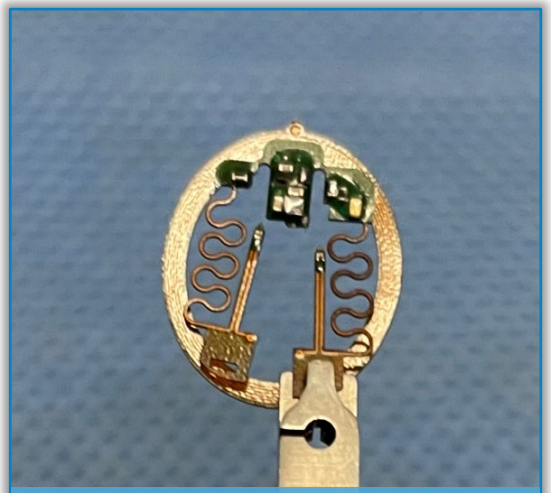


Figure 11 | Step 17

Step 17 | Attach the surgical clip to the first flag using the alligator tooth. Ensure that the tooth is inserted in the flag correctly and that the probe does not deviate laterally when lightly pushed with forceps, then insert surgical clip into the stereotaxic adaptor at the desired orientation. Refer to “Method for inserting probe into the surgical clip” for additional details.

Figure 12 | Steps 18 & 19

Step 18 | Find the desired coordinates using Bregma, then move the probe to the center of the drilled hole.

Step 19 | Slowly lower the probe into the drilled hole until the desired depth is reached. Make sure probe does not bend, which would indicate that it is hitting skull bone fragments. Retract the probe and clean or redrill the burr hole as needed until probe inserts cleanly.

Bilateral Procedure | Remove the surgical clip

Figure 13 | Step 21

Step 20 | Secure probe to skull using Loctite 454, followed by accelerator to quickly harden it (not shown).

! CRITICAL STEP | Refer to ”How to secure implanted probe to the skull” for additional details.

Step 21 | Remove stabilizing pin from the surgical clip and allow device body to drop towards skull.

Step 22 | Apply a second layer of Loctite 454 around the probe to fully secure it to the skull (not shown).

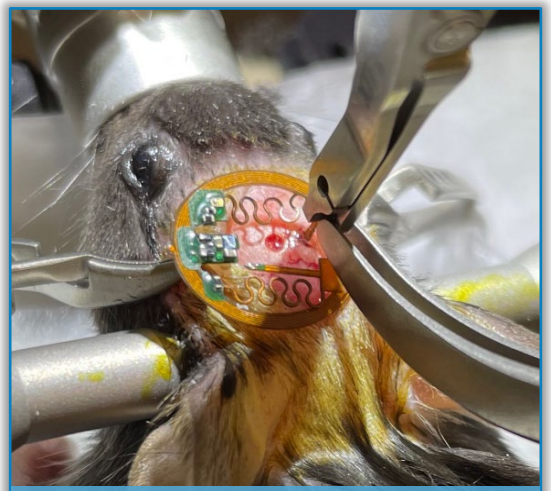


Figure 14 | Step 23

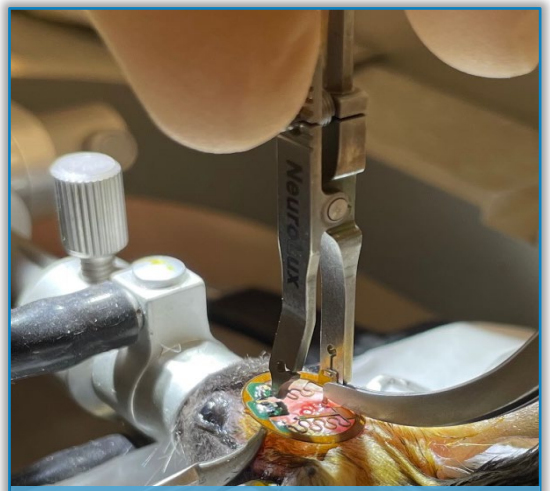


Figure 15 | Step 24

Step 23 | Use forceps to stabilize the flag by securing it the surgical clip.

Step 24 | Slowly open the surgical clip while maintaining pressure on the flag to prevent it from moving.

! CRITICAL STEP | Refer to ”Method for releasing probe from surgical clip” for additional details.

Bilateral Procedure | Secure flag to skull

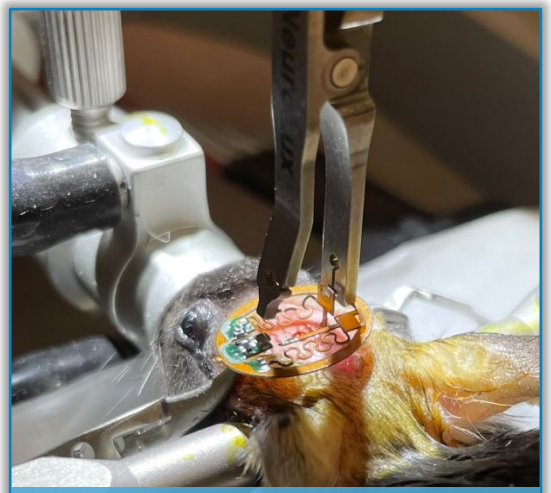


Figure 16 | Step 25

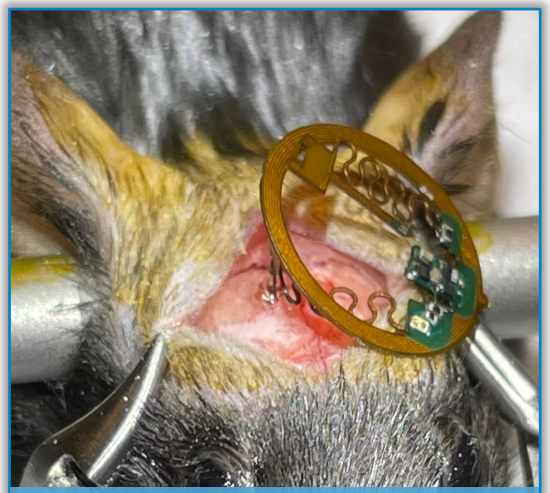


Figure 17 | Step 26

Step 25 | Remove the forceps while the surgical clip remains open.

Step 26 | Retract the surgical clip vertically and remove it from the stereotaxic arm.

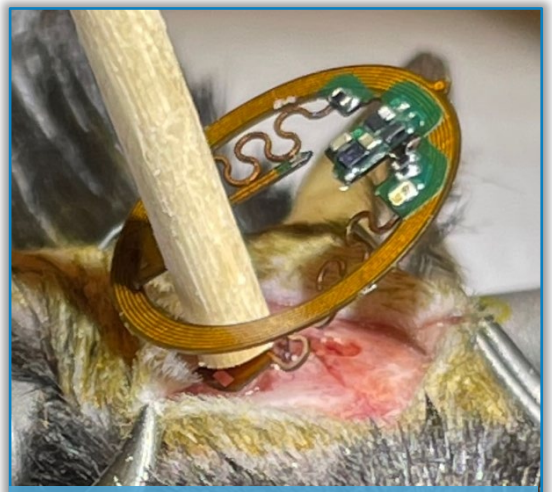


Figure 18 | Step 27 & 28

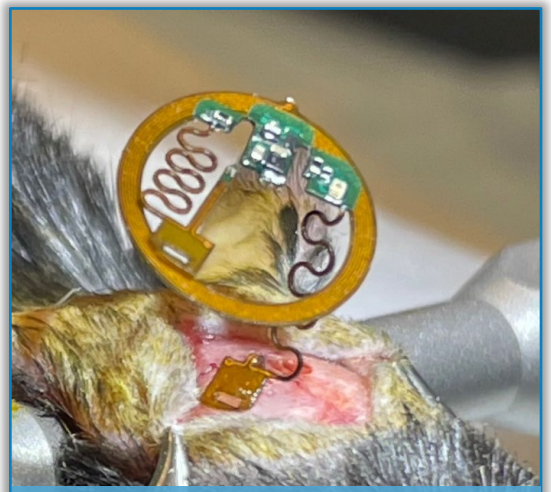


Figure 19 | Step 29

Step 27 | Use a needle to apply Loctite 454 to the flag that will be folded down to the skull (not shown).

Step 28 | Use a blunt wooden stick to fold the flag down until it touches the skull, then apply activator.

Step 29 | The first flag is now secured to the skull.

Bilateral Procedure | Insert probe #2 into brain

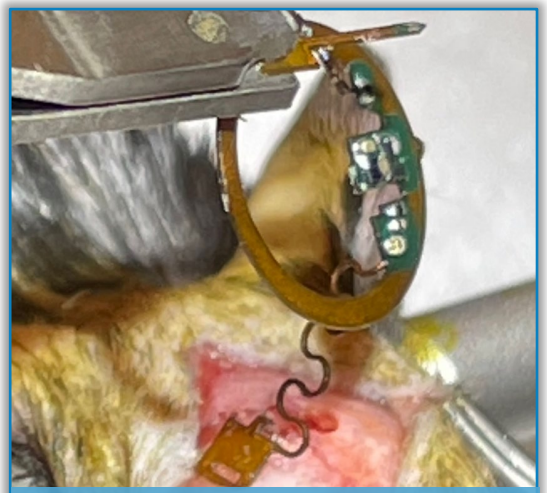


Figure 20 | Steps 27 & 28

Step 27 | Attach the surgical clip to the flag of the second probe using the alligator tooth. Ensure that the tooth is inserted in the flag correctly and that the probe does not deviate laterally when lightly pushed with forceps, then insert surgical clip into the stereotaxic adaptor at the desired orientation. Refer to “Method for inserting probe into the surgical clip” for additional details.

Step 28 | Re-attach the surgical clip to the stereotaxic frame. *Ensure that the probe and µLED are in the correct orientation*.

Figure 21| Steps 29 & 30

Step 29 | Due to the accuracy of our new surgical clip, it is not always necessary to find bregma a second time so long as the surgical clip orientation remains the same. If you reverse the clip direction, re-take bregma and move the probe to the center of the second drilled hole.

Step 30 | Slowly lower the probe into the drilled hole until the desired depth is reached. Make sure the probe does not bend, which would indicate that it is hitting skull bone fragments. Retract the probe and clean or redrill the burr hole as needed until probe inserts cleanly.

Bilateral Procedure | Secure probe to skull

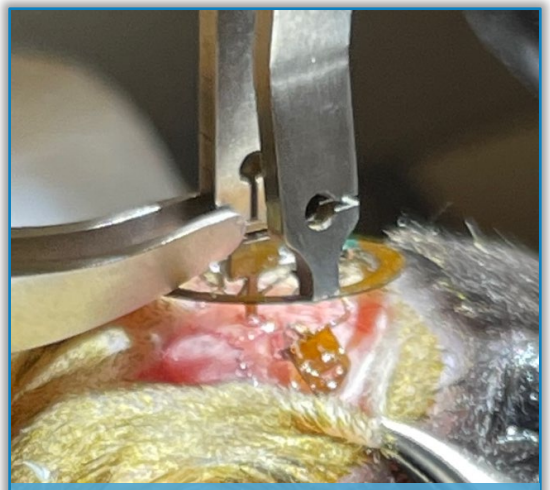


Figure 22 | Steps 31 & 32

Step 31 | Secure second probe to skull using Loctite 454, followed by accelerator to quickly harden it (not shown).

! CRITICAL STEP | Refer to ”How to secure implanted probe to the skull” for additional details.

Step 32 | Use forceps to stabilize the flag by securing it the surgical clip. Slowly open the surgical clip while maintaining pressure on the flag.

! CRITICAL STEP | Refer to ”Method for releasing probe from surgical clip” for additional details.

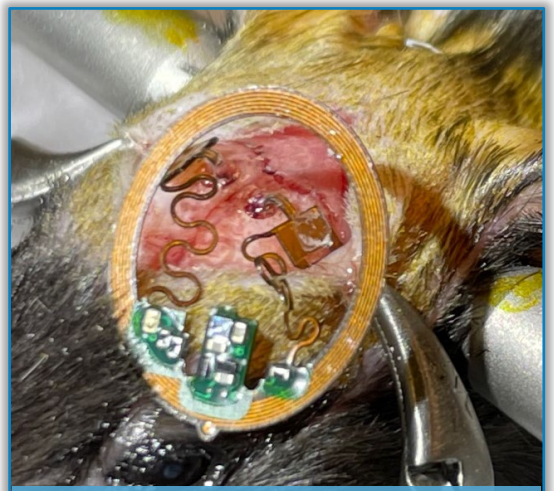


Figure 23 | Steps 33, 34, 35, 36 & 37

Step 33 | Remove the forceps while the surgical clip remains open.

Step 34 | Retract the surgical clip vertically and remove it from the stereotaxic arm.

Step 35 | Use a needle to apply Loctite 454 to the second flag that will be folded down to the skull (not shown).

Step 36 | Use a blunt wooden stick to fold the flag down until it touches the skull, then apply activator. The second probe is now secured.

Step 37 | Add additional superglue to stabilize the flags as needed.

Bilateral Procedure | Close scalp incision

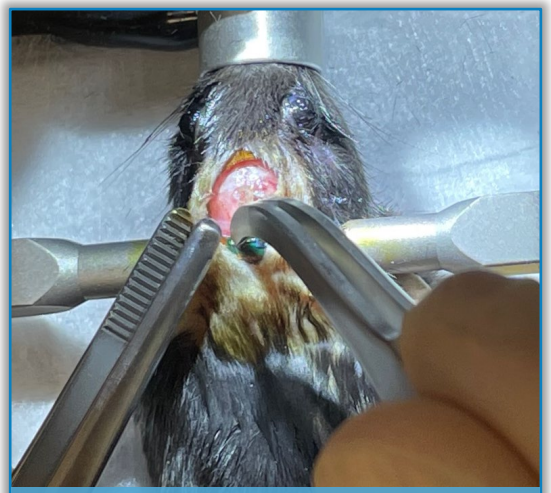


Figure 24 | Step 39

Figure 25 | Step 40

Step 39 | Secure the device to skull using Loctite if needed. Use serrated forceps to pull the skin completely over the device.

Step 40 | Close the incision with sutures. Be careful not to pull the skin too tight or it will promote scratching, which could open the wound and compromise the device if damaged.

SURGICAL TIPS

SCALP INCISION

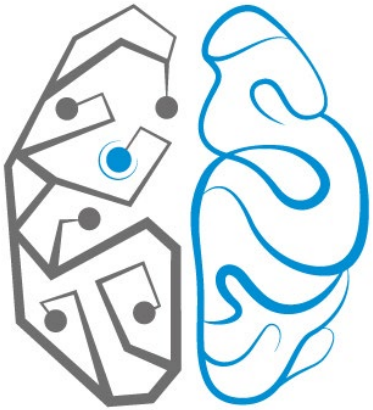
Do not cut the scalp between the animal's eyes. If you suture this area incorrectly, it can prevent them from blinking.

LOCAL SODIUM CHANNEL BLOCKERS

Local anesthetics are routinely injected subcutaneously or intradermal around the surgical incision sites. Refer to your IACUC documentation for specific local anesthetic requirements.

SUTURE TECHNIQUE

When making the first suture knot, ensure that the two sides of the incision just touch each other. Pulling the first suture knot too tightly can create a ridge along the incision line that may inhibit the healing process of the wound. This can also irritate the animal and promote scratching, which can tear the skin and sutures and damage the underlying device. Subsequent suture knots can be tightened over the first knot to lock it in place.



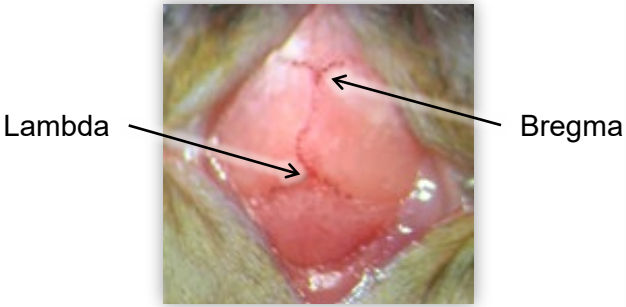
NeuroLux

Supplemental surgical tips and methods

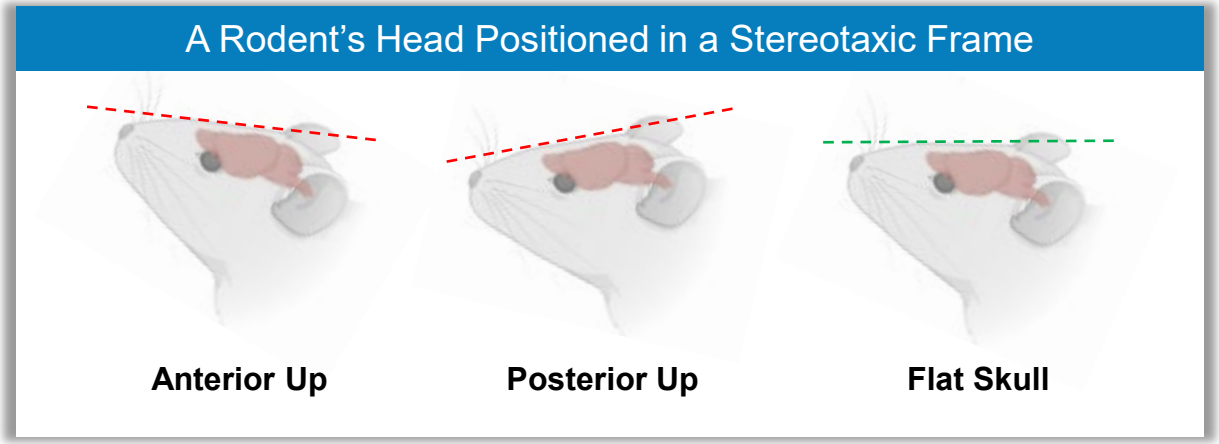
Flat skull procedure

It is *extremely important* for the rodent's skull to be perpendicular to the stereotaxic arm during surgery to ensure accuracy of the implant reaching its desired coordinates.

Head mounted device surgeries operate on a 90° angle principle. It is important to quantify the degree of skull tilt in both the anterior-posterior and medial-lateral planes and correct them to achieve a flat skull. A slight tilt in the rodent's head in the stereotaxic frame may be undetectable to the human eye, yet it could negatively impact the accuracy of your surgical procedure and ultimately your experimental results.



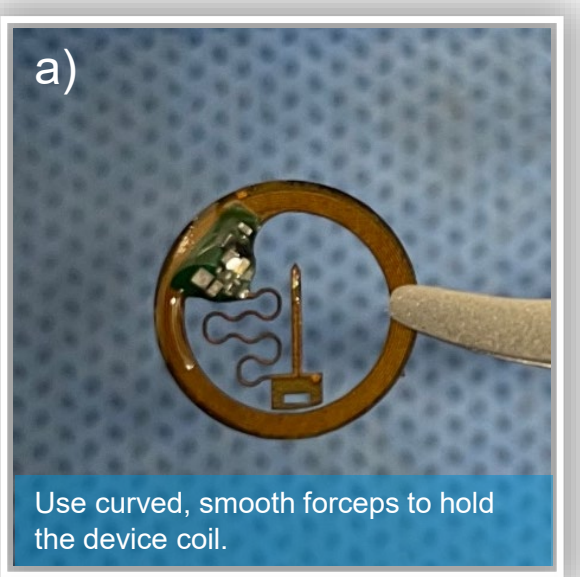
To achieve a flat skull, compare the dorsal/ventral coordinates of two prominent skull landmarks: Bregma and Lambda.

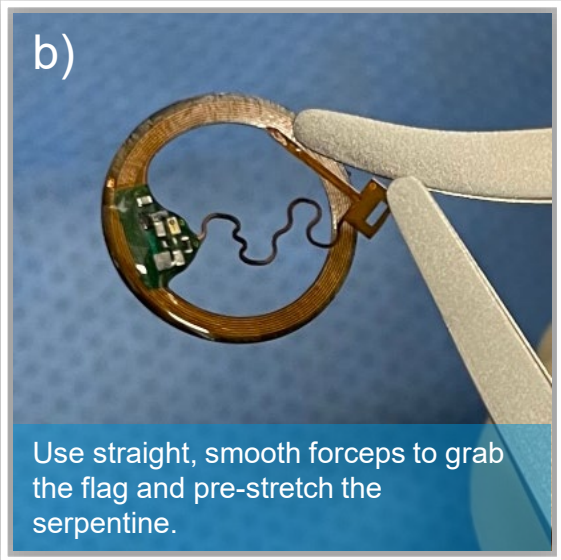


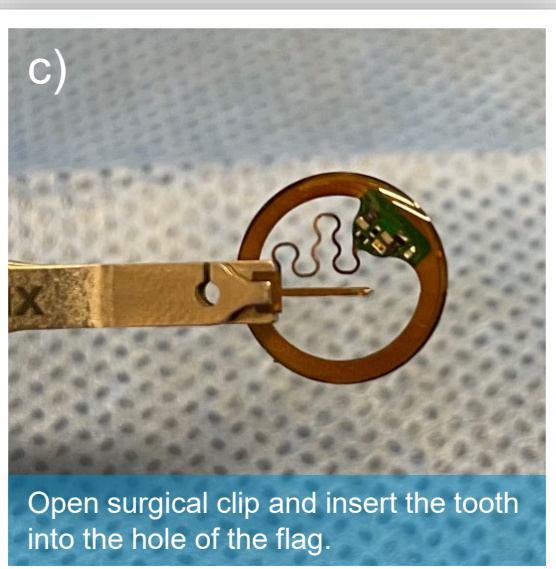
THE FOLLOWING PROCEDURE IS USED TO MEASURE AND ADJUST SKULL ANGLE PRIOR TO PROBE IMPLANTATION

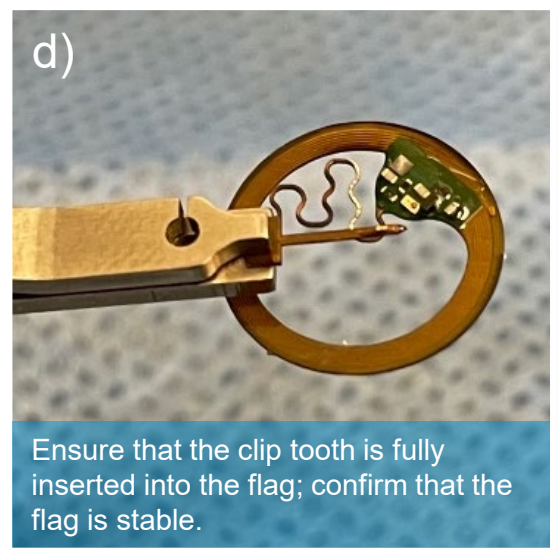
1) Insert a 1" 27 g syringe needle into the PH-300 stereotaxic arm with the open bevel facing away from the surgeon.
2) Navigate the needle to Bregma and slowly lower it until it initially contacts the skull. Record or zero out those coordinates.
3) Next, navigate the needle posteriorly to Lambda and slowly lower it until it initially contacts the skull.
4) Compare the dorsal/ventral coordinates of Bregma and Lambda and adjust the bite-bar as needed. For instance, if Lambda is lower than Bregma (anterior up) you need to lower the bite-bar. Repeat the procedure until a flat skull is achieved.
5) Maximum allowable tolerance between the two locations differs across labs but should always be less than 100 µm.
6) A similar procedure can be performed in the medial/lateral direction to adjust for potential head rotation. Measurements are generally taken at the desired surgical medial/lateral coordinates and the ear bars are used to adjust the angle.

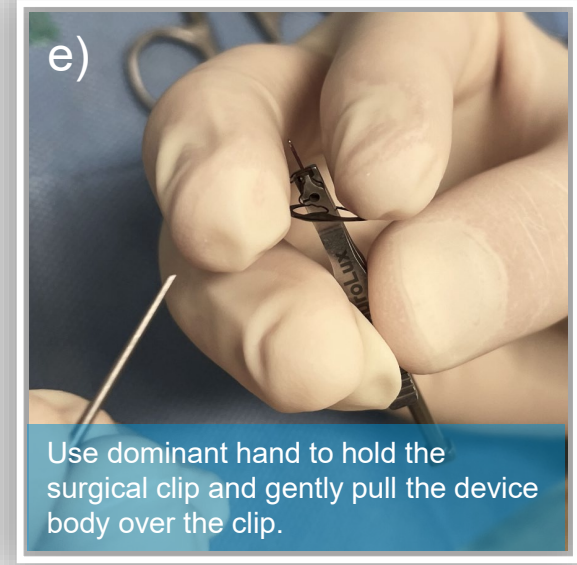
Method for inserting probe into surgical clip





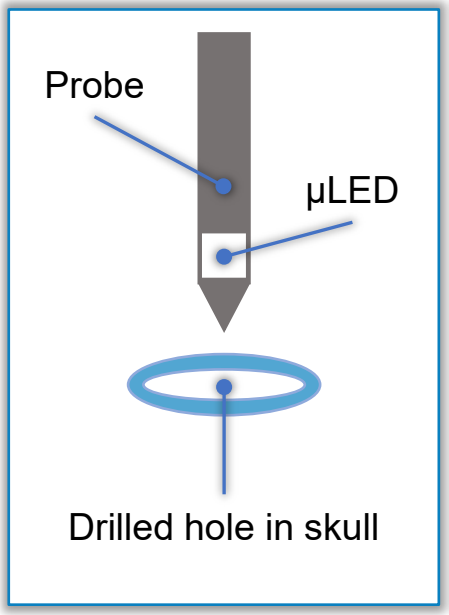




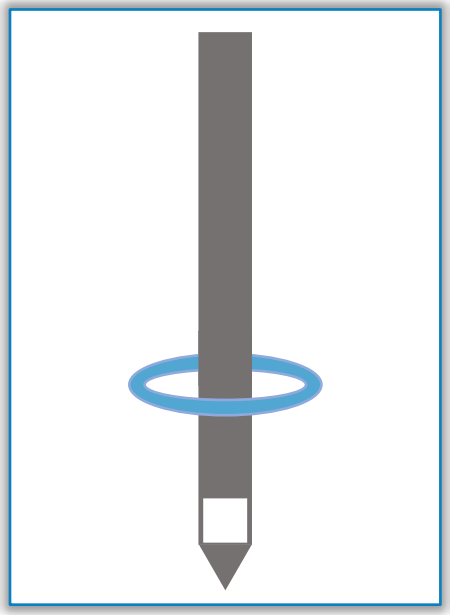


How to secure implanted probe to skull

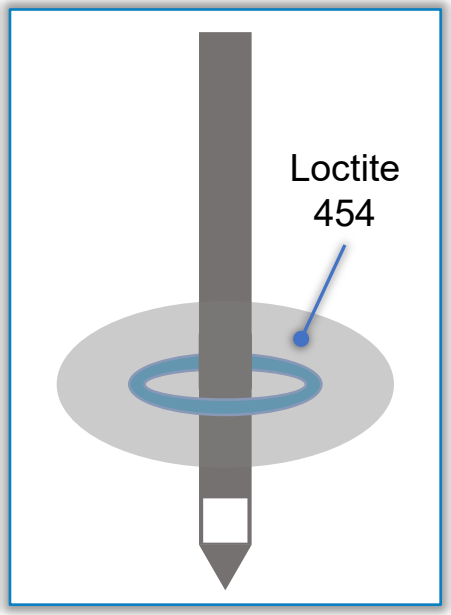
The most common method of securing the probe to the skull is by using superglue and a superglue accelerator. The head mounted device procedures in this document were completed using **Loctite 454** and **Loctite 7452 Accelerator**.



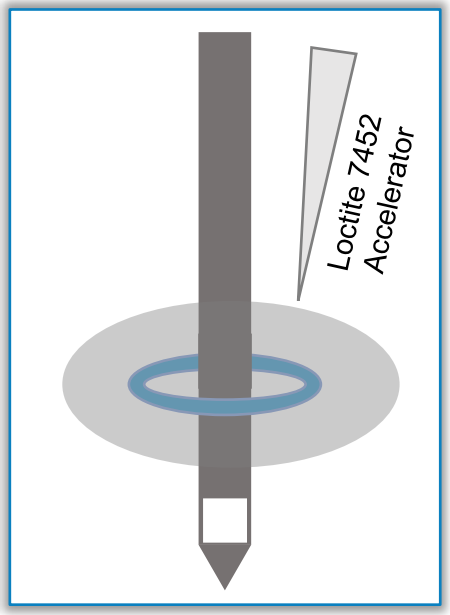
Step 1 | The probe is navigated above the drilled hole in the skull.



Step 2 | The probe is slowly inserted into the brain through the drilled hole and lowered to the desired depth at a rate of 300 - 500 µm per minute

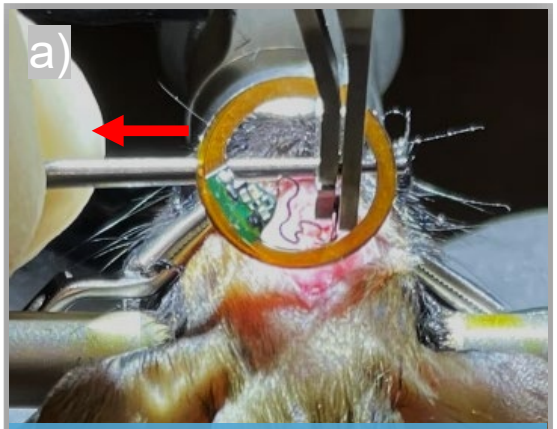


Step 3 | With the surgical clip attached, apply Loctite 454 superglue gel in a ring around the probe to secure it to the skull.

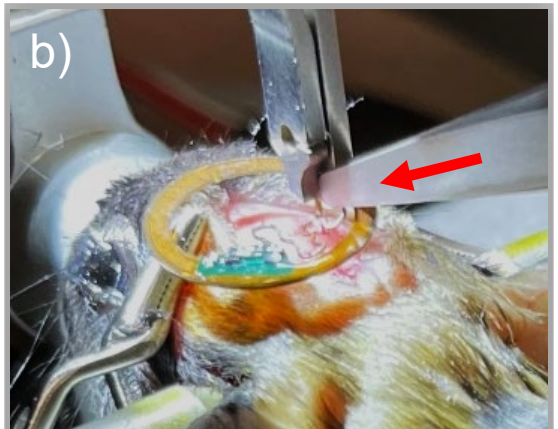


Step 4 | Apply Loctite 7452 Accelerator to the superglue using an insulin syringe. Remove the pin from the surgical clip, then open it and retract vertically.

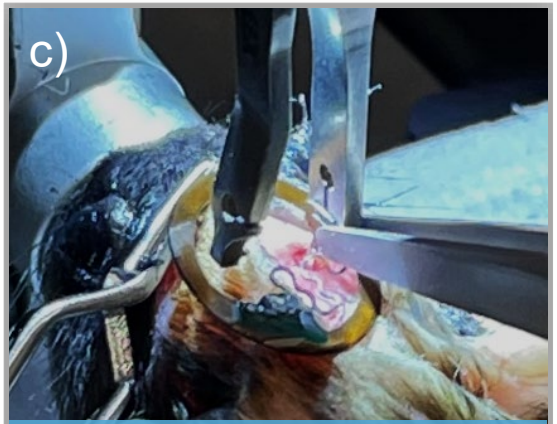
Method for releasing probe from surgical clip



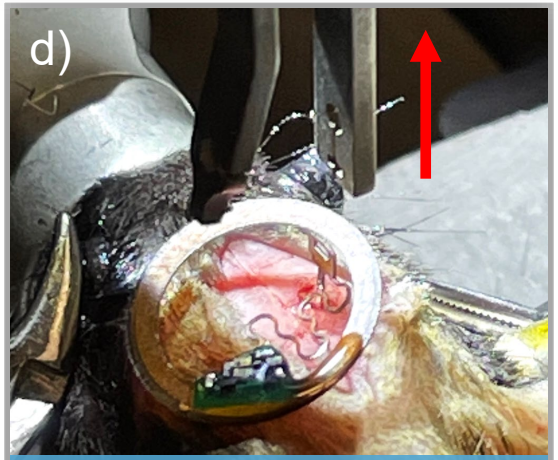
Remove the stabilizing pin from the surgical clip and allow device body to drop (motion indicated by red arrow).



Use smooth forceps to secure the flag to the back of surgical clip (red arrow).



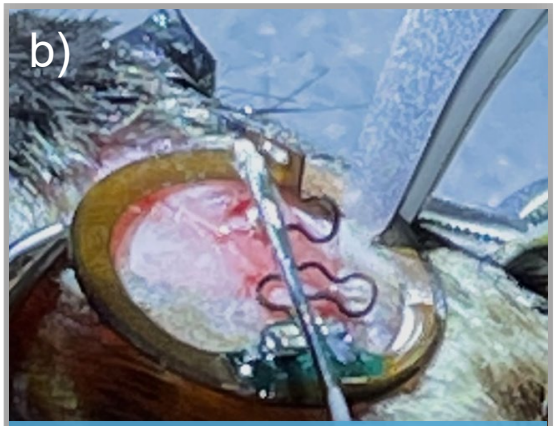
Release the surgical clip from the flag and ensure it remains in an open position while still securing the flag.



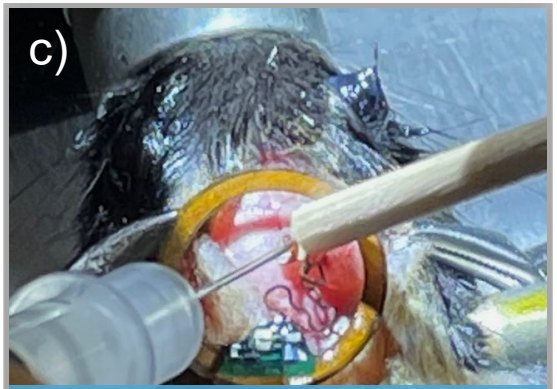
Retract the surgical clip as it remains open (motion indicated by red arrow).

Method for securing flag to skull

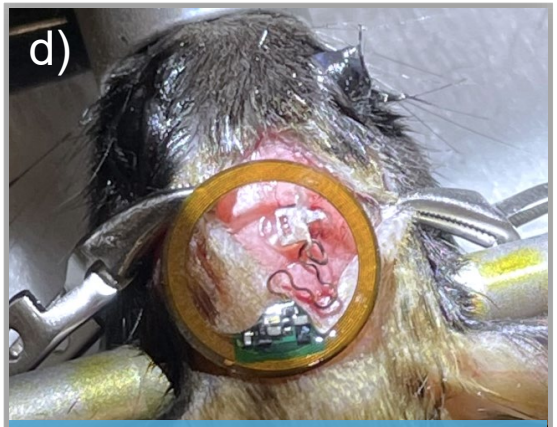
Use a sharp needle to ensure that the glue around probe is dry and that the probe is securely attached to the skull.



Use a 1” 27 g syringe needle to apply a small amount of Loctite 454 gel superglue to the face of the flag.



Use a wooden cotton swab handle to fold the flag over to the skull. Once the flag is flat, use an insulin syringe to apply superglue accelerator.



Apply additional superglue and accelerator on top of the flag, as needed, until stability is achieved.

Alternate methods to secure probe and flag to skull

Based on the desired coordinates and specific needs, alternative methods to secure the probe and flag to the skull may be used. Before using any adhesive, *ensure that the skull is completely dry*. The following is a list of bonding agents successfully used by our customers.

BLUE LIGHT CURABLE DENTAL COMPOSITE

Flowable dental composite is a viable method for securing the probe and flag to the skull. Like other methods, using dental composite requires the skull to be extremely dry. Scoring or scratching of the skull may help the composite adhere more tightly. Eye protection may also be required for the animal to protect against the bright light.

https://tricountydental.com/product/prime-dent-flowable-light-cure-dental-composite-4-syringe-kit/

TETRIC EVOFLOW DENTAL CEMENT AND iBOND

Traditionally, dental composites require specific conditions to bond to bone. By pairing the dental cement (EvoFlow) with iBOND, the cement can more tightly adhere to the skull.

https://www.ivoclar.com/en_us/shop/p/fillingmaterials/tetricevoflowrefill1x2g/p/b601328
https://www.kulzer.com/en/en/for-dentists/products-by-brand/ibond/about-ibond.html

METABOND

Adhesive resin cement such as Metabond can be used for securing the probe and flag to the skull. Like most adhesives, Metabond requires a dry area to adhere to.

https://www.parkell.com/c-b-metabond_3

Differences between disinfection and sterilization

DISINFECTION vs. STERILIZATION

Eliminates **most pathogens** but not necessarily all types of microbes.

Disinfection **reduces** the level of microbial contamination; **does not kill spores**.

Disinfection methods can be divided into *High, Intermediate, and Low activity* levels for killing organisms.

Some common laboratory disinfectants include freshly prepared 10% bleach and 70% ethanol.

The probability of a microorganism surviving on an item subjected to treatment is **less than one in one million**.

Sterilization procedures **kill all microorganisms**.

A sterile surface/object is **completely free** of living microorganisms and viruses.

Methods used in sterilization procedures include heat, ethylene oxide (EtO) gas, hydrogen peroxide gas, plasma, ozone, and radiation.

https://www.cdc.gov/infectioncontrol/guidelines/disinfection/sterilization/index.html
https://labsafety.gwu.edu/sterilization-disinfection-and-decontamination

Device disinfection procedures

ETHYL AND ISOPROPYL ALCOHOL

- Antimicrobial action of alcohol is denaturation of proteins
- Optimum bactericidal concentration is 60%–90% solutions in water (volume/volume)
- Ethyl alcohol, at concentrations of 60%–80%, is a potent virucidal agent inactivating all of the lipophilic viruses.
- Alcohols are **not recommended** for sterilizing medical and surgical materials.
- Isopropyl and ethyl alcohol have been excluded as **high-level** disinfectants because of their inability to inactivate bacterial spores and because of the inability of isopropyl alcohol to inactivate hydrophilic viruses (i.e., poliovirus, coxsackie virus).

UV RADIATION IS DISCOURAGED DUE TO THE FOLLOWING LIMITATIONS

- Is not effective on porous materials that are opaque to the light such as wood or foam.
- Is not effective if a microbe is protected by dust, dirt, or organic matter.
- Is affected by the accumulation of dust and dirt on the bulb surface.
- Does not work in shadowed areas or penetrate into cracks.
- Humidity adversely affects the effectiveness of UV. Above 70% relatively humidity, the germicidal effects drops off precipitously.

https://www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/miscellaneous.html
https://www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/chemical.html
https://labsafety.gwu.edu/sterilization-disinfection-and-decontamination
Burgener, J. 2006. Applied Biosafety. 11(4)228-230

Device sterilization procedures

AUTOCLAVE

- Steam sterilization should be used whenever possible on all critical and semi-critical items that are **heat and moisture resistant**.
- Moist heat in the form of saturated steam under pressure is the most widely used and the most dependable.
- Steam sterilization is nontoxic, inexpensive, rapidly microbicidal, sporicidal, and rapidly heats and penetrates fabrics.
- Recognized minimum exposure periods for sterilization of wrapped healthcare supplies are 30 minutes at 121°C (250°F) in a gravity displacement sterilizer or 4 minutes at 132°C (270°F) in a pre-vacuum sterilizer.

ETHYLENE OXIDE (EtO) GAS

- Used in healthcare facilities to sterilize critical items (and sometimes semicritical items) that are **moisture or heat sensitive** and cannot be sterilized by steam sterilization.
- Four essential parameters (operational ranges) are: gas concentration (450 to 1200 mg/l); temperature (37 to 63°C); relative humidity (40 to 80%)(water molecules carry EtO to reactive sites); and exposure time (1 to 6 hours).
- Main disadvantages associated with ETO are the lengthy cycle time, the cost, and its potential hazards to staff.

https://www.cdc.gov/infectioncontrol/guidelines/disinfection/sterilization/steam.html
https://www.cdc.gov/infectioncontrol/guidelines/disinfection/sterilization/ethylene-oxide.html

Device recommendations

AUTOCLAVE – *NOT RECOMMENDED*

- DO NOT use an autoclave to sterilize NeuroLux devices. The temperature is higher than the rating for some of the electronic components and could render them inoperable.

ETHYLENE OXIDE (EtO) GAS - *RECOMMENDED*

- This low temperature sterilization option is generally found in larger medical universities and can be reliably used to sterilize NeuroLux devices without risk of damaging them. Doing so requires planning prior to surgeries, as the process can take up to a day to complete.

70 % ETHYL ALCOHOL – *RECOMMENDED*

- This level of device disinfection is commonly used by NeuroLux customers. The procedure is quick and dramatically reduces the risk of animal infection following surgery.
- Place the device in a glass petri dish prior to implantation and cover with enough 70 % ethyl alcohol to fully submerge it. Leave it in place for at least 5 minutes, and up to 10 minutes. Remove the device from the petri dish using sterile blunt forceps and rinse with sterile DI water or saline prior to implantation.