NeuroNexus

General Surgical Guide

Acute and Chronic Experiments

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Introduction

This technical document provides important information about performing animal surgery in biomedical research settings. This includes information on pre-operative procedures, anesthesia, analgesia, aseptic/sterile technique, surgical technique, incision closure and post-operative procedures. However, IACUC guideline is a standard procedure. For more information please also check IACUC approved policies of your university.

Principles of Animal Surgery

To learn the principles of rodents surgery please read 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

- Intraperitoneal injection with a cocktail of xylazine (rat and mouse:10 mg/kg) and ketamine (rat: 50-100 mg/kg, mouse: 80 mg/kg)
- Local anesthetics (Lidocaine 1%)
- Inhaled anesthetics using isoflurane setup is recommended. (Note: It is never accepted to use expired anesthetics, euthanasia agents)
    - Mouse: Dose: 2-3% for induction; 1-2% for maintenance
    - Rat, rabbit, guinea pig, ferret: Dose: 3-5 % for induction; 2-3% for maintenance

Sterilization of surgery site (for chronic surgery)

- 70% ethanol
- Iodine-based wash (Betadine)

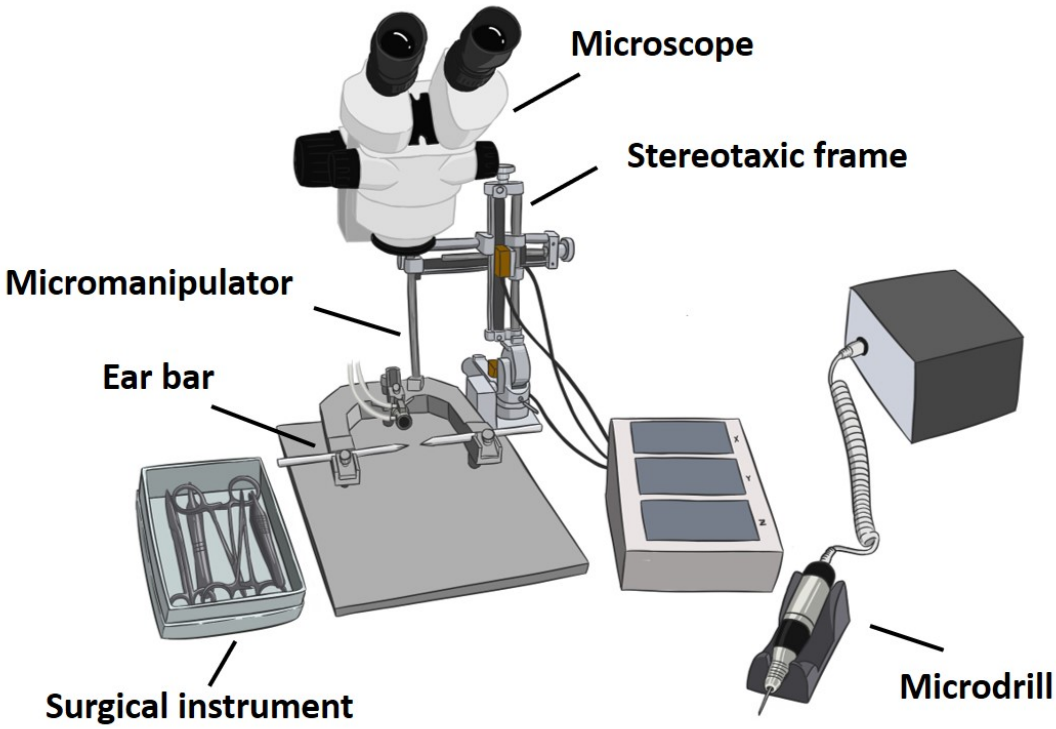
Fluid support

- 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

- Scissor, scalpel, surgical needle with suture, hemostat, forcep, tuberculin syringe with needle, cotton swab, absorption surgical sponge triangle, gel foam
- Note: Expired surgical materials (suture, bandage material, surgical gloves) are not allowed during the procedure

General Considerations

Acclimation (chronic surgery)

- One week period to prevent stress-induced disease in rodent, pig, cat, dog, and ruminants

Fasting (chronic surgery)

- Not necessary for rodents due to their inability to vomit. Necessary for guinea pig, cat, dog, ruminant, and non-human primate
- Never restrict water

Eye protection (chronic surgery)

- In rodents and guinea pig eyes remain open under anesthesia. This can lead to corneal drying and trauma. Apply ophthalmic ointment

Monitoring

- Monitor 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

Heat support

- All species are at risk for hypotension and hypothermia while under anesthesia
- Suggested options are circulating water blankets
- No electric heating pads are allowed
- Regardless of heat source, never place animals directly on the heat

Fluid support

- Consider providing warm subcutaneous (SQ) or intraperitoneal (IP) fluids, particularly for prolonged anesthetic events or animals that are ill, aged, or debilitated

Recovery (chronic surgery)

- Continue to monitor animals until they are fully recovered
- Let animals recover on paper towels and provide heat

Surgery Tips

- For chronic study, use sterile surgical tools
- Apply saline at your surgery site periodically to prevent thermal damage due to drilling and removing the blood at the drilling site
- Use a proper sterile burrs for Micro Drill (Tip Diameter: 0.7 mm for mouse, 0.9 mm for rat, 2 mm carbide burr for primates)
- Choose craniotomy size according to your study needs:
  - Small craniotomy for ephys only
  - Bigger craniotomy for imaging studies (e.g., optogenetics or 2-photon imaging)
- Always remember to create at least 2 extra holes for adding bone screw for attaching your ground and reference wires
- Remove dura before electrode insertion. You do not need to follow this step for mice
- Use microscope and either manual or automatic manipulator for electrode insertion

Surgical Sterilization

For chronic study, the surgery tools need to be sterilized.

Note: You need to perform ETO or VHP sterilization for microelectrode array, insertion tool, vector array probe holder, absorption surgical sponge triangle, and gel foam. The rest you can steam sterilize (autoclave). Please remember that when you are placing your NeuroNexus probe order you can request ETO sterilization of your microelectrodes.

High pressure/temperature (autoclave)

- Effectiveness of autoclaving must be determined by a steam integrator strip placed inside the surgical pack
- It is recommended to seal the pack with autoclave tape, as a second indicator
- Autoclave utilizes steam at high heat and pressure which must penetrate the pack to attain sterilization for surgical tools
- Don’t autoclave microelectrode array, insertion tool, vector array probe holder, absorption surgical sponge triangle, or gel foam
- Don't use aluminum foil or wax paper as a wrapping pack since steam is unable to penetrate these materials
- Exposure time in an autoclave is normally 20 minutes at 121°C (250°F)

Note: Do not use this process for microelectrode array

Dry bead sterilization (dry heat)

- This method is designed to sterilize the tips of surgical instruments in between multiple surgeries
    - Note: Instruments must be fully sterilized by another method between separate surgical sessions

- Sterilizer must be turned on for at least 20 minutes prior to 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 min 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

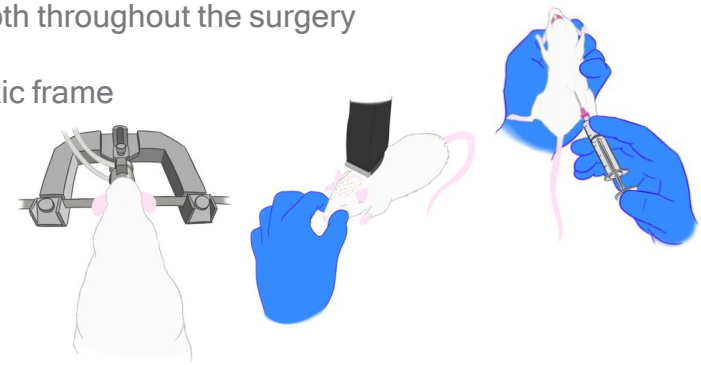
- ETO is designed for items that cannot withstand high temperature. Microelectrode array, insertion tool, vector array probe holder, absorption surgical sponge triangle, and gel foam need to be sterelized using ETO
- The four essential parameters for ETO are:
  - Gas concentration: 450 - 1200 mg/L
  - Temperature: 37 - 63°C
  - Relative humidity: 40% - 80%; water molecules carry ETO to reactive sites
  - Exposure time: 6 - 12 h; these influence the effectiveness of ETO sterilization

Vaporized hydrogen peroxidze (VHP) sterilization

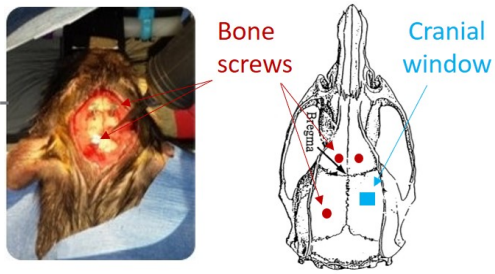
- Also known as hydrogen peroxide gas sterilization, is a low temperature sterilization process commonly used to sterilize heat-sensitive devices. Microelectrode array, insertion tool, vector array probe holder, absorption surgical sponge triangle, and gel foam need to be sterilized using VHP
- A sterilization 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 a hydrogen peroxide
- VHP is maintained at a constant concentration while it is 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

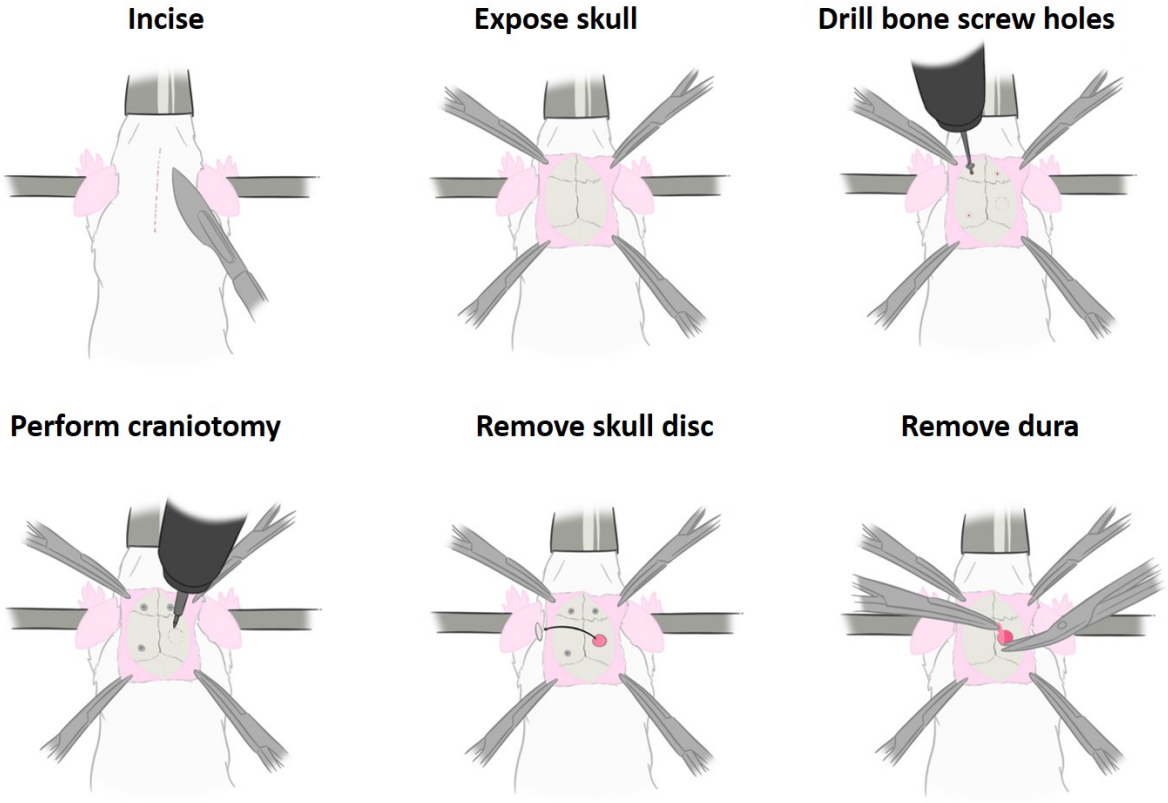
1. Anesthetize animal with a cocktail of xylazine (rat and mouse:10 mg/kg) and ketamine (rat: 50-100 mg/kg, mouse: 80 mg/kg) through ip injection or isoflurane inhale (4% in 100% O_2).During surgery procedure, continue anesthesia with isoflurane (rat:1-2.5%, mouse: 0.8 -1.25%) and apply ventilation with a mixture of oxygen and medical air (50:50)
2. Use toe pinch reflex to measure anesthetic depth throughout the surgery
3. Shave the surgical area
4. Intubate animal and secure them in a stereotaxic frame
5. Artificial tear ointment should be applied to the rodents eye to prevent drying
6. Optional: inject dexamethasone sodium phosphate (2 mg/kg) before surgery to reduce cerebellar edema
7. Apply betadine and 70% alcohol to sterilize the skin of the surgery site in chronic setup.



8. Make a midline sagittal incision along the scalp to expose the skull covering the location of interest
9. Use cotton-tip applicator to remove periosteum from the skull. Apply saline for skull cleaning
10. Apply a thin layer of VetBond adhesive to dry the surface of the skull prior to drilling and provide a supportive grip for a dental cement head cap
11. Use surgical marker to mark the position of three screws and the implant site
12. Drill two or three small holes (1 mm diameter; two over both motor cortices and one over the contralateral site of implant) for stainless steel screws to be used later as a ground and reference
13. Advance screws (mouse: 4 mm long, 0.86 mm diameter) into drilled holes, special care should be taken not to advance the screws beyond the point of contact with the dura



14. Secure the threads of the screw to the bone with a small dab of VetBond



15. Perform craniotomy above the brain region of interest using a high-speed drill
    - Note 1: If you are planning to just do ephys try to make a small craniotomy, while for imaging studies like optogenetics or 2 photon imaging, bigger craniotomy is desired
    - Note 2: During drilling, flush the window regularly with saline to reduce heat buildup and to remove blood and bone shavings
    - Thin the edge of the windows until the underlying pial vasculature becomes visible and continue very carefully until the bone begins to craze. Then, use forceps to genetly seperate the bone flap from the skull without protruding into the brain tissue. Grasp adjacent corners of teh loosened bone flap, and slowly peel it away from the underlying dura mater
16. Remove dura before electrode insertion and control the bleeding using gel foam soaked with sterile saline.You do not need to follow this step for mice
17. Right now you are ready to implant the electrode