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General Setup and Takedown Procedures for Rodent Neurosurgery V.2

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We use this protocol and it's working

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Abstract

This protocol describes the pre-operative setup and post-operative take-down procedures utilized for rodent stereotaxic neurosurgical procedures.

Image Attribution

Gabriel Rodriguez, Allen Institute.

Guidelines

Only perform this procedure in accordance with IACUC and veterinary requirements.

Materials

**Please note, not all supplies and equipment are used for every surgical procedure

Anesthesia and other Drugs

Isoflurane **Patterson Veterinary Catalog #**07-890-8115

1 g Dexamethasone **biorbyt Catalog #**orb134330

Ceftriaxone Injection **MWI Animal Health Catalog #** 094311

Lactated Ringers Injection, USP, Preservative-Free, Baxter **Henry Schein Animal Health Catalog #**059380

Ethiqa XR Buprenorphine Extended-Release Injectable Suspension for Mice and Rats 1.3mg/mL, 3mL **Fidelis Pharmaceuticals Catalog #**099114

1 g Atropine **biorbyt Catalog #**orb322218

1 g Carprofen **biorbyt Catalog #**orb321211

Note

Drugs should only be administered in accordance with IACUC and veterinary requirements. Ensure timing, dosage, and route of administration are accounted for.

Surgical Tools and Supplies:

Tool / Supply	Manufacturer / Supplier	Part Number
Black handle scissors, ToughCut	Fine Science Tools	14058-11
Scalpel handle	Fine Science Tools	10003-12
Iris forceps	Fine Science Tools	11064-07
Dumont #5 45° forceps	Fine Science Tools	11251-35
45° Vanna scissors, 8cm	World Precision Instruments	500260

Tool / Supply	Manufacturer / Supplier	Part Number
45° or 90° Durotomy probe	Fine Science Tools	10066-15
Plastic sterilization container	Fine Science Tools	20810-02
Hemostats	Fine Science Tools	12004-16
large iris forceps	Fisher Scientific	13-820-073
Bulldog clamp	Fine Science Tools	18053-28
PREempt Disinfectant spray	McKesson Corporation	21101
70% Ethanol (Diluted in-house)	Sigma Aldrich	459836
Alchohol wipes	Becton, Dickinson and Company	326895
Sterile Surgical Drape, 18x26	Fisher Scientific	NC9517505
Sterile Multi-well plate, 24 well	Advantor	29443-952
Nair hair removal cream	Arm & Hammer	40002957
Betadine Solution 10%	McKesson Corporation	1073829
Hemostatic Agent Surgifoam	McKesson Corporation	403360
Sterile Gauze, 3x3” squares, (autoclave sterilized)	Patterson Veterinary	07-893-8587
Cotton swabs, double ended, (autoclave sterilized)	Advantor	89133-810
Sugi pointed sterile swabs	Fine Science Tools	18105-01
Insulin syringes, U-100, 0.3 ml, 31G	Advantor	BD328438
Insulin syringes, U-100, 1 ml, 31G	Advantor	BD328418
Luer-Lock Syringe, 20 ml OR	Advantor	53548-025
Luer-Lock Syringe, 10ml	Advantor	75846-756
25G 5/8-inch needle	Advantor	89134-134
32 mm Syringe Filter 0.2 µm Supor Membrane	Advantor	75846-756
Press ‘n’ Seal	Medline	CLO70441
Saran Wrap	GLAD	Amazon B015CLAVU
Sterile Drill Bits, 0.5/0.4, FG1/4 AND/OR	NeoBurr	1734948
Sterile Drill Bits, 1.4/1.1, FG4 AND/OR	NeoBurr	1734214
Sterile Drill Bits 1.0/4.2, EF4	NeoBurr	1730012
Sterile Scalpel blades, #10 OR	Advantor	21909-378
Sterile Scalpel blades, #11	Advantor	21909-380
Systane Eye Ointment	Systane	Amazon ALCON293787
Artificial Cerebrospinal Fluid.V*	Made in-house. Protocol referenced.	http://dx.doi.org/10.17504/protocols.io.besjjecn

Tool / Supply	Manufacturer / Supplier	Part Number
C Universal 4-META Catalyst, 0.7 ml	Parkell	S371
B Quick Base for MetaBond, 10 ml	Parkell	S398
Radiopaque L-Powder, white, 5 gm	Parkell	S396
Radiopaque L-Powder, clear 3 gm	Parkell	S399
Silicone implant coating, SORTA-Clear 18	Renolds Advanced Materials	SORTA-Clear 18
Loctite 4305	Henkel	303389
CS-5R Coverslips, 5 mm	Warner Instruments	64-0700
Vetbond Glue	Patterson Veterinary	07-805-5031
Superglue, Singles	Krazy Glue	Amazon PK4 KG58248SN
3 ml transfer pipette, plastic	Avantor	52947-970
Ortho-Jet BCA Liquid	Lang Dental Maufacturing Company	Ortho-Jet BCA Liquid
Black cement (1) = 4 parts of Ortho-Jet BCA Powder (mixture) AND	Lang Dental Manufacturing Company	Ortho-Jet BCA Liquid
Black cement (2) = 1 part of Powder tempura point, black	Jack Richeson & Co	1# Black 62, Amazon B00JGZ8Q1A
Kwik-Sil Sealant	World Precision Instruments	KWIK-SIL
Kwik-Cast Sealant	World Precision Instruments	KWIK-CAST
Heat-sterilized Glass pipettes AND/OR	Drummond Scientific	3-000-203-G/X
Heat-sterilized Glass pipettes	World Precision Instruments	1B120F-4
“Marker” glass pipette, pulled, broken, and Sharpie mark for measuring coordinates	World Precision Instruments	1B120F-4
Microcapillary Pipette tips	Eppendorf	89009-310
Parafilm	Advantor	52858-000
Lightweight Mineral Oil	Sigma-Aldrich	M8410
30 gauge, 2" Backfilling Needle	Drummond Scientific	3-000-027
Sterile Bone Wax	Central Infusion Alliance, Lukens	CIA2160287, 901
5-0 Monofilament suture with 17mm 1/2C taper needle attached	Penn Veterinary Supply	Monomend MT
Sterilization pouches	Advantor	89140-804
Fiber Optic Cannulae, 200 um fiber core diameter, Black ceramic ferrule	Neurophotometrics	FOC_BF_200um/1.25 mm

All tools / supplies can be substituted with their equivalent.

Key:
AND = Including the tool/supply in row below.
OR = Can use tool/supply in row below instead.

Autoclaved sterilized = Sterilized in-house.
mixture = Mix with tool/supply in row below.
* = **Artificial Cerebrospinal Fluid.V**

Equipment:

Equipment	Manufacturer / Supplier	Part Number
Small Animal Stereotaxic Instrument	Kopf	1900
Adjustable Stage Platform	Kopf	901
Stereo Microscope	Lecia	M80
Gooseneck Illumination	AM Scope	LED-6WA
On-axis Illumination	Lecia	KL2500 LED
Bead sterilizer	Sigma-Aldrich	Z378585
Small Animal Temperature Control System	CWE Inc.	TC-1000
Large Heat plate/pad	Lectro-Kennel	Outdoor Heated Pet Pad
Dental Drill	NSK	Pana-Max2 M4
Oxygen Concentrator	Nidek Medical Products	Nuvo Lite Model 525
Isoflurane with oxygen delivery system	Patterson Scientific	Tec 3 EX
Isoflurane induction chamber	Patterson Scientific	78933385
Ear bars	Kopf	1922
Ultra Fine Point Sharpie	Sharpie	37001
Metabond ceramic mixing dish	Parkell	S387
Xlite LED Curing Light	Independent Dental	Flight Xlight2-CUR
Electrode Holder	Kopf	1970
Sterotaxic Cannula Holder	Inper	-
Galaxy Mini Centrifuge	Avantor	76269-066
P20 Pipettor	Gilson	F123600
Silver wire	Stoelting	50880
Midgard Precision Current Source	Stoelting	51595
Nanoject II Variable Volume (2.3 to 69 nL) Automatic Injector OR	Drummond Scientific	3-000-204
Nanoject III Programmable Nanoliter Injector	Drummond Scientific	3-000-207

All equipment can be substituted with their equivalent.

Key:
OR = Can use equipment in row below instead.

Materials/Equipment designed/made in-house (CAD available upon request):

Material	Part Number
5mm Cranial Window (two 5mm stacked with single 7mm circular cover glass lip)	Tower Optical 18687-2
3mm Cranial Window (3mm coverslip with single 4mm circular cover glass lip).	Tower Optical
CAM Well	0160-200-10
Mesoscope Well	0160-200-20
Neuropixel Well	0160-200-45
Surgical Implant for Whole Hemisphere Craniotomy	0251-110-42
Titanium 42 Headpost	0160-100-42
Titanium AI Straight Bar	1365-6428-001
Titanium VisCtx Headpost	0160-100-10
Titanium LC / Brainstem Headpost	0160-100-52
Whole Hemisphere Well	0160-055-08
2p Whole Hemisphere Headpost	0160-100-45
Well Cap	0160-055-09
Bregma Stylus	0251-900-04
Lambda Stylus	0111-300-01
Dovetail Clamp	0111-200-00
Whole Hemisphere Craniotomy Clamp Tracer	0251-119-00
Whole Hemisphere Craniotomy Hand Tracer	0251-110-45
-6^0 Ear bar Headframe Clamp	0155-100-00
-0^0 Ear bar Headframe Clamp	0155-110-00
Prober Holder	0155-200-00
Titanium MotorCtx Headpost	0160-100-54
Laser Leveling Tool	0111-500-00

All equipment can be substituted with their equivalent.

Personal Protective Equipment (PPE):

Suggested PPE
Gloves
Disposable lab coat
Disposable face mask
Shoe covers / surgery shoes
Scrubs
Surgical cap
Biohazard sharps disposal container
Biohazard waste disposal container
Blue light blocking glasses

Utilize PPE in accordance with IACUC and veterinary requirements. Ensure sterility when necessary.

Safety warnings

- Personal Protective Equipment (PPE) should be used at all times while operating this protocol.
- Isoflurane Warning: Acute over-exposure to waste anesthetic gases (WAG) may cause eye irritation, headache, nausea, drowsiness or dizziness. Repeated exposure may cause damage to cardiovascular system and central nervous system. Refer to MSDS for additional information. Consult the surgical workstation guide to ensure all parts of the dispensation rig are functioning properly.
- Blue-light filter safety goggles must be worn while using LED curing light.

Ethics statement

Research focused rodent neurosurgery must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s).

This protocol has been approved by the Allen Institute Animal Care and Use Committee (IACUC).
PHS Assurance : D16-00781
AAALAC : Unit 1854

Before start

Notice:
Refer to sections via table of contents to view surgery specific setup. Skip any section titled with "Setup specific..." if not applicable.

Prepare Surgical Station for Surgery (all procedures)

1 Disinfect the surgical area.

1.1 Spray area for the surgical drape with PREempt and let sit for at least 00:05:00 . 5m

1.2 Spray all other surfaces - surgical rig, induction chamber, station tools, knobs buttons, and switches you touch during the procedure with 70% Ethanol reapplying after 5 min, so you have a minimum contact time of 00:10:00 . 10m

1.3 Using a non-sterile Kimwipe wipe up any residual PREempt and 70% Ethanol.

2 Cover heating pad on surgical rig with a layer of press ‘n’ seal.

3 Prepare the surgical drape with supplies.

3.1 Open a fresh sterile drape, touching only the blue side to ensure sterility, place it white side up and blue side down, on the area disinfected with PREempt.

3.2 Open sterile packages surgical supplies (Cotton swabs, kimwipes, gauze, and sugi absorbent spears), pouring the items onto the surgical drape to preserve sterility.

3.3 Fill a 10mL or 20mL syringe with ACSF (Artificial Cerebrospinal Fluid), then attach 0.2um syringe filter and 25G 5/8” needle.

3.4 Prepare peri-operative drugs.

Note

Drugs should only be administered in accordance with IACUC and veterinary requirements. Ensure timing, dosage, and route of administration are accounted for.

3.5 Remove autoclaved surgery tools from the sterilization tray and place on the sterile drape, taking care to not touch the instrument tips.

3.6 Obtain titanium headpost with associated well, spray with 70% ethanol and place on drape with the side that will interface with the skull up.

3.7 Prepare additional 0.3ml insulin syringe for vetbond application.

> **Note**
>
> Do not fill syringe until use as it may become clogged.

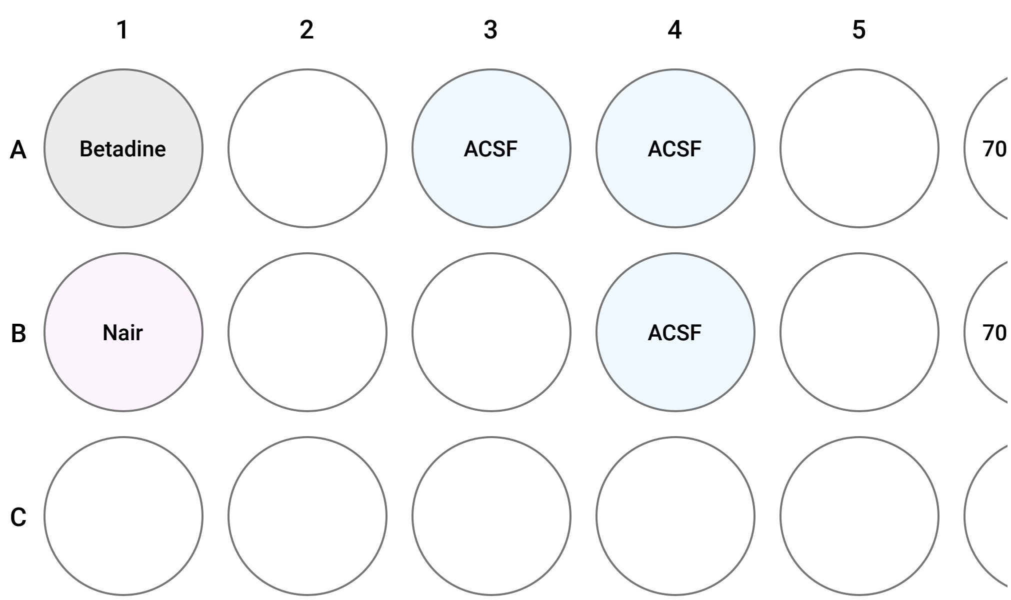
3.8 Obtain #10 Scalpel blade for skin incision.

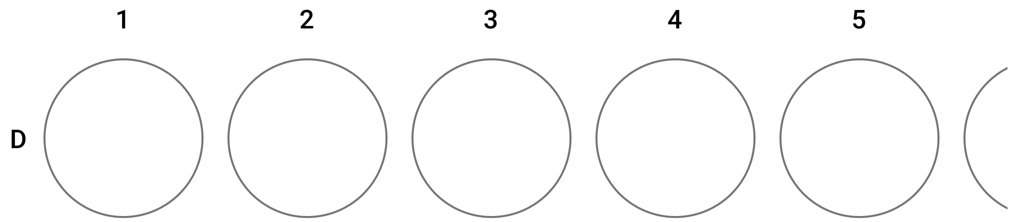
4 Proceed to desired procedure "Specific Setup" section.

Setup Specific to Headpost Only Procedures (no craniotomy)

5

Prepare the 24-well plate with supplies.





5.1 Fill one well with Betadine and one well with Nair.

5.2 Fill three wells with ACSF.

Note

Two wells of ACSF minimum for rinsing 70% Ethanol, 1 well of ACSF for soaking Surgifoam.

5.3 Fill two wells with 70% ethanol.

Note

70% Ethanol can be used for disinfecting any non-sterile supplies or tools used in the surgery typically: Coverslip, Tracers, Stylus, Fiber Implants.

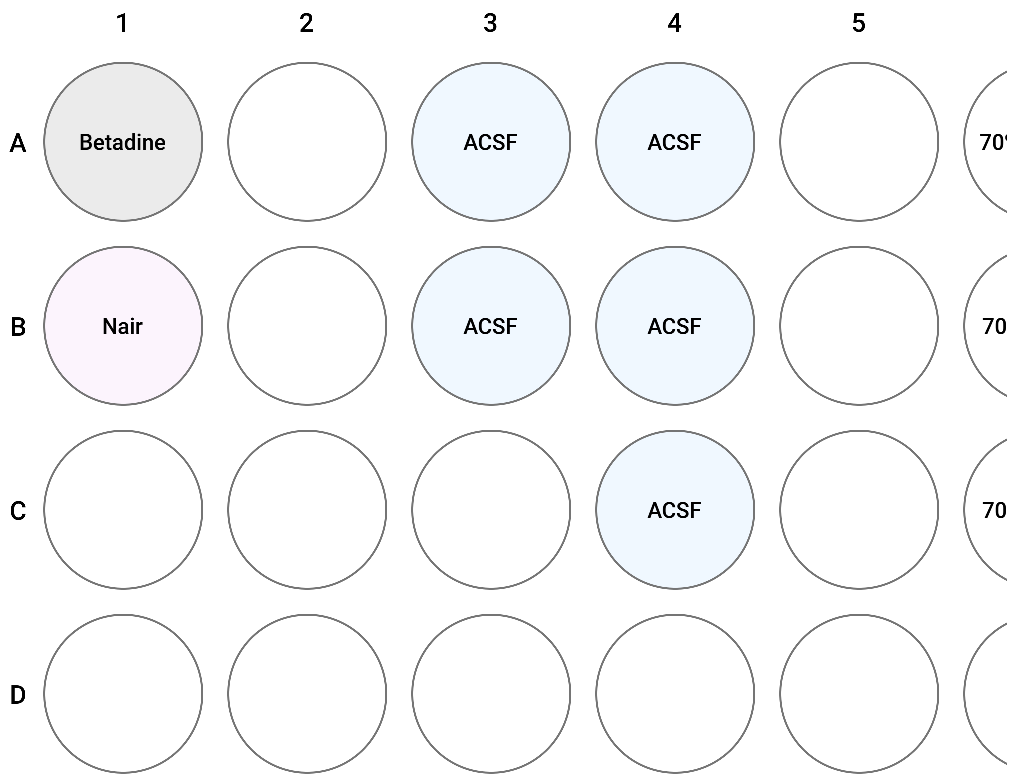
5.4 Soak three cotton swabs in the Betadine well for betadine application to incision site.

5.5 Use sterile forceps to tear off pieces of sterile surgifoam and place in well with ACSF to soak.

6 Place stylus in one of 70% ethanol wells.

Setup Specific to Headpost and Craniotomy Procedures

7 Prepare the 24-well plate:



7.1 Fill one well with Betadine and one well with Nair.

7.2 Fill three to four wells with ACSF.

> **Note**
>
> Two wells of ACSF minimum for rinsing 70% Ethanol, 1 well of ACSF for soaking Surgifoam.

7.3 Fill three wells with 70% ethanol.

Note

70% Ethanol can be used for disinfecting any non-sterile supplies or tools used in the surgery typically: Coverslip, Tracers, Stylus, Fiber Implants.

7.4 Soak three cotton swabs in the Betadine well for betadine application to incision site.

7.5 Use sterile forceps to tear off pieces of sterile surgifoam and place in well with ACSF to soak.

8 For 5mm or 3mm craniotomy procedures: use forceps to place a stacked coverslip in one of the three 70% ethanol wells in the well plate.

Note

Skip this step if the coverslip is coated in silicone.

9 Place the craniotomy tracer in 70% Ethanol well.

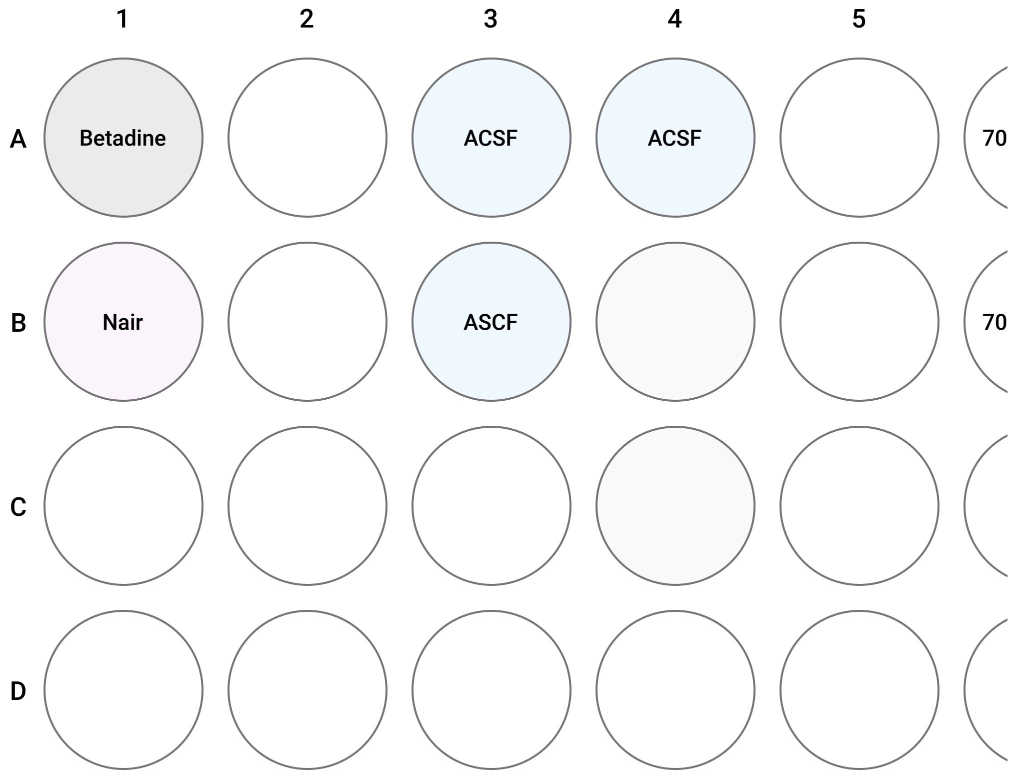
9.1 For 5mm or 3mm craniotomies: place glass coverslip in well.

9.2 For whole hemisphere craniotomies: place the WHC tracer in the well, ensuring the portion that will come in contact with the skull is submerged.

10 Place stylus in one of 70% ethanol wells.

Setup Specific to Iontophoretic Injections (with or without headpost)

11 Prepare 24-well plate:



11.1 Fill one well with Betadine and one well with Nair.

11.2 Fill three wells with ACSF.

Note

Two wells of ACSF minimum for rinsing 70% Ethanol, 1 well of ACSF for soaking Surgifoam.

11.3 Fill 1-2 wells with 70% Ethanol.

Note

70% Ethanol can be used for disinfecting any non-sterile supplies or tools used in the surgery typically: Coverslip, Tracers, Stylus, Fiber Implants.

11.4 Soak three cotton swabs in the Betadine well for application to incision site.

12 Prepare procedure-specific additional supplies:

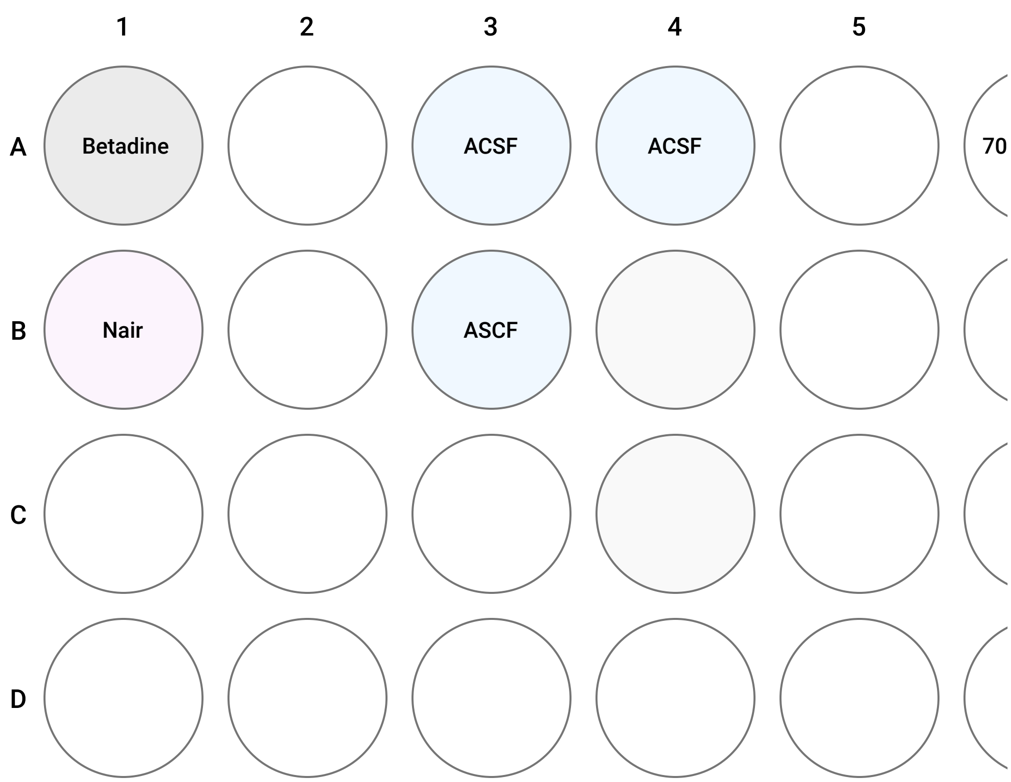
- 5-0 Monofilament suture
- Parafilm square
- Bulldog clamp
- Silver wire

13 Remove one aliquot of virus from -80ºC freezer, thaw at Room temperature , and spin down in the mini centrifuge.

14 Obtain Iontophoretic-specific pipette.

Setup Specific to Nanoject III Injection (with or without headpost)

15 Prepare 24-well plate:



15.1 Fill one well with Betadine and one well with Nair.

15.2 Fill three wells with ACSF.

Note

Two wells of ACSF minimum for rinsing 70% Ethanol, 1 well of ACSF for soaking Surgifoam.

15.3 Fill two wells with 70% ethanol.

Note

70% Ethanol can be used for disinfecting any non-sterile supplies or tools used in the surgery typically: Coverslip, Tracers, Stylus, Fiber Implants.

15.4 Soak three cotton swabs in the Betadine well for application to incision site.

16 Prepare procedure-specific additional supplies:

- 5-0 Monofilament suture
- Parafilm square

17 Remove one aliquot of virus from -80°C freezer, thaw at Room temperature, and spin down in the mini centrifuge.

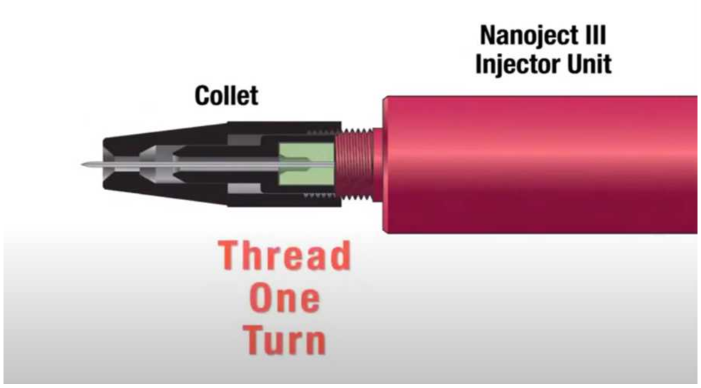
18 Obtain Nanoject-specific pipette.

19 Prepare nanoject-specific pipette.

19.1 Using a 30g, 2” backfilling Hamilton syringe filled with mineral oil, backfill the pipette.

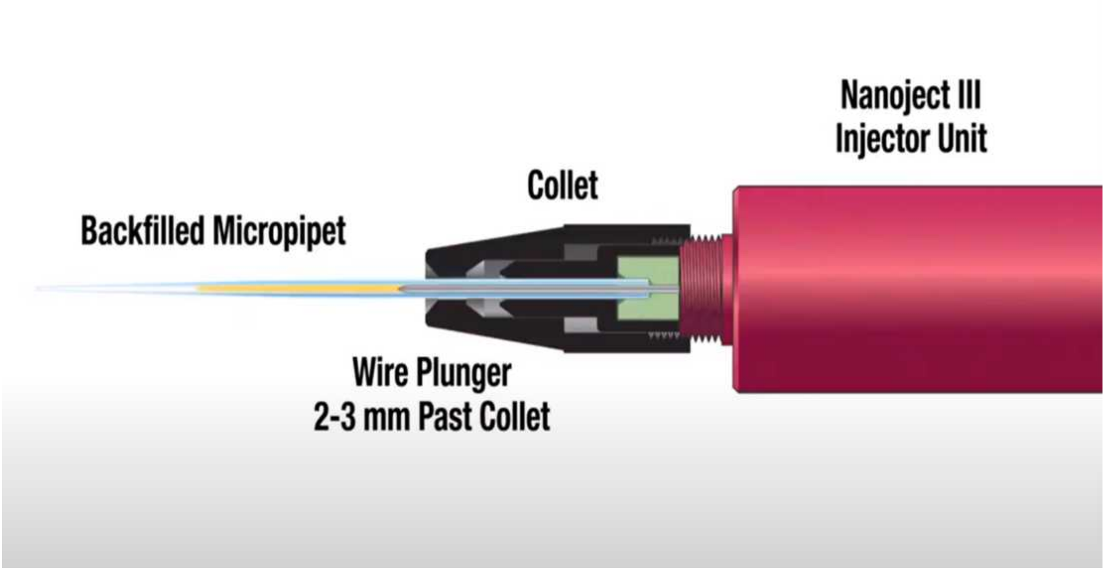
19.2 Insert the tip of the Hamilton syringe into the pulled pipette, all the way to the shoulder, and slowly depress the plunger on the Hamilton syringe, filling the pipette with oil. Be sure not to introduce bubbles into the system or the injection may not be successful.

19.3 Once the pipette is backfilled with oil, loosen the collet on the end of the injector. The wire plunger should be 2-3mm past the collet.



Nanoject III Injection Standard Collet/Chuck/Green Gasket.

19.4 Gently slide the pipette over the wire plunger and push it through the chuck and seating it in the green rubber seal.



Nanoject III with pipette.

19.5 Tighten the collet.

20 Load the virus into the pipette

20.1 Once the pipette is secured to the collet, press, and hold the 'EMPTY' button on the control box until an audible beep is heard. This will drive the wire plunger out forcing oil to the tip of the pipette. Any excess oil will be expelled. Expel about 4-5 drops and work out any air bubbles.

20.2 Take virus aliquot and spin down for 10-15 seconds.

20.3 Use the P20 micropipette with a microfil tip to draw up ~2 µl of virus.

20.4 Using the surgical bed as a platform, aspirate the virus sample onto a clean piece of Parafilm.

20.5 Using the stereotaxic apparatus, lower the tip of the pipette (secured in the Nanoject) into the sample. Be careful to not "bottom out".

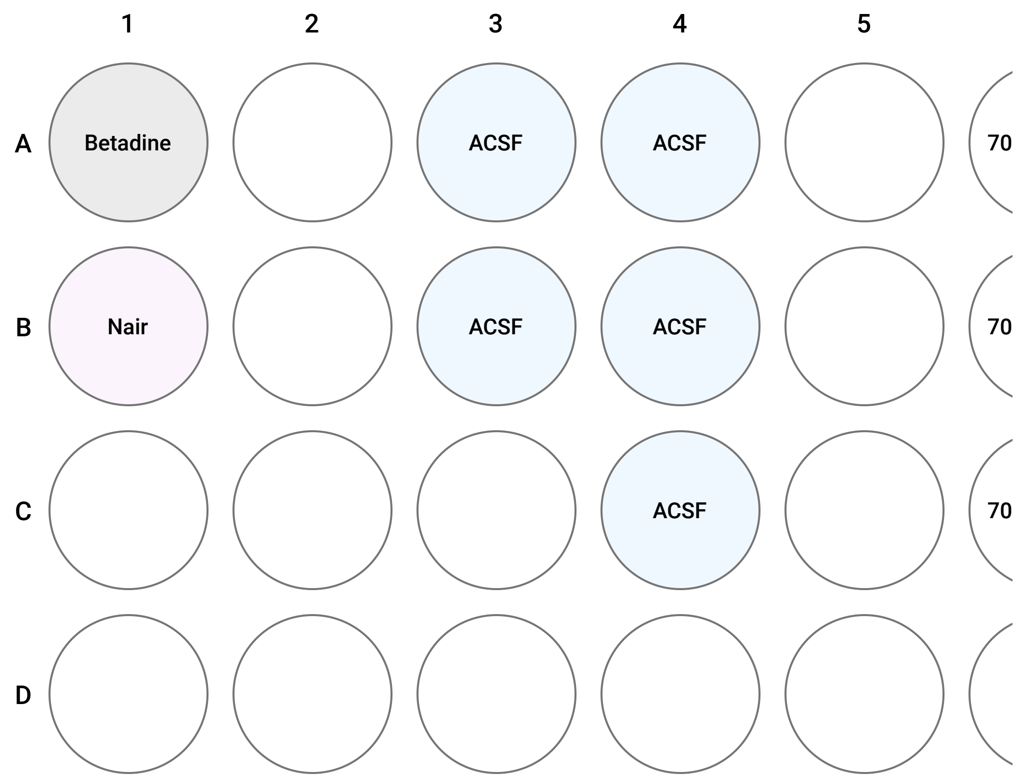
20.6 Press the 'FILL' button and draw up the desired amount of virus (button will change to red). Press the button again to stop the filling of the pipette when finished.

> **Note**
>
> Do not introduce air bubbles into the system as this may result in inaccurate injection volumes. Bubbles will be visible.

21 Carefully set injector aside where pipette will not be disturbed.

Setup Specific to Optic Fiber Implants

22 Prepare 24-well plate:



22.1 Fill one well with Betadine and one well with Nair.

22.2 Fill 3-5 wells with ACSF.

Note

Two wells of ACSF minimum for rinsing 70% Ethanol, 1 well of ACSF for soaking Surgifoam.

22.3 Fill three wells with 70% ethanol.

Note

70% Ethanol can be used for disinfecting any non-sterile supplies or tools used in the surgery typically: Coverslip, Tracers, Stylus, Fiber Implants.

22.4 Soak three cotton swabs in the Betadine well for application to incision site.

22.5 Use sterile forceps to tear off pieces of sterile surgifoam and place in well with ACSF to soak.

23 Gather fiber optic implants and soak them in the 70% ethanol wells.

24 If using a headframe that is not already sterilized, soak headframe in 70% ethanol.

Prepare the Anesthesia System and Anesthetize the Mouse (all procedures)

25 Prepare the anesthesia system.

25.1 Follow anesthesia system's manufacturer guidelines and your facilities environmental health and safety committee regarding the use of an anesthesia system for rodent neurosurgery.

25.2 Turn on the oxygenator and ensure your facilities vacuum lines are functioning properly (i.e vacuum lines are on and gauge displays psi).

25.3 Ensure that all tubes are connected securely and that you can feel the vacuum suction in the scoop underneath the nose cone.

25.4 Ensure the Isoflurane line to the induction chamber is open and the line to the nose cone is closed via their designated stopcocks.

26 Anesthetize the mouse.

26.1 Remove the animal from its experimental cage, obtain a preoperative weight.

27 Place the mouse into the induction chamber, open the vacuum valve (stopcock) and isoflurane line (stopcock) for the chamber, and then turn on the isoflurane vaporizer to 5%.

27.1 Once the mouse is fully unconscious, turn off the isoflurane and wait at least 10 seconds with the vacuum on to allow the chamber to clear before removing the animal.

27.2 Position mouse on surgical rig by placing maxillary incisors in the hole on the bite bar and securing head with ear bars.

27.3 Secure the nose cone over the mouse's snout. Make sure the body of the mouse is on top of the heading pad, resting comfortably. Redirect the gas flow from the induction chamber to the surgical rig via line stopcocks.

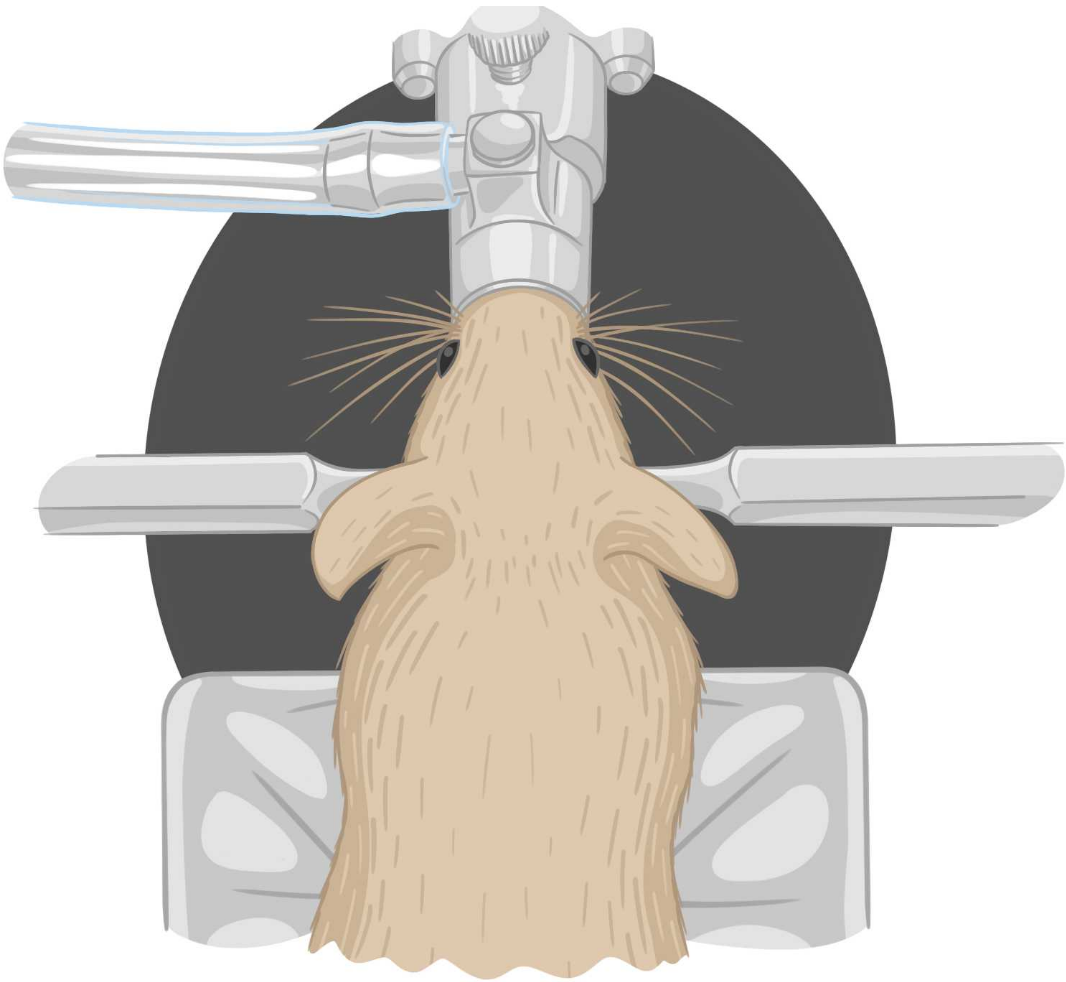


Illustration of mouse fixed to sterotax via ear bars and bite bar.

27.4 Set the isoflurane to ~1.5-2%, turn off the vacuum line to the induction chamber and close the lid.

27.5 Monitor the mouse's breathing throughout the process and adjust gas levels as necessary.

Prepare the Mouse for Surgery (all procedures)

28 Apply Systane to the end of a cotton swab and use to push the whiskers away from the surgical field.

29 Cover the mouse's eyes with a generous amount of Systane. Additional Systane should be added as needed to prevent eye dryness and protection from the scope light.

30 Administer any drugs that have a timing after induction or prior to incision.

31 Use black handled scissors to shorten fur from top of the head. Use caution when trimming hair around the eyes. Avoid cutting off whiskers.

Note

Hair removal is contingent on the type of surgery and placement of headframe, optic fibers, etc.

32 Apply Nair with the pointed end of a non-sterile cotton swab, gently swirling it down to the interface of the skin and hair.

33 Remove all Nair with several alcohol swabs.

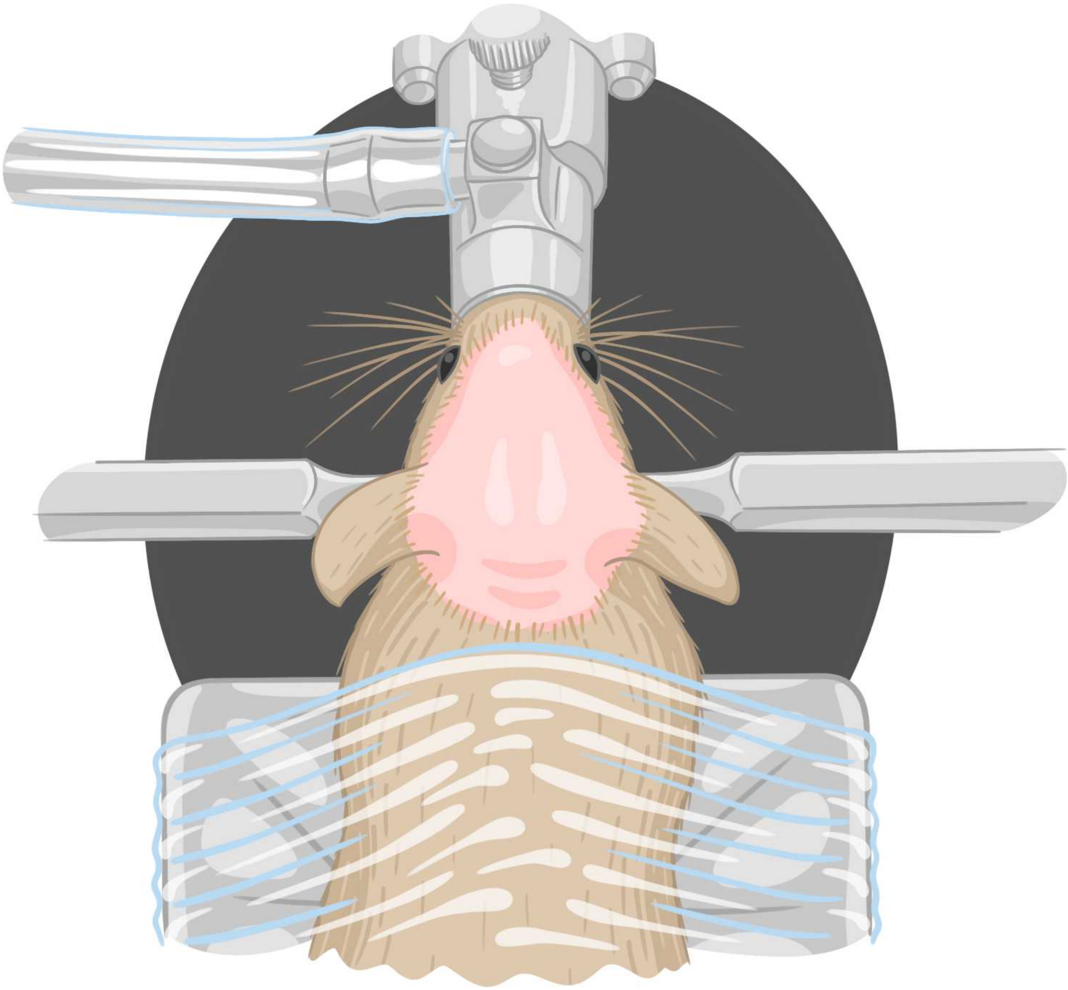


Illustration of mouse with the fur on top of head removed.
Note: Systane not illustrated.

34 Disinfect the surgical site with 3 rounds of alternating Betadine-soaked sterile swabs and alcohol wipes. The last application of Betadine should not be wiped off.

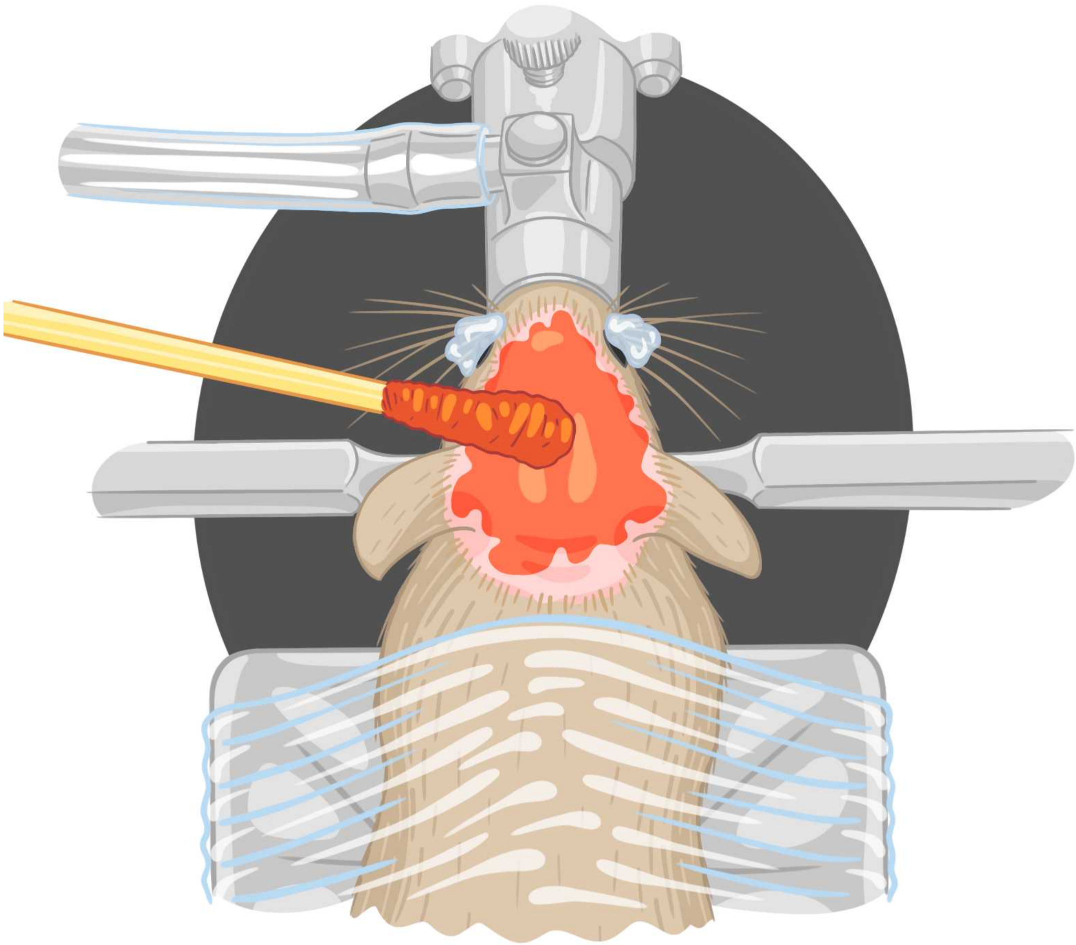


Illustration of Betadine application on mouse head.

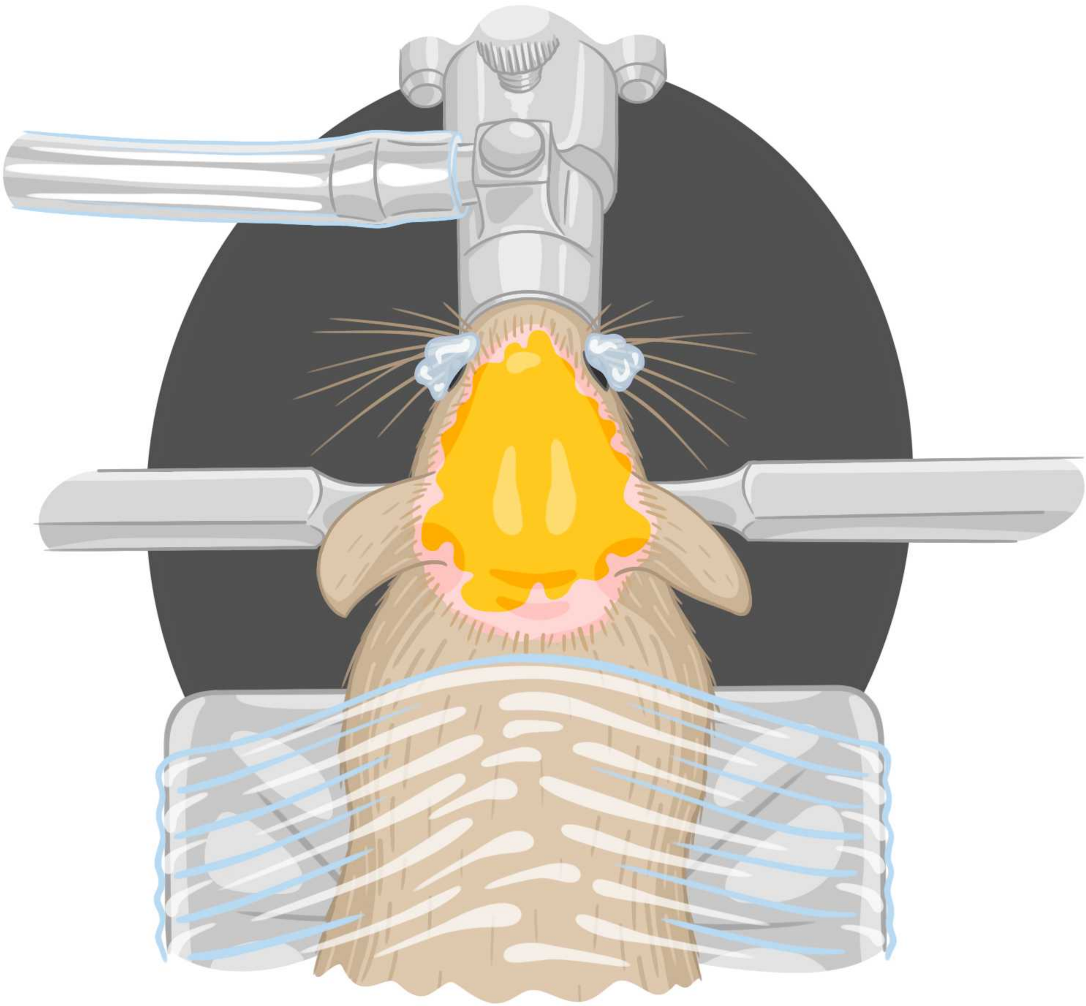


Illustration of dried Betadine on mouse head.

35 Place Saran Wrap over the trunk of the animal and change into new gloves.

Take Down Steps When the Procedure is Complete

36 Dispose of used syringes, blades, drill bits, swabs and needles or anything that could puncture a plastic bag in the biohazard sharps container.

37 Dispose of all disposable materials that came into contact with blood in the biohazard waste container.

38 Spray down surgical tools with pH neutral surgical tool cleaner and wipe with a kimwipe or alcohol swab. Be sure to wipe any blood, skin, or cement residue off the tools. Use caution when wiping down Dumont's.

39 Place tools into tool kit. Place tool kit into sterilization pouch and write your name, department initials and the date on the pack. Bring tool kit to the designated location to be autoclaved.

39.1 If the tools are to be used again the same day, sterilize using the hot bead dry sterilizer.

40 Disinfect ear bars with alcohol swab, then place back on the surgical rig.

41 Turn off the vacuum and oxygen sources by switching the stopcocks to the off position.

42 Release the air from the drill lines by pressing down on the pedals until the pressure reads 0 psi. If the air source is connected to a wall valve, then turn the valve to the off position.

43 Turn off heating pad, bead sterilizer, stereotax, and scope light.

44 Turn off compressed air, vacuum, and oxygen concentrator.

45 Ensure Isoflurane is turned off.

46 Spray down station with 70% cleaning up any debris and detritus from the surgery paying close attention to the nose cone, ear bars, vacuum scoop, and Induction chamber.