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Stereotactic Injections with Headframe Implant

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Protocol status: Working

We use this protocol and it's working

Created: August 08, 2023

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Protocol Integer ID: 86171

Keywords: Headframe, Head post, Rodent Neurosurgery, Stereotactic injections

Abstract

This protocol describes the surgical procedure, instrumentation, and reagents necessary for performing stereotactic injections and securing a headframe to an adult mouse brain for in-vivo recording procedures.

Image Attribution

Gabriel Rodriguez, Allen Institute.

Guidelines

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

Materials

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**

⊗ Ethiq X[®] 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

A	B	C
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
45° or 90° Durotomy probe	Fine Science Tools	10066-15
Plastic sterilization container	Fine Science Tools	20810-02
Bulldog clamp	Fine Science Tools	18053-28
PREempt Disinfectant spray	McKesson Corporation	21101



A	B	C
70% Ethanol (Diluted in-house)	Sigma Aldrich	459836
Alcohol wipes	Becton, Dickinson and Company	326895
Sterile Surgical Drape, 18×26	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, 3×3" 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.besjjech
C Universal 4-META Catalyst, 0.7 ml	Parkell	S371

A	B	C
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
XLite LED Curing light	Independent Dental	Flight Xlite2-CUR
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 Manufacturing 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-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
Sterilization pouches	Advantor	89140-804

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
Electrode Holder	Kopf	1970
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
Titanium 42 Headpost *	0160-100-42
Titanium Al Straight Bar *	1365-6428-001
Titanium Headpost *	0160-100-10
Titanium LC / Brainstem Headpost *	0160-100-52
Dual Hemisphere Headframe *	0160-100-57
Bregma Stylus	0251-900-04
Lambda Stylus	0111-300-01
Dovetail Clamp	0111-200-00
Ear bar Headframe Clamp	0155-100-00

All equipment can be substituted with their equivalent.

* = Optional

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

Reference protocol below for all general setup and takedown procedures for rodent neurosurgery:

Protocol



NAME

General Setup and Takedown Procedures for Rodent Neurosurgery

CREATED BY

Ali Williford

PREVIEW

Reference protocol(s) below if performing injections:

Stereotaxic Surgery for Delivery of Tracers by Iontophoresis V.6:

dx.doi.org/10.17504/protocols.io.14egn8ewzg5d/v6

Stereotaxic Injection by Nanoject Protocol V.6: dx.doi.org/10.17504/protocols.io.bp2l6nr7kgqe/v6

Expose and Prepare the Skull Surface

10m

- 1 After hair removal and disinfection, create a midline incision with a scalpel blade from approximately behind the eyes to the front of the ears.

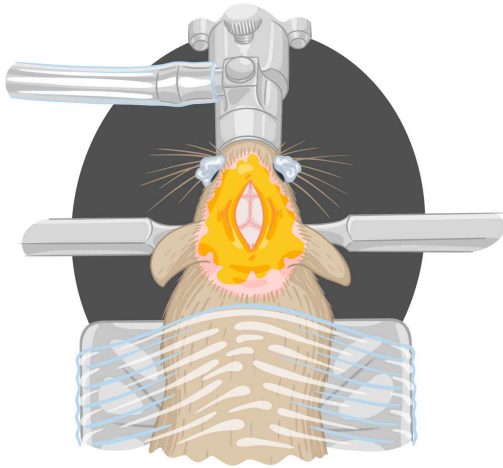


Illustration of initial skin incision on mouse head.

Mouse fixed to stereotaxic instrument base via bite bar and ear bars. Fur from top of head has been removed and skin has been sterilized (see General Setup and Takedown Procedures).

- 2 Using Vanna scissors, cut a teardrop shape of skin away, ensuring enough skull is exposed for headframe placement.

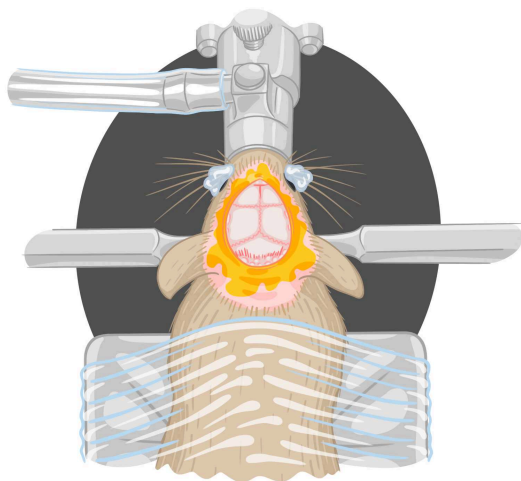



Illustration of skin removal on mouse head.

- 2.1 On the anterior side expose the rostral rhinal vein between the eyes.
- 2.2 On the left and right lateral sides stop at the interface between the skull and cheek muscle.
- 2.3 Posteriorly expose about ± 1 mm - ± 2 mm of neck muscle.
- 3 Remove exposed periosteum by rubbing it apart with cotton swabs and bunching it near the edges of the skin. Then cut away with vanna scissors at the skin edge. Use ACSF to rehydrate if necessary (i.e. periosteum dries out).

Note

Utilize a 10mL or 20mL syringe, 25G 5/8-inch needle, and a syringe filter to store and dispense ACSF.
- 4 Detach visible neck muscles using dumont forceps.
- 5 Score entire skull with scalpel blade to provide greater surface area for bonding agents. Do not obstruct any landmarks. 
- 6 Seal all along incision site with Vetbond. Use a Sugi Absorption Spear to absorb any excess fluid either prior or during. Extend vetbond seal 1-2mm past incision site along undamaged skin.

Note

Extending the vetbond seal over healthy tissue delays the formation of exudate. Additionally, unsealed soft tissue will weep fluid that can compromise the attachment of the headframe.

Align The Skull

5m

- 7 Locate Bregma and Lambda landmarks with Dovetail Clamp and Bregma Stylus, and use them to level the skull in the anterior-posterior axis within 0.1mm.

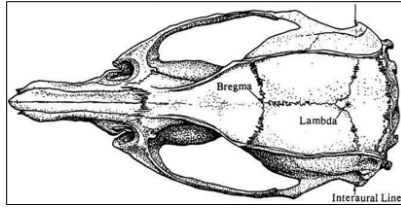


Illustration of Bregma and Lambda landmarks.

- 7.1 If Lambda-Bregma offset is greater than 0.1mm in X, use the yaw adjustment on the stereotaxic alignment system to adjust the yaw within 0.1mm.
- 8 At midline, approximately midway between Bregma and Lambda, measure 2mm laterally on both the left and right hemisphere, ensuring that the skull is level in the medial-lateral axis within 0.15mm.

Perform Stereotactic Injections

10m

- 9 Skip to section "Headframe Placement" if not performing virus injections.
- 10 Reference protocol **Stereotaxic Injection by Nanoject Protocol V.6** for performing stereotactic injections via nanoject:
dx.doi.org/10.17504/protocols.io.bp2l6nr7kgqe/v6

Begin at step **8.2.5 "Mark the Injection Site"**
Stop at step **8.5 "Suturing"**
- 11 Reference protocol **Stereotaxic Surgery for Delivery of Tracers by Iontophoresis V.6** for performing stereotactic injections via Iontophoresis:
dx.doi.org/10.17504/protocols.io.14egn8ewzg5d/v6

Begin at step **8.2.6 "Mark the Injection Site"**
Stop at step **8.5 "Suturing"**
- 12 If target coordinates require an angled injection, rotate mouse before proceeding with the injection. Return to level once injection has been completed and pipette has been removed.
- 13 Fill burr holes with bone wax or Kwick-Cast.

Note

Ensure wax or Kwick-Cast is only within burr hole. Residue from wax or silicone on skull can compromise Metabond seal.

Headframe Placement

20m

14 Seal entire skull with Vetbond.

15 If desired, mark coordinates on skull with Sharpie.

*

Note

It is often useful to mark coordinates indicating either Bregma, Lambda or future location of burr hole craniotomy.

16 Attach Dovetail Clamp to stereotaxic arm and attach stylus. Center stylus over headframe fiducial point and then raise stylus slightly.

Note

Depending on the headframe or head bar desired, the fiducial point may vary. For example the Titanium LC/Brainstem headframe utilizes the Bregma stylus with Bregma as a fiducial point.

17 Replace the stylus with the headframe and lower until there is contact with the skull.

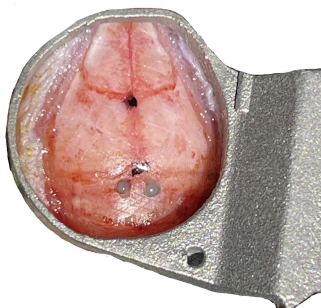




Image of DHC headframe lowered onto skull.

- 18 Apply white Metabond around the outside of the headframe. Metabond should wick under and into the headframe. If it becomes too viscous, remake it. Place Metabond so the majority of the skull within the well remains exposed. Let dry completely (around

 00:05:00).

10m

- 19 Use clear Metabond to seal the rest of the skull within the inside well. Allow all metabond to completely dry (around  00:05:00).

5m

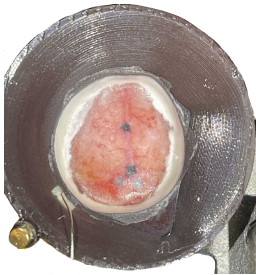



Image of completed surgery with well.

- 20 Remove mouse from stereotaxic instrument base once metabond is fully dry.
- 20.1 Detach Dovetail Clamp from headframe carefully.
- 20.2 Remove earbars.
- 20.3 Remove mouse teeth from bitebar by lightly scruffing mouse in a way that raises the teeth out of the bite bar.

Recover mouse and takedown

- 21 Obtain the mouse's postoperative weight.
- 22 Place the mouse back in a recovery cage and put the cage on the  37 °C heat plate.



- 23 Reference General Setup and Takedown Procedures for Rodent Neurosurgery protocol for takedown procedure.