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Dual Hemisphere Craniotomy for Electrophysiology

DOI

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes the surgical procedure, instrumentation, and reagents necessary for the installation of a stabilizing headframe and cranial window over the cortex of an adult mouse. This protocol should be utilized for neural imaging and experimentation regarding the mouse cortex.

This procedure accommodates two variations of stereotactic injections (Optional 1 and Optional 2). Specifically, this protocol outlines the steps for injections before and after the craniotomy and durotomy. The timing of injections during cranial windowing surgeries depends on the coordinate location. Injections outside of the cranial window should be performed prior to the craniotomy and durotomy to allow for adequate burr hole sealing. Injections within the cranial window should be performed after the craniotomy and durotomy to minimize cortical damage.

The surgical procedure described in this protocol is an extension of the Whole Hemisphere Craniotomy for Electrophysiology. This surgical procedure should be used in cases where access to both hemispheres of the brain is necessary during experiments. If access to a single hemisphere is sufficient, the Whole Hemisphere Craniotomy should be used instead, as it is a less complex surgical procedure.

Image Attribution

Gabriel Rodriguez, Allen Institute.

Guidelines

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

This protocol has been written for review purposes only and should not be utilized for training. Users of this protocol should be familiar with common vocabulary and techniques for rodent neurosurgery.

Materials

Anesthesia and other drugs

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

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

⊗ 1 g Dexamethasone **biorbyt Catalog #orb134330**

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

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

⊗ 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° Vannas 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
	large iris forceps	Fisher Scientific	13-820-073
	PREempt Disinfectant spray	McKesson Corporation	21101
	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	Avantor	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)	Avantor	89133-810
	Sugi Absorption Spears	Fine Science Tools	18105-01
	Insulin syringes, U-100, 0.3 ml, 31G	Avantor	BD328438
	Insulin syringes, U-100, 1 ml, 31G	Avantor	BD328418
	Luer-Lock Syringe, 20 ml OR	Avantor	53548-025
	Luer-Lock Syringe, 10ml	Avantor	75846-756
	25G 5/8-inch needle	Avantor	89134-134
	32 mm Syringe Filter 0.2 µm Supor Membrane	Avantor	75846-756
	Press 'n' Seal	Medline	CLO70441



Tool / Supply	Manufacturer / Supplier	Part Number
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	Avantor	21909-378
Sterile Scalpel blades, #11	Avantor	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.besjjeen
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
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 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-Sil Sealant	World Precision Instruments	KWIK-SIL



	Tool / Supply	Manufacturer / Supplier	Part Number
	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	Avantor	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	Avantor	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 Stereotactic Instrument	Kopf	1900
	Adjustable Stage Platform	Kopf	901
	Stereo Microscope	Leica	M80
	Gooseneck Illumination	AM Scope	LED-6WA
	On-axis Illumination	Lecia	KL2500 LED



	Equipment	Manufacturer / Supplier	Part Number
	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
Well Cap	0160-055-09
Bregma Stylus	0251-900-04
Dovetail Clamp	0111-200-00
0° Ear bar Headframe Clamp	0155-110-00
Prober Holder	0155-200-00
Bilateral SHIELD Implant	0251-170-01
SHIELD Bilateral Implant Tracer	0274-122-00 or equivalent
Bilateral SHIELD Headpost and Well	0160-075-00

All equipment can be substituted with their equivalent.

<https://portal.brain-map.org/explore/toolkit/hardware>

Personal Protective Procedures (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.

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

Avalon Amaya

Preview

Optional 1:

For stereotactic injections prior to performing the craniotomy and durotomy, follow section titled "Optional 1" in addition to the standard sections.

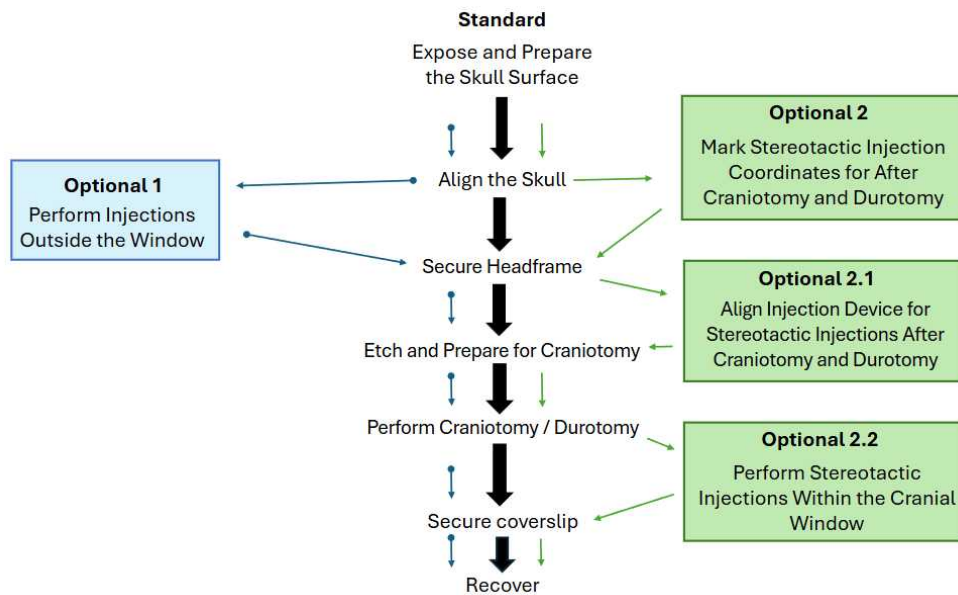
Optional 2, 2.1, 2.2:

For stereotactic injections after performing the craniotomy and durotomy, follow sections titled "Optional 2", "Optional 2.1" and "Optional 2.2" in addition to the standard sections.

Headpost and Headframe will be used interchangeably throughout this protocol.

Graphical Overview of Procedure

1



Map diagram of protocol workflow.

Expose and Prepare Skull Surface

20m

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

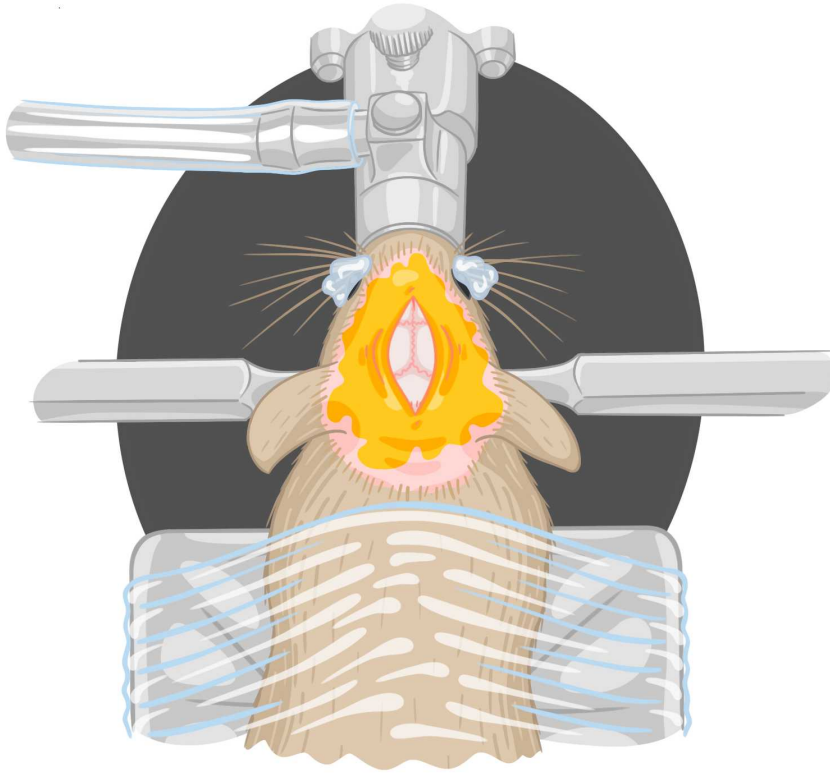


Illustration of initial skin incision on mouse head.

Mouse fixed to stereotactic 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).

- 3 Using Vannas scissors, cut a round teardrop shape of skin away.

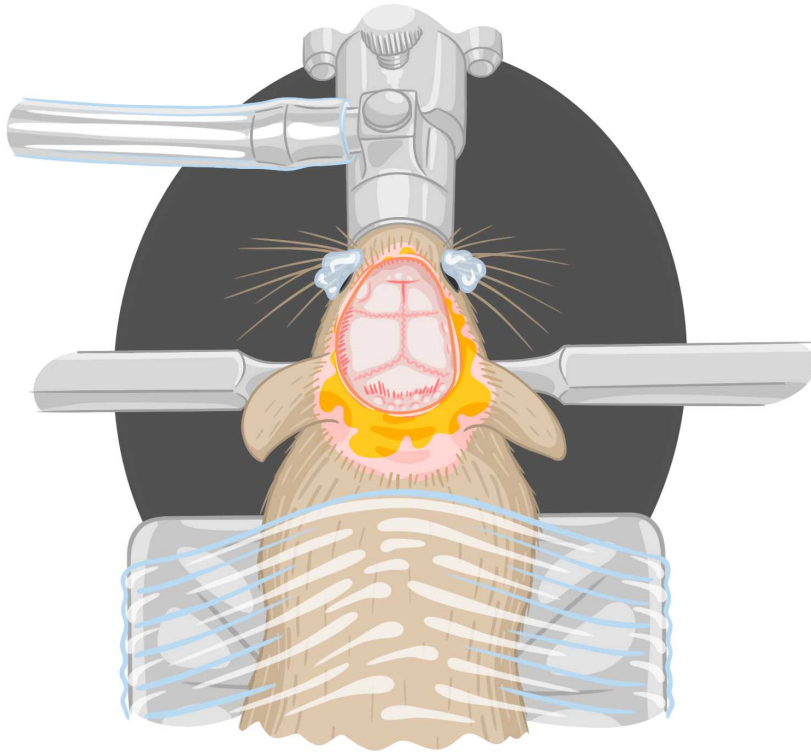


Illustration of skin removal on mouse head.

3.1 On the left and right side remove enough skin to see the interface of the skull and cheek muscle. On the posterior side stop as soon as the neck muscle is exposed. Anteriorly, stop at around 1mm past the rostral rhinal vein.

4 Tear or cut the periosteum and ensure removal at surgical site. Rehydrate with Artificial Cerebrospinal Fluid (ACSF) if needed to finish removal.

Note

Utilize a 10mL or 20mL syringe, 25G 5/8-inch needle, and a syringe filter to store and dispense ACSF.

5 Detach muscles to allow more surface area for headframe securing.

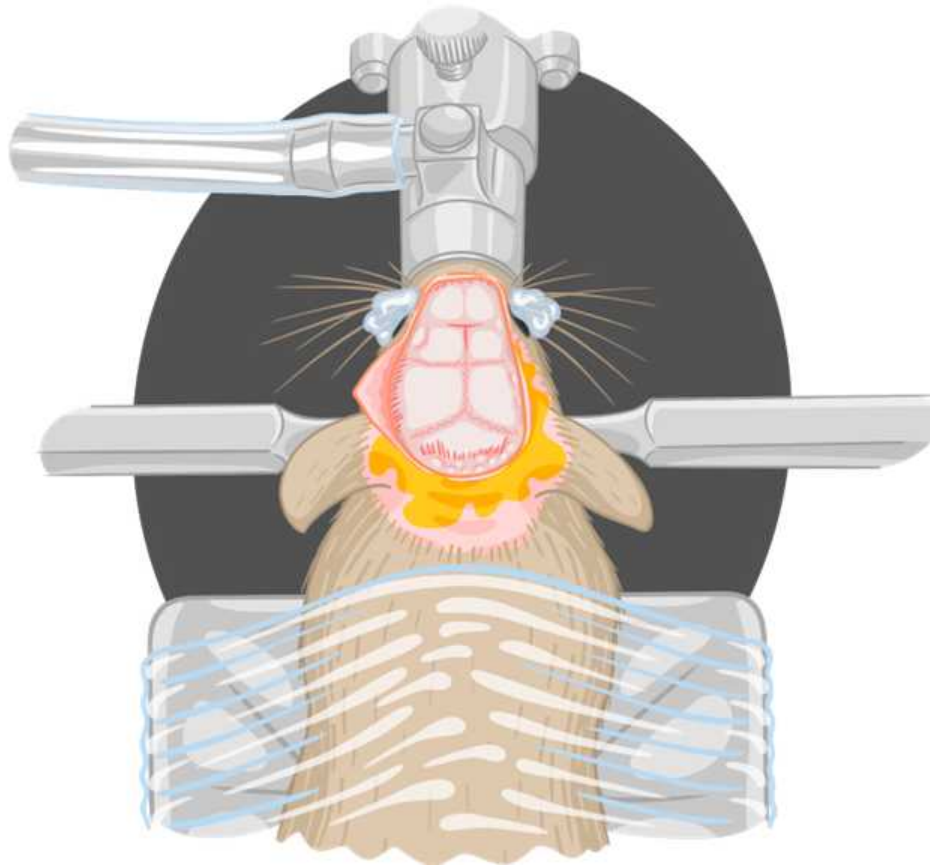


Illustration of skin removal boundaries and muscle removal extent.

- 5.1 Detach the cheek muscle on the left side of the skull by poking into the interface between the muscle and skull, and then drag the Dumont forceps along the length of the cheek. Detach the neck muscles with Dumont forceps by scraping the muscle attachments.
- 5.2 On the left side of the skull, pull separated muscle and skin away from the skull as shown in the illustration. At the posterior edge, tuck the detached neck muscles underneath the skin.
- 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 healthy skin.

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.

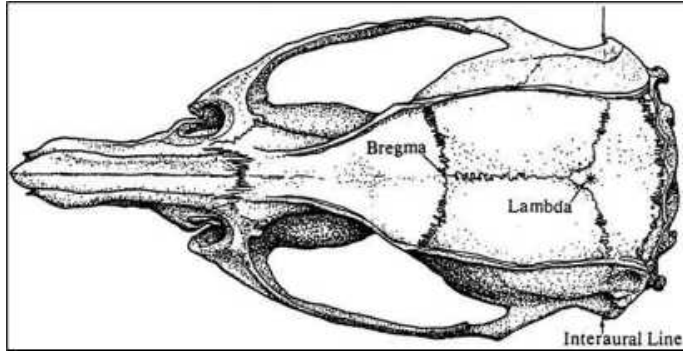


Illustration of Bregma and Lambda landmarks.

- 7.1 If Lambda-Bregma offset is greater than 0.1mm in X, use the yaw adjustment on stereotactic alignment system to adjust the yaw to 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.

Optional (1): Perform Stereotactic Injections Outside of the Cranial Window

10m

9 **Optional 1**

This optional section accommodates injections prior to performing the craniotomy and durotomy. Typically, this is done for injections that will be outside the craniotomy window, as placement of the headframe can obstruct some coordinates. Injection coordinates within the craniotomy window are performed after the craniotomy and durotomy as it will minimize cortical damage.

Skip this section if you will not be performing injections prior to the craniotomy and durotomy.

- 10 Reference protocol **Stereotaxic Injection by Nanoject Protocol V.6** for performing stereotactic injections via nanoject.

**Protocol**

NAME

Stereotaxic Injection by Nanoject Protocol

CREATED BY

Allen Institute

[Preview](#)

Begin at step **8.2.6 "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.

Protocol

NAME

Stereotaxic Injection by Iontophoresis

CREATED BY

Allen Institute

[Preview](#)

Begin at step **8.2.6 "Mark the Injection Site"**.

Stop at step **8.5 "Suturing"**.

- 12 Once completed with the injection, fill burr hole with bone wax with the broken end of a cotton swab.

Note

Do not leave excess wax residue on the skull. Excess wax can contribute to an insufficient seal between the skull and the headframe.

Optional (2): Mark Stereotactic Injection Coordinates for After Craniotomy and Durotomy

7m

- 13 **Optional 2**



This optional section accommodates injections that will be performed after the craniotomy and durotomy. This is the first section out of three required. This first section is specifically for identifying and marking injection coordinates while the skull is level. These markings will be referenced in the second optional section (Optional 2.1).

Skip this section if you will not be marking injection coordinates for injections after the craniotomy and durotomy.

14 **Considerations:**

For injections within the cranial window, it is important to take the injection depth into consideration. Avoid targeting subcortical brain structures, as they cause more brain edema due to the required insertion depth via pipette.

Additionally, ensure you maintain bregma as the fiducial point. Avoid the use of multiple viruses/pipettes as pipettes are not made identically (variance of up to 0.8mm difference).

- 15 Reference protocol **Stereotaxic Injection by Nanoject Protocol V.6** for performing stereotactic injections via nanoject.

Protocol

NAME

Stereotaxic Injection by Nanoject Protocol

CREATED BY

Allen Institute

Preview

Begin at step **8.2.6 "Mark the Injection Site"**.
Stop before step **8.2.7 "Drilling the Burr Hole"**.

- 16 Reference protocol **Stereotaxic Surgery by Iontophoresis V.6** for performing stereotactic injections via iontophoresis.



Protocol

NAME

Stereotaxic Injection by Iontophoresis

CREATED BY

Allen Institute

Preview

Begin at step **8.2.6 "Mark the Injection Site"**.

Stop at step **8.5 "Suturing"**.

- 17 Ensure injection coordinate is marked as precisely as possible. This is best done with a fine point sharpie mark with a notch or point negative made from the marker pipette tip.

Etch Craniotomy and Secure Headframe

20m

- 18 Ensure skull is level.
- 18.1 If you have performed an angled injection, return mouse back to level state.
- 19 Using the dovetail clamp, zero Bregma stylus over Bregma, and replace with the SHIELD Bilateral Implant Tracer.
- 20 Using the SHIELD Bilateral Implant Tracer as a guide, etch the shape of the craniotomy onto the skull using 45° forceps or #11 scalpel blade.

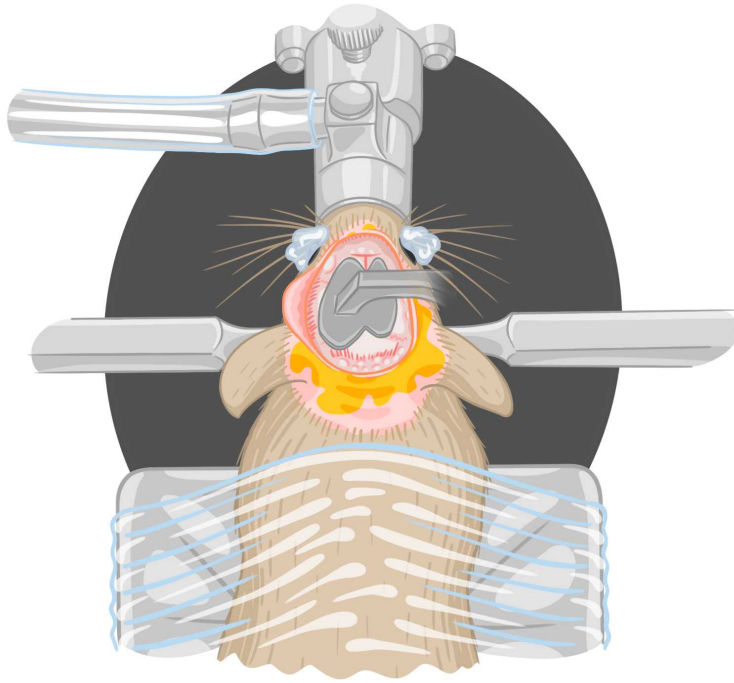


Illustration of SHIELD Bilateral Implant Tracer centered over Bregma.

Note

CAD image for craniotomy shape is available on brain-map.org.

- 21 Remove the dovetail clamp from the stereotaxic arm with the SHIELD Bilateral Implant Tracer still connected. **Do not move the X or Y axis.**
- 22 Using the FG1/4 or EF4 drill bit, drill a shallow trench over the etch.

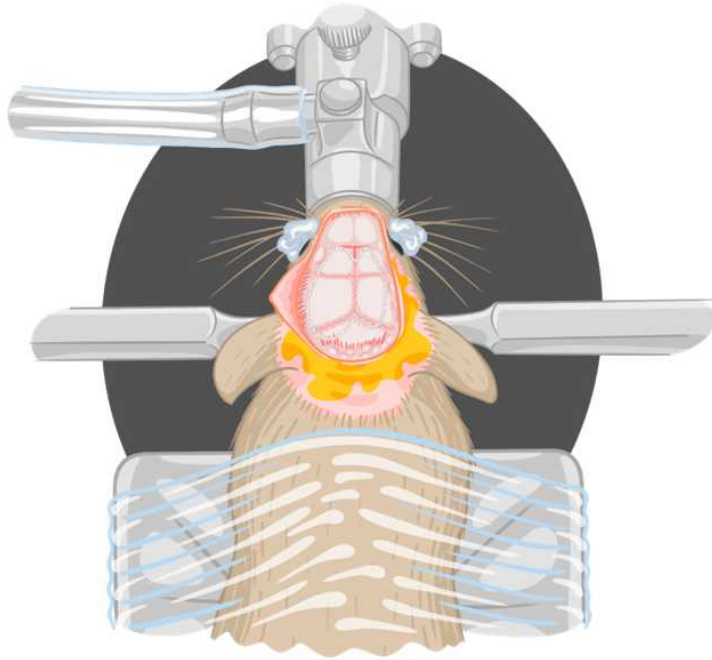


Illustration of dual hemisphere craniotomy etched into skull.

- 23 Return the dovetail clamp with connected tracer back onto stereotax arm. Confirm size and shape of craniotomy. Repeat steps [⇒ go to step #20 - Step #21](#) until the craniotomy etch is accurate.



Note

It is crucial that the craniotomy trace is accurate. The craniotomy shape is specifically designed to avoid disruptions to the sinus confluences.

Secure the Headframe

20m

- 24 With the dovetail clamp still centered over Bregma, raise the Z axis and then replace the SHIELD Bilateral Implant Tracer with the Bilateral SHIELD Headpost.
- 25 Lower the Z axis until the headframe comes in contact with the skull.

Note

The contact point for this headframe is between the eyes. However, the fixed skin by the eyes will likely prevent the headframe from making direct contact with the skull. This is acceptable, but the headframe should be placed as close to the skull as possible without compressing the fixed skin into the eye or disrupting the level plane of the skull.

Note

Lowering the headframe too far down onto the mouse's skull can potentially crack a cranial suture line. If this occurs, your headframe will not attach to the skull due to cerebrospinal fluid leakage.

26 Prepare and apply the first round of Metabond.

26.1 Apply Metabond inside the headpost and around the outside where it's accessible. Place Metabond so that the majority of the skull within the well remains exposed. Let dry ~

 00:05:00 .

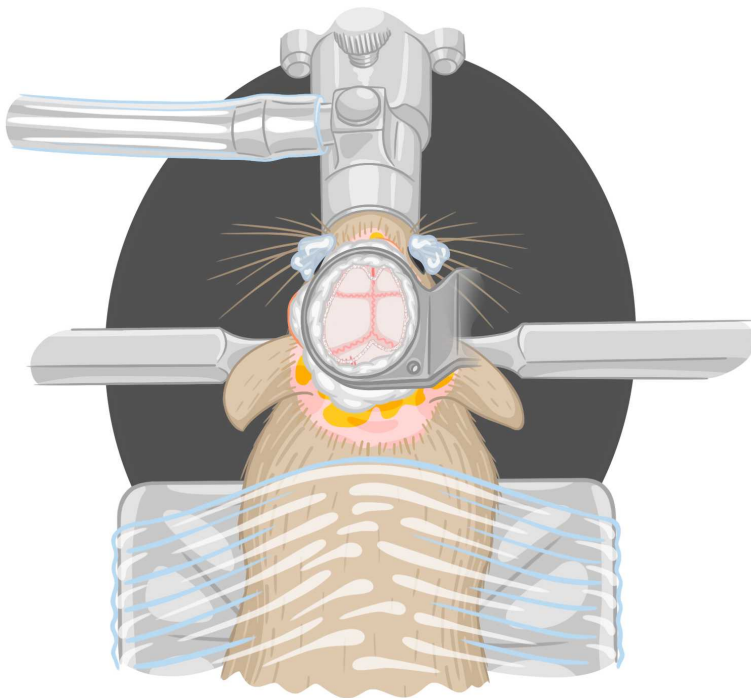


Illustration of Metabond placement for Bilateral SHIELD Headpost.

- 27 Turn off the isoflurane, remove the ear bars, and loosen the nose cone slightly.
- 28 Carefully detach the headframe from the dovetail clamp.
- 29 Prepare another Metabond tray.
- 30 Pull back nose cone completely and remove the mouse from bite bar. Either lay the mouse on the heating pad or hold mouse in hand.
- 31 Apply Metabond to the outside of the headpost to cover all exposed muscle. Extend Metabond to 1-2mm past the exposed muscle and tissue to create a seal.

Note

Be as efficient and quick as possible to ensure mouse stays anesthetized during this step.

Note

Clean up any Metabond close to the eyes and smooth over any sharp points to avoid irritating the mouse.

- 32 Using the 0° earbar headframe clamp, return mouse back onto the stereotax. Ensure headframe is secured in place by ear bar clamp.

Note

Be as efficient and quick as possible to ensure mouse stays anesthetized during this step.

- 32.1 Place headframe plate into the clamp, nose cone to nose, and turn isoflurane on.



32.2 Replace saran wrap over mouse body.

Optional (2.1): Align Injection Device for Stereotactic Injections After Craniotomy and Durotomy

7m

33 Optional 2.1

This optional section accommodates injections that will be performed after the craniotomy and durotomy. This is the second section out of three required. This second section is specifically for aligning the injection device with the desired injection coordinates that were marked in Optional 2. Since the craniotomy and/or durotomy can obscure the coordinate fiducials we need to align the injection device to the precise X and Y (ML and AP) coordinates. After the craniotomy in Optional 2.2, the Z axis (DV) will be manipulated to perform the injections. If there are multiple coordinates, identify one of them and zero the coordinates creating that point as a fiducial to the remaining coordinates.

Note

Oftentimes Bregma may still be visible after drilling the craniotomy; however, it is still recommended to identify the coordinates prior.

Skip this section if you will not be marking injection coordinates for injections after the craniotomy and durotomy.

34 Ensure skull is level.

If headframe has been secured, set the roll and pitch of the mouse to zero degrees.

35 Attach injection device with loaded pipette onto stereotactic arm and center over injection location. This stereotactic coordinate should be pre-marked from Step 17

⇒ [go to step #17](#)

35.1 Place the injection device onto the stereotax arm.

35.2 Zero the X and Y and coordinates over marked injection coordinate.

36 Remove injection device from stereotax arm.



- 37 **DO NOT** move the stereotax arm out of the way. It will stay in this position during the craniotomy and durotomy.

Note

Nanject/Iontophoresis pipettes should not be changed after setting up the stereotax arm. The variation in pipette straightness can cause the injection location to move up to 0.8mm.

Perform Craniotomy

35m

- 38 Using drill and FG1/4 drill bit, drill away any excess Metabond that would interfere with the implant.
- 39 Using the drill and the FG1/4 or EF4 return to the trench and drill until you have cracked through all the way around.

Note

The drilling process will take 30-40 minutes.

- 39.1 Specifically for this dual hemisphere craniotomy, you need to fully drill and separate the skull at the sinus confluences. These are located at the deep curves of the craniotomy etch that sit right along the midline.



Note

The sinus confluence sites will contain skull that is significantly thicker than the majority of the skull.

Note

The anterior confluence sits close to the rostral rhinal vein and is surrounded by cranial vessels. Be sure to note the difference between a cranial bleed and the rostral rhinal bleeding during your drilling.



39.2 If you need to remove debris or blood during the craniotomy you can rinse with ACSF. Be aware that soaking the skull in ACSF will cause it to become soft and can cause the drill to take off more layers than anticipated.

40 Rinse the well using copious amounts of ACSF and sterile gauze/Kimwipes to get rid of debris before removing the skull island.

40.1 Place Hemostatic Agent Surgifoam within the well if anticipating bleeds.

Note

The dual hemisphere craniotomy borders 3 major blood vessels. It is highly likely that you will require anticipatory Surgifoam ready within the window before removing the skull island.

41 Add ACSF to the well. Using 45° forceps, gently pry up the skull at multiple points to ensure the skull island is sufficiently separated.

Note

If skull is still attached (i.e. bone bridge, not cracked), remove ACSF and continue drilling. This is especially crucial at the sinus confluences. Rinse before removal.

42 When the skull island appears ready to be removed (sufficiently detached), use 45° Dumont forceps to carefully remove the skull island, being careful to minimize pressure on the brain (i.e. pressure from skull island or tools).

42.1 With 45° Dumont forceps, gently grab the skull island from the edge. Slowly pull the skull upwards. Watch for any unnoticed bone bridges, the adherence of the dura or sagittal sinus to the skull, bleeding, and bending of the skull island at the skull sutures.

Note

Avoid any rapid movements to prevent the skull from dipping down and damaging the cortex or the ripping/tearing of blood vessels or sinuses.



- 43 Stop all bleeds with Hemostatic Agent Surgifoam, ACSF rinses and/or sterile Sugi absorbent spears. Make sure there is always ACSF covering the brain surface.

Note

Ensure all bleeds are stopped as rapidly and efficiently as possible. Excess blood loss can result in mouse death.

Perform a Durotomy

35m

- 44 Make the initial incision for each of the hemispheres using a durotomy probe.
- 45 Use angled Vannas scissors cut the dura back to the bone line. Remove dura either by cutting along the bone line or using 45° Dumont forceps to pull flaps over the bone line. No dura should remain within the window.
- 45.1 Remove dura by using 45° Dumont forceps to hold the dura taught and the angled Vannas scissors to cut.
- 45.2 Use caution when cutting the dura away near the sagittal sinus and large cranial vessels. The dura is connected to the sagittal sinus as well as many large vessels branching off of the sinus. Excess tugging or pressure can compromise the vessels and cause bleeding.
- 45.3 Two separate durotomies will need to be performed as there are two hemispheres.
- 46 Stop all bleeds with Hemostatic Agent Surgifoam, ACSF rinses and/or sterile Sugi absorbent spears. Make sure there is always ACSF covering the brain surface.

**Note**

Ensure all bleeds are stopped as rapidly and efficiently as possible. Excess blood loss can result in mouse death.

Optional (2.2): Perform Stereotactic Injections Within the Cranial Window

10m

- 47 **Optional 2.2**



This optional section accommodates injections that will be performed after the craniotomy and durotomy. This is the last section out of the three required. This second section is specifically for injecting the desired material at the injection coordinates that were marked in Optional 2.

Skip to next section if not performing stereotactic injections within the cranial window.

48 Ensure mouse's skull is level.

48.1 Set the roll and pitch of the mouse to zero degrees.

Note

Since the headframe was placed on a level skull, having the pitch and roll set to zero with mouse in the ear bar clamp creates a level skull.
Reminder: the visual cortex headframe and ear bar clamp have a pitch of -6° and 6° respectively.

49 Reference protocol **Stereotaxic Injection by Nanoject Protocol V.6** for performing stereotactic injections via nanoject.

Protocol

NAME

Stereotaxic Injection by Nanoject Protocol

CREATED BY

Allen Institute

Preview

Begin at step **8.3 "If Injecting with Nanoject II"** or **8.4 "If Injecting with Nanoject III"**.
Stop at step **8.5 "Suturing"**.

50 Reference protocol **Stereotaxic Surgery by Iontophoresis V.6** for performing stereotactic injections via iontophoresis.



Protocol

NAME

Stereotaxic Injection by Iontophoresis

CREATED BY

Allen Institute

[Preview](#)

Begin at step **8.4 "Inject Virus with Iontophoresis"**

Stop at step **8.5 "Suturing"**.

- 51 Raise and remove injection device from stereotax arm.

Apply and Seal the Implant

10m

- 52 Remove custom made Bilateral SHEILD Implant from packaging and rinse in a well of ACSF.
- 52.1 If there are visible air bubbles, rinse again and/or use the ACSF syringe or a sterile swab soaked in ACSF to detach bubbles.

Note

Air bubbles on the implant occasionally arise from surface tension when implant is being soaked and/or removed from ACSF well. This is to prevent the addition of air into/onto the brain.

- 53 Place the implant so the inside edge sits over the craniotomy and slowly lower the silicone coated probe onto the center of the implant.

Note

If the implant is placed too forcefully or dropped, cortex damage may occur. It is best to have a significant amount of ACSF covering the cortex to cushion it's placement.

- 54 Gently compress until all edges of the implant lip are contacting the full perimeter of the craniotomy.

Note

Excess pressure on the implant can cause detrimental brain compression.

- 55 Apply Vetbond to the perimeter of the implant that is contacting the skull. Be careful not to cover any holes in the implant.

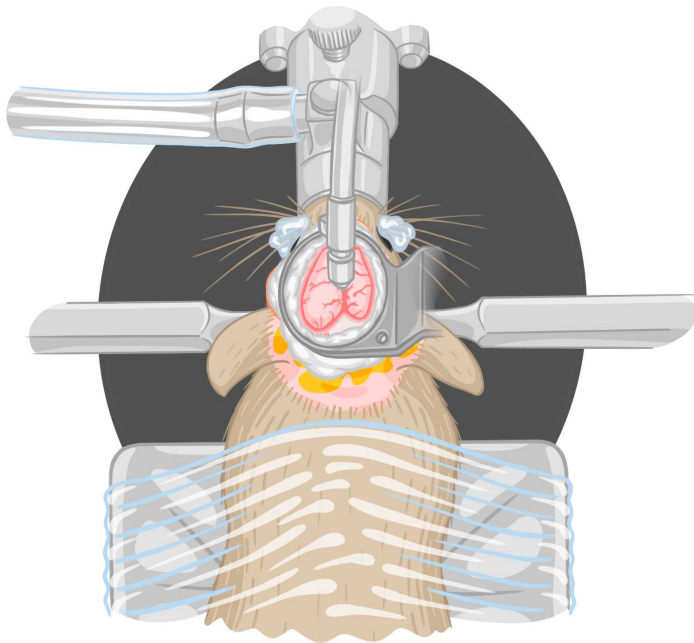


Illustration of pressure tool holding implant in place of craniotomy.

Complete Implant Seal and Attach Well

5m

- 56 Carefully raise and detach the pressure tool holding the implant from the stereotax arm.
- 57 Secure Bilateral SHIELD well to headframe.



57.1 Using Krazy glue or equivalent, apply glue to the top of the headframe ring and place the well in the correct orientation over the headframe, using pressure for 5-10 seconds to allow the glue to adhere.

58 Reinforce Vetbond seal and cover any additional exposed skull with Metabond.

58.1 Apply Metabond within the well halfway up the implant lip with the wooden end of a cotton swab. Be careful not to cover any holes in the implant with Metabond.

Note

If you do get Metabond on the implant, it is best to wipe it away immediately with the cotton end of the swap. The Sorta-Clear easily scuffs.

58.2 With the remaining Metabond, cover the connection point between the well and the metal headframe to reinforce the well attachment. Let dry for ~  00:05:00 .

59 Apply black cement over top of the white Metabond in the well. Be careful not to cover any holes in the implant. Allow to dry completely ~  00:05:00 .

5m



Note

This step is only useful if imaging the animal's brain as it reduces glare and light leak. If only planning on using this animal for electrophysiology, the white Metabond creates a nice contrast to electrodes being lowered into the brain.

59.1 Add equal parts black cement powder and Ortho-Jet BCA liquid to an empty well of the 24 well plate.

59.2 Use the broken end of a swab to layer the black cement.

Note

You will need to work quickly as black cement hardens rapidly.

- 60 Once Metabond and black cement have fully dried, attach a well cap to protect the implant from gathering dirt and debris in cage.

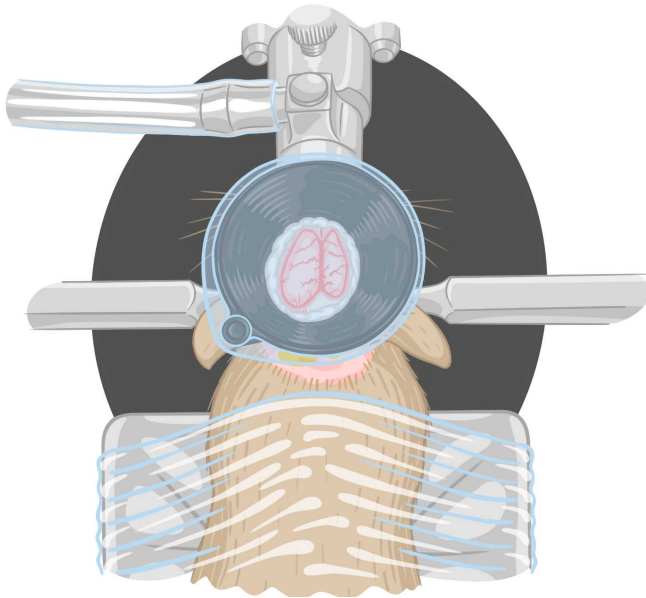


Illustration of complete dual hemisphere surgery.

- 61 Proceed to takedown and recovery steps.

Recover Mouse and Takedown

10m

- 62 Remove mouse from stereotaxic instrument base once Metabond and black cement are fully dry.
- 62.1 Turn off isoflurane.
- 62.2 Detach headframe clamp from headframe carefully.
- 62.3 Remove earbars.
- 62.4 Remove mouse teeth from bitebar by lightly scruffing mouse in a way that raises the teeth out of the bite bar.



- 63 Obtain the mouse's postoperative weight.
- 64 Place the mouse back in a recovery cage and put the cage on the 🌡️ 37 °C heat plate.
- 65 Reference General Setup and Takedown Procedures for Rodent Neurosurgery protocol for takedown procedures.

Protocol



NAME

General Setup and Takedown Procedures for Rodent Neurosurgery

CREATED BY

Avalon Amaya

Preview

Protocol references

SHIELD: Skull-shaped hemispheric implants enabling large-scale electrophysiology datasets in the mouse brain.

Bennett, Corbett et al. Neuron, Volume 112, Issue 17, 2869 - 2885.e8 doi:

<https://doi.org/10.1016/j.neuron.2024.06.015>

Allen Institute for Brain Science. (2023). Stereotaxic Injection by Nanoject Protocol V.6. protocols.io.

[dx.doi.org/10.17504/protocols.io.bp2l6nr7kgqe/v6](https://doi.org/10.17504/protocols.io.bp2l6nr7kgqe/v6)

Allen Institute for Brain Science. (2024). Stereotaxic Injection by Iontophoresis V.6. protocols.io.

[dx.doi.org/10.17504/protocols.io.14egn8ewzg5d/v6](https://doi.org/10.17504/protocols.io.14egn8ewzg5d/v6)