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Visual Cortex Cranial Windowing

DOI

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Protocol status: Working

We use this protocol and it's working

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Keywords: Visual Cortex, Craniotomy, Durotomy, Head Post, Headframe, Cranial Windowing, Rodent Neurosurgery, injections within the cranial window, cranial windowing surgery, visual cortex cranial windowing this protocol, cranial window over the visual cortex, visual cortex cranial windowing, cranial window, cranial window coverslip, slope of the cranial window coverslip, variation in mouse skull shape, craniotomy, mouse skull shape, level skull, stereotactic injection, mouse visual cortex, stabilizing headframe, variations of stereotactic injection, headframe, surgical procedure, steps for precision laser leveling, precision laser leveling, cortical damage, visual cortex, procedure, timing of injection, level coverslip, neural imaging

Abstract

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

This procedure accommodates for 2 variations of stereotactic injections (Optional 1 and Optional 2). Specifically this protocol accommodates for injections before and after the craniotomy and durotomy. Timing of injections during cranial windowing surgeries is typically dependent on their coordinates. 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.

Additionally, this protocol includes accommodating steps for precision laser leveling (Optional 3). Due to the variation in mouse skull shapes, oftentimes the cranial window coverslip will be seated at different slopes. To avoid imaging limitations related to the slope of the cranial window coverslip, the headframe will be secured based off a level coverslip rather than a level skull.

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 technique for rodent neurosurgery.

Materials

Anesthesia and other Drugs

⊗ 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° 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
	large iris forceps	Fisher Scientific	13-820-073
	PREempt Disinfectant spray	McKesson Corporation	21101



Tool / Supply	Manufacturer / Supplier	Part Number
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 Spear	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
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



Tool / Supply	Manufacturer / Supplier	Part Number
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
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



	Tool / Supply	Manufacturer / Supplier	Part Number
	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 Stereotaxic Instrument	Kopf	1900
	Adjustable Stage Platform	Kopf	901
	Stereo Microscope	Leica	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



	Equipment	Manufacturer / Supplier	Part Number
	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
	5mm Cranial Window (two 5mm stacked with single 7mm circular cover glass lip)	Tower Optical 18687-2
	CAM Well	0160-200-10
	Mesoscope Well	0160-200-20
	Neuropixel Well	0160-200-45
	Well Cap	0160-055-09
	Bregma Stylus	0251-900-04
	Lambda Stylus	0111-300-01
	Dovetail Clamp	0111-200-00
	-6° Ear bar Headframe Clamp	0155-100-00
	-0° Ear bar Headframe Clamp	0155-110-00



	Material	Part Number
	Prober Holder	0155-200-00
	Titanium VisCtx Headpost	0160-100-10
	Laser Leveling Tool	0111-500-00

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

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

Ali Williford

Preview

Optional 1:

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

Optional 2, 2.1, 2.2:

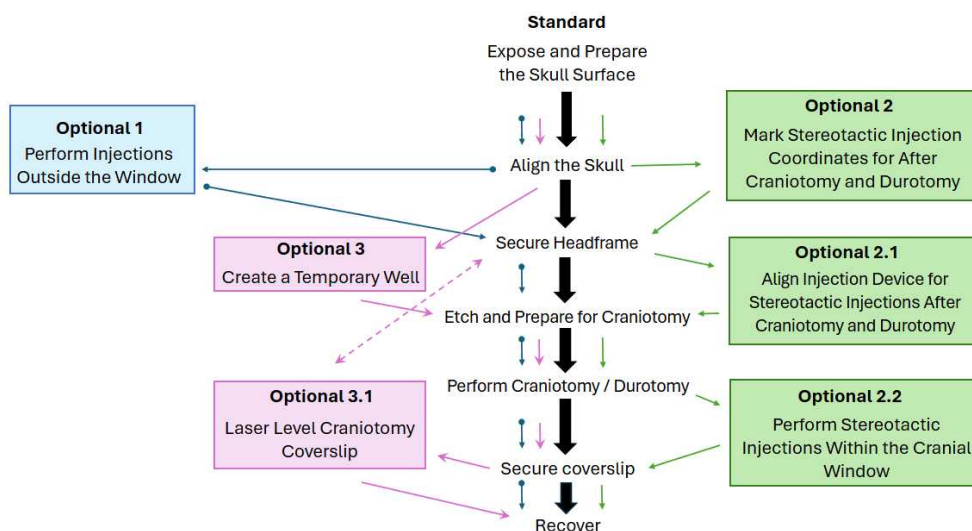
For stereotactic injections after performing the craniotomy and durotomy, follow sections titled with "Optional 2", "Optional 2.1" and "Optional 2.2" in addition to the standard sections.

Optional 3, 3.1:

For precision laser leveling that requires the cranial window coverslip to be level with the headframe, follow sections titled with "Optional 3" and "Optional 3.1" along with the standard sections.

Graphical Overview of Procedure

1



Map diagram of protocol workflow.

Expose and Prepare the 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.

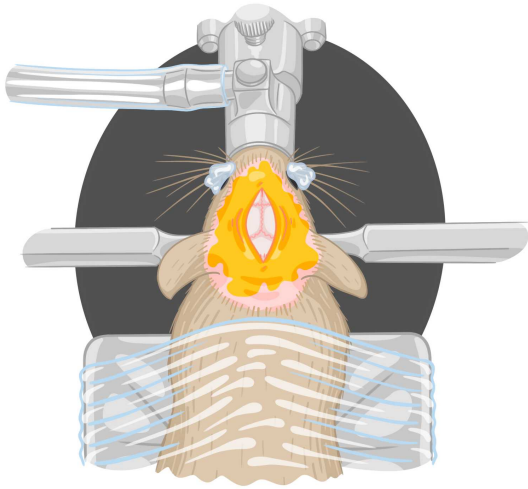


Illustration of initial skin incision on mouse head.

Mouse fixed to 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).

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

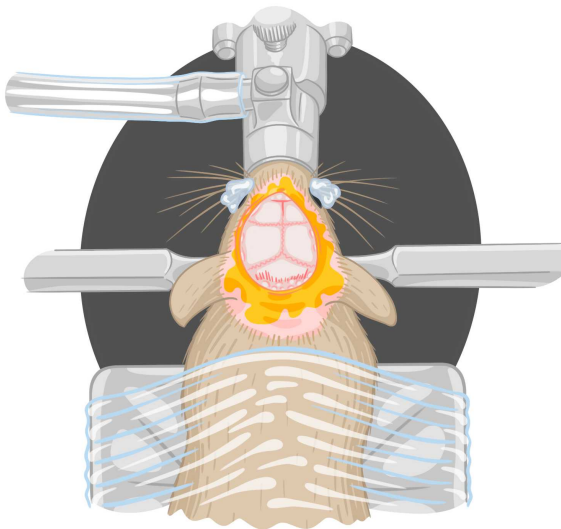


Illustration of skin removal on mouse head.

- 3.1 On the left side remove enough skin to expose 1-2mm of cheek muscle. On the right stop before muscle is exposed. Anteriorly, stop at the rostral rhinal vein.

- 4 Remove exposed periosteum by rubbing apart with cotton swabs and bunching near the edges of the skin. Then cut away with Vanna scissors at the skin edge. Use Artificial Cerebrospinal Fluid (ACSF) to rehydrate if necessary (i.e. periosteum dries out).

Note

Utilize a 10mL or 20mL syringe, 25G 5/8-inch needle, and a 0.2um syringe filter to store and dispense ACSF.

- 5 Detach the muscle on the left side of the skull with Dumont forceps and then press the tissue down and laterally.

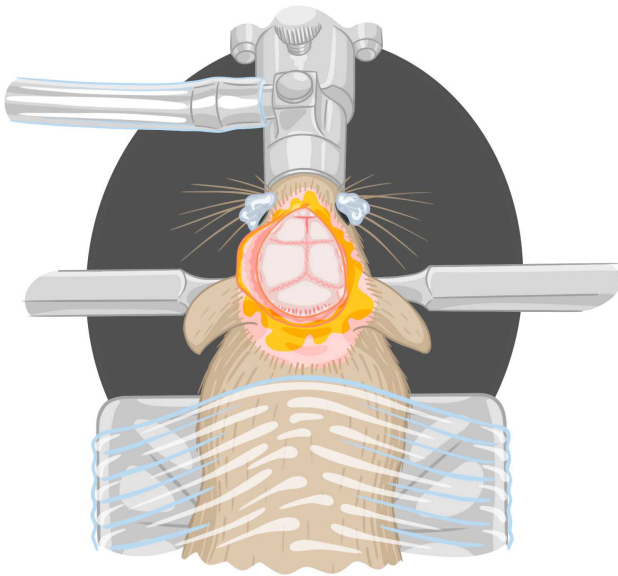


Illustration of mouse with detached and fixed left cheek muscle.

Note

Due to the angle that the headframe sits over the visual cortex, you will need to push the detached muscle on the left side down until it just meets the earbar.

- 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.

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.

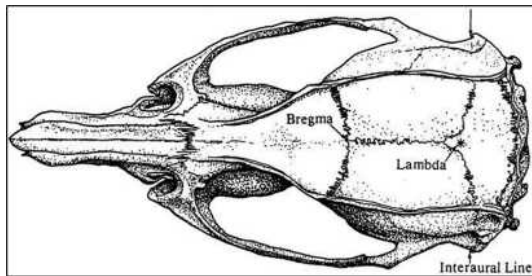


Illustration of Bregma and Lambda landmarks.

- 7.1 If Lambda-Bregma offset is greater than 0.1mm in X, use the yaw adjustment on stereotactic alignment system to adjust the yaw to within 0.1 mm.
- 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 will obstruct most 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:
dx.doi.org/10.17504/protocols.io.bp2l6nr7kgqe/v6
- 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:
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 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 this will 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:

dx.doi.org/10.17504/protocols.io.bp2l6nr7kgqe/v6

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 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"**.

- 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.

Optional (3) Create a Temporary Well

6m

18 Optional 3

This optional section accommodates for precision laser leveling, which involves a non-standard operation order where the headframe is leveled based off the craniotomy coverslip rather than the skull. This is the first section out of the two required. This first section is specifically for creating a temporary well that will be utilized in place of the headframe. This will be removed and replaced with the headframe once the skull has been laser leveled (Optional 3.1).

Skip this section if not utilizing precision laser leveling. [➡ go to step #22](#)

- 19 Create a temporary Kwik-Cast well around the craniotomy area.

- 19.1 Dispense Kwik-Cast into an empty well within your well plate. Mix and let it sit for

00:01:00 to allow it to slightly stiffen.

1m

**Note**

Kwik-Cast has an ideal working time within 2-4 minutes of mixing. As it solidifies, it will become increasingly stringy. Do not use once it becomes rubbery.

20 Once slightly stiff, use the broken end of a swap to create a temporary well around the craniotomy area. It will settle a little, so be sure to place it sufficiently away from craniotomy coordinates and areas with excess fur.

20.1 Let Kwik-Cast set for about  00:05:00 before proceeding.

5m

21  [go to step #33](#) to skip the headframe placement. You will return to that section in Optional 3.1.

Secure Headframe

20m

22 Ensure skull is level.

22.1 If you have performed an angled injection, return mouse back to level state.

23 Using the dovetail clamp, zero Lambda stylus over Lambda, and replace with the desired visual cortex headframe.

24 Lower the headframe until there is contact with the skull. Ensure that the headframe is not pushing down on the skull.

Note

The Vetbond seal can often break during the placement of the headframe. Before proceeding to the next step, check for compromised areas and re-seal with Vetbond if necessary.

**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.

25 Prepare and apply the first round of Metabond.

25.1 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).

5m

26 Turn isoflurane off, remove earbars, and loosen the nose cone slightly.

27 Carefully detach the headframe from the earbar headclamp and/or earbars.

28 Prepare another Metabond tray.

29 Pull back nose cone completely and remove the mouse from bite bar. Either lie the mouse on the heating pad or hold mouse in hand.

30 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

Ensure Metabond is not left too close to eye and does not have any sharp points. This is to avoid any irritation to the mouse.



- 31 Using the 6° 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.

Note

Visual cortex headframe and ear bar clamp have a pitch of -6° and 6° respectively. This is to accommodate for the natural head tilt of a mouse.

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

- 31.2 Replace saran wrap over mouse body.



32 **Optional 3.1**

If following precision laser leveling steps in Optional 3.1 section, proceed to

 [go to step #90](#) to complete.



Skip this step if not utilizing precision laser leveling.

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 headframe has obscured the coordinate fiducials and a craniotomy will remove the coordinate markings, 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.

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.

If using temporary well, the mouse will remain in the ear bars so no leveling adjustments will need to be made due to the angle of the headframe.



Note

Since the visual cortex 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.

35 Attach injection device with loaded pipette onto stereotactic arm and center over injection location. This stereotactic coordinate should be premarked from .

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

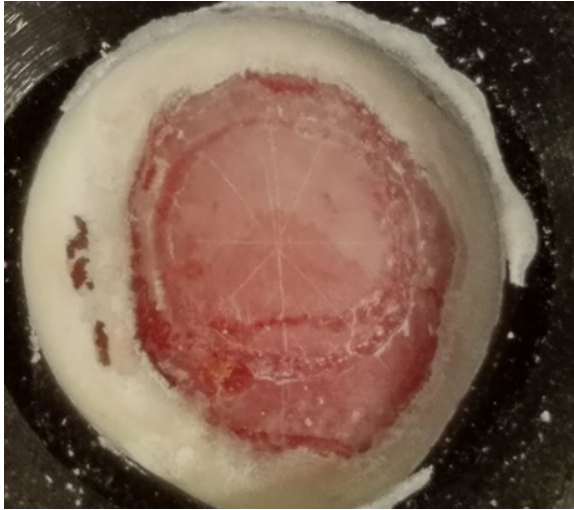
Nanoject/Iontophoresis pipettes should not be changed after setting up the stereotax arm. The variation in pipette straightness can cause the injection location to move up to 0.8mm.



Etch and Prepare for Craniotomy

15m

- 38 Place angle finder on the roll arm of the stereotactic instrument base, and roll mouse 23 degrees so that the visual cortex is level.
- 39 If headframe has been secured, pitch the skull up to zero to level the skull in the 6° earbar clamp. Skip this step if the mouse has a temporary well as the mouse is in earbars and not the angled headframe clamp.
- 40 Remove 5mm coverslip from 70% Ethanol and rinse in two wells of ACSF.
- 41 Place coverslip within the well of the headframe, and secure with broken sterile swab in probe holder.
- 42 Using 45° dumont forceps or #11 Scalpel blade make an etch around the 5mm coverslip.
- 43 Remove swab and confirm etch with 5mm coverslip, then remove from well.
- 44 Using the drill with the FG1/4 or EF4, create a shallow trench over the etch.
- 44.1 Ensure trench is the correct diameter and shape by confirming with the 5mm coverslip.
- 45 Using the drill and the FG4, buff the 2mm around the outside of your trench.



5mm craniotomy drilled etch and buffed surrounding Metabond.

- 45.1 Using the drill remove any high points of Metabond that could cause a coverslip to break, or will cause the window to sit incorrectly (not level) within your craniotomy.

Perform Craniotomy

30m

- 46 Using the drill and the FG1/4 or EF4 return to the trench and drill until you have cracked through the skull all the way around.

20m

Note

The drilling process will take 20-40 minutes.

- 46.1 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.
- 47 Rinse the well using copious amounts of ACSF and sterile gauze/kimwipes to get rid of debris before removing the skull island.
- 47.1 Place Hemostatic Agent Surgifoam within the well if anticipating bleeds.

**Note**

The visual cortex craniotomy contains the transverse sinus within the window. It is highly likely that you will require anticipatory Surgifoam ready within the window before removing the skull island.

- 48 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 on the posterior edge. Rinse before removal.

- 49 When the skull island appears ready to be removed (sufficiently detached), use 45° forceps to carefully remove the skull island, being careful to minimize pressure on the brain (i.e. pressure from skull island or tools).
- 50 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.

Perform a Durotomy

10m

- 51 Ensure brain is always covered with a layer of ACSF.
- 52 Make the initial incision using a durotomy probe.
- 53 Remove dura either by cutting along the bone line or using 45° forceps to pull flaps over the bone line. Other than around the sinus or vessels connected to dura, no dura should remain within the window (unless connected to sinus or vessel).

Note

Avoid tugging or pulling of the dura as this can cause vessel bleeds, sinus bleeds and/or "piano wire" lacerations.

**Note**

Use caution around the sagittal and transverse sinuses.

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

10m

54 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.

55 Ensure mouse's skull is level.**55.1** 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.

56 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 **8.3 "If Injecting with Nanoject II"** or **8.4 "If Injecting with Nanoject III"**.
Stop at step **8.5 "Suturing"**.

57 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 **8.4 "Inject Virus with Iontophoresis"**



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

- 58 Raise and remove injection device from stereotax arm.
- 59 Place angle finder on the roll arm of the stereotax, and roll mouse 23° so that the visual cortex is level.

Secure Coverslip

10m

- 60 Depending on the desired experimentation, the securing of the coverslip may vary. Reference one of the following sections titled with "Secure Coverslip (_____ Experimentation)".

Note

For example:

- Optical physiology experiments require a permanent coverslip surrounded by a dark cement to aid with contrast and to prevent light leakage during scope recordings. The variability in scope sizes also determines the size of the headframe well attachment.
- Electrophysiology experiments (specifically regarding this visual cortex surgery) require a temporary removable coverslip. This requires a silicone coated glass coverslip, and utilizes Vetbond and Kwik-Cast instead of a hard cement to temporarily hold the coverslip in place. This coverslip will be replaced with another specialty coverslip later within the experimental timeline.
- Laser level optical physiology experiments require a permanent and precise alignment between the coverslip and the headframe.

- 61 Rinse the desired coverslip within the two rinse wells of ACSF.

- 61.1 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 coverslip occasionally arise from surface tension when coverslip is being soaked and/or removed from ACSF well. This is to prevent the addition of air into/onto the brain.

- 62 Carefully place the coverslip into the craniotomy with Dumont forceps.

Note

If the coverslip 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 its placement.

- 63 Load the broken end of a sterile cotton swab into the probe holder and center it above the craniotomy.

Note

The end of the sterile swab should be as flat as possible to ensure even pressure while securing the coverslip. This can be done by cutting the end of the swab and hitting it against a flat and sterile surface. If accessible, a metal probe with a drop of silicone at the tip can be used as a substitute for a broken sterile swab.

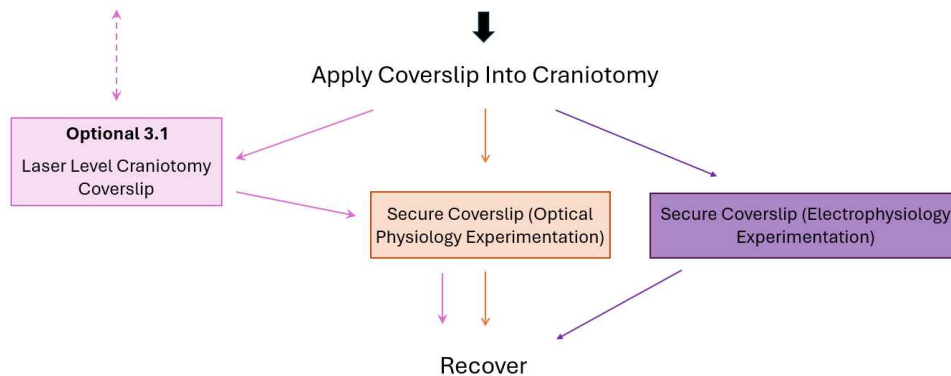
- 64 Lower the broken side of the sterile cotton swab very gently onto the center of the coverslip to secure it in the craniotomy, being careful to not crack the coverslip.

Note

Excess pressure on the coverslip can cause detrimental brain compression.

- 64.1 Ideally the 7mm edge of the coverslip should contact the full perimeter of the craniotomy. This may vary between mice due to skull shape and age.
- 65 Remove ACSF from the well with Kimwipes or Sugi Absorbent Spears until the surface tension of the ACSF is held between the 5mm and 7mm edge of the coverslips.
- 66 Seal coverslip by placing small drops of Vetbond using an insulin syringe around the coverslip via capillary action, forming a seal between the coverslip and skull surface. Let dry completely. Absorb extra ACSF with sugi.
- 67 Proceed to one of the coverslip securing sections below, which will be determined by the intended use of the animal (e.g. **Secure Coverslip (Optical Physiology**

Experimentation), Secure Coverslip (Electrophysiology Experimentation), or the optional step **Optional (3.1) Laser Level Craniotomy Coverslip.**



Graphical overview of securing coverslip workflow options.

Optional (3.1) Laser Level Craniotomy Coverslip

5m

68 Optional 3

This optional section accommodates for precision laser leveling, which involves a non-standard operation order where the headframe is leveled based off the craniotomy coverslip rather than the skull. This is the second section out of the two required. This second section is specifically for removing the temporary Kwik-Cast well and laser leveling the coverslip.

Skip this section if not utilizing precision laser leveling. [go to step #90](#).

69 Carefully raise and detach the probe holder from the stereotactic arm.

69.1 Watch the Vetbond seal/coverslip closely to identify any ACSF leaks or seal breaks. Lower probe and re-Vetbond if leaks occur. Proceed to next steps once complete seal is confirmed.

70 Replace probe with the laser leveling tool and turn laser on.

71 Align laser onto the coverslip and adjust the pitch and roll of mouse until the laser reflects back up to the center of the laser leveling tool.



- 72 Once laser leveled, place digital angle finder on the roll arm of the stereotax and zero. Roll the mouse to -23° .
- 73 Pitch the mouse up 6° using the stereotax markings.
- 74 Turn off the laser level and remove from stereotax arm.
- 75 Secure headframe by following steps  [go to step #23 - #32](#) within "Secure Headframe" section.
- 76 Proceed to "Secure Coverslip."

Secure Coverslip (Optical Physiology Experimentation)

7m

- 77 Optical physiology experiments require a permanent coverslip surrounded by a dark cement to aid with contrast and to prevent light leakage during scope recordings. The variability in scope sizes also determines the size of the headframe well attachment.  
- 78 Prepare white Metabond in a cold ceramic dish.
- 79 Surround the coverlip edges with Metabond.
- 79.1 Apply Metabond within the well up halfway to outer 5mm coverslip edge with the wooden end of a cotton swab. Let dry for ~  00:05:00 . 

Note

Ensure Metabond does not cover 5mm cranial window. Edge of 5mm lip should be visible.

- 80 If necessary, attach Mesoscope or desired well onto headframe using superglue. Ensure headframe is free of debris and excess Metabond. 



80.1 Align Mesoscope well to the well of the titanium headframe. The wells are not circular and contain a narrower perimeter near the lower right.

81 When attaching a well, apply black cement to cover white Metabond and potential areas that could cause light leak.

If following laser leveling steps, only place black cement on the outside of the well where the titanium meets the well.

Note

Potential sources of light leak can include:

- Translucency of the Metabond surrounding the coverslip.
- Seal between metal headframe and plastic well.
- Translucency or gaps in the plastic well itself.

81.1 In your 24 well plate mix up black cement powder and Ortho-Jet BCA liquid.

81.2 Apply within well and/or on the outside of the well where the titanium meets the well using the broken end of a sterile swab. Allow to dry around  00:02:00

2m

Note

Black cement cures extremely fast. Work quickly. If cement starts becoming stringy, make a new batch.

82 Carefully raise and detach the probe holder from the stereotax arm.

83 Proceed to takedown and recovery steps  [go to step #90](#) .

Secure Coverslip (Electrophysiology Experimentation)

5m

84 Electrophysiology experiments (specifically regarding this visual cortex surgery) require a temporary removable coverslip. This requires a silicone coated glass coverslip, and utilizes Vetbond and Kwik-Cast instead of a hard cement to temporarily hold the





coverslip in place. This coverslip will be replaced with another specialty coverslip later within the experimental timeline.

85 Prepare Kwik-Cast in a 24 well plate. Add black cement powder.

85.1 The Kwik-Cast should have a matte finish with the added black cement powder.

Note

Too much black cement will increase the amount of time the Kwik-Cast takes to set. Typically, a 4:1 ratio of Kwik-Cast to black cement is ideal.

86 Apply Kwik-Cast within the well up halfway to the 5mm coverslip edge with the wooden end of a cotton swab. Let set ~  00:07:00 .

Note

The Kwik-Cast will likely settle over your 5mm craniotomy window. To avoid this, you can gently push the Kwik-Cast to the sides of the coverslip with the broken end of a cotton swab as it dries.

87 If desired, once the Kwik-Cast mixture is set, lightly cut away any excess Kwik-Cast covering the 5mm window using the tip of a scalpel blade. 

88 Carefully raise and detach the probe holder from the stereotax arm.

89 Proceed to takedown and recovery steps  [go to step #90](#) .

Recover Mouse and Takedown

10m

90 Remove mouse from stereotactic instrument base once Metabond and black cement are fully dry.

90.1 Turn off isoflurane.

- 90.2 Detach headframe clamp from headframe carefully.
- 90.3 Remove earbars.
- 90.4 Remove mouse teeth from bitebar by lightly scruffing mouse in a way that raises the teeth out of the bite bar.
- 91 Obtain the mouse's postoperative weight.
- 92 Place the mouse back in a recovery cage and put the cage on the  37 °C heat plate.
- 93 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

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