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Acute LC Craniotomy and Skull Thinning for Neuropixels Recordings and Optogenetic Stimulation

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We use this protocol and it's working

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Abstract

This protocol details the acute craniotomy procedure to target locus coeruleus (LC) *in vivo* with a Neuropixels 2.0 single shank probe, and includes the skull thinning procedure to better expose regions of cortex for antidromic laser stimulation. Given that LC is a small structure that is difficult to target precisely, leaving the skull and surrounding vasculature intact allows for more accurate targeting. The surgery should result in a cranial window in both hemispheres with an exposed inferior colliculus, through which LC is targeted, as well as an anterior portion of cerebellum. The sagittal sinus should also be relatively clear in order to orient the probe in relation to all of the aforementioned structures.

Image Attribution

Gabriel Rodriguez, Allen Institute

Guidelines

This procedure should only be performed in accordance with IACUC and veterinary requirements.

Materials

Surgical Tools and Supplies:

	Tool/Supply	Manufacturer/Supplier	Part Number
	Dumont #5 45° forceps	Fine Science Tools	11251-35
	Vanna scissors	World Precision Instruments	500260
	45° or 90° Duratomy probe	Fine Science Tools	10066-15
	Alcohol swabs	Becton Dickinson Medical Supplies	326895
	Hemostatic Agent Surgifoam	McKesson Corporation	403360
	Sterile gauze 3×3"	Patterson Veterinary	07-893-8587
	Kimwipes	Fisher Scientific	06-666
	Sugi pointed sterile swabs	Fine Science Tools	18105-01
	Insulin syringes, U-100, 1mL, 31G	Advantor	BD328418
	Sterile Drill Bits FG4 (coarse drill bit)	NeoBurr	1734214
	Sterile Drill Bits FG1 (fine drill bit)	NeoBurr	1734948
	Artificial Cerebrospinal Fluid.V	Made in-house	
	Well with grounding pin and 3D printed cap	Made in-house	
	Duragel	Ellsworth Adhesives	DOW DOWSIL™ 3-4680
	5mm microruler	TDI International	TDI MRM100H
	C Universal 4-META Catalyst, 0.7mL	Parkell	S371
	B Quick Base for Metabond, 10mL	Parkell	S398
	Radiopaque L-Powder, white, 5g	Parkell	S396
	Superglue	Krazy Glue	Amazon PK4 KG58248SN

All tools/supplies can be substituted with their equivalent.

Artificial Cerebrospinal Fluid.V

Equipment:

	A	B	C	D
	Small Animal Stereotaxic Instrument	Kopf	1900	
	Adjustable Stage Platform	Kopf	901	
	Stereo Microscope	Leica	M80	
	Gooseneck Illumination	AM Scope	LED-6WA	
	On-axis Illumination	Leica	KL2500 LED	
	Small Animal Temperature Control System	CWE Inc.	TC-1000	
	Large Heat 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	
	Headframe clamp	Made in-house		

Safety warnings

Personal Protective Equipment (PPE) should be worn 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

This surgery is a secondary procedure that should only be performed after the headframe has been attached and necessary viruses have been injected during the Stereotactic Injections with Headframe Implant procedure. Allow the mouse at least 2 weeks to recover from initial surgery before attempting this procedure.

Protocol

NAME

Stereotactic Injections with Headframe Implant

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Avalon Amaya

Preview

Preparation of surgical rig

1 Reference following protocol for surgical rig setup:

Protocol

NAME
General Setup and Takedown Procedures for Rodent Neurosurgery

CREATED BY
Avalon Amaya

Preview

Begin at step 1 **"Disinfect the surgical area"**
Stop at step 3.5 **"Remove autoclaved surgery tools"**

Anesthetization and preparation of animal

2 Reference following protocol for anesthetization of animal:

Protocol

NAME
General Setup and Takedown Procedures for Rodent Neurosurgery

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Preview

Begin at step 25 **"Prepare the anesthesia system"**
Stop at step 30 **"Administer any drugs"**

3 Secure mouse to surgical rig via the headframe attached during initial surgery.

3.1 Swab the exterior of the clear Metabond with an Alcohol swab.

Skull thinning procedure

4 Perform skull thinning procedure to enable sufficient light transmission for later optogenetic antidromic stimulation. For all regions of interest (ROIs), thin the skull over both hemispheres.

4.1 Using a 5 mm micro ruler, measure the regions of interest (prelimbic cortex, primary somatosensory cortex, and primary visual cortex) from the bregma marking made in the previous surgery.

Note

These measurements indicate distance from bregma and are consistent across all mice.
Prelimbic cortex (PrL): AP: 1.8, ML: ±0.5
Primary somatosensory cortex (S1): AP: -0.5, ML: ±2.35
Primary visual cortex (V1): AP: -3.3, ML: ±2

4.2 Using a dental drill with the coarse drill bit, remove the layer of Metabond that was applied during the previous surgery.

4.3 Continue to drill a 1.5 mm area at all ROIs until the blood vessels are easily visible but ensure that the skull remains intact.

Fig. 1 The black dot in the image denotes bregma as it is marked in initial surgery. The gray dashed circles denotes the thinned skull regions.

Perform craniotomy

5 Perform acute craniotomy at LC on both hemispheres.

Note

It is recommended to flush the surface of the skull with ACSF before beginning the craniotomy for better visibility of surrounding vasculature (such as the sagittal sinus) and to remove the Metabond debris from previous steps. This will result in a surgical area that has better clarity as illustrated in Fig. 2.

5.1 Using a 5 mm micro ruler measure the location of LC from bregma and drill at measured location to thin the skull covering both hemispheres. Expose enough of the skull to see where the sagittal sinus is located. Most of the craniotomy should be over the inferior colliculus (IC) for accurate targeting and include enough of the cerebellum to see where the boundary of the IC ends.

Note

For male mice, coordinates from bregma are AP: -5.4, ML: ±0.85
For female mice, coordinates from bregma are AP: -5.2, ML: ±0.85

Fig. 2 The black dot in image denotes bregma as it is marked in initial surgery. The SS denotes the sagittal sinus that runs along the edge of where the craniotomy will take place.

5.2 Flush the area with several drops of ACSF such that the entire craniotomy is covered. Remove the ACSF with enough Sugi spears to soak up the liquid. This ensures all debris is removed before performing craniotomy.

5.3 Using a fine drill bit, drill a 1 mm skull island over both LC regions.

5.4 Once there are visible cracks around the skull island and some CSF has started leaking out, cover the entire area with ACSF and, using Dumont forceps, carefully pry up the skull at multiple points to ensure the skull island is sufficiently separated. If blood starts leaking out, stop immediately and staunch the bleeding with Sugi spears and surgifoam soaked in ACSF.

5.5 Remove the skull island using Dumont forceps and ensure all bone fragments are removed. To minimize bleeding, tilt the bone fragment away from the sagittal sinus when detaching from rest of skull.

5.6 Rinse the area with ACSF and gently swab area with surgifoam soaked in ACSF to clean the craniotomy.

Perform duratomy

6 Perform the duratomy.

6.1 Make an initial cut in the dura using a Duratomy probe or a sterile 1 mL Insulin syringe, such that there is a flap that can be peeled back using forceps or lifted off the brain with the Duratomy probe.

6.2 Lift up the flap of dura using Dumont forceps and pull with enough force to remove the dura covering the brain. Alternatively, trim away with Vanna scissors.

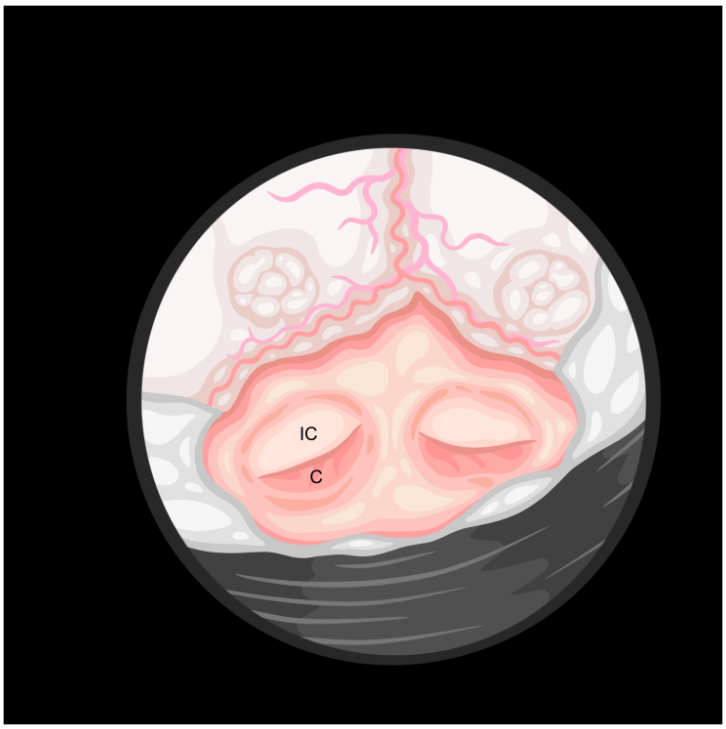

Fig. 3 In above image, IC denotes inferior colliculus and C denotes cerebellum.

Attach well

7 Place well onto the headframe.

> Note
>
> This step may be omitted if well was attached with the headframe.

7.1 Apply a thin layer of Superglue to bottom of the well and attach to the headframe.

7.2 Secure well with Metabond inside the headframe and around the outside of the headframe. Ensure the well does not leak by filling it with ACSF and checking for leaks.

7.3 Remove the ACSF with a Kimwipe or cotton tipped applicators and ensure inside of well is dry.

8 Using a fine drill bit, drill a small burr hole (<0.2 mm) until the brain surface is exposed and some CSF leaks out.

Note

Depending on what type of headframe is used, the location of the grounding hole will differ. For the whole hemisphere headframe, place the grounding hole at anterior right hemisphere and for the dual hemisphere headframe place the grounding hole at posterior left hemisphere. Drill the hole away from any major blood vessels or brain regions of interest.

8.1 Gently place grounding pin in the small burr hole such that it is secure but is not depressing the brain tissue or causing bleeding.

Fig. 4 Final product of this procedure with 6 skull thinned areas, 2 craniotomies, and the grounding pin (GP) inserted into the skull.

8.2 Mix the Duragel in a sterile weigh boat with the 1 mL insulin needle in a 1:2 ratio of Duragel A and Duragel B. Scrape up a drop of the mixed Duragel with the needle and apply a thin layer to both craniotomies, the thinned skull regions, and the hole for the grounding pin. Ensure there is no exposed brain tissue.

9 Attach a well cap to the well.

Take down of surgical rig

10 Turn off the isoflurane vaporizer and remove mouse from surgical rig.

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

12 Obtain postoperative weight of animal and return mouse to cage. Keep the cage on the 37°C heating pad until mouse regains consciousness and resumes normal behavior. Return mouse to vivarium.

> Note
>
> Follow post-operative veterinary guidelines.

13 Take down the surgical rig once surgery is complete as described in General Setup and Takedown Procedures for Rodent Neurosurgery V.2

Protocol

NAME

General Setup and Takedown Procedures for Rodent Neurosurgery

CREATED BY

Avalon Amaya

Preview