ALM Window Surgery

Forked from ALM Window Surgery

Kayvon Daie[1], Tim Wang[1], Amrita Singh[1], Arseny Finkelstein[1], JJ Kim[2], Marton Rozsa[1], Karel Svoboda[1]

[1]Janelia Research Campus;
[2]HHMI Janelia Research Campus, Johns Hopkins University School of Medicine

M Marton Rozsa

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This is the standard protocol for ALM window surgeries in the Svoboda lab.

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ABSTRACT

Protocol for Head Post and Cranial Window Surgery developed at Janelia Research Campus in the Svoboda Lab

Developed by Karel Svoboda for Trachtenberg et al 2002
Improved by Anthony Holtmaat for Holtmaat et al 2006 and Holtmaat et al 2009
Improved by Daniel Huber for Huber et al 2012
Compiled by Bryan Maclennan (2013), Abridged version by Courtney Davis (2015)
Compiled to protocols.io by JJ Kim (2019)

Note: This outline only provides practical step-by-step advice. All surgeons should be familiar with and carefully adhere to the official animal protocol and current procedures posted on the Svoboda lab wiki.

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ATTACHMENTS

ALM Imaging Window.pdf

GUIDELINES

ALM Craniotomy Considerations

- Coordinates for ALM are 1.5mm lateral and 2.5mm anterior and to Bregma. Medial edge of circle falls over the midline.
- Head post placement for ALM surgeries is just posterior to the outer edge of the glass window. A slot needs to be drilled out for it to sit in. Be careful when drilling for HP because the perietal bones of the skull is (relatively) thin.
- May need to rotate skull when drilling and measuring circle.
- Thinning down for ALM surgeries requires you to drill over 3 major blood vessels. There will be bleeding, but just do your best to stop it. ALM craniotomies are more difficult than M1, S1, V1 craniotomies, so it's important not to be discouraged! Drill slowly (as in, only remove tiny layers of bone at a time, not reduce speed of the drill because that may cause it to get caught on the bone) may help to minimize vascular disruption of the vessels in the skull
- Use 2.5mm/2.5mm/3 mm triple glass window.
- Skull is very thick in all parts except posterior edge - be careful drilling over posterior edge! The lateral edge especially is surprisingly thick because it's right next to one of the sutures and the brain curves.
- Be very careful when thinning down in the anterior region - You have to thin down right over the inferior cerebral vein (the giant blood vessel perpendicular to the sagittal sinus), and while completely rupturing this vessel may not necessarily kill the mouse, blood loss is a serious concern.
- Tearing brain sinus is not common for ALM craniotomies, though edema can be severe. Administering dexamethasone or buprenex will reduce the edema, but slighting raising the iso will also help reduce the edema (just be sure to lower it again).
- Besides being a difficult surgery to begin with, dural thickening is common for ALM craniotomies - consequently success rate can be low. This is why it's important to thin down the skull enough and to keep pressure on the window while the cement is drying so that the window puts enough pressure on the brain to keep the dura from regrowing and thickening.
- Biood takes ~90 seconds to fully coagulate, so if you accidentally drill through a blood vessel, do your best to slow down the bleeding using cotton swabs and gauze, and then leave it alone for a while to so It can clot. You can easily drill through dried blood, but be careful to not drill through the same blood vessel again.

Animal Preparation

1 Weigh the mouse and transfer it to a new cage if needed (i.e. if it's group housed, or if its cage is dirty.)
If mice do not have ear tags - denote first mouse removed as the first animal number etc.and cross out number on the original cage.

If the mice have ear tags, use the animal number that corresponds to the ear tag on the mouse you removed
Fill out a temporary cage card and write the weight on the temp cage card. Add and fill a post-op card as well.

2 Give the mouse a new cage several hours before the surgery if possible so that it can build a nest and become familiar/comfortable with the cage. If placed In a different cage after surgery, the mouse will become stressed out by the new environment and unfamiliar scents (and also the mouse probably won't feel like building a nest while recovering from surgery.)

3 Remove wirebar from cage and place several pellets of food into cage. Add container of dietgel. Can also give sunflower seeds and yogies to the mouse before and/or after the procedure.

Drug Prep

4 Remove buprenex dilution from locked drug box. Calculate volume (dose 0.1mg/kg), draw up with insulin syringe, and fill out controlled substance log. Draw the appropriate amounts of marcaine (50uL), dexamethasone (2mg/kg), and ketoprofen (5mg/kg) with insulin syringes - denote which syringe has what drug by marking syringe with sharpie. Make sure to return buprenex to the locked drug box and the schedule VI drugs (keto, dexamethasone, and marcaine) to a drawer. Administer dexamethasone 1M to mice (about an hour or more before start of surgery)

4.1 Dexamethasone is optional. If surgery typically has a lot of bleeding and edemas aren't much of a concern, it's advisable to not administer the dexamethasone.

Preperation and mounting

5 Surgical Prep:

5.1 Put on gloves, gown. and face mask. Rinse gloved hands with ethanol frequently

5.2 Prepare viruses and pipettes if needed (see "Virus Injections" below)

5.3 Tum on lights, heating pad, and microscope, and open the oxygen tank.

5.4 Spray surgery area with Virkon-s. Wipe up with paper towels

5.5 Wipe down surgery rig with alcohol swabs or kim wipe soaked in ethanol making sure heating pad, nose cone and all handles/parts you will touch are serialized. Wipe down lights, microscope handles and hand drill

5.6 Set up the surgical station with the needed supplies.

- surgical tools (sterilize them in glass bead sterilizer)
- kimwipes and/or gauze
- cotton swabs
- 3ml syringe, and a 25 gauge (blue) needle filled with sterile cortex buffer
- insulin syringes (orange caps) filled with drugs (dexamethasone optional, marcaine, ketoprofen, and buprenex)

5.7 Ensure vetbond, crazy glue, and artificial tears are accessible and within reach.

5.8 Obtain 2 insulin syringes and place with other sterile supplies on benchtop near surgical rig

5.9 Replace drill bit if dull.

5.10 Place the vial of cortex buffer in ice to keep it cold for later use.

5.11 Close isoflurane to rig and open tube to induction chamber (open is colinear to tube)

6 Tum on isoflurane to 3.0% with flow at ~1L/min before putting mouse in chamber.

7 Place mouse in chamber and record start time.

Note

Start time:

8 Once breathing has slowed and animal is deeply anesthetized (about 3 minutes), open both tubes and then close the tube to the induction chamber. Lower isoflurane to 1.5% - 2% with O_2 flow at ~0.6L/min. Adjust iso throughout surgery, ensuring that breathing and anesthesia plane remain stable. Mouse should have around 1 breath per second.

9 Remove mouse from chamber and secure in nose cone.

9.1 Pull back gently on ears and beneath neck to display teeth - gently place upper teeth in the hole of the nose cone. Be sure the tongue is to the side

9.2 While holding the head of the mouse higher then the nose cone, secure the nose cone over snout.

9.3 Make sure body is on top of heating probe and resting comfortably - head should not move when touched.

Window and headbar

10 Cover eyes with artificial tears.

11 Inject marcaine just below the scalp and massage the injection site so marcaine is evenly distributed below the scalp.

12 Place scissors in glass bead sterilizer.

13 Generously apply betadine to mouse scalp using cotton swab - using mass amounts of artificial tears will help you avoid getting betadine in eyes. Then clean with ethanol. Repeat 3 times ending with ethanol.

14 Pull skin margins on the base of the skull taunt with blunt forceps and make a clean incision with scissors from the bead sterilizer (hot scissors cauterize skin). Excise scalp - be very careful while removing as much skin as possible near left eye without cutting off the eyelid.

14.1 Typically cut scalp starting just between the ears to midway between the eyes (be very careful around eyes!)

15 Using a cotton swab, wipe away as much periosteum as possible. Make sure skull is dry and free of hair - push back skin and any remaining periosteum. Only use each cotton swab once, otherwise you risk reintroducing the hair you just removed.

15.1 Remove periosteum as quickly as possible after removing scalp. It dries very quickly and once it's dry it's much more difficult to remove. If it does happen to dry, a few drops of cortex buffer will help.

15.2 Snap cotton swabs in half. If one end is tapered well, set it aside for later use.

16 Fill an empty insulin syringe with vetbond (don't need much - about 20 microliters) and place drops around the skin margins, over exposed muscle and skull edges. Make sure vetbond completely seals wound - open wounds can become infected.

16.1 Close the eyelid as much as possible while the vetbond is drying; it's better to glue the eye closed than open.

17 On the left hemisphere, using surgical ruler, measure 2.5mm anterior to bregma

17.1 Etch a light horizontal line with #5/45 (sharp and angled) forceps. Measure 1.5mm lateral from midline and etch a light vertical line with #5/45 forceps; the resulting cross marks the center of the craniotomy. Tum the mouse's head so that the area you just marked is flat(ish). Lightly mark the center with the hand drill.

18 Place a 2.5mm window where you want to drill. Keep in mind all of the blood vessels nearby (can see them better with a drop or two of cortex buffer).

18.1 You can slightly adjust the window in any direction to avoid make the surgery easier for you, but no more than 0.5mm at max.

18.2 It is useful to take an image of the location of the cross in regards to the blood vessels (visible after a minute of cortex buffer on the skull) so if an injection is needed it will be centered around ALM and not the center of the window.

19 Use a sharpie to outline the window. Keep the window (relatively) in place by pressing on it with forceps.

20 Use the sharpie marks as a guide when drilling.

21 Zoom in on the microscope and begin drilling around the circle. Drill very lightly, gently and carefully. Adjusting the tilt of the skull can improve visuals and help with drilling. Use cotton swabs soaked in cortex buffer, kimwipes, gauze, and/or bone wax' to stop all major bleeds and any bleeds that may impede your ability to see where you're drilling.

22 Drill until all sides of the skull island move independently of the rest of the skull when lightly touched - don't press on the skull, but rather tap it to gauge how much more to drill.

22.1 Do not drill through skull! Instead thin the skull until it is very flexible - cortex damage will result in edema [aka swelling] and bleeding.

22.2 You can use drops of cortex buffer to look at the vasculature and estimate how much more you should drill.

23 Pre-fit window over the skull (dip it in cortex buffer first!!) island to see how much farther out you have to drill to thin down the skull for the outer window.

24 Thin the skull around the circle by gently drilling - thin until you can see brain vasculature throughout- a the skull and the skull bends when pressure is applied with forceps or the drill

head. This weakens the skull surrounding the craniotomy and creates a groove or slot for the outer glass circle. The thinned down, weakened skull allows the window to put the necessary pressure on the brain to help minimize brain movement in z during imaging experiments and also lessens the chance of dural thickening.

Side view, the outer glass circle sits in the groove surrounding the craniotomy.

25 Gently scrape the skull (with the exception of the circle) with the drill bit of the hand drill (or drill very lightly to create shallow grooves) - this creates a rough dry surface allowing glue and cement to better adhere to skull

26 Pre-fit head post just posterior to the edge of outer glass window. Thin the area of bone where threadbare will sit so that it fits nicely to the skull and is flush with the window. Do not glue the headbar down yet though!

27 Before performing the craniotomy, cover this area with gelfoam that has been soaking in cold cortex buffer and let it sit for about 10 minutes, or until the skull has completely absorbed the liquid (skull will be transparent when this happens).

28 When the bone has become translucent, use #5 (straight and pointed) forceps to carefully remove skull island. Be careful/gentle so as to not tear blood vessels or cortex - try to get forceps just under the skull (without damaging brain, or dura, or piercing any blood vessels) and then carefully and gently pry open skull and detach thinly attached edges.

29 Once skull island is off, stop all bleeds with cortex buffer rinses, gelfoam, and gauze.

29.1 Don't let the gelfoam dry! It will stick to the dura and could potentially cause cortex damage

when removed! Just be sure that the brain and dura stays moist at all times.

30 Once bleeding has stopped, place the glass window in a well with cortex buffer to rinse off ethanol and then place glass window on the brain. Lower the pressure applicator (cotton swab with pipette tip) gently and slowly onto the glass window - check pressure on glass with forceps (allows you to gauge how much lower you need to lower the wooden applicator). It's better to put too much pressure on the window than not enough, but be aware that too much pressure can break the cover glass.

30.1 If doing virus injections it's a good idea to check and make sure that the window will fit in to the craniotomy at this point. If craniotomy is too small for the window (when there's excess glue around the edges of the inner glass, it can make it difficult to fit the window, so try scraping off any excess glue first), very carefully chip away pieces of the skull until the window fits.

31 Remove excess cortex buffer with gauze or kimwipes. Leave layer of liquid just underneath the lip of the outer glass coverslip. The rest of the skull should be dry.

32 Using an insulin syringe, place small drops of vetbond around the edge of the coverslip helping to form a seal closing off the craniotomy to the air and gluing the the coverslip to the bone. Let the vetbond dry completely before proceeding to the next step! (3-5 minutes)

33 Zero the micromanipulator, and then raise the cotton swab.

34 Pace a small dab of krazy glue on bottom of head bar and hold head post onto skull in the desired position (flush with the window). Make sure not to glue fingers to head bar!

35 Let glue dry for a few seconds, and then quickly lower the cotton swab to reapply firm pressure on the window.

36 Mix dental cement with solvent and place a thin layer around the window and over skull/skin-margins. Be careful not to glue or cement wooden cotton swab to the glass. Also be careful not to cement the mouse's eyelids open.

36.1 The dental cement thickens quickly so you will probably need to mix more than one batch.

37 Lower iso to 1%.

38 Once dry (wait until cement is rock hard around the window; -10-15 minutes) slowly remove tapered cotton swab. Clean up window if necessary; scrape off any vetbond or cement with #5/45 forceps and clean glass with cotton swabs soaked in ethanol.

Recovery

39 Tum off oxygen and iso and remove the mouse from the nose cone

40 Leave mouse on heating pad until mouse exhibits righting reflex and starts to move around/attempt to escape. Administering buprenex too early can stop heart or elongate recovery process.

41 Administer ketoprofen and buprenex and record end time. If there was significant bleeding during the surgery - administer 1 to 2 mL of lactated ringers with dextrose subcuteneousty. Make sure to note end time and administration of drugs in notebook.

Note

Record end time:

42 Place mouse in back cage. Add procedure ("head post + cranial window and date") to the [temp] cage card and fill out blue post-op card.

43 Email -JF VivTechRequests to request that mouse receive 2 days of post-op ketoprofen. Send them the animal number, weight, and the date and time of all post-operative treatments thus far (i.e., buprenex, keto, and ringers) - use the provided vivarium post op. request email format.

Virus Injections

44 Place virus aliquot from -80 freezer into insulated box filled with ice.

45 Before starting the surgery - prepare an injection pipette.

46 Fasten metal plunger (with rounded edge pointed out) into injector.

47 Backfill a beveled pipette with mineral oil: insert micropipette with mineral oil into opening of glass pipette and move orange tip all the way down the shaft.

47.1 Start expelling mineral oil. Do not introduce bubbles! Do not move micropipette tip away from the end of the glass pipette until you see mineral oil climbing up the shaft. Slowly move micropipette tip out of glass pipette - all the while expelling mineral oil - do not pass the mineral oil that is climbing up the pipette.

48 Once pipette is completely filled with mineral oil and the micropipette has been removed - insert glass pipette onto the rounded edge of the metal plunger and slide pipette up the plunger. Fasten

glass pipette once glass end has passed into the space between the glass holder and the plunger holder

48.1 Use the canned air to blow away excess mineral oil from the glass holder on the injector. If there's too much oil, the glass won't be property secured in place and will move up and down along with the metal plunger, making the pippette appear to be blocked.

48.2 Make sure the tip of the glass pipette does not hold any air bubbles or blocked by dust. Expelling a bit of mineral oil with narshige wheel and wicking away excess mineral oil droplets carefully does the trick.

49 Fasten injector onto the stereotactic arm of the injector. Always be aware of the beveled tip of pipette - be careful not to break it!

50 Spin down virus. If doing any dilution of virus -add cortex buffer into virus aliquot, vortex and spin down.

51 Place parafilm over the tooth palate bar of the nose cone.

52 Pipette out ~2ul of virus onto parafilm covered palate bar.

53 Tum on sutter micromanipulator and press move. Position tip of pipette over the expected place of a drop of virus and lower until tip is just above the parafilm. Use fine setting on manipulator controller -look through the microscope focus on tip and slowly lower tip of pipette into drop of virus (be careful not lower tip too far and break tip against metal bar - as tip is lowered, always adjust focus so you can see tip - especially important once when tip is in the drop of virus.

54 Once tip is submerged in virus - tum Narishige wheel towards you - this will suck up the virus - do not move wheel too fast. Keep an eye on virus drop - make sure drop is getting smaller and pipette tip is always submerged in virus - do not suck up air!

55 Once virus is completely sucked up (or until no more virus can be sucked up without breaking tip or sucking up air), check pipette and look for meniscus of the virus against the mineral oil. Move injector upwards and move manipulator arm out of the way - be careful not to break pipette tip!

56 After skull island is removed and bleeding has been controlled, start injections.

56.1 Note: Before each injection make sure pipette is not clogged and virus can still be expelled. Before lowering pipette into the brain take note of zed value and use to adjust final depth accordingly (this will vary from injection to injection due to brain curvature and brain swelling).

57 Keep brain under cortex buffer or use gelfoam soaked in cortex buffer to keep brain moist. Position injector/pipette over brain. Lower until pipette is just over brain. Use fine movement setting on manipulator controller. Position pipette so tip is at the center of the craniotomy. Lower pipette to surface of the brain - zero manipulator. If injecting more in more than one location or in a grid, this is the center.

58 Slowly lower pipette into brain until the appropriate depth. Slowly tum wheel away to expel virus into brain. Recommended injection rate is 15 nl/min (25 ticks or 0-50 is 10nl). Wait about 40 sec before slowly raising pipette out of brain. If injecting in multiple sites, be sure pipette is all the way out of the brain before moving it in x/y. Avoid puncturing blood vessels.

58.1 if injecting multiple z positions start with the deeper sites