Aspiration Surgeries: dCA1

1. Supplies
	1. Autoclave bags
	2. Autoclaved tools/supplies
		1. 5mL syringes
		2. Scissors
		3. Fine-tip tweezers
		4. Drill bit - fine tip
		5. Scalpel holder
		6. Scoop
		7. Hemostats
		8. Yellow, green and purple needles - for aspirating
		9. 28 gauge needle (gray; bend tip for duratomy needle)
		10. Cotton swabs
		11. Dental applicators
		12. Set of gloves - to be sterilized
		13. Diaper pads (x2) - only use the ones that can be autoclaved
	3. Paper towels
	4. Rectal thermometer/heating pad
	5. Large gauge needles (blue) for 5mL syringes
	6. Nair/Veet
	7. Sterile saline - to fill 5mL syringes
	8. Gel foam
	9. Ice box - for syringes
	10. Isoflurane
	11. Drugs
		1. Meloxicam (0.2mL/mouse) - end of surgery
		2. Buprenorphine (0.1mL/mouse) - end of surgery once the mouse is partially awake
		3. Dexamethasone (0.1mL Dex: 0.1mL saline) - beginning of surgery
2. Once things are autoclaved, you can begin!
3. Fill 8-10 x 5mL syringes (large gauge; blue needles) with sterile saline
	1. Put in -80C for 5-10 minutes to chill them quickly
	2. Place them submerged into the ice box to keep them cold
4. Prep mouse (take weights, fill surgery log, etc.)
5. Administer dexamethasone (0.1mL Dex + 0.1mL sterile saline diluted in the syringe) I.P. and place the mouse in the induction chamber.
	1. This will take about 20-30 minutes to start working
6. Induce mouse at 3-3.5% - really knock them out
7. Place mouse in ear bars on a folded paper towel below their belly
8. Paralube eyes - big clump needed on stereotax #4 so the lights don’t blind them
9. Nair off any remaining hair from the previous surgery - it may be difficult to remove so just do your best!
10. Alcohol/betadine Q-tips x 3 alternating to sterilize skin
11. Dry out skull with Q-tips to remove any moisture
12. Tweezer to scrape a little of the periosteum away
13. Scratch up the skull with sharp tweezers or the drill bit on low speed - avoid bregma so you don’t make yourself sad.
	1. Clean up all this bone dust before drilling your craniotomy
14. Look for the craniotomy divots from surgery #1
15. Place drill in the drill sterotax attachment and level the skull
	1. Bregma with your drill bit within 0.05 front to back
	2. Check left to right flatness of the skull as well.
16. Go to your holes from surgery #1 and increase the depth of the four outer holes to make it easier for you to see
	1. -2.0AP, +1.4ML -this is the center hole
	2. Four external holes are 0.5 in each direction (north, east, south, west)
	3. Let the drill do the work, following the donut shape you’ve created with the tip of the bit.
17. Remove the entire arm of the stereotax to allow for the stereoscope to be placed directly above the skull.
18. Hand drill craniotomy using the four divots to guide your circle
	1. Ideally, you will be able to remove the circular bone disk using tweezers
	2. Use canned air to blow bone dust out of the area.
	3. Use duratomy needle to remove any remaining bone chips
19. Remove bone chips - wipe - remove - repeat
	1. Take your time with bone removal to save time later!
20. Once your craniotomy is clear and beautiful, check their breathing and begin
21. Grab your sterile, cold saline syringe in your left hand and the vacuum line in your right hand
	1. Open the vacuum on the wall to your desired whoosh noise
	2. Select your desired needle gauge (yellow, green or purple) depending where you are in the aspiration
22. Spiral clockwise outside of the hole to the inside, hovering gently over the tissue - then move counterclockwise back to the outside of the hole.
23. Make sure there is ALWAYS saline on the tissue before trying to suck it out
	1. Keep that bubble of saline constant as you remove liquid with the vacuum line
24. If the mouse is an early bleeder, it may be good to stop and apply gently pressure with a Q-tip for 1-2 minutes
25. Needles may clog with dura, bone chips, etc.
	1. Pause and clear the line out
	2. Try to remove the item with duratomy needle or tweezers or increase your needle size
26. ALWAYS check the paper towel to make sure the mouse isn’t swimming in saline and getting too cold.
27. If the mouse is bleeding further down, you can use a bit of gel foam
	1. Gel foam must be ‘activated’ by placing the tiny bit in sterile saline
	2. You can then put the gel foam above the craniotomy and use a tweezers/q-tip to push it into the hole gently.
	3. Before removing gel foam, wet the top of it with saline so it doesn’t dry and rip your tissue out.
28. Aspirate until you begin to see horizontal thick fibers (corpus callosum), these will transition to diagonal fibers quickly and the goal is to stop at the vertical thick fibers.
	1. To remove these, focus on the outside of the aspiration - you will be able to suck up the thick fibers and ‘pull them off like a bandaid’ unlike the tissue.
	2. Once you start seeing thick fibers, avoid the middle of the aspiration site and DON’T touch down with the needle.
29. Once you’re happy with your FOV, make sure they stop bleeding first
30. Place gel foam and wait 5-10 minutes to remove
	1. Make sure to hydrate the gel foam well before removing
	2. As long as bleeding has stopped and it’s relatively clear, allow the aspiration to air-out for 2-3 minutes.
31. Check breathing, add more paralube, etc.
32. Prep your lenses (dCA1) in the holder with the dummy camera
	1. Screw in gently like you would for recordings
	2. Screw in sterotax arm and attach holder
	3. Screw should be on the left side of the holder - left side of the animal.
33. Bregma the lens without damaging it
34. Move it over to above the craniotomy - (coordinates: -2.0AP, +1.4ML, -1.30/-1.35DV)
35. Drop a little saline bubble into the hole
36. Lower the implant VERY slowly into the hole and watch for bleeding
37. Lower to -1.35DV to account for bregma not being perfect
38. Use Q-tip or kim-wipe to mop up extra saline around the implant and the skull
39. Build your head cap
	1. Use Kwik-Sil to create a donut of silicone around the implant and the skull - prevents metabond from falling into the brain tissue.
		1. Let this dry a few minutes
		2. You don’t want too much of this all over the skull, isolate like a little ant hill around the implant
	2. Use a lot of metabond - like A LOT - and catalyst to start building the head cap.
		1. Build metabond up to the black portion of the bottom of the baseplate and cover the entire skull surface evenly
	3. You can switch to dental cement + mixed with black ink to finish the rest of the headcap.
40. Wait to dry completely - remove from the dummy camera carefully using tweezers and a will to live.
41. Place a dust cap on the base plate to keep everything clean and safe
42. Lower iso level to 0% to allow them oxygen
43. Admin your post-op drugs once the mouse has started to regain some movement.
44. Weigh mouse - finish surgical log book.
45. Place mouse in a clean recovery cage
46. Prep new cage with clean bedding, Dox diet (mixed into regular food), hut, nestlet
47. Administer post-op drugs in following days.