GRIN Lens Implantation Surgery

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Scientific Support Webinar Series

February 2023

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Webinar Series

- Deep dive of relevant topics for success with Mightex products such as OASIS Implant, Macro, Polygon 1000
- Current and soon-to-be users, distributors, core facility management teams, etc.
- Offer opportunity to meet with Mightex scientific team members and ask questions/ learn new tips and tricks to optimize data collection and experiment success
- Meet other Mightex product users (e.g., OASIS Implant, OASIS Macro, Polygon, etc.)

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Webinar Topics

- ❑ **OASIS Implant GRIN lens implantation surgery - today!**
- ❑ Headmount surgery and behaviour optimization
- ❑ Virus selection and infusion for calcium imaging
- ❑ Experimental data collection and analysis using PolyScan3
- ❑ Pattern generation methods for the Polygon 1000
- ❑ Closed loop control of the Polygon 1000

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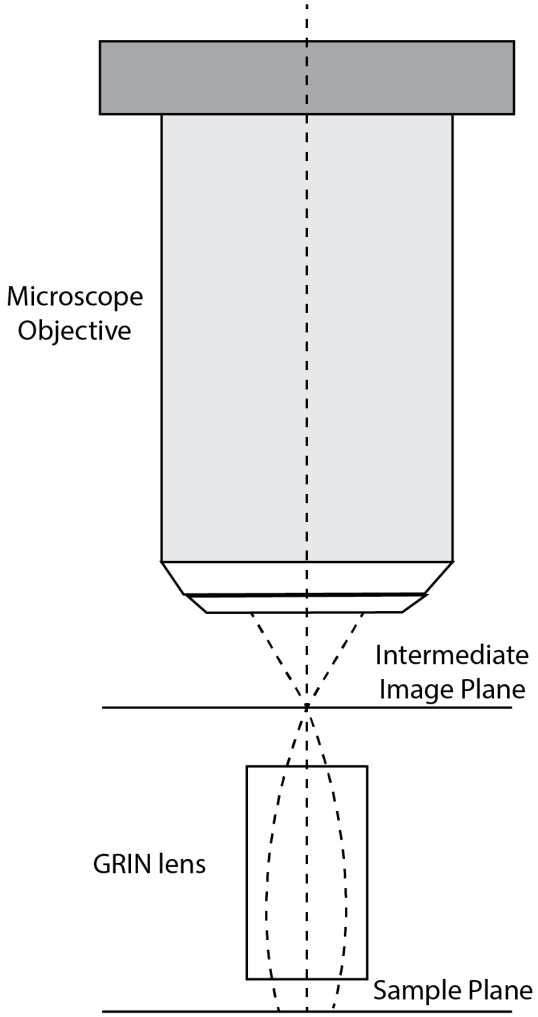
Purpose of GRIN lens



- Allows high magnification visualization of tissues that is not readily accessible from a regular objective
- GRIN lenses have a flat surface that allows them to be optically aligned with a fiber bundle to collimated output to a camera sensor for in vivo imaging applications
- Usually used for imaging below the surface of the brain
- **OASIS Implant works with 1:1 relay GRIN lenses**

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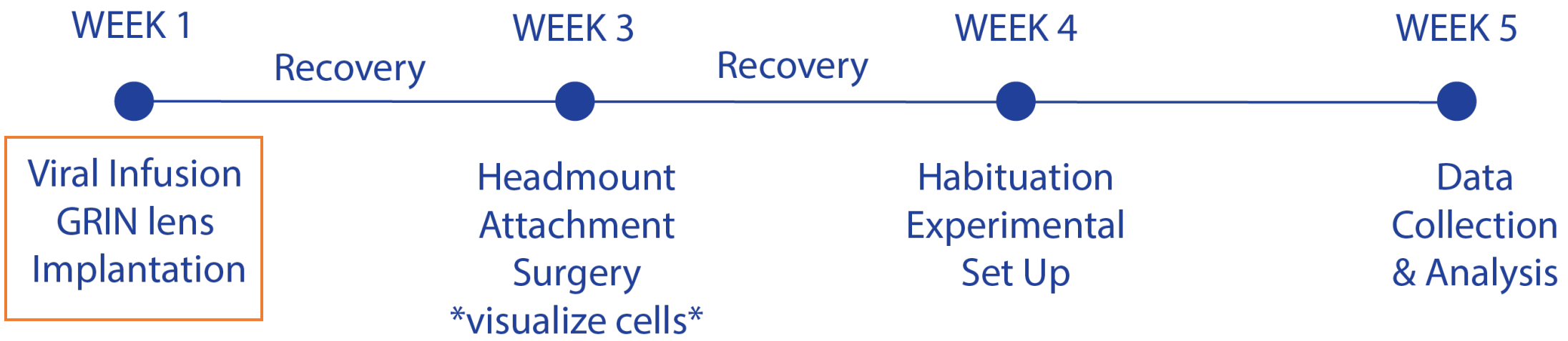
Experimental timeline



NB duration of recovery and/or transfection will vary across applications

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3.8mm length

1mm diameter GRIN
200um working distance

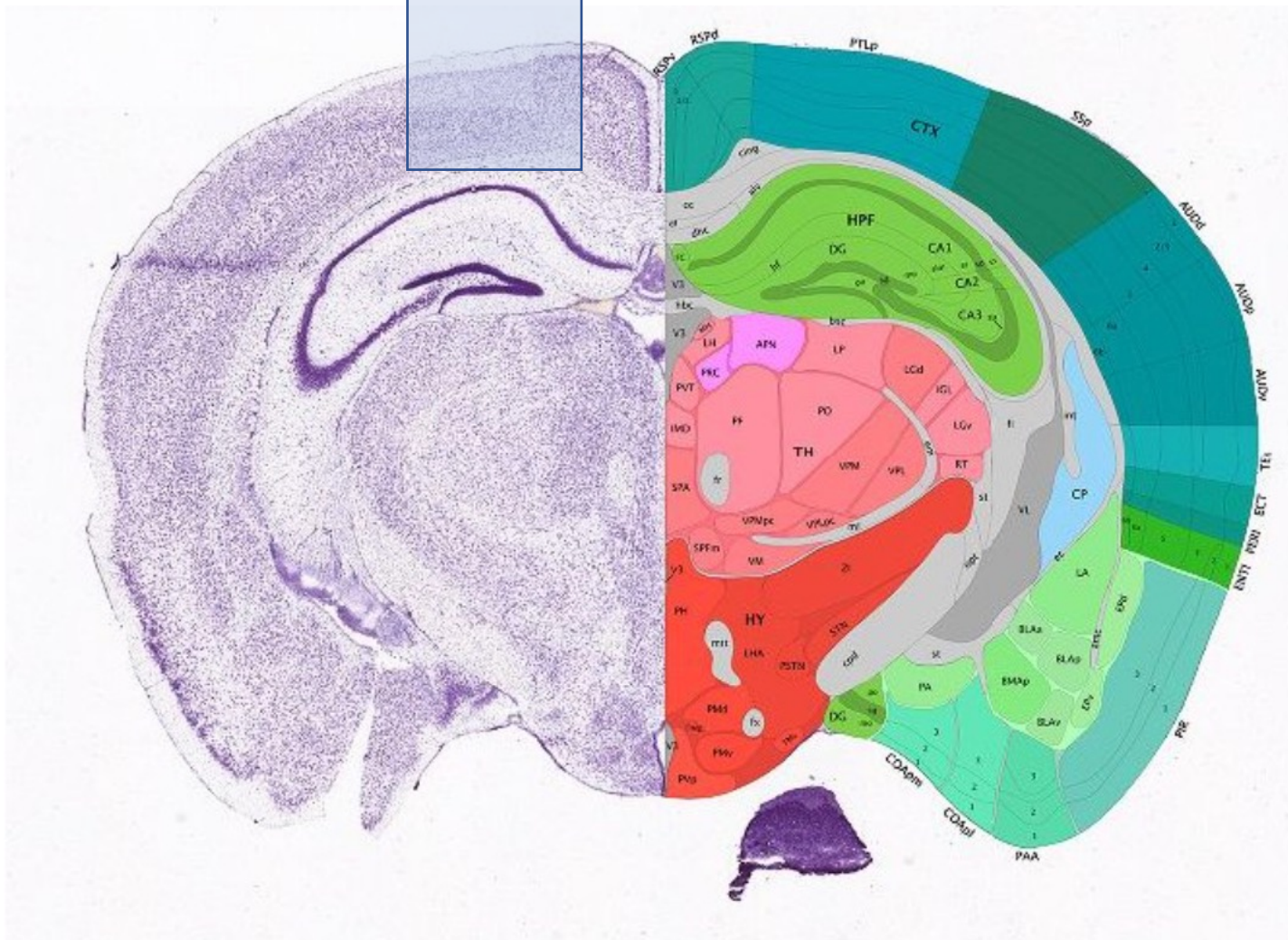

Image courtesy of
Allen Brain Atlas

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0.5mm diameter GRIN
200um working distance

7mm length

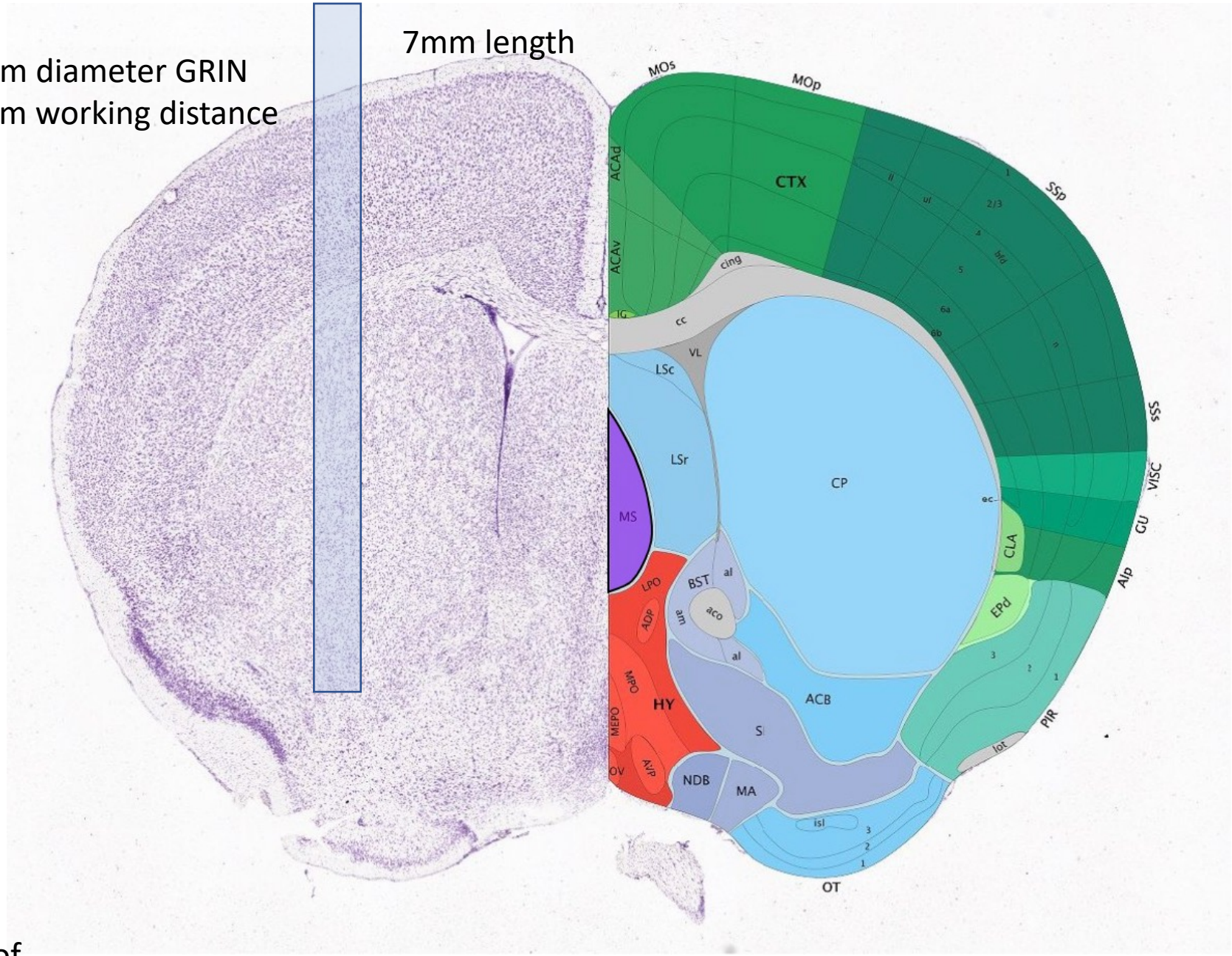

Image courtesy of
Allen Brain Atlas

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Standard Headmount

OASIS Implant

Ideally 2-3mm outside of skull

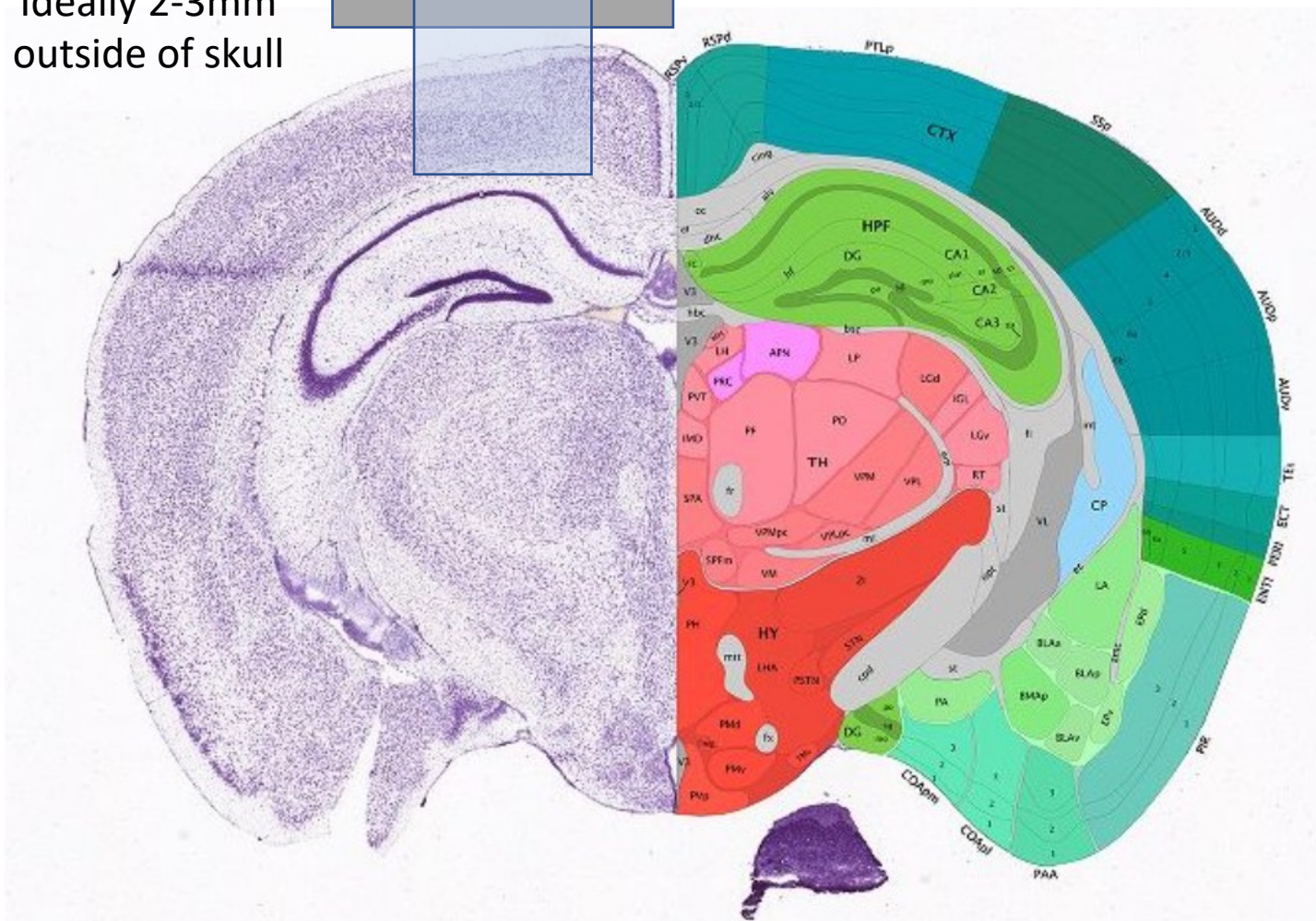

Image courtesy of Allen Brain Atlas

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Overview of GRIN lens surgery

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Overview of GRIN lens surgery

- Everyone does surgery slightly differently
  - Equipment and surgical set up
  - Brain region of interest
  - Additional hardware or techniques

- Best way to optimize your surgery is to practice, practice, practice!

- Important Notes:
  - Sterility is vital
  - Securing your headcap appropriately will ensure longevity of recording possibilities
  - Single house vs. pair housing (this will impact head cap and recovery)
  - Always follow AUP and surgical policies for your own institution/ lab; steps outlined here are for educational purposes and may not match your own AUP requirements

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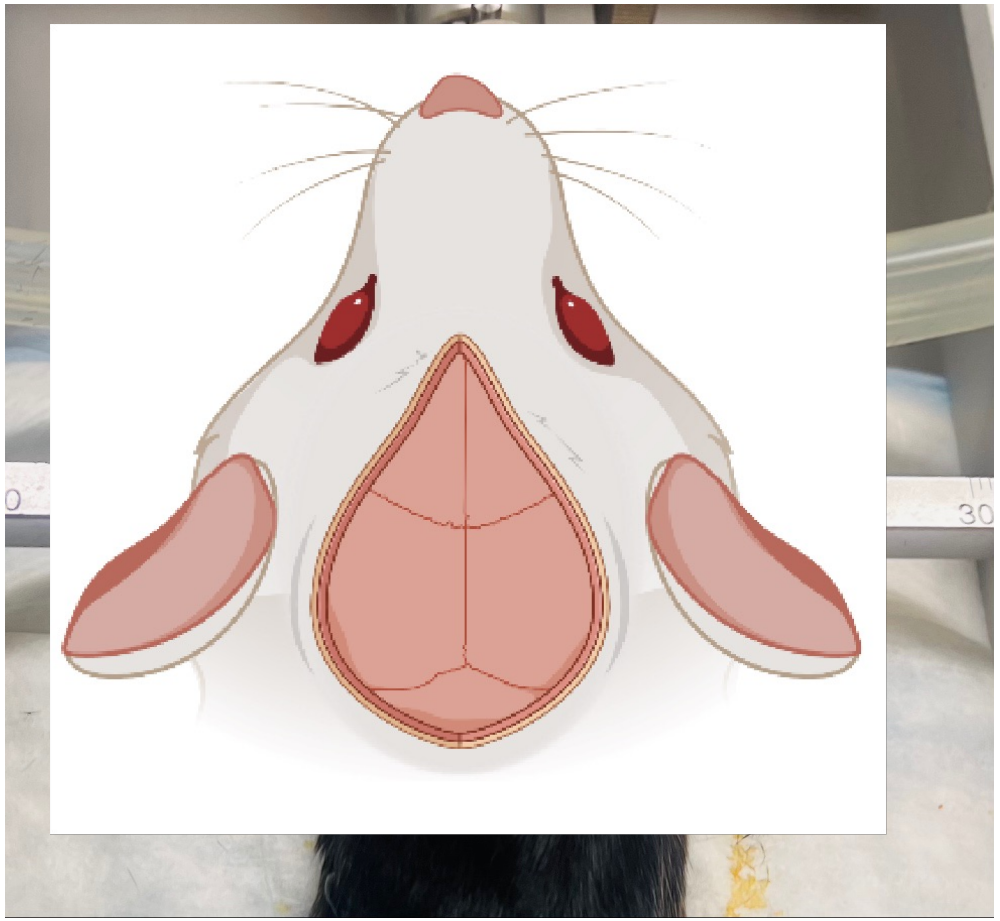
Clearing and preparing the skull



- Anaesthetize animal (isofluorane ideally)
- Head fix and level skull
- Administer IP saline and preoperative analgesic
- Remove fur and clean skin of head in circular motion (diameter ~2cm)
- Use surgical scissors and forceps to remove small circle of skin (1cm in diameter)
- Use surgical Q tips to scrub away top layers of fascia to reveal skull
- Apply cortex buffer* or saline solution to the skull and continue to clear away tissue

*** Recipe will be provided in downloadable resource from MyPage**

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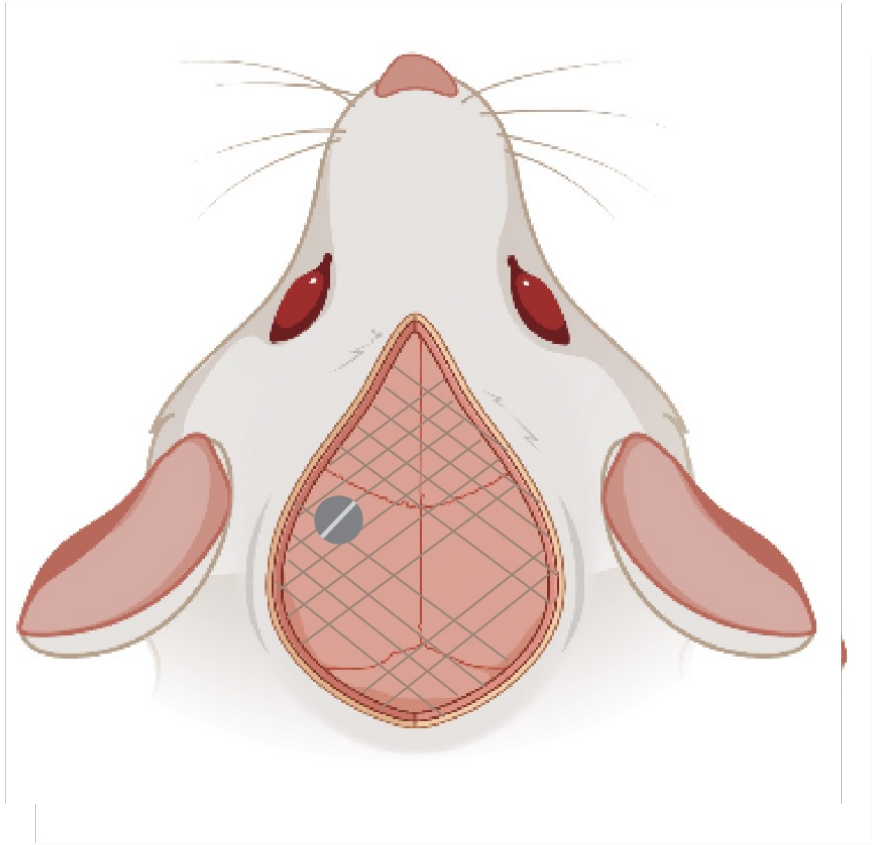
Clearing and preparing the Skull



- Apply1-2 drops of H_20_2 to remove stubborn fascia (leave for 30s then remove with saline and dry with Kim wipe)
- Score skull to create rough surface
  - Create some small dents with the drill for extra traction
- Contralateral skull screw for anchor point
  - Optional→ depends on location or duration of headcap

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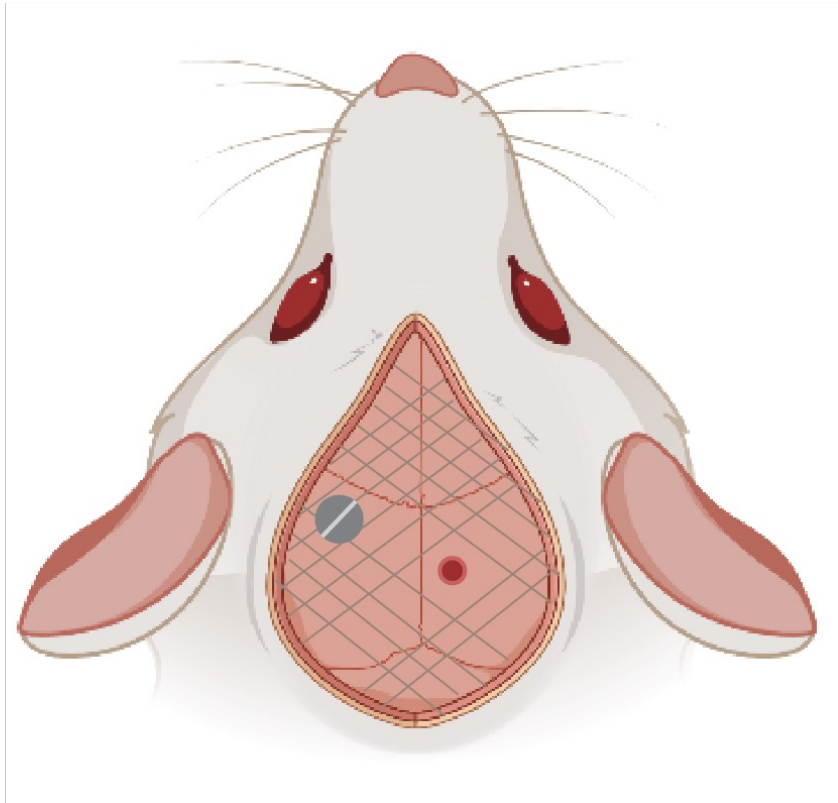
Viral Infusion (optional)



Viral transfection typically produces stronger fluorescent signal than transgenic mice BUT require additional wait time for viral transfection (~3weeks) and surgical steps.

- Can do viral infusion before GRIN surgery in separate surgery or as an early step in the GRIN lens surgery

- More information will be provided in separate webinar on how to choose as virus and transfection

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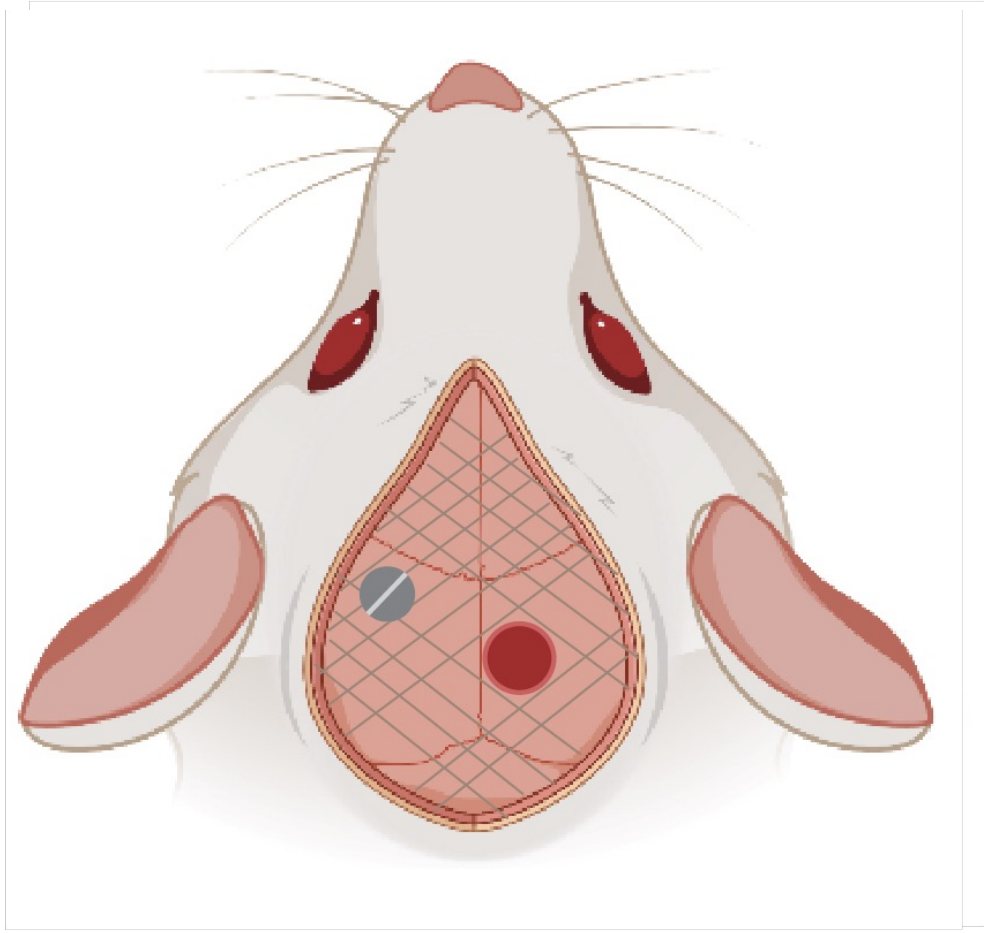
Craniotomy – large GRIN



- Locate coordinates of the center of your desired GRIN location
- Create small burr holes +/- 0.5mm of the center in AP and ML directions creating a small X shape
- Connect 4 burr holes together and remove center piece of skull; remove blood with Kim wipe, buffer, and saline
- Smooth jagged edges of craniotomy with drill bit leaving a circular smooth craniotomy that is very slightly bigger than your GRIN

NB make sure to drill directly down in DV direction, avoid sutures and midline

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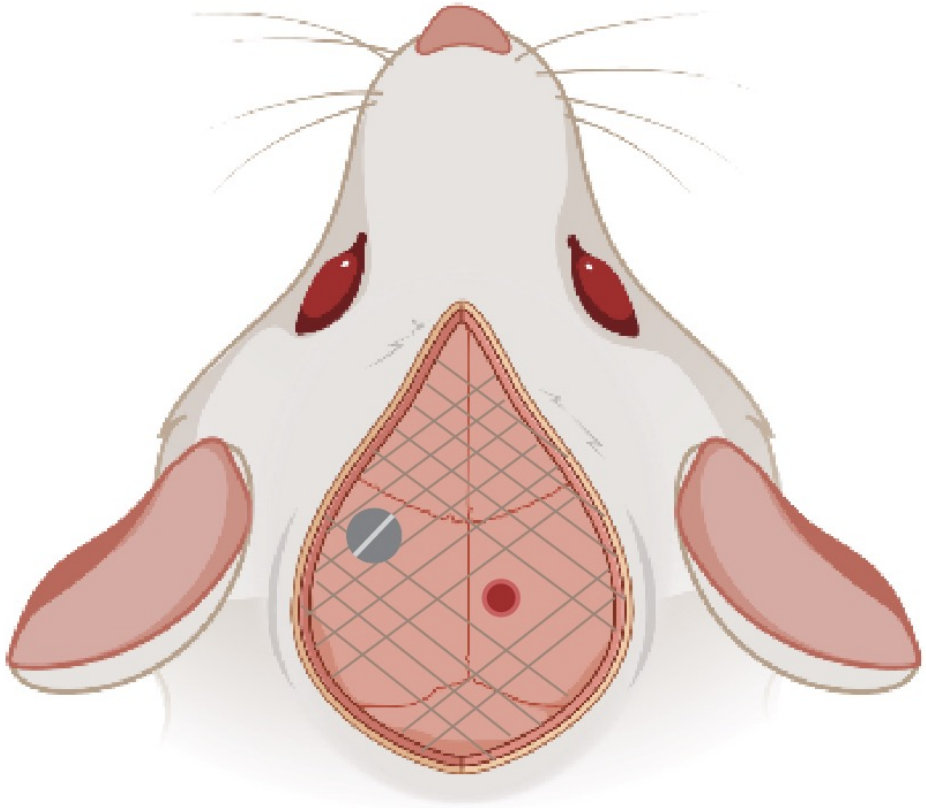
Craniotomy – small GRIN



- For small GRIN lenses you can make a center burr hole above the coordinates of choice and then expand by making smooth circular motions with your drill bit in the hole
- Again, make sure to smooth any jagged edges of the craniotomy and drill straight down

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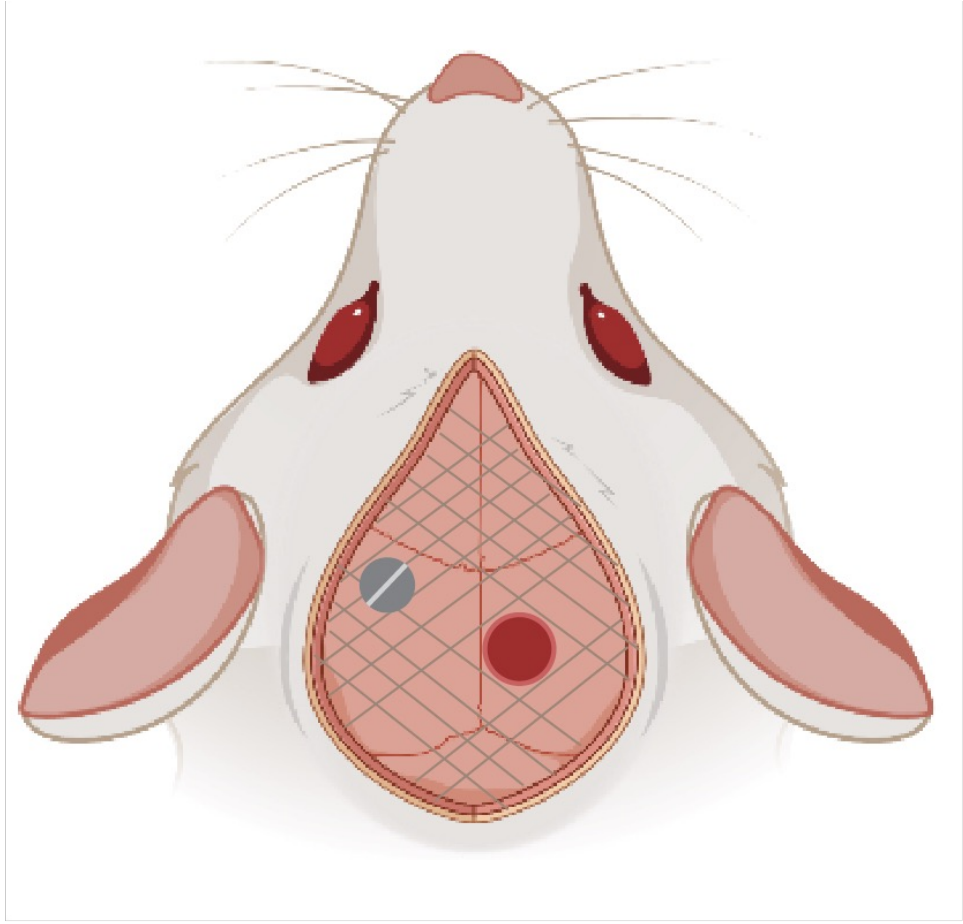
Craniotomy



- Using fine surgical forceps, clean the craniotomy and break through the dura
- Avoid touching any major blood vessels
  - E.g., at VTA there is usually a lot of bleeding
- If you see bleeding, you can use the corner of a Kim wipe to mop up. Apply saline to keep the tissue moist and to maximize visualization as you work

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For CA1 imaging, you can use the corpus callosum as a marker for your aspiration/tunneling

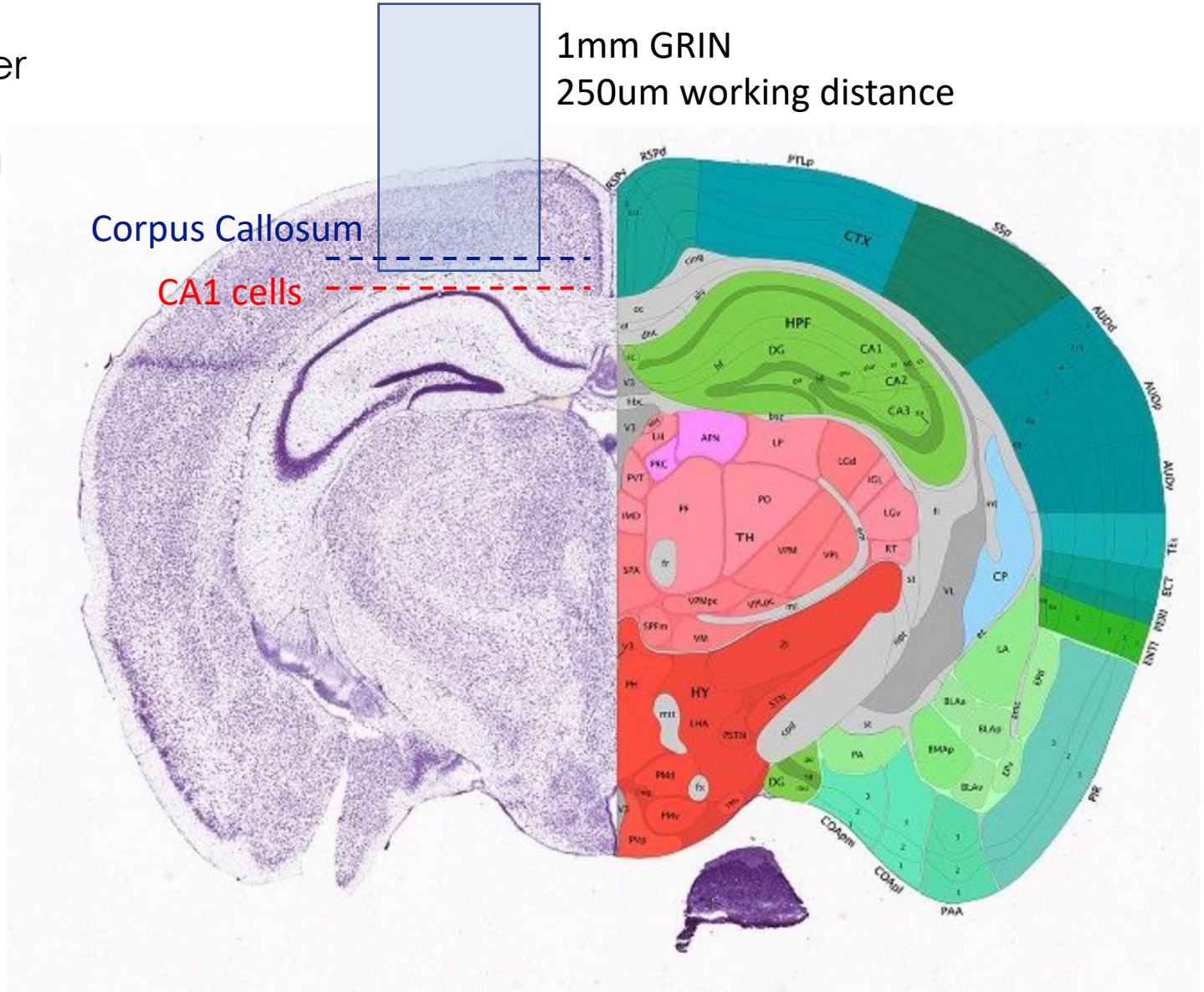

Image courtesy of Allen Brain Atlas

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Targeting deep brain structures

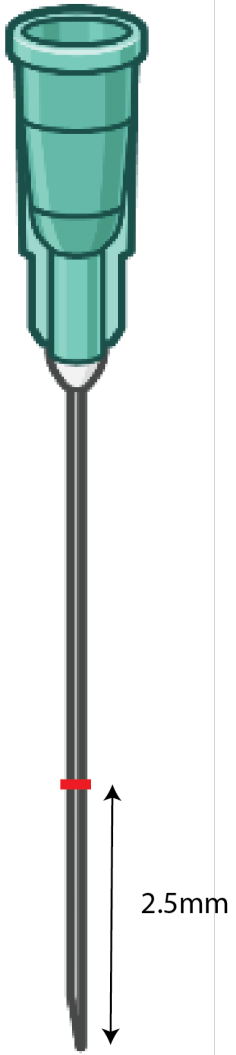
- Calculate the distance you will need to aspirate down and then mark this distance on your needle tip
- DV coordinates of target area minus 1mm above injection site

For example:

Target cells: -4.0mm

Infusion site: -3.5mnm

Aspiration: -2.5mm



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Aspiration vs. Tunneling

Aspiration:

- Removal of tissue
- More invasive
- Longer recovery/tissue clearing
- Necessary for larger size GRINs
- Can be beneficial at superficial level for targeting deeper structures
- More well-established technique for other applications (e.g., in vivo ephys)
- Provides better visualization of target area

Tunneling:

- Does not involve removal of tissue
- Less invasive, shorter recovery
- Only useful for smaller GRINs
- Can be used for superficial structures or when there is large range for DV coordinates

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What tools to use for aspiration?

- Vacuum line or pump
- Tubing and liquid trap
- 1ml syringe with small hole added
- Blunt end needles of various gauges (start with larger and move smaller)
- Hot glue and stopper (if building in lab)

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Aspiration process

- Ensure vacuum system is working (away from animal's head)
- Maximize light onto sample area
- Flood region with saline, holding a larger syringe (5ml) of saline in your less dominant hand to continually add saline as needed throughout aspiration
- Proceed slowly and with caution! Release vacuum if needed
- Begin moving the needle down slowly in circular motions while applying additional saline
- Move down in a straight DV direction (not on an angle)
- Stop and start, checking the visualization until you see a lighter colour, striped tissue of the corpus callosum
- Switch to a smaller pointed or angled needle tip, carefully peel back the corpus callosum with the needle tip and flush with saline; there should be no more bleeding and the surface of the brain shiny pale pink

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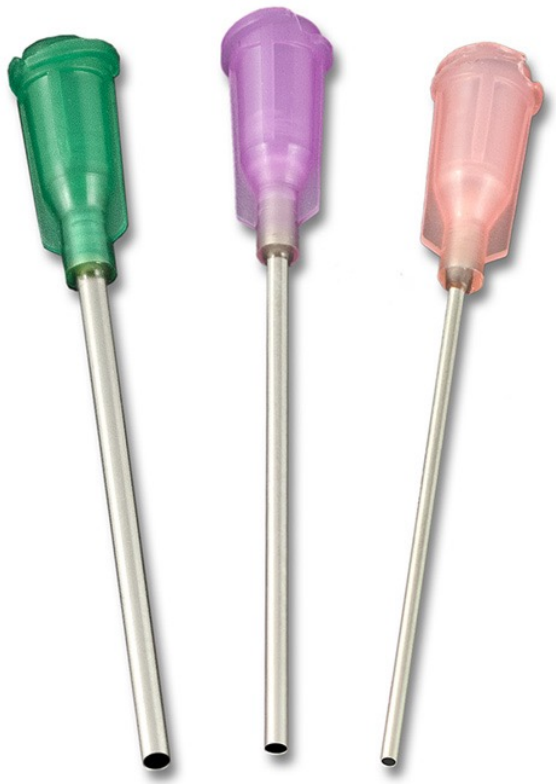
Tunneling process



- Use a mid-range blunt or cone-shaped needle tip (depends on GRIN size)
  - Start with larger then switch to smaller size when getting closer to target area
- Attach needle and needle tip to stereotax arm after breaking through dura
- Zero stereotaxic readout at top of brain and slowly lower the needle tip to your desired depth (as per previous calculation) while applying saline to surface
- Be careful to avoid major vessels
- Leave in place for 1-2 mins then slowly raise

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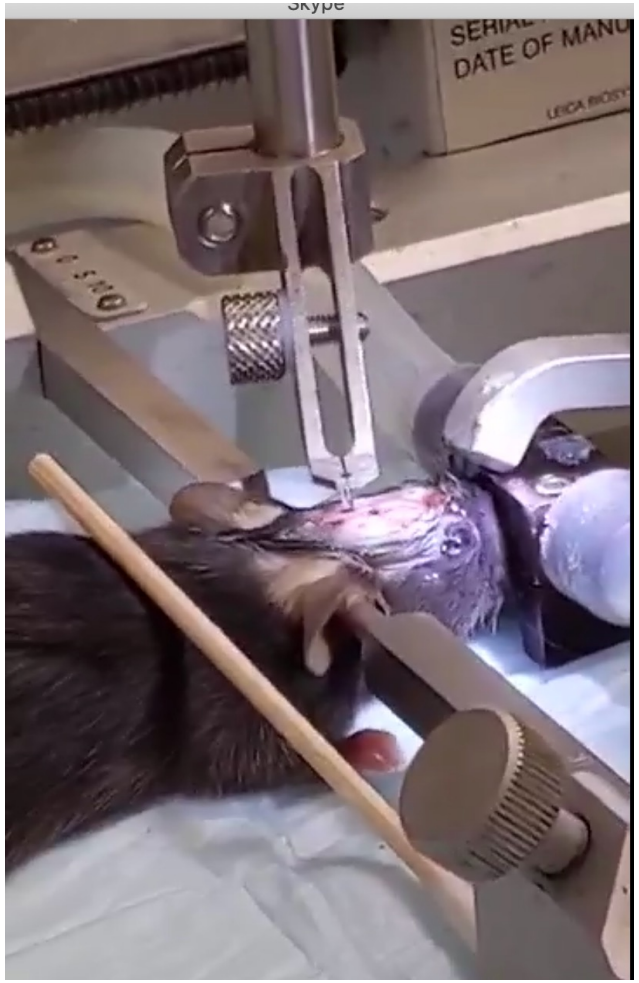
Lens preparation



- Clean GRIN in 100% ethanol to ensure sterility
- Use forceps to position lens in GRIN holder arm
- Tighten with Allen key screw once aligned vertically
- Affix arm to stereotax and lower to near top of skull

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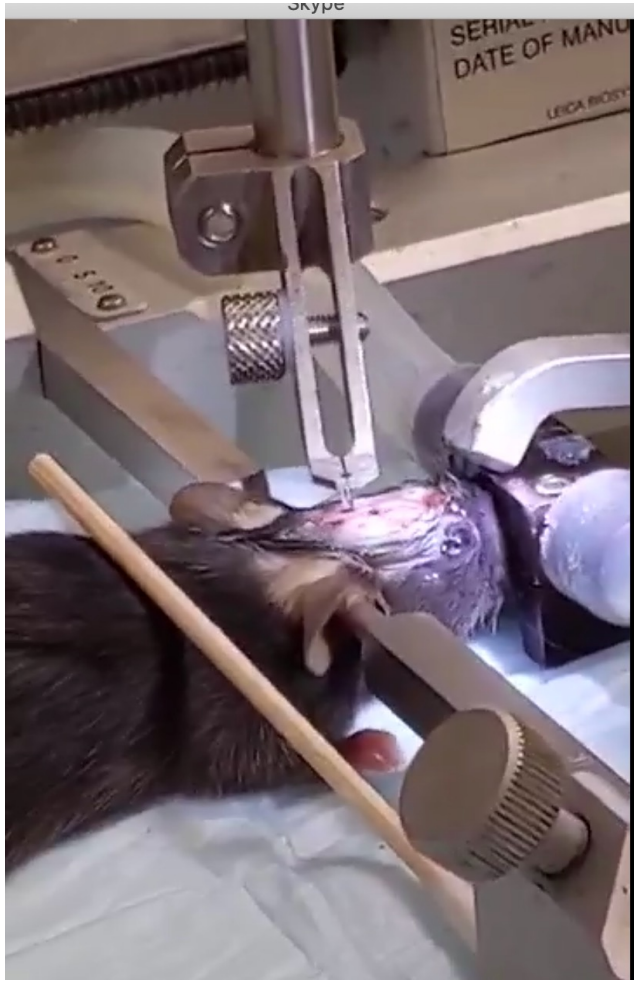
Lens insertion



- Position stereoscope so that you are looking at the AP direction of the skull (back view instead of top view)
- Clean craniotomy and dry out upper most surface
- Position GRIN centrally above craniotomy
- Zero DV coordinates of stereotax at top of brain (make sure your GRIN is in contact with tissue) and then lower by 100um
- Apply a tiny drop of glue around edge of GRIN (not touching the holder)
- Slowly and smoothly lower to your DV coordinates
- Apply thin layer of metabond around the GRIN; connecting to set screw if using
- Allow to dry completely (or cure with UV light)

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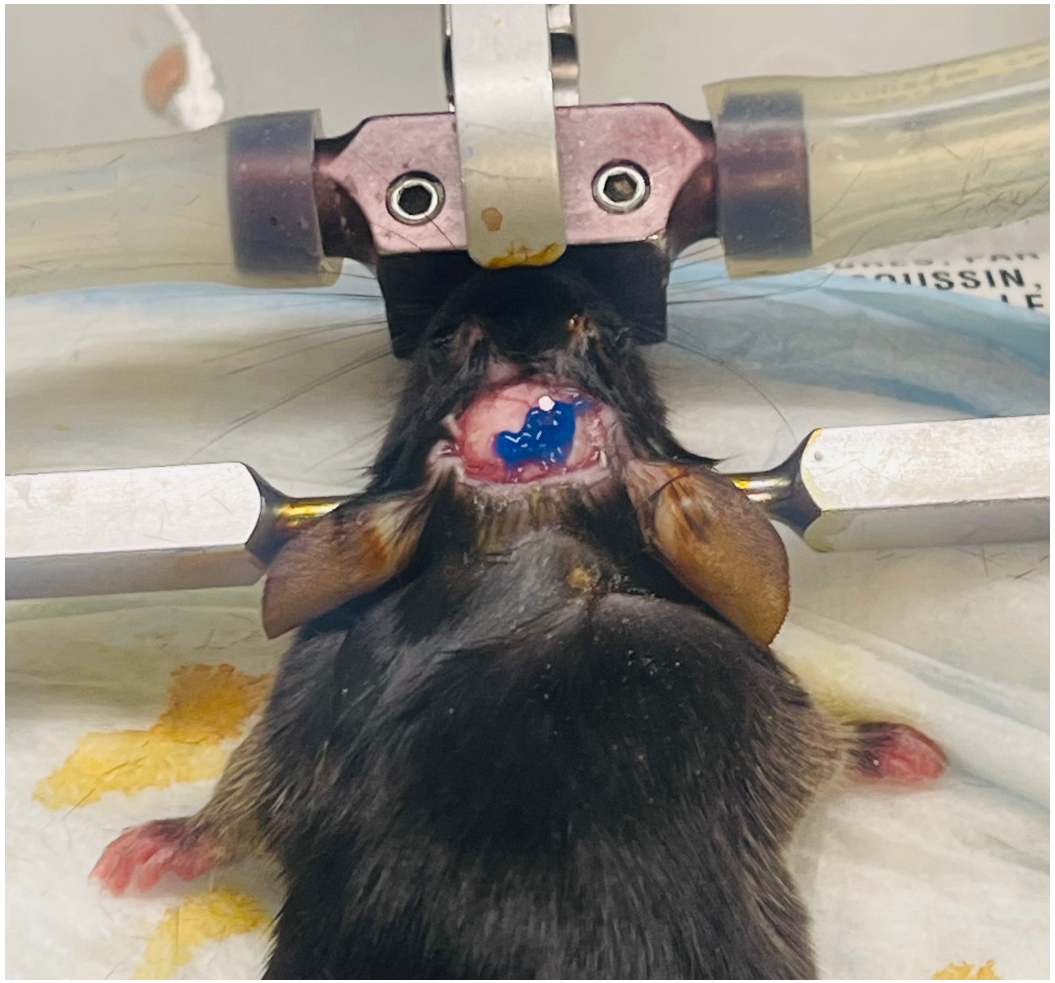
Securing the lens and gluing



- Once metabond and glue is dry, gently loosen the GRIN lens holder and remove; make sure to retract in DV plane first and then remove from stereotax once far away from the the skull
- Flip stereoscope back to top view
- Apply second coat of metabond to build up support further up GRIN sides and cover all exposed skull, make sure to not obscure the surface of the GRIN
- The whole surface of the skull should now be covered in metabond and the GRIN held in place up to 1mm of exposed GRIN above skull

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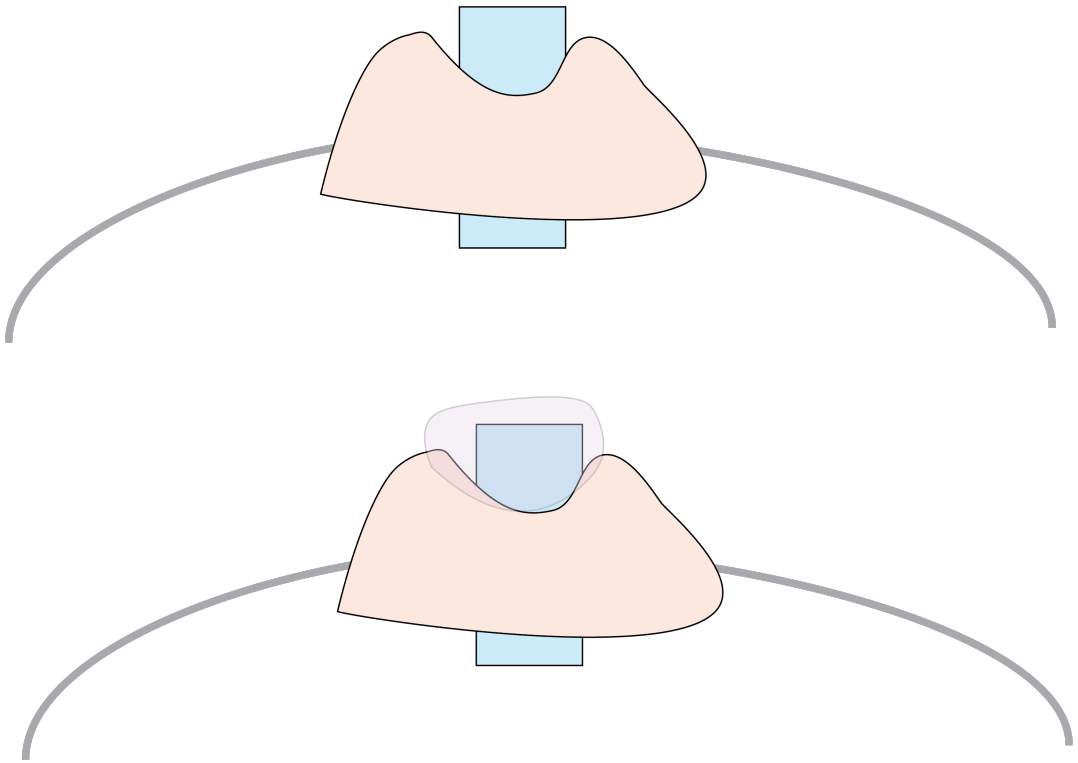
Creating a head cap



- Add dental cement over the existing layers of metabond. Build up a wall around GRIN to create a volcano-like shape with a well that includes the open surface of the GRIN. ~2.5mm of GRIN should be exposed
- You can then protect the exposed GRIN surface with Qwik-Sil
- Wait for all cement to dry completely before removing animal from stereotax

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Recap

- Skull preparation
  - Clean, score, screw
- Craniotomy
  - Approach dependent on GRIN size
- Aspiration or Tunnelling
  - Approach dependent on target areas
- Inserting the GRIN
  - Store in ethanol, use stereotaxic holder
- Securing and cementing the headcap
  - Metabond, dental cement, QwikSil

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Post-operative Care

- 3 days of analgesic (e.g., meloxicam i.p.)
- Monitor weight loss/ signs of stress for 7 days
- Hand feed or provide wet food in cage immediately after surgery
- Antibiotics if needed or part of your AUP
- Dexamethasone for clearing imaging window (if part of your AUP)

- Recovery 7-10 days
- Visualize cells 14+ days after surgery (once gliosis has decreased)
- Conduct next surgery to affix headmount

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Summary

- GRIN lenses allow cellular resolution calcium imaging of neuronal populations not visible from surface of brain
- GRIN lens surgery involves:
    1. Skull prep and craniotomy (viral infusion optional)
    2. Lens insertion
    3. Lens securing and headcap
    4. Recovery
- Sterile surgical field is important
- Individual differences with each experimental design
- Practice, practice, practice!

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Available Resources

- Surgery Primer – will be available on MyPage
- Webinar recording

- Calcium Imaging Guide
- OASIS Implant product focused webinars via website and MyPage
- Future webinars
- Support team – contact us!

Webinar on headmount surgery (next!)

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Thank you!

Webinar and accompanying primer will be available on MyPage

Further questions: Catherine.Thomas@mightex.com

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