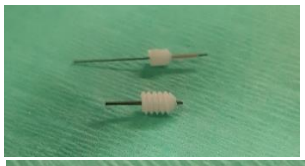
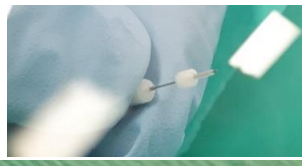
Technical material

Stereotactic viral injection

Current approaches of in vivo Calcium-imaging use viral expression of gene-encoded Calcium indicators to mark active neuronal populations in a region of interest. This technique achieves particular specificity by linking the transcription of the exo gene to the cell line-specific expression of Cre-recombinase in gene-modified model organisms. Below we provide a short guide to viral infusion. The procedures described to transfect the brain region of interest are to be performed under Biocontainment Level 2 precautions. All surfaces, tools and materials have to be efficiently disinfected or autoclaved if the material permits.

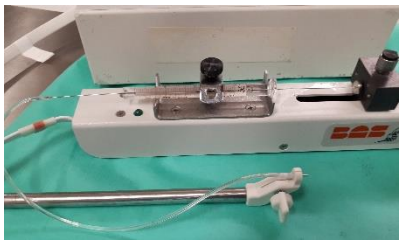
Viral loading to microinjection pipette (Under BCL II BSC)

1. Glue Cannula to guide cannula with viscous superglue. Leave to dry overnight.


2. Cut two fingers length of tubing and flush with saline using a 3ml syringe with 27 G needle.
3. Attach tubing gently to the cannula (by tweezing).
4. Flush again with saline check flow.5. Prepare a 10 µl Hamilton syringe by checking smooth movement of piston (If piston does not move smoothly, soak it in cornoil, insert in syringe and move it slowly back and forth until a smooth movement is established).
6. Fill Hamilton syringe with 10 µl Saline and gently attach the tubing (Use a 1.5ml Eppendorf tube to draw saline).
7. Push piston to 1µl mark and check if excess saline leaves injection cannula.

8. Draw 1 µl of air into tubing (The piston is now on 2µl mark). Check that an air bubble form formed inside tubing (Separation between virus and saline).
9. CL2 hood: Draw 5µl of virus into tubing (Make sure cannula is immersed into virus).10. Conseal virus by drawing in 1µl of air into tubing, check bubble.
11. Attach cannula to unattached stereotactic arm with cannula holder.
12. Insert Hamilton syringe in precision microinjection pump.



Preparation of aspiration set up

GOAL:
REQUIRED INSTRUMENTS AND MATERIAL:

HOW TO SETUP:
1. Connect a Büchner flask (vacuum filtration flask) to a vacuum pump (Thermofisher Model 420-1901-0001 or similar) or to laboratory valve to prevent biological substances entering pump.
2. For flow regulation connect Buchner flask to infusion port (piggy back) or infusion roller clamp for with adequate tubing. It is also possible to control the aspiration strength by introducing a hole in the side of the syringe and manipulating it with the finger. [ADD PHOTOS]

3. Connect infusion port to 1ml syringe. [ADD PHOTO].
4. Prepare a good dozen of 27 G blunt needles by bending them to a working angle of approximately 22 deg. Keep them ready on sterile surface.

5. Alternative: If using 21 G Hamilton needles it is possible to directly fit them into the tubing without using the 1 ml syringe.

Pre-surgical preparation of subject

1. Measure and note mouse weight for proper dose of analgesic and post-surgical control of weight loss.

   Recommanded analgesic is ketoprofen (dose: 5mg/kg at a concentration of 1mg/ml Exp: mouse 30g. 0.03kg x 5mg/kg = 0.15 mg, 0.15mg x 1ml/1mg = 0.15ml).

   The animal care requires the mouse weight before surgery to be able to monitor the animal recovery after surgery.

2. Anesthetize mouse in induction chamber:

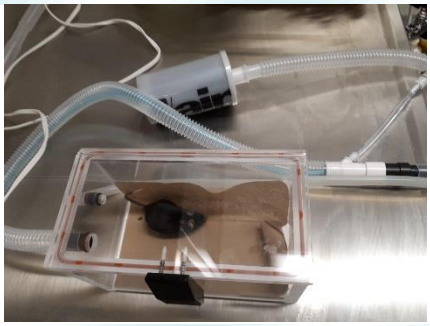
   Add tissue to the induction chamber to decrease animal stress and also keep the chamber clean.

   Start oxygen flow at rate 1l/min before releasing isoflurane [ADD PHOTO].

   Check to ensure Oxygen is flowing into the chamber by ensuring the tubes are properly connected and the oxygen valves are open.

   use isoflurane vaporizer at a flow rate of 1l/min with 4% isoflurane [ADD PHOTO].

3. Check animal being anesthesized by pinching the toe. Animal should be responsive. It usually takes 2 to 3 minutes for animal to be fully anesthesized. Do not leave animal unattended.



4. When fully anesthetized, switch isoflurane flow to nose cone. Place mouse on sterile pad and keep anesthetized at flow rate of 1l/min with 2% isoflurane. [ADD PHOTO]

5. Apply lubricant to protect the eyes.

6. Inject 0.5 ml warm saline (s.c.) for hydration and ketoprofen (5mg/kg s.c.) for analgesia during the surgery. [ADD PHOTO]

4. Shave mouse head from between the eyes to hind head. [ADD PHOTO] Remove lose hair with sterile gaze. Three alternating swipes with 70% ethanol and betadine may be applied at this point or after mouse is fixed to stereotactic frame (See below).

Craniotomy

1. Switch isoflurane flow to nose cone of the stereotactic frame.
2. Transfer mouse to stereotactic frame, and attach bite bar and nose cone.
3. The mouse must be placed on a heat pad.
4. Keep mouse anesthetized under 1l/min, 2% isoflurane. Continuously pay attention to the breathing of the animal and change the level of isoflurane accordingly [ADD MORE EXPLANATION]

5. Attach ear bars. Ensure the head is properly fixed by gently pressing on the head of the mouse. There should be minimal movement of the head.

5. Make sure the eyes remain well lubricated with eye gel throughout the surgery.

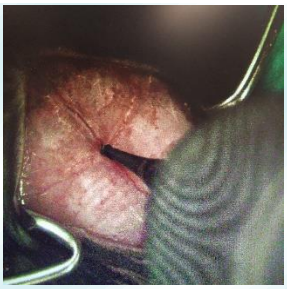
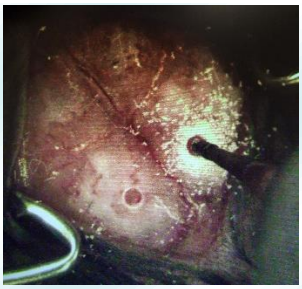
7. Control the body temperature by letting the body rest on a heat pad (using a temperature probe is suggested, though not necessary).

8. Wipe the head of the mouse with 3 alternating swipes of betadine and 70 % ethanol with a sterile cotton swap (This step is done here because the transfer to the stereotactic frame may lead to breach in sterility).

9. Test full anesthesia by checking unresponsiveness to foot pinch.

10. Expose the skull by making an incision along the midline with a scalpel then use retractors to keep the surgical area free from skin. (If deemed necessary, swipe the skull with a cotton swab dipped in 3% hydrogen peroxide to better expose bregma).

5. Move the stereotactic arm to place the tip of the sterilized drill to bregma. Make a first visual adjustment of the skull position both in medial lateral as well as anterior posterior direction.
6. Set bregma coordinates to zero then move posteriorly to lamda and measure the Y axis coordinates. If bregma and lamda are not leveled change the position by manipulating the bitebars vertically. Repeat process until planarity of axis is established. The usually accepted difference is below 100micron.

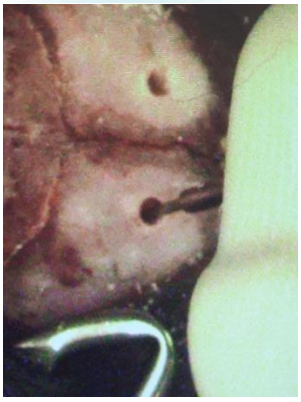



7. Raise the drill off the skull surface to mark bregma with fine sterile mark.
8. Move the drill above the injection coordinates of the CA1 region of the dorsal hippocampus (dHPC CA1; AP: -1.9 mm and ML: ±1.4 mm). Set the drill in motion before making contact to the skull! Then lower the stereotactic arm and perform craniotomy without damaging the dura mater. Use sterile saline soaked cotton tips to remove the debris.
9. Remove the drill from the frame.

Intracerebral viral infusion

1. Install microinjection pipette in stereotactic frame and move pipette tip to the mark on bregma by manipulating the stereotactic arm. Set coordinates to zero then move micropipette above one of the respective injection sites (AP:- 1.9, ML: ±1.4).
2. Carefully exhaust air from mircropipette until virus reaches the tip. Remove virus from the tip with sterile tissue paper, spray with virox and dispose in biohazard bag.


3. Gently lower the micropipette into the brain to the depth of CA1 (DV: -1.38 mm below bregma).

4. Infuse 1µl of the virus into the dHPC CA1 at rate of 0.2 µl/min for 5 min. During the infusion track the bubble in the tubing between virus and mineral oil/saline to confirm that the infusion is working properly and the volume of virus in the micropipette is decreasing.
5. Leave the micropipette in the brain for an additional 10 min after the end of infusion to avoid drawing back the virus in to the tissue above.
6. Raise the micropipette gently, move it to next coordinates after checking that it is not clogged.
7. After repeating the infusion procedure at the second hemisphere remove micropipette from the brain. Use cotton swabs to clean the skull around the craniotomy.
8. Close the incision with 3 simple interrupted sutures (nonabsorbable #4-0) and apply antibiotic ointment to the sutured incision.

Post-OP

1. Hydrate the animal by injection of 0.5 ml warm saline (0.9%, s.c.).

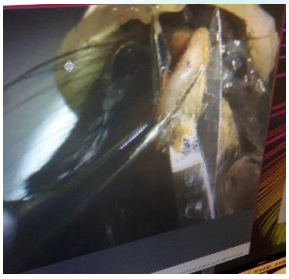
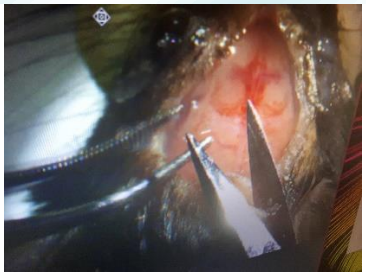
2. Place mouse in a clean cage on a heating pad and monitor until the animal is fully recovered from anesthesia.
3. Monitor mouse postsurgically for 3 days, if mouse is not recovering well, hydrate the mouse. If the animal exhibits signs of distress administer analgesic (ketoprofen 5mg/kg s.c.).Remove sutures 10-14 days after surgery.

Bilateral lens implantation

Here we provide a short guide to the implantation of GRIN lenses in the brain. The details may vary depending on the brain region. Here we use describe the procedure as used for the CA1 region of the dorsal Hippocampus. To ensure the good health of the animal sterile working procedures have to be met. This procedure is done two weeks or more after virus injection to ensure good expression of GCaMP in the target region.

Pre-surgical preparation

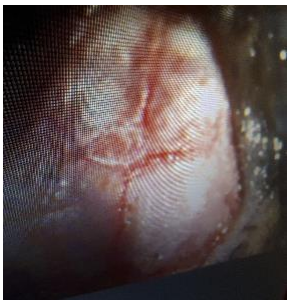
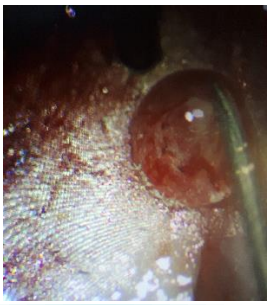
1. Follow pre-surgical steps as described above with the following addition, administer dexamethason (2 mg/kg, s.c.) in order to suppress inflammatory tissue responses during surgery.
2. Install the mouse in the stereotactic frame, as described above for the virus injection, sterilize the shaven head with 3 alternating swipes of 70% ethanol and betadine.
3. Excise a round area of skin of approximately 5 mm in diameter from the skull exposing bregma, lambda and the target region.
4. Remove the periosteum by cutting it off with scissors or scrubbing with a scalpel and then clean with saline soaked cotton swaps.

5. Delicately bond the edges of the skin to the skull with VETBOND tissue adhesive using a pipette tip and let it dry for 5 min (Applying Vetbond to the whole skull while leaving out the area around the craniotomy site increases the strength of dental acrylic adhesion to the skull).

Craniotomy

1. Ensure bregma and lambda are planar using a thin needle attached to the stereotactic arm as described for the virus injection above (WHERE EXACTRLY), if necessary adjust AP axis.
2. In order to perform craniotomy move the stereotactic arm above the target coordinates and mark the position on the skull (Example of coordinates AP: -1.9, ML: ±1.4).
3. Use a hand held drill to draw a superficial outline of the craniotomy of about 1.5mm in diameter. Use of a ruler is recommended. Proceed under visual control using a surgical binocular microscope.

4. Following the outline gently weaken the bone with the drill leaving a thick area in the center. Regularly apply saline to the site as this helps soften the bone.
5. Check if bone is equally weak and thin all around by applying mild pressure on the edges of the center then pull off the central part with a fine curved tweezers in a single move.
6. After completion of the bilateral craniotomy clean the debris with sterile cotton swabs soaked in saline without contact to brain tissue.

Leading track (0.5 or below lens diameter)

1. To create a leading track in the brain tissue for a 0.5 mm lens load a 10 µl syringe (with 30-G blunt needle) with 5 µl saline.
2. Move the needle tip to bregma and set coordinates to zero or write down the coordinates. 3. Set the needle to the stereotactic coordinates above the cranial overture (AP: -1.9, ML: ±1.4).
4. Gently lower the needle at a speed of 1mm/min to 0.1mm above the depth of lens implantation (DV: -1.18).
5. Remove needle from the brain at 1mm/min

Ablation procedure (above 0.5 mm lens diameter)

Note: An adequate ablation procedure has to be adopted according to your best scientific judgement. Anatomical and veterinarian considerations need to be met in order to ensure the survival, behavioral viability of the animal and the reproducibility of the experimental paradigm.

1. For the 1 mm diameter lens use 21 G blunt needle attached to pump in order to create mild negative pressure (~13.5 psi).
2. Apply constant drops of saline to the edge of the ablation site (By hanging a saline bag or a 60 ml syringe overhead connected to a 1ml syringe with a regular 21G needle via infusion tubing).

3. Gently lower the ablation needle at a speed of 1mm/min. Slightly retract the needle after each 20 micron step in order to let saline unclog the needle tip. Continue to insert the needle into the tissue in 20 micron steps until reaching 0.1mm above the depth of virus injection (DV: -1.18).

4. Turn off the pump.

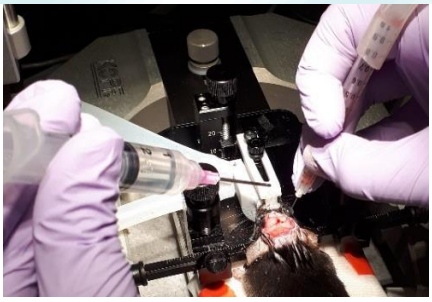
5. After 1 min, retract the needle from the brain at 1mm/min.

6. Cover ablation site with a drop of saline or saline-soaked sterile foam).

The preferred ablation method is performed by using a hand-held 27 G blunt needle connected to the pump to gently aspirate the tissue under visual control. It necessitates the use of a surgical binocular.

1. Ensure to remove the meninges with a needle or a fine-tipped tweezers before proceeding with the ablation. The meninges will otherwise impede a proper ablation of the cortical tissue.

2. The tissue will be ablated by picking at it with the 27 G aspirating needle in a ~1mm circle around the center of the ablation



3. Constantly administer of drops of saline or aCSF above the ablation site to facilitate aspiration and to prevent cortical damage beyond the actual ablation site.

4.If clogging of the needle tip occurs replace needle (Always have plenty of replacement needles at hand. Cleaning it by using a second 1ml syringe and blowing the debris out is also a possibility).

5. The appearance of the white matter of corpus callosum serves as the visual landmark for ablation.

6. In case the lens is supposed to sit below the corpus callosum keep on removing the white matter until slightly darker tissue appears.

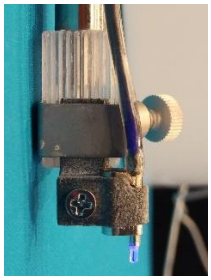
7. Stop the procedure there, the removal of the white matter exposes the surface of the tissue below that needs to stay intact.

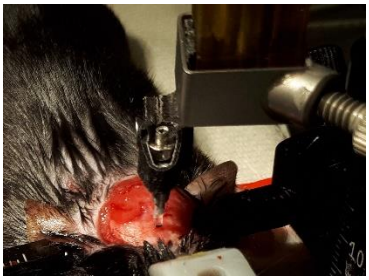
8. After the procedure, apply saline or aCSF to the ablation site. Do not allow brain tissue to dry. Remove excessive fluid with sterile cotton swab avoiding direct contact to ablation site.

9. If bleeding occurs, repeat application of saline or aCSF and remove fluids with cotton swap or aspiration needle until incision site is free of blood (using ice-cooled saline will constrict cerebral vessels and help quench the bleeding).

Lens implantation

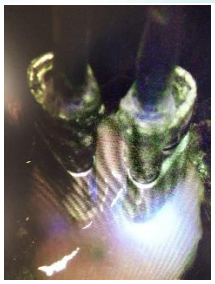
1. Attach the lens via its connector to the electrode holder (Model 1770, Kopf) of the stereotactic arm using the Piccollo surgery accessory tool. Ensure lens is aligned to the stereotactic arm.





2. Locate the lens tip to bregma, set to zero or write down the coordinates. Move the lens to the stereotactic coordinates (AP: -1.9, ML: ±1.4) above the insertion site. Insert the lens very slowly into the leading track (at 1 mm/min) towards target coordinates (DV:-1.28).

3. If lens is connected to the imaging device the insertion depth can be fine-tuned by monitoring GCaMP fluorescence intensity while lowering the lens below the final coordinates of the leading shaft. Delicately remove excess artificial cerebrospinal fluid and blood from around the lens with sterile tissue paper without touching the cortical surface.

5. Let the skull dry for appr.5 min.
6. It is suggested to use bone wax to close gap between lens and skull.

5. Apply a layer of dental cement over the exposed skull and all sides of the lens connector. Wait for adequate time to allow cement to cure. Check hardness of cement with a tweezers before proceeding.

6. It is suggested to use an additional a layer of black dental acrylic cement to prevent external illumination of GCaMP.

7. Loosen the Picollo surgical accessory holder diligently by opening the screw on the back of the holder. Spread the band of the holder slightly open in order to release the connector from the stereotactic arm. Make sure that the holder does not stick to the connector. At no moment any pull should be applied to the implant while retracting the stereotactic arm.

8. Cover the lens connector with the custom-made protective cap. Repeat the implantation procedure for the other hemisphere.

Post surgical

1. After completing the surgery administer 0.5ml of warm saline (0.9% s.c.). Apply antibiotic ointment (Polysporin) and local anesthetic ointment (Lidocain) to the edges of the skin.

2. Remove the animal from the stereotactic frame into a clean cage on a heat pad and monitor closely until fully recovered from anesthesia. Keeping the half of cage on a heat pad overnight enables the animal to warm itself in case its body temperature drops during recovery.

3. Perform post-surgical monitoring and administration of analgesic (Ketoprofen; 5mg/kg and 1-2 mg/kg Dexamethason each 24 hours for 3 days.

Materials:

Mouse:

Wild type C57/B6 or Cre-recombinase-dependent strain
(e.g. VGAT- Cre, Pv-Cre, CCK-Cre, Gad-Cre e.c.t.)

Adeno-associated virus:

For example; AAV1.CAG.Flex.GCaMP6s.WPRE.SV40, 10^{13} GC/ml titer, 300 - 500 nl,
(University of Pennsylvania core facility)

Drugs, fluids and desinfectant:

- Isoflurane (Henry Schein, 1182097)

Technical material

- 100% oxygen
- Lubricating ophthalmic ointment (Rugby, 0536-1086-91)
- Surgical scrub: povidone-iodine (Betadine)
- 70% ethanol
- 2% lidocaine (Fresenius Kabi, 63323-486-57)
- Antibiotic ointment (Polysporin)
- Ketoprofen (5mg/kg)
- Dexamethasone (Henry Schein, 002459)
- Sterile saline (NaCl 0.9%)
- Artificial cerebrospinal fluid (aCSF;)
- Tissue glue (VetBond)
- Pre-Empt disinfectant 1:20
  ement

- 3M RelyX Unicem, Aplicap (resin based dental adhesive) mixer and activator kit

  or :
- Metabond kit (Parkell Inc., S371, S396, S398)
- Dental acrylic cement (BASi, MD-1300)
- Carbon (Sigma-Aldrich, 484164-50g)

Equipment:

- Isoflurane vaporizer (VetEquip)
- Induction chamber
- Plastic-backed benchtop pads
- Shaver
- Heating pad (TCAT-2DF, Physitemp)
- Dissection microscope or camera
- UltraMicroPump (UMP3) with SYS-Micro4 Controller (World Precision Instruments, UMP3-1)

Stereotax:

- Stereotaxic frame for mouse with nose cone for anesthesia (Model 963, Kopf)
- Stereotaxic instrument (Model 963, Kopf)
- Standard electrode holder with clamp (Model 1770, Kopf)

Ablation (Aspiration) syringe and needle:

- syringe with a Hamilton 21-G blunt-needle connected to laboratory vacuum or pump (pressure _13.5 psi).

Administration of analgesics and flushing of injection pipette and tubing, aspiration needles

- 1-ml syringes (BD, 329654),
- 27 G syringe needles (BD, 305109)
- 10 µl syringe (Hamilton, 7653-01)
- 21 G syringe needle (Hamilton, 7804-12)

Autoclaved materials:

- Fine-tipped scissors (one; Fine Science Tools, 14081-09)
- Fine-tipped thumb forceps (two; Fine Science Tools, 11370-42)

Technical material

- Micro-drill (Ideal Micro Drill Kit, CellPoint Scientific)
- 0.5-mm drill bit (one; Fine Science Tools, 19007-05)
- 1.2-mm diameter drill bit (one; Widget Supply, D-EK09)
- 4-0 wax coated braided silk suture (Roboz Surgical Store, SUT-1074-31)
- Surgical retractors

Quartet™
- Neurescence lenses with lens connector: 1.0 mm or 0.7 mm diameter
- LED driver
- Phuiyong
- Controller with screen

Others:

- Sterile paper towels
- Surgical benchtop pads
- Paper towels
- Lens cleaning paper