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Gradient-index (GRIN) lens implantation surgery in non-human primates

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Adriana Galvan[1,2], Thomas Wichmann[1,2]

[1]Emory University; [2]ASAP

ASAP Collaborative Rese…

Johnson Agniswamy
Emory University

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We use this protocol and it's working

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Abstract

These procedures are done in the context of calcium imaging experiments in non-human primates. We inject a solution containing AAV-GCaMP6 into the supplementary motor area (SMA) of the animal to be tested and then insert the GRIN lens into the injected area during the same session. The GRIN lens assembly is protected by a custom-made 'chamber' with a lid that can be closed for protection between uses. Details regarding the preparation of the virus solution are not included in this description, as they are immaterial to the GRIN lens implantation itself.

Materials

Required specialty items:

- Virus solution
- Micromanipulator
- Micromanipulator needle holder
- Hamilton syringe (5 or 10 µl), with 25-ga needle
- Syringe pump that can be mounted to the micromanipulator (e. g., Stereotaxic injector, Stoelting ™)
- Surgical stereo-microscope
- GRIN lens attached to metal baseplates (e.g., Inscopix™ ProView™ M Prism Integrated lens, 1.0 mm diameter, ~12.0 mm length (product code 1050-006024) or ProView™ M Prism Integrated Lens 1.0mm x 9.1mm (product code 1050-005303)
- Cranial chamber system for non-human primates (Inscopix™ product code 1000-005574). The system includes one cranial chamber (16 mm ID, one chamber cap, and screws).
- Circular metal disc fitting into the chamber, with cutout ('slot') sized to accommodate the GRIN lens width. We recommend to have various diameters (5, 7, 9, 11 and 13 mm) with slot widths of 2 or 4 mm.
- Base plate holder (Inscopix™).
- Dummy mini-endoscope
- Duragen™ dural repair graft
- UV-curable dental cement
- Veterinary glue (e.g., Dermabond™)
- UV light source
- Eye protection (to wear during use of UV light)
- Dental acrylic
- 8-0 or 6-0 absorbable surgical suture material
- 4-0 absorbable surgical suture material
- Fine surgical tools: Surgical blade #10, forceps #3 and #5, dura hook.

Prepare the animal (assumed to be anesthetized throughout the entire procedure)

1 Shave the animal's head.

2 Place an endotracheal tube and secure it with umbilical tape.

3 Secure the animal in a stereotaxic frame.

4 Cover the animal with sterile surgical drapes, leaving the head accessible.

Surgery 7m

5 **Preparatory steps**

5.1 Make an incision in the scalp and clear/clean the surface of areas of the skull that will subsequently be used for the craniotomy.

5.2 Use a stereotaxic manipulator to determine the center of the planned craniotomy and mark it (either with marker pen or small drill hole).

6 **Craniotomy**

6.1 Draw an outline of the planned craniotomy on the skull (10 mm diameter).

6.2 Make sure that the available space on the skull accommodates all hardware to be placed; mark chamber locations and locations of screws that will be used to secure the chamber to the skull.

6.3 Perform craniotomy (10 mm diameter), using an electrical drill with 2 mm drill bits.

6.4 Place saline-soaked gel foam atop the exposed dura until the durotomy.

6.5 Identify the location of injection and lens placement inside of the craniotomy.

7 Durotomy and preparation for virus injection

7.1 Perform the following steps using a surgical microscope.

7.2 Once the dura is exposed, insert 6-0 or 8-0 suture through the dura parallel to the location of the planned durotomy. Using the same suture, thread the needle in the opposite direction, keeping a ~4 mm distance between the sutures.

7.3 Hold the edges of the sutures and gently pull up to 'tent' the dura (alternatively, a dura hook can be used to lift the dura).

7.4 Make an incision (~2 mm) in the tented dura with #11 blade. Subsequently, #3 and #5 forceps can be used to slightly increase the durotomy size (if needed). Be aware of the location of small blood vessels.

7.5 Use the tip of a dura hook or the tip of a 23- or 30-ga needle to gently remove the pia in the durotomy location from the underlying surface of the brain.

8 Virus injection

8.1 Prepare for virus injection while the durotomy is performed.

8.2 Place soaked gel foam atop all portions of the exposed dura that are not currently used for injections.

8.3 Load the Hamilton syringe with 5 µL - 10 µL of virus solution. Mount the loaded syringe on the pump (which itself is mounted alongside the stereotaxic micro-manipulator).

- Subsequent injections require positioning of the needle tip over the area to be injected and lowering the tip of the needle to the desired depth.

8.4 Place one injection track at the anterior-posterior and medio-laterallocation where the lens will be placed. Lower the injection needle into the brain and wait for 00:03:00 before starting the infusion. 3m

- Plan to inject the virus solution at various depths ventral and dorsal to the location chosen for lens implantation (e. g., +1, +0.5, and -0.5 from the lens implantation location).

8.5 Inject solution using 0.2 to 0.4 µl/min. Wait 00:01:00 after each deposit and 00:03:00 after the last deposit. 4m

9 **Lens implantation**

9.1 Place the lens on the lens holder,

9.2 Use the micromanipulator with the attached baseplate holding rod to maneuver the baseplate and attached lens to the correct location.

9.3 Verify that the baseplate will be correctly oriented within the protective chamber and make note of the required height of the chamber.

9.4 If needed, place an 18-ga injection needle into the baseplate holding rod, and generate an incision/path in the brain.

9.5 Secure the rod and holder with the baseplate attached lens on the manipulator arm.

9.6 Thread spacers (if needed) and one chamber up the rod and up to the manipulator's arm and secure them with twisty ties.

9.7 Verify that the screw holes by which the chamber lid will be secured face up.

9.8 Lower the lens into the brain while monitoring the entry with the surgical microscope.

9.9 Once the lens is at the required depth, suture the dura around the lens, using the suture segments inserted in the dura earlier.

9.10 Cut a round piece of dry dural repair graft (Duragen™) of the approximate diameter of the craniotomy (10 mm). Cut a slit approximately halfway through the circular piece, and place this around the lens.

9.11 Add a few drops of saline on top of the dural repair material with a syringe and a 25-ga needle. This step may not be needed as the CSF leaking from durotomy may be sufficient to moisten the dural repair graft.

9.12 Carefully place the slotted disc so that the slot is located around the GRIN lens shaft atop the skull.

9.13 Lower the chamber that was placed on the micromanipulator arm and attach it to the skull around the craniotomy with a few drops of dental acrylic. Make sure the chamber location relative to the baseplate leaves enough room for docking of the calcium imaging microscope.

9.14 Secure the implant to the slotted disc and the skull within the chamber using UV-cured dental cement.

9.15 Cover the remaining bottom of the chamber with dental acrylic. Build the acrylic up to the bottom of the GRIN lens baseplate.

10 **Securing the chamber and building an acrylic 'cap'.**

10.1 Mark the locations of screws around the chamber on the exposed skull, drill holes at these locations, and insert bone screws.

10.2 Generate a single acrylic head cap containing the implanted hardware(bone screws, base of the chamber, head holders etc.).

10.3 If needed, suture the skin around the remaining portions of the incision using 4-0 surgical suture material.

10.4 It may be useful to generate images or drawings of the baseplate configuration and orientation.