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Virus injections and lens placement (Mendonça et al 2024)

Marcelo D Mendonça[1,2,3]

[1]Champalimaud Research, Champalimaud Foundation, Lisbon 1400-038, Portugal;
[2]Champalimaud Clinical Centre, Champalimaud Foundation, Lisbon 1400-038, Portugal;
[3]NOVA Medical School | Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisbon 1169-056, Portugal

ASAP Collaborative Research Network

c.weber-schmidt
Allen Institute, ASAPCRN

ABSTRACT

Protocol for viral injections and GRIN lens placement used in Mendonça et al 2024.

GUIDELINES

All procedures were done in aseptic conditions.

Experiments were done on male mice-C57BL/6 or B6.SJL-Slc6a3tm1.1(Cre)Bkmn/J (Jax labs stock #006660).

MATERIALS

-Stereotaxic surgery set up required (this protocol uses Kopf insturments)
-GCaMP6f stock viral solution -AAV2/5.SYN.FlexGCaMP6fWPRE.SV40 - University of Pennsylvania
-GRIN Lens is A 500-um diameter, 8.2-mm long gradient index (GRIN) lens (GLP-0584, Inscopix).

BEFORE START INSTRUCTIONS

Mice were kept in deep anesthesia using 1-3% isoflurane and oxygen (at 1L/min flow rate).

Protocol status: Working
We use this protocol and it's working

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Stereotaxic Surgery

1 Place the anesthetized mouse in the stereotaxic apparatus then stabilize the head.

2 Make a skin incision to expose the skull.

3 Carefully remove any connective and muscle tissue from the exposed area.

4 Level the skull surface to be at less than 0.05mm by comparing the height of bregma and lambda, and also in medial-lateral directions.

AAV Virual Injections

4.1 Find the injection coordinates and mark area for drilling: Substantia nigra compacta was injected at the following coordinates: -3.16 mm anteroposterior, 1.40mm lateral from bregma and 4.20 deep from the brain surface.

4.2 Drill Burr-holes in marked areas carefully.

4.3 Load glass pipette into the pipette holder (Nanojet II or Nanojet III (Drummond Scientific)) and affix to the stereotaxic frame.

4.4 Set rate of injection to 4.6 nL per 5 seconds.

5 Inject GCaMP6f stock viral solution (AAV2/5.SYN.FlexGCaMP6fWPRE.SV40 - University of Pennsylvania). Note-The virus solution is kept at -80 ºC and thawed at room temperature just before the injection.

STEP CASE

Optogenetics Injections 12 steps

Virus volume of 1.5 nL was injected in the substantia nigra compacta at the following coordinates: -3.16 mm anteroposterior, 1.40mm lateral from bregma and 4.20 deep from the brain surface.

6 After the injection finishes, leave the pipette in place for 10-15 minutes.

GRIN Lens Placement (if desired)

7 Please initially follow stereotaxic surgery section.

8 A blunt 28 G needle was lowered to 3 mm deep from the brain surface to facilitate the lowering of the GRIN lens.

9 The GRIN lens was then lowered at the same coordinates as the AAV injection coordinates (-3.16 mm anteroposterior, 1.40mm lateral from bregma and 4.20 deep from the brain surface.)

9.1 GRIN Lens is A 500-um diameter, 8.2-mm long gradient index (GRIN) lens (GLP-0584, Inscopix).

10 The lens is then fixed in place using cyanoacrilate, quick adhesive cement (C&B Metabond) and black dental cement (Ortho-Jet).

11 Mouse recovers for 3 weeks post-GRIN placement, then a baseplate was positioned.

11.1 Mouse is anesthetized with isoflurane and fixed with head bars.

11.2 A baseplante (BPC-2, Inscopix) attached to a mini epifluorescence microscope (nVista HD, Inscopix) is then positioned above the GRIN lens.

11.3 To correctly position the baseplate, brain tissue was imaged through the lens to find the appropriate focal plane using 40% LED power, a frame rate of 10 Hz and a digital gain of 4.

11.4 Once the focal plane was set, the baseplate was cemented to the rest of the cap using the same dental cement.

11.5 Imaging with the GRIN lense started 2–3 days after this final step.