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Omar Jaidar[1], Eddy Albarran[2], Eli Nathan Albarran[1], Yu-Wei Wu[3], Jun B. Ding[1]

[1]Stanford University; [2]Columbia University; [3]Academia Sinica

Omar Jaidar
Stanford University

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We use this protocol and it's working

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Abstract

This protocol details the step-by-step process for the construction of guide cannulas, the administration of viral injections, and the subsequent insertion of cannulas in approximately 5-week-old mice. It also provides a detailed behavioral protocol used in this study. These procedures are designed to prepare the mice for long-term two-photon imaging studies.

Materials

Isoflurane anesthesia (1.5-2.5% in 0.5L/min of O2)
AAV1-syn-Flex-GCaMP6f-SV40 (Addgene, 100833-AAV1)
Pulled glass pipette
UV curable glue (Norland #81)
#0 glass coverslip (2mm-diameter)
18G stainless steel tube (1.27mm OD, 1.06mm ID, 3.8mm long, McMaster-Carr)
Ultratec basic polisher with 30mic silicon abrasive disc
27G blunt needle
n-butyl cyanoacrylate adhesive (Vetbond, 3M)
Dental cement (Metabon, Parkell)
Aluminum head bar
Polishing wheel

GRIN lens & animal preparation for long term striatal imaging.

19m

1 Fabrication of GRIN lens Guide Tube

1.1 Glue a 2mm-diameter #0 glass coverslip to the tip of a 3.8mm long 18G (316 Stainless Steel TubingMiniature, 0.05" OD, 0.004" Wall Thickness, 89935K66, McMaster-Carr), using UV curable glue (Norland #81). 5m

1.2 Remove excess glass using a polishing wheel (ULTRAPOL Basic Polisher) equipped with a 30mic silicon abrasive disc. 2m

2 Anesthesia and animal preparation

2.1 Redirect the anesthesia system to the induction chamber and turn on oxygen flow at 0.5L/min. Turn on isoflurane to 4% for induction, and place mouse inside. Wait until mouse's breathing has slowed down and has not withdraw pinch reaction.

2.2 Redirect the anesthesia system to the stereotaxic apparatus and change the isoflurane to 1.5% and fix the animal head with the nose and ear holders. Cover eyes completely with artificial tears (Puralube Ophthalmic ointment) to prevent the eyes from drying. Turn on the heat pad and set it to: 37.5 °C

2.3 Apply Nair on top of the head of the animal for no longer than 00:02:00, wipe off and clean the skin alternating ethanol 70% and Iodine 3 times. 2m

3 Injection of Viral Construct.

3.1 To express GCaMP6f in the striatum of A2a or Drd1-Cre mice, inject 400nl of AAV1-syn-Flex-GCaMP6f-SV40 into the dorsolateral striatum using the following stereotaxic coordinates (from bregma): AP: +1mm, ML: +2.25mm, V:-2.45mm from dura.

3.2 Inject the viral construct at a rate of 40nl/min using a pulled glass pipet. Wait for 00:10:00 post-injection before withdrawing the pipette. 10m

3.3 Suture the incision and allow the animals to recover overnight in their cages.

4 **Implantation of GRIN lens Guide Tubes**

4.1 Anesthetize the previously injected animals with isoflurane as described in **step 2.**

4.2 Once the animal's head is secured in the stereotaxic apparatus, use the same coordinates as the initial injection to create a new craniotomy with a 1.4 mm diameter drill bit (F.S.T.). Carefully remove all small pieces of bone from the edge of the craniotomy.

4.3 Aspirate the cortex with a 26G blunt needle until the striatum is visible (dorsoventral: -20mm from dura).

4.4 Lower the guide tube to -2.35mm from dura. Seal the gap between the cannula and the skull with n-butyl cyanoacrylate adhesive (VetBond, 3M).

4.5 Cover the expose area with dental cement (Metabon, Parkell), and attach an aluminum head bar to the cranium using a second batch of dental cement.

5 **Recovery**

5.1 Allow the animals to recover and express GCaMP6f for at least 3-4 weeks before starting behavioral and imaging experiments.

Behavioral Training

20m

6 **Water-Restriction Protocol**

6.1 2-3 days before training begins start water-restriction protocol for the mice by limiting their daily water intake to approximately 1ml .

6.2 Monitor each mouse's weight daily. Ensure that weight does not drop more than 20% from their starting weight. Provide supplementary water if necessary to maintain animal welfare.

7 **Imaging and Training Protocol**

7.1 Before each behavioral session, secure the animals in a head-restricted configuration on top of the behavioral apparatus. Ensure their paws are resting on the foam wheel, allowing them to run at will.

7.2 Carefully clean the guide tube on the head of the animal with distilled water to remove any debris. Dry the guide tube by carefully inserting a filter paper. **Note:** You only need to do this once before fixing the GRIN lens to the guide cannula. Once is fixed, you only need to clean the surface of the GRIN lens before each imaging session.

7.3 Insert a clean GRIN lens to the guide tube.

7.4 Place the behavioral apparatus on the transitional stage of the 2-photon microscope. **Important:** To ensure consistent field of view (FOV) across sessions, ensure the transitional stage clamps the behavioral apparatus in the same orientation each time.

7.5 Use the micromanipulator of the transitional stage of the microscope to find the desired FOV. Once GCaMP expression is confirmed, fix the GRIN lens to the guide tube using UV curable glue (Norland #81). **Note:** After the first session, use a reference mean projection image of the calcium signal from the first session to get back to the same FOV.

7.6 After finding the desired FOV, position the water spout in front of the mouse's mouth and begin the recordings. Expose mice to the running wheel for 8 consecutive days for approximately 00:20:00 each day.

20m