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Triple Anterograde Tracing

Hongwei Dong¹

¹USC Mark and Mary Stevens Neuroimaging and Informatics Institute

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Hongwei Dong



ABSTRACT

Stereotaxic surgeries are performed to inject neural circuit tracers into different target regions in the brain. Animals are allowed one week to habituate following their arrival at the host vivarium prior to the start of any experimental procedures.

GUIDELINES

All experiments are conducted according to the regulatory standards set by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and by the institutional guidelines set by the Institutional Animal Care and Use Committee at USC.

- 1 Instruments are washed with soap, wiped down with alcohol, and autoclaved for the first surgery. Instruments are wiped down with alcohol and sterilized via a glass bead sterilizer in between surgeries.
- 2 Animal is deeply anesthetized. The surgeries are performed under isoflurane anesthesia. Mice are initially anesthetized in an induction chamber primed with isoflurane and are maintained under deep anesthetic state via a vaporizer throughout the duration of the surgery.
- 3 4% lidocaine gel is applied to the ear bars of the stereotaxic frame, and the animal is mounted to the stereotax on top of a homeothermic blanket
- 4 Analgesics are delivered to alleviate any persistent pain following surgery. Ketoprofen (5 mg/kg) or buprenorphine-SR (1 mg/kg) is administered subcutaneously
- 5 Bland ophthalmic ointment is placed on the eyes
- 6 The scalp is prepared with three alternating betadine swabs and alcohol wipes
- 7 A midline incision approximately 1.5 cm is made above the scalp with a sterile scalpel blade, the underlying periosteum is dissected using blunt dissection techniques, and the skull is cleaned using sterile saline
- 8 Bregma is marked under a surgery microscope and a dental drill is used to carefully drill a small hole on the bone over the desired brain nuclei (positions determined from x, y, z coordinates of the Allen Reference Atlas for each region of interest)
- 9 A glass micropipette (tip diameter 10-20 μ m) filled with neural tract tracers is placed stereotaxically through the hole into the desired brain nucleus
- 10 Tracers are iontophoretically injected by applying a positive current (5 μ A, 7 seconds on/off intervals) for 7-15 minutes, before the glass micropipettes are removed. For triple anterograde tracing, Phaseolus vulgaris leucoagglutinin (Phal; 2.5%; Vector Laboratories) and adeno-associated viruses encoding green fluorescent protein (AAV-GFP; pENN.AAV.CB7.Cl.eGFP.WPRE.rBG; Addgene) and red fluorescent protein

(AAV-RFP; pENN.AAV.CAG.tdTomato.WPRE.SV40; Addgene) are injected in three different regions of interest

- 11 To avoid backflush, the micropipettes will be left in situ for an additional 10 minutes
- 12 After tracer infusions, the skin incision (~1.5 cm) is closed using Nylon sutures
- 13 A nystatin-neomycin sulfate-thiostrepton-triamcinolone acetonide ointment is applied to the wound to provide anti-inflammatory, antipruritic, antifungal, and antibacterial protection
- 14 The animal is then released from the stereotaxic frame and put back to the host cage on a warm pad (one animal per cage)
- 15 The animal is closely monitored until it fully recovers from anesthesia (i.e., achieves sternal recumbancy and is independently mobile) before it is returned into the host vivarium
- 16 Three weeks is allowed for tracer transport after which animals are humanely euthanized via an overdose injection of sodium pentobarbital prior to perfusions. Brains are extracted and sectioned at 50- μ m thickness using a compresstome. One of four series of sections is immunostained for Phal. All sections are counterstained with a fluorescent Nissl solution (NeuroTrace 435/45; Invitrogen), mounted, coverslipped, and imaged as high-resolution virtual slide image (vsi) files using an Olympus VS110 high-throughput microscope.



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