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TRIO tracing

Hongwei Dong[1]

[1]USC Mark and Mary Stevens Neuroimaging and Informatics Institute

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Hongwei Dong

ABSTRACT

TRIO (Tracing the Relationship of Inputs and Outputs) experiments systematically inject complementary viruses into different target brain regions to monosynaptically label inputs to a specific projection neuron population. TRIO experiments use 3 different viruses (AAVretro-Ef1a-Cre, AAV8-hSyn-FLEX- TVA-P2A-GFP-2A-oG helper virus, and EnvA pseudotyped G-deleted Rabies mCherry (EnvA RVdG-4mCherry).

EXTERNAL LINK

https://cic.ini.usc.edu/protocols/trio-tracing

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.

SAFETY WARNINGS

All surgeons and study personnel are vaccinated against rabies virus.
Rabies virus is kept in a BSL2-certified biosafety cabinet until needed.
Following all rabies surgeries, experimental surfaces and tools are sprayed down with 10% bleach solution and left for 15 mins of contact time before wiping up with ethanol.

BEFORE STARTING

Animals are allowed one week to habituate following their arrival at the host vivarium prior to the start of any experimental procedures. 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.

Day 1 (after habituation)

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. 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

3 Ketoprofen (5 mg/kg) or buprenorphine-SR (1 mg/kg) is administered subcutaneously

4 Bland ophthalmic ointment is placed on the eyes

5 The scalp is prepared with three alternating betadine swabs and alcohol wipes

6 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

7 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)

8 A glass micropipette (tip diameter 10-20 µm) filled with AAVretro-Ef1a-Cre is placed stereotaxically through the hole into the desired brain nucleus. AAVretro-Ef1a-Cre is iontophoretically injected by applying a positive current (5 µA, 7 seconds on/off intervals) for 5 mins, before the glass micropipette is removed. Following AAVretro-Ef1a-Cre injection, 500nl of AAV8-hSyn-FLEX- TVA-P2A-GFP-2A-oG helper virus is filled into a micropipette and pressure-injected into a different target brain region using a Nanoject II injector.

9 To avoid backflush, micropipettes are left in situ for an additional 10 minutes following injection

10 After tracer infusions, the skin incision (~1.5 cm) is closed using Nylon sutures

11 A nystatin-neomycin sulfate-thiostrepton-triamcinolone acetonide ointment is applied to the wound to provide anti-inflammatory, antipruritic, antifungal, and antibacterial protection

12 The animal is then released from the stereotaxic frame and put back to the host cage on a warm pad (one animal per cage)

13 The animal is closely observed until it fully recovers from anesthesia (i.e., achieves sternal recumbancy and is independently mobile) before it is returned into the host vivarium

Day 14 (2 weeks after AAV injection)

14 Animals that received AAV injections undergo surgical injections of EnvA RV dG-4mCherry. All surgeons and study personnel are vaccinated against rabies virus. Aliquot of rabies virus is thawed from -80°C storage and kept on ice. During surgery, rabies virus is kept in a BSL2-certified biosafety cabinet until needed.

15 Instruments are washed with soap, wiped down with alcohol and autoclaved for the first surgery. Instruments are wiped down with alcohol and sterilized via 10% bleach and a glass bead sterilizer in between surgeries.

16 Animal is deeply anesthetized. 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

17 Ketoprofen (5 mg/kg) or buprenorphine-SR (1 mg/kg) is administered subcutaneously

18 Bland ophthalmic ointment is placed on the eyes

19 The scalp is prepared with three alternating betadine swabs and alcohol wipes

20 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

21 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)

22 Within the BSL2 biosafety cabinet, a glass micropipette (tip diameter 10-20 µm) attached to a Nanoject II injector is filled with EnvA RVdG-4mCherry and placed stereotaxically through the hole into the desired brain nucleus. 200nl of EnvA RVdG-4mCherry is pressure-injected using the Nanoject II injector.

23 To avoid backflush, micropipettes are left in situ for an additional 10 minutes following injection

24 After tracer infusions, the skin incision (~1.5 cm) is closed using Nylon sutures

25 A nystatin-neomycin sulfate-thiostrepton-triamcinolone acetonide ointment is applied to the wound to provide anti-inflammatory, antipruritic, antifungal, and antibacterial protection

26 The animal is then released from the stereotaxic frame and put back to the host cage on a warm pad (one animal per cage)

27 The animal is closely observed until it fully recovers from anesthesia (i.e., achieves sternal recumbancy and is independently mobile) before it is returned to a BSL2 room within the host vivarium

28 Following all rabies surgeries, experimental surfaces and tools are sprayed down with 10% bleach solution and left for 15 mins of contact time before wiping up with ethanol.

Day 21 (one week after rabies injection)

29 TRIO mice are transcardially perfused with saline and 4% PFA by rabies vaccinated personnel within a BSL 2 biosafety cabinet. Brains are dissected and removed from the skull and post-fixed within 4% PFA for 1-2 days.