Stereotaxic Injections and Implants V.1

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ABSTRACT

Methods for Stereotaxic injections and implants from Tang et al 2023.

MATERIALS

Please see references for similar materials to what was used.

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

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VTA AAV Viral Steriotaxic Injections

1 Using a standard mouse surgical suite (see references for example), 750nl of rAAV.EF1a.DIO.hChR2(H134R).eYFP or rAAV.EF1a.DIO.eYFP (3-4 x 10^12 vg/ml, AAV5, University of North Carolina Vector Core; 1-2 x 10^13 vg/ml, AAV1, Addgene, 27056-AAV1 and 20298-AAV1) were injected into each hemisphere of the VTA of 3−4-month-old DAT-Cre mice.

1.1 For viral injections, the coordinates are AP - 3.52 mm, ML - +/- 0.35 mm, DV − 4.3 mm. Injections were made at 0.2 Hz pulses. Each pulse injects 4.6 nl volume.

1.2 Injected needles were kept in place in the injection site for ~15 minutes before withdrawal.

Dual Optic Fiber Cannula Implant

2 For each mouse, a dual optic fiber cannula (200/240 µm diameter, 6 mm length, 0.7 mm center-to-center FLT, 0.22 NA; Doric, DFC_200/240-0.22_6mm_DF0.7_FLT) was placed 200 µm above the injection site and fixed to the skull.

3 Next, a 4-position receptacle connector (Harwin Inc., M52-5000445) was fixed anteriorly to the dual optic fiber cannula, with its posterior edge set at -0.6 mm.

3.1 Skull implants are then fixed with dental cement.

3.2 A 4-position connector (Harwin Inc., M52-040023V0445) with pins removed from one end was used to cap the receptacle connector.

Photometry Experiments

4 The conditions used for VTA injections and implants were as above, and 3–5-month-oldDAT-Cre males were used.

4.1 Additionally, 1 µl and 500 nl of AAV9-hSyn-GRAB-rDA1m (2 x 10^13 vg/ml; RRID:Addgene_140556) were injected into the dorsal striatum (AP 0.5 mm, ML +2.1 (right), DV 2.3 (from brain surface)) and ventral striatum (AP 1.15mm, ML +1.65 (right),DV 4.2 (from Bregma)), respectively.

5 For photometry fiber implants, mono fiberoptic cannula were used (400/430 µm diameter, 4 mm length (dorsal striatum) and 6 mm length(ventral striatum), 0.37 NA, 1.25 diameter ferrule, flat; Doric, MFC_400/430-0.37_6mm_MF1.25_FLT (ventral striatum) and MFC_400/430-0.37_4mm_MF1.25_FLT (dorsal striatum)). Implants were inserted at a 22 degrees angle.