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Stereotaxic virus injection and fiber optic probe implantation

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We use this protocol and it's working

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Abstract

Stereotaxic virus injection and fiber optic probe implantation

Stereotaxic injections

- 1 Stereotaxic injections were performed in mice under isoflurane anesthesia (4% for induction and maintained at 1–1.5% air mix v/v).
- 2 After being placed in a stereotaxic apparatus, mice received subcutaneous injection of analgesic and anti-inflammatory drugs (buprenorphine, 0.1 mg/kg and Carprofen, 5 mg/kg) as well as a local analgesic (lidocaine, 7 mg/kg) before the incision of the skin.
- 3 Mice were bilaterally injected in the STN/pSTN with a virus containing either a Cre-dependent Channelrhodopsin (ChR2) construct coupled with an eYFP reporter (rAAV2/EF1a-DIO-hChR2(H134R)-eYFP), or only the eYFP reporter (rAAV2/EF1a-DIO-eYFP), respectively, 3.8×10^{12} virus molecules/mL and 4.6×10^{12} virus molecules/mL (viruses purchased from UNC Vector Core, Chapel Hill, NC, USA) at the following mouse brain coordinates (from Paxinos and Franklin, 2013): anteroposterior (AP) = 1.90 mm, mediolateral (ML) = ± 1.70 mm from the midline and 250 nL of virus was injected with a NanoFil syringe (World Precision Instruments, Sarasota, FL, USA) at two dorsoventral levels (DV) = 4.65 mm and 4.25 mm from the dura matter at 100 nL.min⁻¹.

Optic cannula implantation

- 4 Optic cannulas (Doric Lenses) were implanted directly after completion of virus injections in mice prepared for behavior analysis.
- 5 Two skull screws were implanted in the skull to hold the optic cannula-cement-skull complex.
- 6 Optibond FL Prime 1 and 2 Adhesive (Kerr) were then applied and hardened using Elipar LED polymerisation lamp (3M Espe).
- 7 Optic cannulas were implanted bilaterally above the STN (coordinates: AP = 1.90 mm, ML = ± 1.70 mm from the midline DV = 4.30 mm) or the VP (coordinates: AP = +0.45 mm, ML = ± 1.55 mm from the midline, DV = 4.00 mm), and fixed with dental cement.
- 8 1 mL of saline was injected subcutaneously at the end of the surgery.

For ex vivo electrophysiology experiments

- 9 Mice were injected into the LHb and the STN.
- 10 A glass pipette (30–50 mm tip diameter) filled with rgAAV2-hSyn-DIO-EGFP and was lowered at the LHb coordinates: AP: 1.55 mm; ML: 0.50 mm; DV: 2.65 mm depth and 120 nL were injected using a picospritzer III (Parker).
- 11 Similarly, a glass pipette filled with AAV2-EF1a-DIOChR2(H134R)-mCherry was lowered at the STN coordinates: AP: 1.90 mm; ML: 1.60 mm; DV: 4.65/-4.30 mm and 2 times 120 nL were injected.
- 12 5 min after the last injection, the glass pipette was withdrawn
- 13 After stitching, mice were transferred to a cage placed on a heating pad until waking up.