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Histology, Immunohistochemistry and Imaging

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ABSTRACT

Sample preparation, histology, immunohistochemistry (IHC) and imaging of brain sections in Tang et al 2023.

MATERIALS

Standard perfusion setup
Vibrotome
Zeiss Axio ImagerM2 microscope and accessories (see steps for exact details)

Antibodies/Stains
-Rabbit anti-GFP 488 conjugate (1:1000;Molecular Probes A21311, RRID:AB_221477)
-Mouse Anti-TH (1:5000;Immunostar Th 22941, RRID:AB_572268)
-Goat Anti-Mouse - IgG (H+L) Highly cross-adsorbed secondary antibody - Alexa Fluor647(1:1000; ThermoFisher, A-21236, RRID:AB_2535805)
-DAPI (1:1000 of 20 mg/mL stock; Sigma, D9542).

BEFORE START INSTRUCTIONS

Process begins after behavioral sessions were completed.

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

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Sample Preparation

1 Deeply anesthetized mouse with isoflurane.

2 Perfuse transcardially in PBS and then 4% PFA/PBS.

3 Dissect brains with skulls attached and submerged in 4% PFA in PBS at 4 degrees Celsius overnight

4 Next day-brains were rinsed 3 times in PBS.

5 Brain regions including VTA and implants were sectioned by vibratome into 50 or 100 µm slices (depending on desired output).

Histology/Immunohistochemistry

6 Slices were stained with the following reagents by the Champalimaud Histopatholgy Platform (core facility) using their standard protocol.

6.1 -Rabbit anti-GFP 488 conjugate (1:1000;Molecular Probes A21311, RRID:AB_221477)
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-DAPI (1:1000 of 20 mg/mL stock; Sigma, D9542).

Imaging

7 Using a Zeiss Axio Imager M2 microscope-10x tiled images were taken through the relevant fluorescent channels.

7.1 The M2 is equipped with a fast Colibri.7 LED illumination for excitation of fluorophores. Images are captured with a high-sensitivity monochromatic sCMOS camera(Hamamatsu Orca Flash 4.0 v2). The objective used for the images is a ZEISS Plan-ApoChromat 10x/0.45, which allowsto resolve up to 577 nm when using a wavelength of observation of 520nm and it is fully corrected for chromatic and spherical aberrations. Implant locations were determined using Paxinos and Franklin's the Mouse Brain in Stereotaxic Coordinates.