Jan 11, 2024

Histology, Immunohistochemistry and Imaging

DOI
dx.doi.org/10.17504/protocols.io.eq2lyjbdelx9/v1

Jonathan Tang[1,2,3]

[1]Department of Neuroscience, Zuckerman Mind Brain Behavior Institute, Columbia University, New York, NY 10027, USA;
[2]Seattle Children's Research Institute, Center for Integrative Brain Research, Seattle, WA, USA;
[3]University of Washington School of Medicine, Department of Pediatrics, Seattle, WA, USA

Christine Weber-Schmidt
Allen Institute

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DOI: https://dx.doi.org/10.17504/protocols.io.eq2lyjbdelx9/v1

Protocol Citation: Jonathan Tang 2024. Histology, Immunohistochemistry and Imaging . **protocols.io** **https://dx.doi.org/10.17504/protocols.io.eq2lyjbdelx9/v1**

Manuscript citation:
Tang, J.C.Y., Paixao, V., Carvalho, F. *et al.* Dynamic behaviour restructuring mediates dopamine-dependent credit assignment. *Nature* (2023). https://doi.org/10.1038/s41586-023-06941-5

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

Created: January 11, 2024

Last Modified: June 01, 2024

Protocol Integer ID: 93347

Keywords: ASAPCRN, imaging of brain section, immunohistochemistry, brain section, histology, imaging, ihc

Funders Acknowledgements:
Life Sciences Research Fellowship
NINDS K99/R00 Award
Grant ID: 1K99NS112575
NIH
Grant ID: 5U19NS104649
Aligning Science Across Parkinson's (ASAP)
Grant ID: ASAP-020551

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).

Troubleshooting

Before start

Process begins after behavioral sessions were completed.

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.

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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.