Mar 11, 2025

Histology Fushiki et al 2024

DOI
https://dx.doi.org/10.17504/protocols.io.5qpvo9o49v4o/v1

Akira Fushiki[1,2,3]

[1]Allen Institute; [2]Zuckerman Mind Brain Behavior Institute, Columbia University;
[3]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network

Christine Weber-Schmidt
Allen Institute

DOI: https://dx.doi.org/10.17504/protocols.io.5qpvo9o49v4o/v1

Protocol Citation: Akira Fushiki 2025. Histology Fushiki et al 2024. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5qpvo9o49v4o/v1

Manuscript citation:
A Vulnerable Subtype of Dopaminergic Neurons Drives Early Motor Deficits in Parkinson's Disease
Akira Fushiki, David Ng, Zachary R. Lewis, Archana Yadav, Tatiana Saraiva, Luke A. Hammond, Christoph Wirblich, Bosiljka Tasic, Vilas Menon, Joaquim Alves da Silva, Rui M. Costa
bioRxiv 2024.12.20.629776; doi: https://doi.org/10.1101/2024.12.20.629776

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

Created: December 19, 2024

Last Modified: March 11, 2025

Protocol Integer ID: 116216

Keywords: ASAPCRN, fushiki et al 2024 histology protocol, histology protocol, histology, cell, dorsal, tier of snc dan, snc dan, histology fushiki et al 2024 histology protocol, histology fushiki et al

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: ASAP-020551

Abstract

Histology protocol for Fushiki et al 2024 , to quantify Th+ cells and visualize the dorsal-tier or ventral-tier of SNc DANs.

Materials

24-well tissue culture plates
Normal Goat Serum (Abcam #ab7481) or Donkey Serum (Millipore Sigma, #S30)
PBS (prepared from tablets; Sigma, #P4417)
4% Paraformaldehyde in PBS (prepared from granules; use fresh; EMS, #19208)
1% Triton X-100 (Fisher Scientific, #BP151)
Bovine Serum Albumin (BSA; Sigma, #A2153)
Primary antibodies:
To quantify Th+ cells:
anti-GFP antibody (Chicken, Aves labs Inc, GFP-1020) or TH antibody (Mouse, ImmunoStar, 22941) at 1:2000 dilution
To visualize the dorsal-tier or ventral-tier of SNc DANs:
Calbindin D28K 1:2000 dilution (Rabbit, Synaptic Systems, 214002) and/or an Aldh1a1 antibody 1:1000 dilution (Goat, R&D Systems, AF5869)
Secondary antibodies all diluted at 1:2000 for use:
Donkey anti-Chicken Alexa Fluor 488 (Jackson ImmunoResearch, 703-545-155)
Donkey anti-Goat Alexa Fluor 568 (Invitrogen, A11057)
Donkey anti-Mouse Alexa Fluor 647 (Invitrogen, A31571)
Donkey anti-Rabbit Alexa Flour 647 (Invitrogen, A31573)
DAPI (Sigma, #D9542; 1:1000)
Used as a counterstain in all experiments

Safety warnings

Wear appropriate PPE as required by your institution.

Ethics statement

This protocol was approved by Columbia University IACUC. Please do not perform any of these procedures unless there is prior approval from the institution's animal ethics committee.

Before start

Read through protocol before starting.

Perfusion

1 Deeply anesthetize the mouse using 5% isoflurane.

2 Shave the abdominal hair and make an incision in the abdominal skin. Expose the heart by removing the rib cage, snip the right atrium, and insert a fine-gauge needle into the left ventricle.

3 Perform transcardial perfusion with ice-cold PBS until the fluids run clear—indicated by the liver and ears turning pale—ensuring proper perfusion.

4 Then, switch to fresh, ice-cold 4% paraformaldehyde and perfuse 25 mL at a flow rate of approximately 5 mL/min. During fixation, the limbs, tail, and head may move, and the mouse should become stiff if the fixation is successful.

5 Dissect the brain and incubate it overnight in 4% PFA at 4°C.

Prepare Tissue Sections

6 (**Optional**) Before sectioning, it is recommended to embed the brain in 4% agarose, as this helps stabilize the sample and ensures even slices. This is particularly useful when sectioning the whole brain, as certain regions, such as the caudal part of the hippocampus, may become separated during the process. The agarose helps maintain their position.

7 Using a Leica VT1000 vibratome, cut coronal sections at either 30 µm or 75 µm thickness, with 75 µm primarily for whole-brain 3D reconstruction. Arrange the sections sequentially in a 24-well plate with PBS for further processing.

Permeabilization

8 Permeabilize tissue sections in PBS containing 0.4% Triton X-100 (PBST) for two rounds of 10–15 minutes each.

Blocking

9 Replace PBST with blocking buffer (1x PBS, 0.4% Triton X-100, 3% BSA, and 2% Goat or Donkey Serum) and incubate at room temperature for 1 hour on a shaker.

Primary Antibody Labeling

10 Dilute the primary antibodies desired in incubation buffer (1x PBS, 0.4% Triton X-100, and 2% Goat or Donkey serum). Refer to the Materials section for dilution details.

11 Add the diluted primary antibodies to each well and incubate overnight at 4°C on a shaker, ensuring the tissue sections remain suspended in the antibody solution.

11.1 (Example) For DAT-Cre slices, use anti-TH (Mouse, ImmunoStar, #22941) at a 1:2000 dilution.

12 After overnight incubation, transfer the plate to the bench, replace the solution in each well with PBS, and wash twice for 30 minutes each.

Secondary Antibody Labeling

13 Dilute the secondary antibody in incubation buffer (1x PBS, 0.4% Triton X-100, and 2% goat or donkey serum) as per the manufacturer's guidelines.

14 Add the diluted secondary antibodies to each well and incubate overnight at 4°C on a shaker, ensuring the tissue sections remain suspended in the antibody solution.

Secondary Antibody Labeling

14.1 (Example) For DAT-Cre slices, use Donkey anti-Mouse Alexa Fluor 647 (Invitrogen, #A31571) at a 1:2000 dilution.

15 After overnight incubation, transfer the plate to the bench, replace the solution in each well with PBS, and wash twice for 30 minutes each.

DAPI Staining

16 Prepare a working DAPI solution (following the manufacturer's guidelines, Sigma, #D9542) by diluting the stock to 1:1000 in PBS.

17 Replace the secondary antibody solution with the DAPI solution in each well and incubate for 15 minutes at room temperature on a shaker, ensuring the plate is protected from light (typically by covering with aluminum foil).

DAPI Staining

18 Rinse sections with PBS for 15 minutes at room temperature on a shaker, ensuring the plate is protected from light (typically by covering with aluminum foil).

Mounting

19 Transfer sections to histology slides and mount in mounting media, avoid creating bubbles and protect from light once completed.

20 Allow mounting media to dry before imaging.