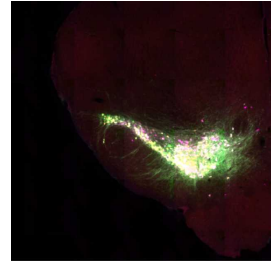


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Semi-Quantitative Histology Scoring

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We use this protocol and it's working

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ASAP

Abstract

This protocol describes our procedure for semi-quantitative scoring of midbrain mCherry histology in a cell type specific AAV-based alpha synuclein mouse model. In this model, the midbrain of DAT-Cre animals was injected with AAV encoding Cre-dependent mCherry (control group) or asyn-IRES-mCherry (synuclein group) to achieve expression in midbrain dopamine neurons. This type of scoring was not used to quantitate any readouts of pathology, but only to include/exclude animals for further physiological and/or behavioral assays. Animals were sacrificed at different timepoints after injection of AAVs, once physiological and/or behavioral assays were completed. Data from these animals was then included or excluded based on postmortem mCherry histology. This protocol includes steps to section, immunostain, image, and score brains on a 0-5 scale. Animals with a score of 2 or more on each side of the midbrain were included for additional analysis.

Attachments



[SNC_a-syn_close up.p...](#)

662KB

Sectioning Brains

- 1 After completing experiments, sacrifice animals, perform transcardial perfusion with paraformaldehyde (PFA, 4% in phosphate-buffered saline, PBS), store in PFA overnight, then transfer to sucrose (30% in PBS) for additional storage, minimum overnight.
- 2 Section brains into 30 μ m coronal sections on a freezing microtome.
- 3 Place every 6th section in PBS 1X in a 24-well plate for immunohistochemistry.
- 4 Store the remaining 5 sections as replicates in cryoprotection solution in a 24-well plate.
- 5 If immunohistochemistry is not performed the same day, cover the PBS plate and store at 4°C overnight. Store the cryoprotection plate in a -20°C freezer.
- 6 To cover the entire SNc/VTA region (Bregma -3.88 mm to -2.54 mm), approximately 7 good slices are required.

Immunostaining

- 7 Select your sections if necessary.
- 8 Wash sections 3 times with PBS 1X to remove any debris or residual solutions.
- 9 Incubate sections in 3% Normal Donkey Serum (NDS) with 0.1% Triton X-100 (NDST) in PBS 1X to block nonspecific binding sites.
- 10 Prepare primary antibodies in 3% NDS and PBS 1X: Rabbit anti-mCherry (1:500, M11217, Rat anti-mCherry clone 16D7); Rat anti-alpha-synuclein (1:300, ALX-804-258L001, Enzo Life Sciences); Chicken anti-tyrosine hydroxylase (TH) (1:1000, P40101-150, Pel-Freez Biologicals).
- 11 Incubate sections with the antibody mix on a shaker at 4°C for at least 1–2 days.



- 12 Wash sections 3–5 times in PBS 1X for 10 minutes each on a shaker at room temperature.
- 13 Prepare secondary antibodies in 3% NDS and PBS 1X: 568-conjugated AffiniPure Donkey Anti-Rabbit (1:300, 712-575-150, Jackson ImmunoResearch Laboratories) for mCherry; 488-conjugated AffiniPure Donkey Anti-Rat (1:300, 712-545-150, Jackson ImmunoResearch Laboratories) for alpha-synuclein; 647-conjugated AffiniPure Donkey Anti-Chicken (1:500, 703-605-155, Jackson ImmunoResearch Laboratories) for TH.
- 14 Incubate sections with the secondary antibody mix on a shaker at 4°C for 2 days.
- 15 Wash sections 3–5 times in PBS 1X for 10 minutes each on a shaker at room temperature.

Mounting and Imaging

- 16 Mount sections onto slides using Vectashield hardset mounting medium with DAPI.
- 17 Allow sections to dry in the dark.
- 18 Image all sections using a Zeiss Axioscan at 20X epifluorescence with 4 channels for DAPI, Alexa 568, Alexa 488, and Alexa 647.
- 19 Maintain consistent lighting and exposure settings for reliable comparisons and subsequent analysis.

Scoring

- 20 Two independent raters score each slide. Raters are blinded to whether the sections are from an animal treated with AAV-DIO-mCherry (control group) or AAV-DIO-asyn-mCherry (synuclein group).
- 21 Use software capable of opening images and adjusting contrast (e.g., Zeiss Microscopy Software or QuPath-0.5.1).
- 22 Open images with the software and adjust the intensity of mCherry staining for visualization.



- 23 Assess the percentage of neurons expressing mCherry in midbrain regions (VTA and SNc) out of the total presumed dopamine (TH-expressing) neurons.
- 24 Assign scores per side: 1 = 0–20%; 2 = 20–40%; 3 = 40–60%; 4 = 60–80%; 5 = 80–100%.
- 25 Repeat scoring on each selected section for the mouse.
- 26 Average the scores for each side.

Inclusion/Exclusion

- 27 A minimum score of 2 (20–40% of cells expressing mCherry) bilaterally is required for inclusion.
- 28 Mice with asymmetric scores (difference > 2 between sides) are excluded.
- 29 Compare results between two raters and discuss discrepancies.