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Immunohistochemistry on mouse brain sections

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Abstract

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1. Mice used for immunohistochemistry were anesthetized with 200 mg/kg Avertin and perfused with TBS/Heparin and 4% PFA.

2. Brains were collected and post-fixed in 4% PFA overnight, cryoprotected in 30% sucrose, frozen in a solution containing 2 parts 30% sucrose and 1-part O.C.T. (TissueTek), and stored at −80°C.

3. Floating coronal tissue sections of 30 µm, 40 µm or 100 µm thickness were collected and stored in a 1:1 mixture of TBS/glycerol at −20°C.

4. For immunostaining, sections were washed in 1× TBS containing 0.2% Triton X-100 (TBST), blocked in 10% NGS diluted in TBST, and incubated in primary antibody for 2-3 nights at 4°C with gentle shaking.

5. Primary antibodies used were anti-LRRK2 (Rabbit, 1:500; ab133474, Abcam), phospho-ERM (Rabbit, 1:500; #3141, Cell Signaling), Sox9 (Rabbit, 1:500; AB5535, Millipore), GFAP (Rabbit, 1:500; Z0334, Agilent DAKO), VGluT1 (Guinea pig, 1:2000; 135304, Synaptic Systems), PSD95 (Rabbit, 1:300; 51-6900, Invitrogen), VGAT (Guinea pig, 1:1000; 131004, Synaptic Systems), and GEHPYRIN (Rabbit, 1:1000; #14304S, Cell Signaling).

6. Following the primary incubation, sections were washed in TBST, incubated in Alexa Fluor conjugated secondary antibodies diluted 1:200 (Life Technologies) for 2-3 hours at room temperature, washed with TBST, and mounted onto glass slides using a homemade mounting media (90% Glycerol, 20 mM Tris pH 8.0, 0.5% n-Propyl gallate) and sealed with nail polish.

7. For DAPI staining, DAPI (1:50,000) was added to the secondary antibody solution for the final 10 minutes of incubation.

8. Images were acquired with an Olympus FV 3000 microscope.