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Mouse synapse imaging and analysis

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Abstract

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1 **Tissue Sectioning and Preparation**

1.1 - Prepare coronal sections of 30 µm thickness containing the anterior cingulate cortex (ACC) and primary motor cortex (MOp) from WT and LRRK2 G2019Ski/ki mice.

2 **Synaptic Staining**

2.1 - Perform synaptic staining using the following antibody combinations:

2.2 - Excitatory (Intracortical): VGluT1 (pre-synaptic) and PSD95 (post-synaptic).

2.3 - Inhibitory: VGAT (pre-synaptic) and Gephyrin (post-synaptic).

3 **Antibody Incubation**

3.1 - Dilute primary antibodies (anti-VGluT1, anti-PSD95, anti-VGAT, anti-Gephyrin) appropriately in blocking solution.

3.2 - Incubate tissue sections overnight at 4°C with primary antibodies.

4 **Secondary Antibody Incubation**

4.1 - Wash sections with 1x TBS containing 0.2% Triton X-100 (TBST).

4.2 - Block with 10% normal goat serum (NGS) diluted in TBST.

4.3 - Incubate sections with Alexa Fluor-conjugated secondary antibodies (Life Technologies) for 2-3 hours at room temperature.

5 **Confocal Microscopy**

5.1 - Acquire high-magnification images using an Olympus FV 3000 inverted confocal microscope.

5.2 - Use a 60x objective with 1.64x optical zoom.

5.3 - Acquire z-stack images consisting of 15 optical sections spaced 0.34 µm apart.

6 **Image Processing**

6.1 - Convert each z-stack into 5 maximum projection images (MPI), each corresponding to a 1 µm section of the z-plane, using FIJI.

7 **Synaptic Puncta Analysis**

7.1 - Analyze synaptic puncta using FIJI plugin Puncta Analyzer153.

7.2 - Identify synapses by the colocalization of pre and postsynaptic puncta (VGluT1/PSD95 for excitatory synapses, VGAT/Gephyrin for inhibitory synapses).

8 **Data Collection**

8.1 - Analyze 15 MPIs per mouse (5 MPIs per tissue section, 3 tissue sections per mouse).

8.2 - Analyze between 4 and 5 age- and sex-matched mice per genotype and condition, as specified in the figure legends.

9 **Data Handling**

9.1 - Ensure all animals appear healthy at the time of collection.

9.2 - Include all data collected without exclusions, as per experimental design.

10 Notes:

11 - Maintain consistency in tissue processing and staining protocols.

12 - Perform all image analysis using standardized settings and procedures to ensure data reproducibility.