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Mouse synapse imaging and analysis

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Shiyi Wang[1]

[1]Duke University

ASAP Collaborative Rese…

Shiyi Wang
Duke University

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Abstract

Mouse synapse imaging and analysis

1. Staining, image acquisition, and analysis of synaptic density were performed as described previously with slight adjustments.

2. Synaptic staining was performed in coronal sections (30 µm thick) containing the ACC and MOp from WT and LRRK2 G2019S$^{ki/ki}$mice.

3. To label pre and postsynaptic proteins, the following antibody combinations were used: VGluT1 and PSD95 (excitatory, intracortical), VGAT, and GEPHYRIN (inhibitory).

4. High magnification 60× objective plus 1.64x optical zoom z-stack images containing 15 optical sections spaced 0.34 µm apart were obtained using an Olympus FV 3000 inverted confocal microscope.

5. Each z-stack was converted into 5 maximum projection images (MPI), each corresponding to a 1 µm section of z plane, by using FIJI.

6. Synapses were identified by the colocalization of pre and postsynaptic puncta. The number of co-localized synaptic puncta of excitatory intracortical (VGluT1/PSD95) and inhibitory (VGAT/GEPHYRIN) synapses were obtained using the FIJI plugin Puncta Analyzer.

7. 15 MPIs were analyzed for each mouse (5 MPI/tissue section, 3 tissue sections/mouse).

8. Between 4 and 5, age and sex-matched mice/genotype/condition were analyzed for each synaptic staining combination, as indicated in the figure legends.

9. All animals appeared healthy at the time of collection. No data were excluded.