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Assessing Astrocyte Territory Volume and 3D Sholl Analysis

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We use this protocol and it's working

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Abstract

Assessing Astrocyte Territory Volume and 3D Sholl Analysis



1 ****Section Collection****

- 1.1 - Collect 100 μm -thick floating sections containing the anterior cingulate cortex (ACC) and primary motor cortex (MOp) from mice.
- 1.2 - Ensure astrocytes are sparsely labeled via PALE with mCherry-CAAX.

2 ****Imaging****

- 2.1 - Use an Olympus FV 3000 microscope with a 60x objective to acquire high-magnification images (50-60 μm z-stack) of individual astrocytes.
- 2.2 - Criteria for inclusion: Capture the entire astrocyte within a single brain section, located in layer 2/3 of ACC or MOp.

3 ****Surface Reconstructions****

- 3.1 - Analyze imaged astrocytes using Imaris Bitplane software.
- 3.2 - Generate surface reconstructions of the astrocytes.

4 ****Territory Volume Measurement****

- 4.1 - Use Imaris Xtensions "Visualize Surface Spots" and "Convex Hull" to create additional surface renders representing the territory of each astrocyte.
- 4.2 - Record the volume of each astrocyte territory.

5 ****Statistical Testing****



- 5.1 - Analyze astrocyte territory sizes from biological replicates across experimental conditions.
- 5.2 - Use a nested two-way ANOVA followed by the Bonferroni posthoc test for statistical comparisons.
- 6 ****Surface and Filament Creation****
- 6.1 - Load astrocyte images onto Imaris and create a surface representation for each astrocyte.
- 7 ****Filament Generation****
- 7.1 - Create filaments using 'Add new filament (leaf icon)' in Imaris.
- 8 ****Quantification of Complexity****
- 8.1 - Use the gear tool on Imaris to display Sholl intersections for each astrocyte.
- 8.2 - Quantify the complexity of astrocytes based on Sholl intersections.
- 9 ##### Experimental Details
- 10 #### Notes:
- 11 - Maintain consistency in imaging and analysis methodologies across experimental conditions.
- 12 - Consider biological variability and replicate experiments to ensure robust conclusions.