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Mouse astrocyte territory volume analysis

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Abstract

Mouse astrocyte territory volume analysis

1. To assess the territory volume of individual astrocytes in the mouse cortex, 100 μm -thick floating sections containing anterior cingulate cortex (ACC) and primary motor cortex (MOp) astrocytes labeled sparsely via PALE with mCherry-CAAX were collected.
2. High-magnification images containing an entire astrocyte (50–60 μm z-stack) were acquired with an Olympus FV 3000 microscope with the 60 $\times$ objective.
3. Criteria for data inclusion required that the entirety of the astrocyte could be captured within a single brain section and that the astrocyte was in layer 2/3 of the ACC or MOp.
4. Astrocytes in which the entire astrocyte could not be captured within the section or was in other layers or outside of the ACC or MOp were not imaged.
5. Imaged astrocytes were analyzed using Imaris Bitplane software.
6. Surface reconstructions were generated, and the Imaris Xtensions “Visualize Surface Spots” and “Convex Hull” were used to create an additional surface render representing the territory of the astrocyte.
7. The volume of each territory was recorded, and astrocyte territory sizes from biological replicates were analyzed across experimental conditions using a nested two-way ANOVA followed by Bonferroni posthoc test.
8. For 3D Sholl analysis of individual PALE astrocytes, we first loaded images onto Imaris and then created a surface.
9. After generating the surface of astrocytes, we created filaments using ‘Add new filament (leaf icon)’.
10. For the quantification of complexity, we clicked on the gear tool on Imaris to display Sholl intersections.
11. The number of animals and cells/animals analyzed are specified in the figure legend for each experiment.