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Abstract

Immunocytochemistry

1. Astrocyte-neuron co-cultures on glass coverslips were fixed on DIV 12 with warm 4% PFA for 7 minutes, washed 3 times with PBS, blocked in a blocking buffer containing 50% normal goat serum (NGS) and 0.4% Triton X-100 for 30 minutes, and washed in PBS.

2. Samples were then incubated overnight at 4°C in primary antibodies diluted in blocking buffer containing 10% NGS, washed with PBS, incubated in Alexa Fluor conjugated secondary antibodies (Life Technologies) for 2 hours at room temperature, and washed again in PBS.

3. Coverslips were mounted onto glass slides (VWR Scientific) with Vectashield mounting media containing DAPI (Vector Labs), sealed with nail polish, and imaged on an AxioImager M1 (Zeiss) fluorescence microscope.

4. Images of healthy astrocytes with strong expression of fluorescent markers that did not overlap with other fluorescent astrocytes were acquired at 40× magnification in red, green, and/or DAPI channels using a CCD camera.

5. Astrocyte morphological complexity was analyzed in FIJI using the Sholl analysis plugin, as described previously(https://github.com/Eroglu-Lab/In-Vitro-Sholl).

6. Statistical analyses were conducted using a custom code in R (https://github.com/Eroglu-Lab/In-Vitro-Sholl).

7. A mixed-effect model with multiple comparisons made using the Tukey post-test was used for Sholl analysis to account for the variability per experiment as a random effect to evaluate differences between the conditions.

8. The exact number of independent experiments and the exact number of cells analyzed are indicated in the figure legend for each experiment.

9. To ensure the health of astrocyte-neuron co-cultures, the peak for the number of astrocyte intersections must be greater than or equal to 25 in the control condition.

10. We imaged non-overlapping astrocytes that contained a single nucleus (DAPI stain) and expressed consistent fluorescent markers and EZRIN constructs according to the experimental conditions.