Electron microscopy (EM) and quantification

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ABSTRACT

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Electron microscopy (EM)

1

Hippocampal neurons were plated at a density of 60,000 cells/cm^2. DIV-3 neurons were infected with lentiviruses carrying α-syn tagged at the C-terminus to mScarlet, or mScarlet alone (multiplicity of infection or MOI=2.5). The transduced neurons were cultured to maturity (DIV17-DIV21) before imaging. The ~100 % infection efficiency of the mScarlet-tagged constructs was confirmed before using the coverslip for EM experiments.

2 EM embedding protocol for monolayer cultured cells was performed on ice. The coverslips were fixed in 2 % glutaraldehyde and in 0.1 M sodium cacodylate (SC buffer) buffer for at least 60min at 4 °C, washed 3 min with 0.1 M SC buffer (5 times), and postfixed in 1% osmium tetroxide (OsO4) diluted in 0.1 M SC buffer for 30 min to 1hr.

3 The coverslips were then washed for 3 min (5 times) with 0.1 M SC buffer, followed by a water rinse, sequential ethanol dehydration (20%, 50%, 70%, 90%, 2x100%) 1min each, and subsequent incubation with dry acetone for 1 min at room temperature (2 times).

4 The samples were then incubated with a Durcupan:Acetone solution (50:50%) for 1hr in rotation, followed by incubation with 100% Durcupan for 1 hr (2 times). The cells were then embedded using foil plates along with wood sticks in the embedding oven (overnight incubation).

5 60 nm ultrathin sections were cut with a diamond knife and mounted on 300 mesh grids. Grids were post-stained with 2% of uranyl acetate for 5 min and lead for 1 min. JEOL 1400 Electron Microscopy and Gatan digital Camera were used to obtain images.

quantification

6

For quantification of inter-vesicular (IV) distances between vesicles, we considered all the vesicles in the synapse encompassing 1µmx1µm. To mark each vesicle, a line was drawn across the center of the vesicle, recording its diameter and its position.

7 These parameters were then fed into our algorithm on MATLAB (RRID:SCR_001622)(http://www.mathworks.com/products/matlab/), which gave the output of IV distances for a particular image. The IV distance for all images per condition was pooled together. This algorithm is available upon request.