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Electron microscopy (EM) and quantification V.1

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Protocol status: Working

We use this protocol and it's working

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Abstract

Electron microscopy (EM) and quantification

Troubleshooting

Electron microscopy (EM)

- 1 Hippocampal neurons were plated at a density of 60,000 cells/cm². 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 postfixes 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%, 2×100%) 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 1 μ m×1 μ m. 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.