Guide to source data for: Journal of Cell Biology article DOI: 10.1083/jcb.202112101

Title: Drosophila SPG12 ortholog, Reticulon-like 1, governs presynaptic ER organization and Ca^{2+} dynamics

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Article Description:
Neuronal endoplasmic reticulum (ER) appears continuous throughout the cell. Its shape and continuity are influenced by ER-shaping proteins, mutations in which can cause distal axon degeneration in Hereditary Spastic Paraplegia (HSP). We therefore asked how loss of *Rtnl1*, a *Drosophila* ortholog of the human HSP gene *RTN2* (*SPG12*), which encodes an ER-shaping protein, affects ER organization and the function of presynaptic terminals. Loss of Rtnl1 depleted ER membrane markers at *Drosophila* presynaptic motor terminals and appeared to deplete narrow tubular ER while leaving cisternae largely unaffected, thus suggesting little change in resting Ca^{2+} storage capacity. Nevertheless, these changes were accompanied by major reductions in activity-evoked Ca^{2+} fluxes in the cytosol, ER lumen, and mitochondria, as well as reduced evoked and spontaneous neurotransmission. We found that reduced STIM-mediated ER-plasma membrane contacts underlie presynaptic Ca^{2+} defects in *Rtnl1* mutants. Our results show the importance of ER architecture in presynaptic physiology and function which are, therefore, potential factors in the pathology of HSP.

Dataset Description:
Files are organized within folders and subfolders, ordered by Figure and Figure Panel, containing progressive steps in data processing, from raw data to final version used in Figure Panels.

Original Figures shown in the article, in **.pdf** formats, were made using Adobe Illustrator or Affinity Designer from the images described below.

Confocal microscopy images:

- **.nd2** files are the raw data collected with Nikon EZ-C1 acquisition software; **.tif** files, generated with Fiji from **.nd2** files, are progressive steps in data processing.

- Each different date indicates an independent experiment. The data processing performed is described in the corresponding file name. In some cases, we provide microscopy images from additional examples and/or controls to those shown in the article.

Widefield microscopy calcium videos:

- Raw data collected with Micro-Manager acquisition software saved as multilayer **.tif** files; further steps in data processing, generated with Fiji, are also saved as multi-layer **.tif** files. These multi-layer **.tif** files were downsized from 16-bit 512x512 pixels to 8-bit 256x256 pixels in Fiji for repository upload.

- Example video files were generated by splitting and merging the GFP and RFP channels of the multilayer .tif files with the Fiji Cairn Image Splitter plugin. Further steps in video

processing were also saved as multilayer .tif files; final videos were then exported with JPEG compression as .avi files at 100 frames per second.

- Each larvae indicates an independent experiment; each larvae has its own folder, containing sub folders, one for each stimulation frequency tested. Each sub folder contains original and processed videos in **.tif** files; ROIs used in **.roi** files; time traces graphs showing raw graphs, along with graphs showing bleach corrected, delta F/F, and rolling average transformations in **.pdf** files exported from R; raw time traces data exported from R in **.csv** files; bleach corrected and rolling average transformed data in **.txt** files; extrapolated intensity and time data exported from these time traces from R in **.csv** files; and R files used to quantify this data in **.R** files.

Quantifications and statistical analysis:

- The quantifications performed are annotated in **.xlsx** or **.pzfx**, generated with Microsoft Excel or Prism; **.txt** files, generated from the **.xlsx** files, were used for statistical analysis using RStudio. All the R libraries and the specific functions used are indicated in the scripts, **.R** files. Graphs were exported as **.pdf** files from RStudio or Prism.

Analysis of nucleotide and amino acid sequences:

- .dna files were generated by SnapGene software from reference genome sequences. The subsequent annotations, selections or actions performed are described in the corresponding file name. Alignments were save as .dnaln files. Genomic maps were exported as .png files.

Electron microscopy images:

- TIFF files acquired with FEI MAPS software were imported to Fiji and aligned and reconstructed with TrakEM2 plugin. The resulting files were imported to Meshlab to generate .ply files.

All the information about each Figure can be found in the article "DOI: 10.1083/jcb.202112101" (open access).

Software

- <u>Windows OS Software</u>:
  Nikon EZ-C1 acquisition software.
  Micro-Manager acquisition software.
  FEI MAPS acquisition software.
- <u>Mac OSX Software</u>:
  Adobe Illustrator CC 2017
  Affinity Designer 1.10.0
  Fiji 2.1.0/1.53c
  Microsoft Excel 2019
  Prism 9.1.2 2021
  RStudio Desktop 1.3.1093
  SnapGene 4.2.11
  Meshlab 2022.02

File Formats:

.dna - SnapGene file containing annotated nucleotide sequence and its products.
.dnaln - SnapGene file containing an alignment of nucleotide sequences.
.fa - Fasta format
.nd2 - Nikon confocal files, open with Fiji - LOCI BioFormats.
.pdf - Layered Adobe pdf format. Keep edition capabilities from Adobe Illustrator.
.png - png image file.
.R - R script file.
.tif - tif image file. Note that some tif files are multilayered.
.txt - text document.
.xlsx - Spreadsheet file created by Microsoft Excel.
.csv - Spreadsheet file created by R (also compatible with Microsoft Excel).
.pzfx - Spreadsheet and graph file created by Prism9.
.zip - Files are zipped because their filenames contain characters disallowed on some file systems, or due to file size. You will need to decompress before accessing them.
.roi - Region of interest file created by Fiji.
.ply - 3D rendering of EM sections generated with Meshlab.