

Supplemental Material

Research Articles | Cellular/Molecular

Presynaptic vesicles supply membrane for axonal bouton enlargement during LTP

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Figure S1: Mitochondria containing boutons (previous).

All boutons containing mitochondria that were measured for this study are shown in descending order from smallest to largest surface area for control (left) and LTP (right) conditions. Each bouton is shown as a pair with its 3D rendering containing presynaptic vesicles (green), mitochondria (purple), and synapses (red) on the left and the selected region of the bouton used for quantification on the right (orange).

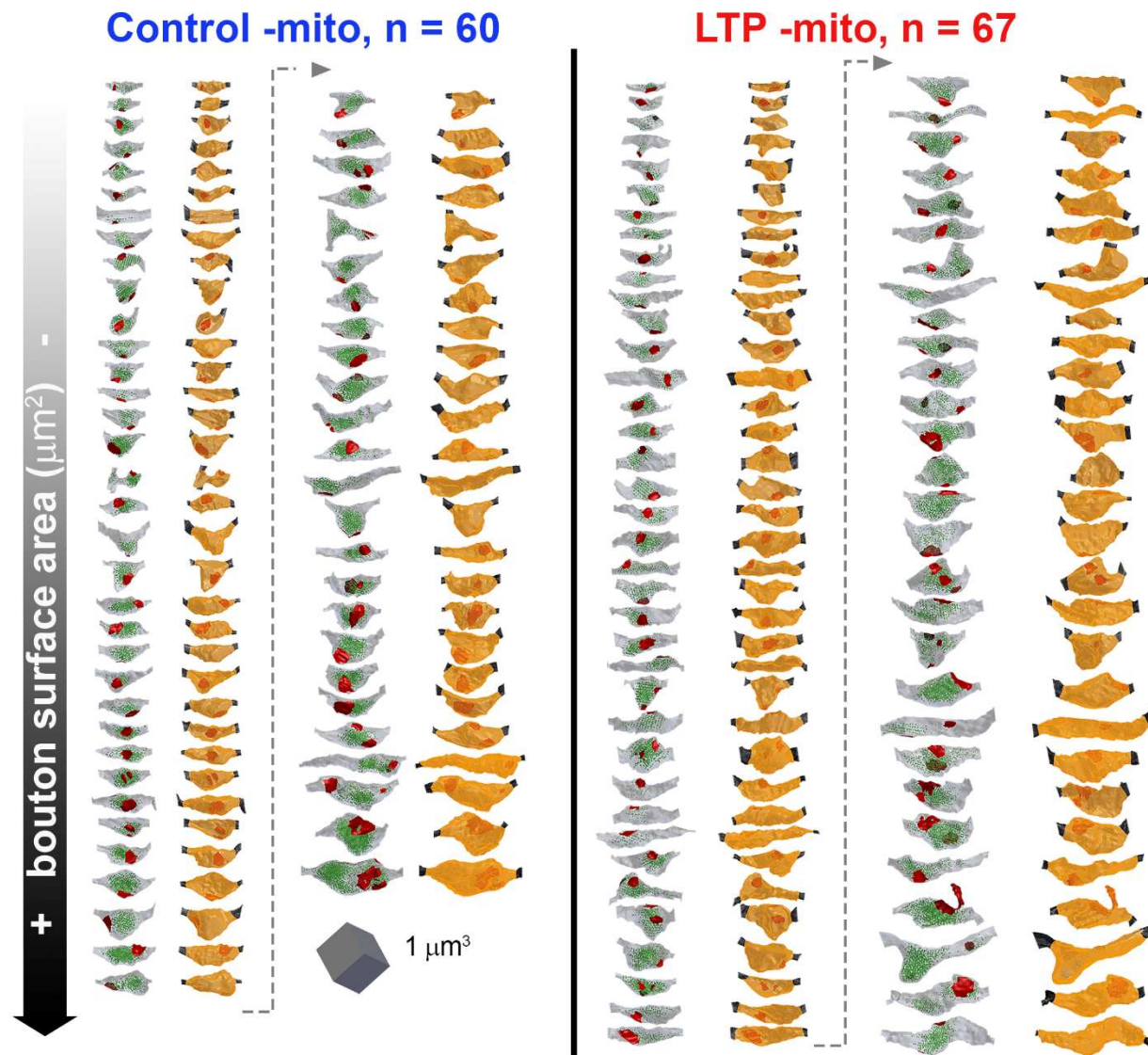


Figure S2: Mitochondria lacking boutons.

All boutons lacking mitochondria that were measured for this study are shown in descending order from smallest to largest surface area for control (left) and LTP (right) conditions. Each bouton is shown as a pair with its 3D rendering containing presynaptic vesicles (green) and synapses (red) on the left and the selected region of the bouton used for quantification on the right (orange).

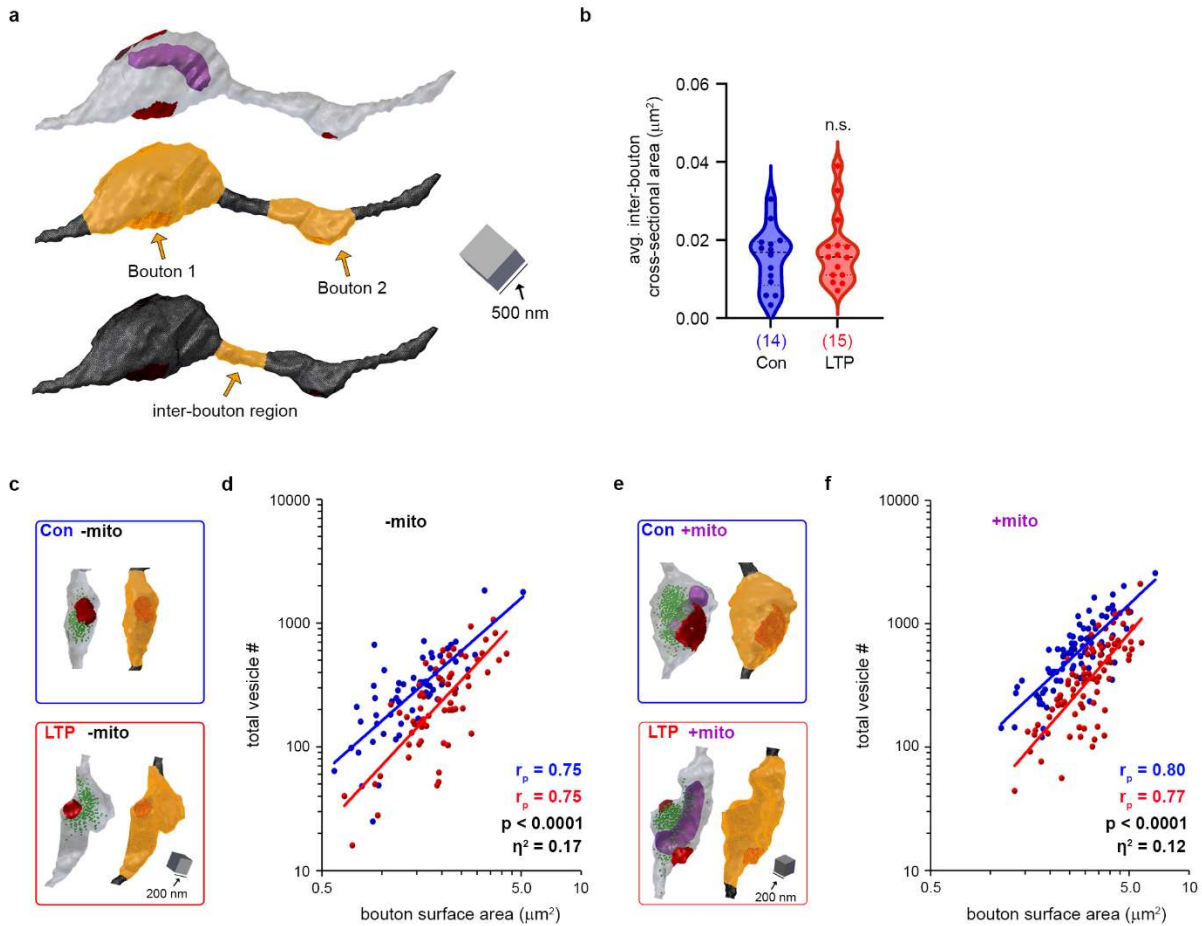


Figure S3: Boutons with and without mitochondria show vesicle loss with LTP.

a) Representative 3D reconstruction of an axonal segment containing two complete boutons (middle, orange) and the inter-bouton region (bottom, orange). **b)** The mean cross-sectional area of the inter-bouton region did not differ between control and LTP ($p = 0.81$). **c)** 3D reconstructions representing the median vesicle number and surface area of control and LTP boutons lacking mitochondria. **d)** In mitochondria lacking boutons, presynaptic vesicle numbers are also positively correlated with bouton surface area in both control and LTP conditions (Pearson's R , $p < 0.0001$). Similarly, LTP boutons lacking mitochondria have fewer presynaptic vesicles across bouton sizes compared to control, with an effect size of 17% attributed to condition (ANCOVA: $F_{\text{condition}} = 26$; $df = 1$; $p < 0.0001$; $n_{\text{Con}} = 60$; $n_{\text{LTP}} = 67$). **e)** 3D reconstructions representing the median vesicle number and surface area of control and LTP boutons containing mitochondria. **f)** In mitochondria containing boutons, presynaptic vesicle numbers are positively correlated with bouton surface area in both control and LTP conditions (Pearson's R , $p < 0.0001$). LTP boutons containing mitochondria have fewer presynaptic vesicles across bouton sizes compared to control, with an effect size (partial eta-squared) of 12% attributed to condition (ANCOVA: $F_{\text{condition}} = 23$; $df = 1$; $p < 0.0001$; $n_{\text{Con}} = 81$; $n_{\text{LTP}} = 87$).

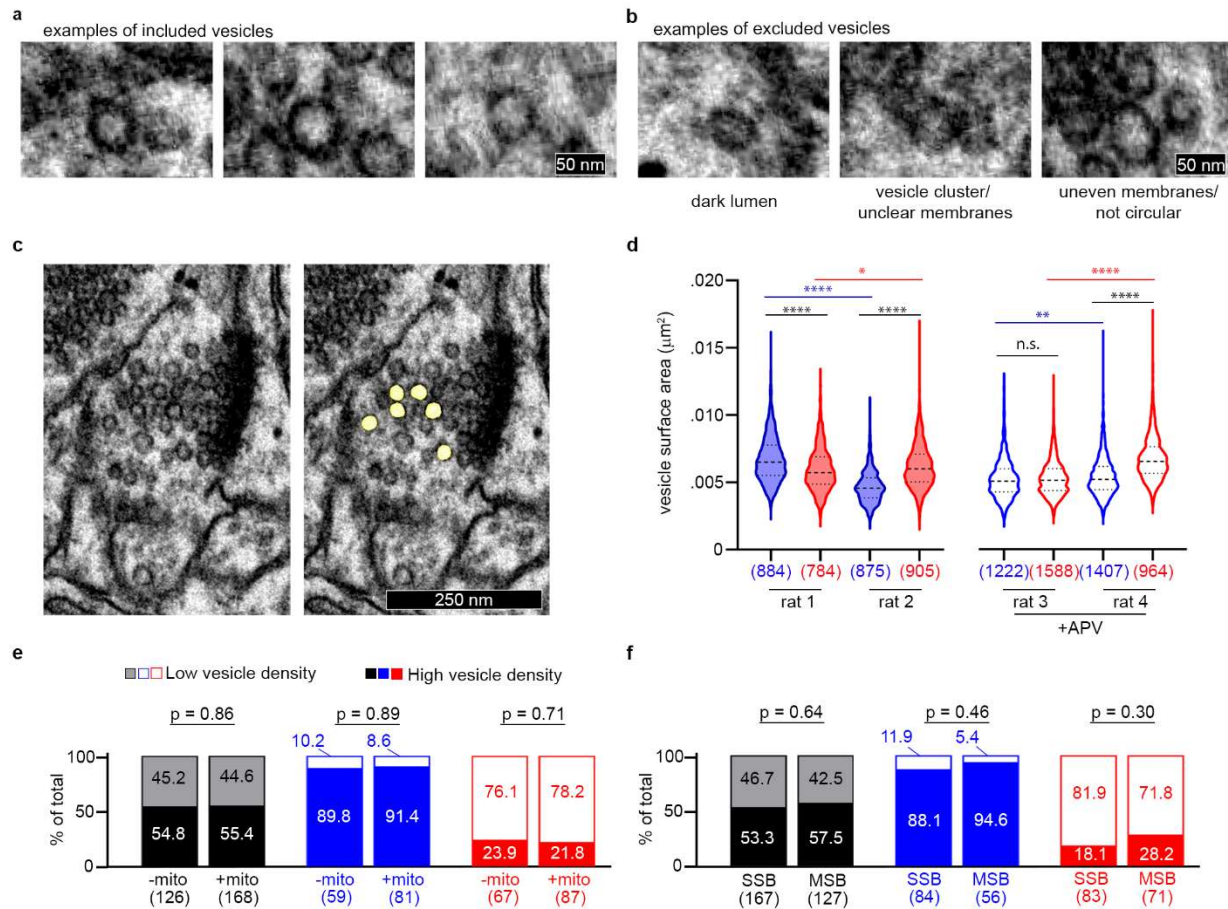


Figure S4: Vesicle surface area measurements and densities.

a) Examples of presynaptic vesicles that met inclusion criteria for segmentation: clearly distinguishable from other vesicles, circular in shape, and evenly stained membrane with clear lumen. **b)** Examples of synaptic vesicles that did not meet our criteria. Reasons for exclusion are listed below each image. **c)** Example bouton that was selected for vesicle surface area measurement. The vesicles that passed our conservative inclusion criteria and used for quantification are indicated in yellow (right). **d)** Median vesicle surface areas are shown for each series +/- IQR, with (n= number of vesicles) in parenthesis below each group. The Kruskal-Wallis test showed a statistical difference between groups ($p < 0.001$), and Dunn's multiple comparisons test was used to test differences between conditions in the same animal (black), and within condition across animals (blue= control, red= TBS). **e)** When looking at all boutons in our data set (grey/black, left), the presence of mitochondria did not affect the proportion of boutons that had high or low vesicle densities (chi-squared, $p = 0.86$). Similarly, there was no difference between -/+ mitochondria boutons in either control (blue, $p = 0.89$) or LTP (red, $p = 0.71$) conditions. **f)** Similarly, whether a bouton was single synaptic (SSB) or multi-synaptic (MSB) had no effect on the proportion of high/low vesicle densities in all boutons ($p = 0.64$), control ($p = 0.46$), or LTP ($p = 0.30$) conditions.