Mitochondria are Distributed Closer to Pre-synapses than Post-synapses

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Introduction: Mitochondria are essential organelles that generate ATP through aerobic respiration, supplying the energy required for neuronal processes such as synaptic transmission and action potential initiation (Harris et al., 2012). Their dysfunction is linked to various neurological diseases, including Parkinson's Disease, where mutations in mitochondrial dynamics-related genes contribute to pathology. Understanding mitochondrial distribution in neurons is crucial, as it can inform targeted therapeutic strategies.

Both pre-synaptic and post-synaptic compartments rely on ATP-dependent membrane pumps to maintain ionic gradients. However, pre-synaptic terminals have additional energy demands, as they must synthesize, transport, and release synaptic vesicles, processes that consume significant ATP (Rangaraju et al., 2014). Harris and Attwell (2012) estimate that approximately 55% of a neuron's total ATP is consumed within axonal terminals, suggesting that pre-synaptic regions require more energy than post-synaptic counterparts.

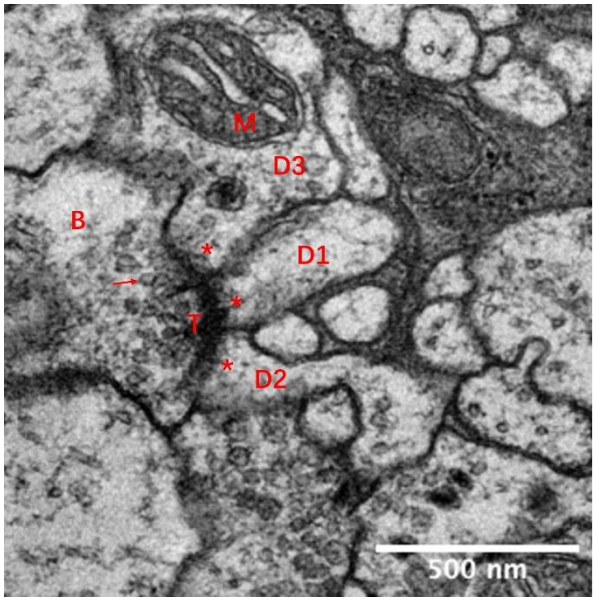
Given that mitochondrial proximity ensures efficient energy delivery, we hypothesize that mitochondria are preferentially distributed closer to pre-synaptic terminals. To test this, we analyzed mitochondrial localization in neurons and measured their distances from pre- and post-synaptic specializations. Our goal was to determine whether mitochondria are distributed statistically significantly closer to pre-synapses than post-synapses.

Method:
To analyze mitochondrial distribution, we examined electron micrographs (voxel size: $4.6\ nm \times 4.6\ nm \times 45\ nm$) from a small region of the neuropile of the third instar Drosophila larva with *Fiji* software. The sample was stained and segmented for electron microscopy.

First, we classified subcellular features in 20 micrographs (each corresponding to one slice) based on their shapes and locations. Then, we measured the areas, from which the diameters were calculated, and mean grey values of 25 dense core vesicles, synaptic vesicles, and microtubules. This characterization enabled us to distinguish pre-synaptic and post-synaptic specializations. Next, we manually traced mitochondria and neuronal structures across the 20 slices to generate 3D reconstructions. From these models, we calculated the centroid coordinates of mitochondria within 10 neurons. Using a dataset from Schneider-Mizell et al. (2016), we then quantified the distances between mitochondria and both pre- and post-synaptic terminals.

Finally, we assessed whether mitochondrial distances differed significantly between pre- and post-synaptic regions. We plotted the frequency distributions of these distances in a histogram and performed a Kolmogorov-Smirnov test to determine statistical significance. Our conclusions were drawn based on the test results and distribution patterns.

Result:
We first classified subcellular structures in electron micrographs. An example classification on



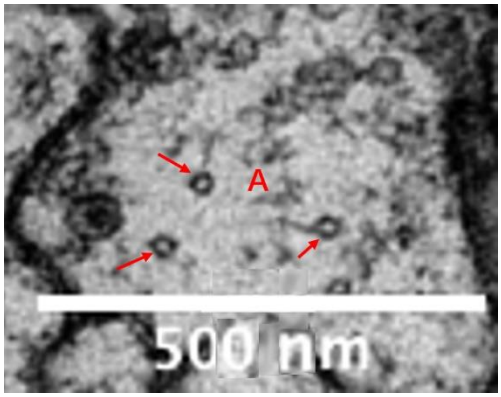


Figure 1 Example of classified subcellular features

1A: Axon terminal is forming a bouton (B) presynaptic to three dendrites (D1, D2, and D3). The synaptic density (T) and post-synaptic densities (asterisks) are darkly stained. One mitochondrion (M) is presented in one of the dendrites. See synaptic vesicles present in the bouton (arrow). Scale = 500 nm.

1B: Microtubules (arrows) distribute evenly in an axon (A). Scale = 500 nm.

one electron micrograph is shown (Figure 1). Synaptic densities and post-synaptic densities are characterized by darkly stained regions. Synaptic vesicles, which are small white circles clustering near the synapse, can be used to distinguish synaptic densities from post-synaptic ones. Axons were distinguished by the presence of microtubules, which are small white circles evenly distributed in the cell. Based on the locations of synaptic and post-synaptic densities, the pre-synapses and post-synapses can be identified. A mitochondrion, identified as a large, gray, wave-like structure, can be observed in one of the post-synaptic terminals.

Based on our classification, we measured the diameters (denoting sizes) and mean grey values (denoting electron densities) of 25 dense core vesicles, synaptic vesicles, and microtubules to quantify their distinct characteristics (Table 1). Dense core vesicles had the largest average diameter (63.6 nm) and the lowest average mean gray value (27.5), while synaptic vesicles and microtubules were more similar in size and electron density. Synaptic vesicles, however, had a slightly larger average diameter and mean gray value than microtubules. These distinctions allowed for reliable identification of pre- and post-synaptic structures, enabling precise measurements of mitochondrial positioning relative to synapses.

Feature	Dense Core Vesicles	Synaptic Vesicles	Microtubules
Sample Size	25	25	25
Average Diameter (nm)	63.6	36.7	30.6
Standard Deviation of Diameter (nm)	8.4	4.3	1.3
Average Mean Gray Value (units of intensity)	27.5	106	89.3
Standard Deviation of Mean Gray Value (units of intensity)	9.0	12	9.6

Table 1 Average and standard deviation of diameter and mean gray value for subcellular features

Using reconstructed 3D representations of each neuron and its mitochondria, we identified mitochondrial centroids and calculated their coordinates. Applying the same method to pre- and post-synaptic specializations, we then measured the distances between mitochondria and synaptic structures on a per-neuron basis.

Neuron Name	X coordinate (nm)	Y coordinate (nm)	Z coordinate (nm)
Adele	287.923	655.738	15.886
Bobolink	668.66	695.686	14.674
Cassie	870.902	919.273	15.609
EE	747.274	283.875	4.015
Figaro	942.54	568.543	9.103
Gideon	382.013	658.468	8.689
Horatio	660.913	816.524	1.378
Igor	369.682	439.942	2.257
Jackalope	812.888	535.58	12.983
Kragg	141.498	800.362	2.309

Table 2 Locations of the calculated centroids of mitochondria in 10 neurons

In the final step, we analyzed the distance frequency distributions of pre-synapses and post-synapses provided in the Schneider-Mizell et al. (2016) dataset. The frequency histogram (Figure 2) revealed that mitochondria were generally positioned closer to pre-synaptic terminals than post-synaptic ones. To statistically validate this observation, we conducted a Kolmogorov-Smirnov test, which is suitable for non-parametric distributions. The test yielded $\mathrm{ks} = 2.236$ and $p = 9.1 \times 10^{-5}$, indicating a statistically significant difference between the two distributions ($p<0.05$). Based on these results and the histogram, we confirmed that mitochondria are preferentially distributed near pre-synaptic terminals.

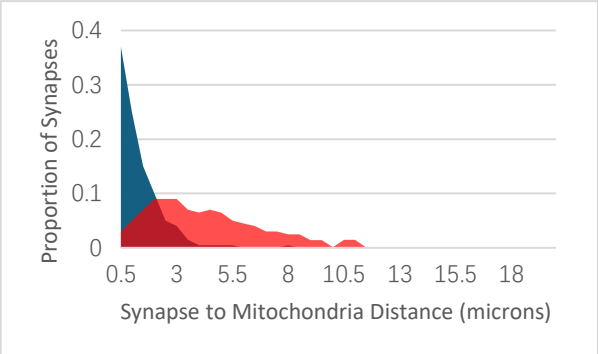


Figure 2 Frequency Distribution of Synapses based on their distance from mitochondria

The blue curve represents the distance distribution of pre-synapses, while the red curve represents post-synapses.

Discussion: Mitochondrial dysfunction is a key contributor to many neurological diseases, including Parkinson's and Alzheimer's, where impaired energy metabolism disrupts synaptic function. Understanding mitochondrial distribution within neurons is essential for developing targeted therapeutic strategies. While pre-synaptic terminals have been shown to demand more energy than post-synaptic ones, it remains unclear whether mitochondria are preferentially distributed to accommodate these energy needs. Addressing this question is crucial for optimizing interventions that rely on mitochondrial localization.

In this study, we investigated whether mitochondria are positioned closer to pre-synaptic terminals than post-synaptic ones. Our findings confirmed a significant bias in mitochondrial distribution toward pre-synapses, suggesting that mitochondrial localization aligns with synaptic energy demands. This insight enhances our understanding of neuronal energy dynamics and informs strategies for targeted drug delivery. Therapies aimed at restoring mitochondrial function should prioritize pre-synaptic regions to maximize efficacy.

References

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