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Dissecting early steps in the authophagy machinery to understand the molecular basis of neurodegeneration

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Autophagy is involved in degrading long-lived proteins such as aged/oxidized cell components including alpha-synuclein, SOD1 huntingtin in addition to organelles, such as mitochondria. Autophagy is involved in a variety of neurodegenerative disorders and the ability to detect autophagy alterations represents a seminal point in clinical anatomy. Despite such a critical role it remains unclear how autophagy starts at sub-cellular level. In detail, the ability to dissect the fine morphological correlates of the autophagy machinery remains a critical point to unravel the early molecular and morphological events in neurodegeneration.

In the present study we used transmission electron microscopy both in baseline condition and following neurodegeneration-related autophagy-dependent triggers at various time intervals. Plain transmission electron microscopy was paralleled by immunocytochemistry for LC3, beclin-1 and Rab24 to localize early autophagy structures.

The present data provide morphological evidence for the occurrence of the so-called phagophore during early steps in autophagy induction. The phagophore appears on nuclear and endoplasmic membranes as a beclin1 positive membrane bulging. We found a correlation between the number of altered mitochondria and phagophore-like labelled membranes.

These data suggest that immunogold-based TEM provides a useful tool to detect early autophagy activation during neurodegeneration allowing to dissect early vs late autophagy compartments such as autophagolysosomes and stagnant autophagy vacuoles.

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Key words
Autophagy, PC12 cell line, immunoelectron microscopy.