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Long time-lapse imaging reveals unique features of PARK2/Parkin-mediated mitophagy in mature cortical neurons

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Keywords: : mitochondria Parkin PARK2 lysosome autophagosome autophagy depolarization mitochondrial mobility neuronal mitophagy mitochondrial membrane potential mitochondrial quality control

This article refers to:

Mitochondria are essential organelles for neuronal function, development and survival. Throughout a neuron's lifetime, aged and damaged mitochondria undergo dynamic recycling via fusion/fission or are ultimately eliminated via mitophagy, an autophagic pathway specific for mitochondrial degradation. Dysfunctional mitochondria not only produce energy less efficiently, but also release harmful reactive oxygen species and initiate apoptotic signaling cascades, which have been linked to the pathogenesis of several major neurodegenerative diseases. Proper sequestration of damaged mitochondria into autophagosomes and subsequent degradation within the lysosomal system constitute a key cellular pathway in mitochondrial quality control mechanisms. Mutations in PINK1 and PARK2 are linked to autosomal recessive early onset Parkinson disease. Recent studies demonstrate that the PINK1-PARK2 pathway mediates mitophagy, and that PARK2 translocation onto mitochondria induces mitochondrial degradation via mitophagy in several non-neuronal cell types where PARK2 is overexpressed, such as in HEK293, HeLa, MEF and neuroblastoma cells. However, evidence nd degrada missing or controve gy has some unique f focused on addressi (2) whether this tran ochondrial mobility geted mito on through the auto

First, we us to observe ndrial membra pled mitocho be observed. We estal s such that they survive long enough to exhibit PARK2 translocation. CCCP treatment for 24 h

induces 26.67% of neurons to undergo PARK2 translocation onto depolarized mitochondria. Co-treatment with CCCP and lysosomal inhibitors (LIs) results in a doubling in the percentage (55.87%) of neurons with PARK2 translocation. Thus, proper lysosomal function is critical to avoid the accumulation of PARK2-associated mitochondria in neurons upon dissipating $\Delta\psi_m$. Second, we examined PARK2 translocation kinetics by imaging neurons at various time points during CCCP treatment. PARK2 translocation between 0.5–6 h was exceptionally rare, occasionally observed as early as 12 h, and became increasingly frequent after 18 h of CCCP treatment. We further imaged the dynamic recruitment of endogenous PARK2 to mitochondria in mature neurons following CCCP/LIs treatment. To confirm this, we alternatively isolated the mitochondria-enriched membrane fraction from mature cortical neurons following the same treatment. While the majority of PARK2 is present in the cytosolic fraction, mitochondrial depolarization results in the association of substantial levels of endogenous PARK2, along with autophagic markers LC3-II and SQSTM1/p62 with the mitochondrial membrane. These results consistently indicate the recruitment of both endogenous and exogenous PARK2 onto depolarized mitochondria in mature cortical neurons.

Next, we utilized a $\Delta\psi_m$-sensitive dye, TMRE, to examine whether PARK2-targeted mitochondria display dissipated $\Delta\psi_m$. Healthy mitochondria will sequester and accumulate TMRE, thus resulting in a higher TMRE fluorescence intensity. In control neurons,
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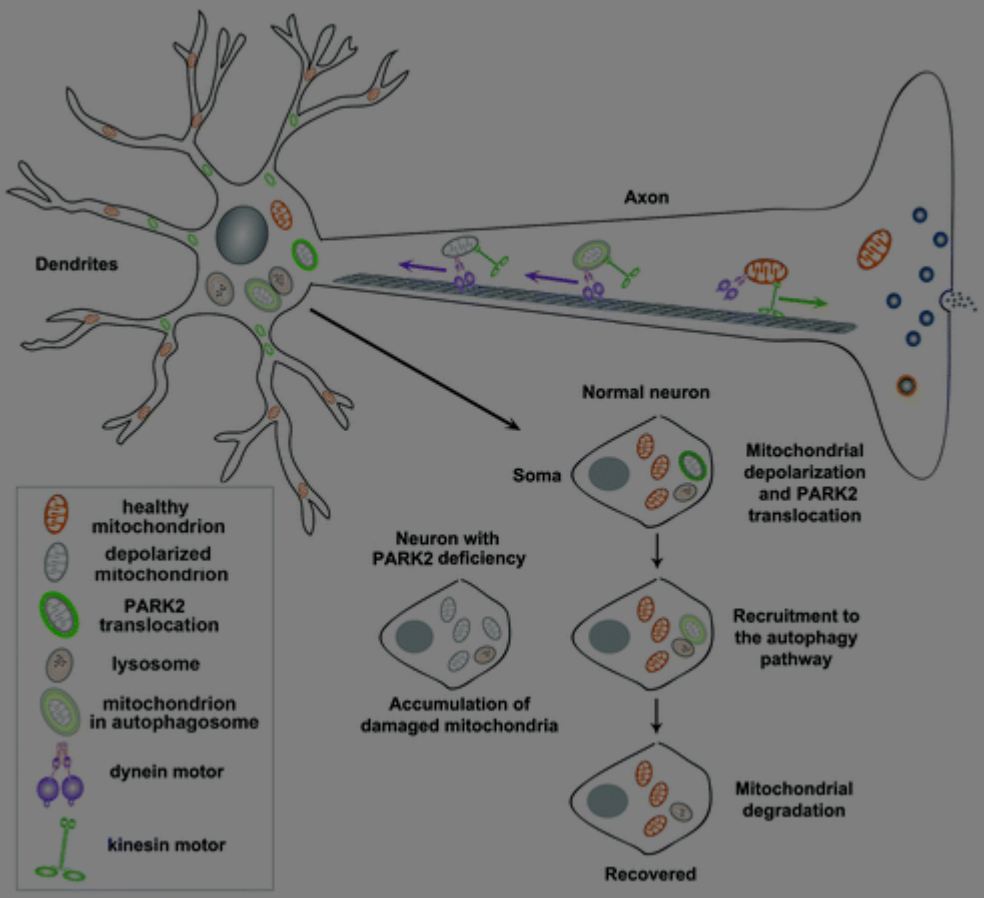
be attributed to the unique distribution pattern. PARK2-tagged depolarized

mitochondria are restricted to the soma for degradation, where lysosomes are predominately located, while healthy mitochondria are distributed distally to support synaptic functions. To test this hypothesis, we utilized syntaphilin, an axonal mitochondria docking protein, to artificially immobilize mitochondria in distal processes. To our surprise, we found that PARK2 is recruited to stationary mitochondria anchored by syntaphilin in distal processes. Therefore, the PARK2-mediated process prevents dysfunctional mitochondria from traveling peripherally, leading to their accumulation in somatodendritic regions. In addition, our long time-lapse imaging exhibits dynamic PARK2 recruitment and degradation of depolarized mitochondria within the autophagy-lysosomal system. CCCP exposure results in LC3-labeled ring-like structures surrounding fragmented mitochondria in the somadendritic regions and enhances the recruitment of mitochondria to lysosomes for degradation in live cortical neurons.

In summary, our study reveals several unique features of PARK2-mediated mitophagy in mature cortical neurons. First, PARK2 is selectively recruited to depolarized mitochondria to form ring-like structures, a process occurring more slowly than in non-neuronal cells. Second, following 24 h CCCP incubation, PARK2 translocation only occurs in a small percentage of neurons. Third, PARK2 translocation is restricted to the somatodendritic regions, where mature lysosomes are predominantly located. This spatial and dynamic process allows neurons to efficiently eliminate dysfunctional mitochondria via the autophagy-lysosomal pathway (Fig. 1).

Figure 1. control via PARK2-m itochondria to form a oximal dendritic distal dendritic al transport of mitoc ased. Alter while healt ARK2-targeted gions, where mature l cess allows neurons ysosomal pathway mination of dysfunct



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